Equine Protozoal Myeloencephalitis 2012

Course materials. A PDF copy will be provided upon request. The links to topics are provided. An overview of our work is found in

http://pathogenes.com/blog/2012/12/06/the-death-of-the-neuronacentric-model-of-epm/

1. Introduction and some basics
   a. An introduction to the disease equine protozoal myeloencephalitis. A major point is the definition of EPM as a clinical diagnosis. A second point is that EPM is a syndrome that involves protozoal infection and inflammation. Effective treatment must address both issues.  

2. There are 2 studies, full effectiveness INAD 012092 decoquinate/levamisole HCl for the treatment of clinical signs associated with S. neurona encephalomyelitis and INAD 012219, Levamisole HCl for the treatment of inflammatory encephalitis associated with EPM due to Sarcocystis neurona myeloencephalitis. CE Participants are encouraged to choose the treatment, licensed or investigational, that makes sense to them based on their experiences. The forms for EPM trial:
   http://www.pathogenes.com/clinical_trials/clinical_trial_documents

3. Definitions…learning the language
   a. There are some misconceptions about EPM that are based on semantics. This section will provide definitions that will help with interpreting literature.

Licensed therapy for EPM

INAD 012092 INAD 012219

Pilot effectiveness
4. Five myths that render EPM incomprehensible and untreatable
   This section will list the five myths that render EPM incomprehensible and illustrate why these ideas are perpetuated using peer reviewed literature.
   http://pathogenes.com/blog/2011/10/28/one-thing/

5. Myth 1: Prevalence of *S. neurona* is high but disease is low
   The prevalence of *S. neurona* antibodies in horses versus the prevalence of equine protozoal myeloencephalitis is discussed. It is important to recognize when the antibody is related to disease. EPM horses are a subset of horses with antibody. Inflammatory encephalitis is a disease in horses that are a subset of EPM horses.
   http://pathogenes.com/blog/2011/06/15/27003-horses-have-epm/

6. Myth 2: Many strains of *S. neurona* cause disease
   a. There are those that believe that there are limited strains that cause disease and those that think there are many strains, many unidentified, that cause disease. The best test to facilitate treatment decisions depend on your view on this topic. We believe there are few pathogenic phenotypes and determining a titer to SAG 1, 5, 6 can facilitate treatment. When inflammatory encephalitis is recognized, treatment decisions are simple.

7. Myth 3: *Sarcocystis neurona* is neurotropic
   This section will review older data across species to illustrate all *Sarcocystis* induce neurological signs in the definitive and intermediate hosts. Therefore, *S. neurona* behaves like any other *Sarcocystis* and is not unique. It is the host response to the parasite that can be genetically determined and the reason that some horses get “EPM” and most don’t.
   http://pathogenes.com/blog/2011/06/13/what-makes-s-neurona-different/

8. Myth 4: Horses have a genetic predisposition for EPM
   The use of data from published in vivo models as well as sophisticated molecular tools will be used to illustrate that should horses have a genetic predisposition toward EPM it would be in the adaptive immunity of the host.
   http://www.pathogenes.com/about_epm/epm_interactive_page

9. Myth 5: Disease is due to parasites in the central nervous tissues
   a. This position will be supported using literature that describes challenge model infections and immunological data post treatment.

10. A decision tree is used to illustrate a treatment strategy for a horse with signs consistent with EPM.
    a. EPM is a rare disease but a suspect-EPM horse is common diagnosis. Sometimes ascertaining a response to treatment is a reasonable objective. *Sarcocystis neurona* leaves
some horses with inflammatory disease (encephalomyelitis). Inflammatory encephalomyelitis is present in a subset of horses that have or had *S. neurona*, but can follow viral, bacterial, or protozoal infections. We are concerned with inflammatory encephalomyelitis (IE) as a result of *S. neurona* infections. Inflammatory encephalomyelitis can be treated with levamisole HCl for horses (INAD 012219, undergoing FDA approval). Horses with infections due to *S. neurona* are identified by specific antibody tests that yield a titer or an increasing titer to specific antigens. A discussion of our ELISA test is available at: [http://pathogenes.com/blog/2012/04/29/figuring-it-out/](http://pathogenes.com/blog/2012/04/29/figuring-it-out/)

b. Decision tree:

Exam shows signs compatible with EPM:
Treat with an anti-protozoal drug (Marquis, Protozil, Orogin®). Only Orogin® has an immune modulator to address inflammatory encephalomyelitis. Treat acute disease immediately with Orogin® and institute further diagnostics (C-reactive protein) if the titer is <8. Look for a response to treatment with Orogin®, apparent in the 10 days of treatment. If the titer remains <8 ten days after treatment, seek an alternative diagnosis. A positive treatment response with Orogin® does not rule out inflammatory encephalomyelitis.
Treatment response

No treatment response, look for an alternate diagnosis or prostaglandin mediated disease. Mild response or relapse within 4 weeks—treat for inflammatory encephalomyelitis. Clinical assessment in 4 weeks and repeat SAG 1, 5, 6 titer in 8 weeks and 6 months.

Serum C-reactive protein (CRP) can indicate a positive treatment response to IL6 mediated (levamisole responsive) inflammatory encephalomyelitis. CRP values are normal at 0-4 µg/ml, high normal 7-10 µg/ml, and elevated at > 10 µg/ml. CRP, when successfully treated, will drop in 2 weeks. CRP is a non-specific inflammatory protein, thus additional causes can result in elevated values.
EPM strategy:

11. Current limits to our understanding of the pathogenesis of *S. neurona* infections
   a. The issues that halt further understanding and treatment of EPM include research funds, patents, and economics. The changing scientific platform is one that demands discovery and commercialization of intellectual property. Even teaching colleges require funds through invention and licensing. An avenue for continued discovery is the use of field veterinarians in the licensing of products rather than a few institutions.
   b. [http://pathogenes.com/blog/2012/01/08/chicken-or-the-egg/](http://pathogenes.com/blog/2012/01/08/chicken-or-the-egg/)

12. The limits and benefits of diagnostic tests
   a. The commercially available diagnostics are compared and contrasted using published literature.
   b. [http://pathogenes.com/blog/2012/07/02/arsenal/](http://pathogenes.com/blog/2012/07/02/arsenal/)
13. “Managing relapses”
   a. The key to managing relapses is recognizing when the clinical signs are due to infection (protozoa) and inflammation (secondary to infections). Infection is detected by the presence of IgG. Treatment with licensed anti/protozoal drugs decreases the antibody in 70% of the animals. A decrease in antibody is detected in 93% of decoquinate-treated horses. Inflammation is part of the pathogenesis of EPM-syndrome. Inflammation is best controlled during treatment with a protocidal drug. Once the infectious cause of the process resolves, approximately 10% of horses will have recurrent inflammation. Inflammation is an innate, non-specific reaction to infection (as opposed to antibody mediated reaction) and in some animals, once stimulated, it is a continuing issue. That means that the presence other inflammatory stimuli will result in clinical signs (one confounding infection we are investigating is intestinal parasites). Vaccination is associated with "relapse" in 0.03% of cases. De-worming can result in "relapse" signs.
   b. Generally, relapsing horses will manifest the same signs as if focal areas are more sensitive to inflammatory molecules. Chronic relapsing horses will require a longer treatment (with levamisole) to resolve inflammation. The horses that we have discussed with veterinarians are eventually weaned from treatment. A few horses were treated for several months.
   c. http://pathogenes.cm/blog/2012/02/20/the-road-to-recovery/

14. Treatment success
   a. The etiology of EPM is S. neurona. The goal in treating a horse with EPM is elimination of the parasite. Another goal is recognizing inflammatory disease.

15. Managing chronic exposure, prevention strategy by vaccination
   a. Chronic exposure can be managed with decoquinate.

16. Rehabilitation
   a. The rehabilitation of horses that have clinical signs and those horses that have neurological deficits due to inflammation will be reviewed. There are no published references for this section and veterinarians will be encouraged to provide input.
   b. http://pathogenes.com/blog/2013/01/rehabilitation/

Method of recording participation  The participation will be documented as part of the FDA protocol database. A determination of inclusion/exclusion for a horse in the field trial will not be the basis for participation in the continuing education portion of the program. The satisfactory documentation of once case by inclusion of 2 tested samples and communication logs will be the method to record completion of the outlined program.
Developing patent protected technologies for the diagnosis and treatment of disease since 1999

Siobhan P. Ellison DVM PhD
EPM 2012
EQUINE PROTOZOAL MYELOENCEPHALITIS

Understanding EPM
developed by Dr. Ellison/Pathogenes Inc.

Identify, treat, and prevent EPM in horses
Goals

- Principal based understanding of EPM
- Email any questions to sellison@pathogenes.com
- Topics
  - Our strategy
  - Understanding the semantics
  - The role of inflammation in EPM
  - Treatment of parasites and inflammation
  - Rehabilitation
  - Cure, re-infection, relapse
Siobhan P. Ellison graduated from UF

- BS 1972 Agricultural microbiology
- MS 1974 *Equine Leptospirosis and Recurrent uveitis*
  - Periodic ophthalmia – chronic relapsing inflammation
- DVM 1983-current - practice equine medicine Ocala, Florida
- PhD 1998-2000 *Clone and Express S. neurona SAG 1*

- Founded Pathogenes, Inc. in 1999 — EPM Strategy
  - Developed *S. neurona* antibody tests using SAG 1, 5, 6 genes
  - Developed Equine Model to produce EPM in horses
  - Used model to test antiprotozoal drugs -- efficacy
  - Used model to test efficacy of SAG 1 vaccine
  - Re-discovered Decoquinate as an effective therapy for EPM
  - Field study to test the efficacy of decoquinate/levamisole in horses
  - Recognized the role of inflammation in EPM
Pathogenes develops *patented* tools for a treatment strategy.

1- *antibody detection test* that identifies phenotypes 1, 5, 6 of *S. neurona* that infect horses.
2- *rapid detection of antibody* for point of care decisions—treat quickly
3- *decoquinate/levamisole* treats inflammation and parasites
4- *prevention* following treatment

A necessary part of treatment and prevention strategy is *immune modulation*. 
Peer Reviewed Publications

Ellison, SP., Witonsky, S. Evidence that antibodies against recombinant SnSAG1 of Sarcocystis neurona merozoites are involved in infection and immunity in Equine Protozoal Myeloencephalitis. Ca J Vet Res 2009 July:73(3): 176-183


We're basically agreed; let's not quibble over semantics.

EPM: Horse with parasites in CNS (could be obtained by culture).

Presumptive EPM: Horse with neurological signs
   Response to treatment

Sarcocystosis has signs of disease and Sarcocystiasis does not.

Sarcocystiasis: Sarcocystis (neurona, falcatoria, fayeri) in gut or organs
   infection and induces immune response but no signs

Inflammatory encephalitis due to immune reaction (cytokine) to infections

Phenotype: Mutually exclusive display of SAG 1, SAG 5, or SAG 6  Horse only “sees” phenotype

Genotype: Phenotype & variable SAG 2, 3, 4, and microsatellite markers

S. neurona: One of 3 phenotypes, or 6 genotypes or 12 antigen types

Strains: Defined by genotype  Horses can’t distinguish genotype

Virulence: Infects horses, neurovirulent can invade CNS
EPM is an uncommon neurological disease of horses in the United States and South America. *Sarcocystiasis* is a common infection in horses—horses commonly have antibodies.

Inflammation causes the signs associated with EPM.

USDA has strict definitions for EPM, diagnostic tests, kits, and antibody tests. There are no USDA licensed EPM diagnostic tests.

Since 1970 EPM cases have been reported in 48 States.

Horses are infected by the parasite when ingesting hay, feed or grass contaminated by opossum feces.
Our strategy to identify, treat and prevent disease

Identify horses that have or are at risk for EPM
Identify presence of antibody in serum

Identify phenotype of S. neurona causing EPM
ELISA – quantitative
Point of care identification at 1:20 dilution

Treat parasite and inflammation for response
Signs and antibody= institute treatment
Treatment response in horses in 5 days or less

Vaccinate
phenotype specific
prevents inflammatory encephalitis
not commercially viable

Prevention to stimulate protective immunity
phenotype independent
triazine shown not to prevent infections
Sarcocystosis-disease

- Morbidity of EPM is 0.88% of the population (63,360 horses) with *active disease* that need treatment/year.

Frank Andrews LSU

Sarcocystiasis-exposure

- 62% of the equine population (4,464,000) have *infections* and are at risk to develop EPM.

USDA 2001 Consensus statement

**Antibodies against *Sarcocystis* in Normal Horses**

- Mixed: 11%
- Negative: 11%
- SnSAG1: 29%
- SnSAG5: 11%
- S. falcatula: 38%
Two antigen types are responsible for most of animal disease. Only SAG 1 and 5 phenotypes cause EPM in horses. *Sarcocystis neurona* is defined by 35 genotypes, 12 antigen types and three (phenotypes).

Drug treatment is difficult unless the phenotype is identified. Treatment fails in triazine resistant strains. Peptide 1, 5, 6 ELISA’s identify phenotypes and can assist therapy decisions.

Genotyping involves sequence of SAG’s (1, 3, 4, 5, 6) 9 microsatellite markers, and 33 microsatellite types. SAG’s 2, 3, 4 are common to all *Sarcocystis*
It takes **10x** more triazine drugs to kill **SN2** and **SN138** strains than the **SN6** genotype.

Severe clinical EPM cases are 92.8% **SnSAG 1** phenotypes.
Pathogenes model
- used SnSAG 1 phenotype
- induced moderate to severe disease 93% of horses (n=75)
- *S. neurona* SnSAG 1 enters CNS recover by isolation
- All animals produced a titer that increased over time

- 93% of the published isolates from horses are SnSAG 1 phenotypes.
  - Neurovirulent in the horse
- Studies confirm the resistance of some SAG 1 and SAG 5 strains to triazine drugs. Field trial, experimental trials, *in vitro*

- Published study showed the laboratory effectiveness of vaccination against rSnSAG 1 challenge prevents the clinical signs of EPM
Ohio model
- used SnSAG 5 phenotype (SN138)
- induced mild disease in the transport studies
- *S. neurona* SnSAG 5 didn’t enter CNS in their studies
  - Inflammatory encephalitis associated with *S. neurona*
  - Some horses resolved infections by the end of the trial

Only 7% of the published isolates from horses are SnSAG5 phenotypes. SAG 5 phenotypes were neuro-virulent in those 7%

Studies by Furr showed 40% of horses on Marquis therapy (5 mG/kG) prior to infection were not protected against disease.
SnSAG 6

- Not yet found in horses...a hint that cross-protection exists?

- Responsible for epizootic in sea otters killing 1/3 of the California population in 2004

- Horses have antibodies to SnSAG 6

- Some horses with pure SnSAG 6 antibody evidence of infection are ataxic and these horses respond to treatment
Phenotype SnSAG 6 is prevalent in horses. Nov. 2010

Phenotype Distribution in EPM Horses

- SAG1 & 5: 14%
- SAG6: 31%
- SAG1, 5 & 6: 17%
- SAG1: 24%
- SAG5: 9%
- SAG1 & 6: 5%
Antibody titer increase over time correlates with active disease.

No vaccine is on the market yet, not commercially viable

Effective therapy can induce protective immunity

Treatment with FDA licensed drugs fail in 61% of cases

Successful treatment with FDA licensed drugs is less than 25%  Rob MacKay UF

Relapse is 10% in horses successfully treated with FDA approved drugs. Some say parasites may encyst…feeding horse tissue to opossums did not result in oocyst shedding—(Cutler UF)
Peptide ELISA detects the presence of antibodies, three phenotypes of *S. neurona*, in serum

**ELISA Results for 8 horses with signs of EPM**

- SnSAG1
- SnSAG5
- SfSAG6

**Clinical exam is the most important factor for identification of EPM and treatment efficacy for EPM suspect horses**

There is no statistical advantage to determine active infections by CSF analysis.

The Peptide ELISA test determines:

- presence of parasites by phenotype
- triazine resistant infections
- pyrimethamine/sulfa resistant strains
- endpoint to treatment
- outcome
- prevention strategy

Clinical exam is the most important factor for identification of EPM and treatment efficacy for EPM suspect horses.
Pathogenes Multiplex *Sarcocystis neurona* Antibody Detection Strips
detects antibodies to three phenotypes of *S. neurona*

Fill the pipette with the serum sample.

Express the sample into the buffer tube and mix.

Hold the strip at the blue end and add the test strip to the buffer tube. Leave the strip in the tube for 20 minutes.

Remove the strip after 20 minutes and make sure the control line is present.

Components of the test are two strips, two tubes with buffer, and two tiny pipettes. A serum sample is obtained from the horse.
Sarcocystis neurona antibody detection strip, serum transferring pipette, and buffer tube are needed to run the test. Make sure the control line is present after the test is run.

Use the second strip if the control line is absent.

1. To read the test, align the strip with image in 1.

   Line up the control line.

   If there is a line that corresponds with the black arrow, the test is positive for SAG 6 antibodies.

   If there is a line that corresponds to the blue arrow, the test is positive for SAG 5 antibodies.

   If there is a line that corresponds to the red arrow the test is positive for SAG 1 antibodies.
EPM drugs comparing efficacy, treatment duration and safety

Decoquinate/levamisole in 150 horses with no adverse events.
Legend is an 11 YO gelding that went from severe ataxia to good with 10 days treatment with Oroquin-10.

This horse had complicating inflammatory encephalitis.
Trial results for 141 horses

The “60%” Group

Chronic disease
Unresponsive to Triazine or pyrimethamine/sulfa

Levamisole therapy alleviates signs in this group
Six percent, with mild signs, opted for low dose treatment. There were 7.7% of horses that responded to anti-inflammatory drugs (DMSO, steroids, or levamisole) after day 10 indicating that inflammatory encephalitis is a component of disease.
Levamisole has a place in the treatment of EPM if parasites are effectively killed

Ongoing trials: 012092 & 012219

Enrollment criteria 012092:
Veterinarian trained on the protocol—complete EPM 2012 course
Horse has antibody 1, 5, or 6 and clinical signs of EPM

Enrollment criteria 012219
Veterinarian trained on the protocol—complete EPM 2012 course
Horse has inflammatory encephalitis—“relapse” with conventional treatment
Identify horses that have or are at risk for EPM
Identify presence of antibody in serum

Identify phenotype of *S. neurona* causing EPM
Peptide 1, 5, 6 ELISA –quantitative
Point of care identification at 1:20 dilution

10 day therapy
Signs and antibody= institute treatment
Treatment response in horses in 5 days or less
Identify inflammatory encephalitis

Vaccinate
phenotype specific
prevents allergic encephalitis

Prevention by low dose therapy goal is by stimulating protective immunity
phenotype independent
**Decision Tree**

1. **EPM suspect**
   - ELISA Titer
     - **Orogin**
       - OK within 10 days
       - ELISA titer 4-6 weeks
         - Re-exposure possible? Prevention
           - Antibody still elevated
             - Low dose prevention
               - Antibody decreases, slow imp.
                 - Levamisole, Quest
               - no progress, plateau, worse
                 - DMSO IV, Steroids, Levamisole
                   - Inflammatory Encephalitis, vaccinate
               - 13%
             - Continue diagnostics
               - No clinical or Ab change
               - 3%
We are working on the diagnosis and mechanism of inflammatory encephalitis in EPM

Inflammatory encephalitis—diagnostics and mechanism of disease

CRP

Encysted parasites—and the effects on inflammation
Pathogenic protozoa

Equine Protozoal Myeloencephalitis
  - EPM = parasites + INFLAMMATION

Three unique phenotypes: SAG 1, 5, 6
  - SAG 1 & SAG 5 are responsible for the majority of animal disease (Wendte 2011)
Genotypes (strains)
- defined by 35 molecular markers
- Can’t discern genotype clinically

Phenotypes are defined by 6 surface antigens
- SAG 1, 5, 6 are unique to *S. neurona*
- Discern phenotypes by antibody ELISA
Antibody is made to phenotypes

- Antibody is detected by serum antibody test
- SAG 1, 5, 6 ELISA is reported as a titer
- A change in titer can identify active disease
- Antibody can indicate resistance to treatment

- 17 years was dedicated to the parasite...and none to inflammation
S. Neurona induces inflammation

- Signs of EPM are due to inflammation
  - Established in 1985, proven in 2001-2007
- Inflammation in CNS +/- parasites
- Inflammation can be reversed quickly
  - Inflammation is mediated through several pathways
    - Prostaglandin mediated inflammation responds to NSAID’s and steroids
    - S. neurona induces an alternate inflammatory path so NSAID’s won’t work well
- Remove parasites
  - Requires a protocidal drug
- Stop inflammation with levamisole
- Chronic inflammation
  - Inflammation longer than 30 days
  - Also responds to levamisole—takes longer to reverse inflammatory disease
Is S. Neurona neuroinflammation mediated through the cytokine IL6?

Circumstantial evidence:

We determined that levamisole treatment works even in chronic, relapsing cases of EPM.

Levamisole $\rightarrow$ IL6?
These results confirm that treatment with ponazuril at 5.0 mg/kg minimizes, but does not eliminate, infection and clinical signs of EPM in horses.

Identify the cause and then treat inflammation

- Test to identify parasite antibody SAG 1, 5, 6
  - Eliminate the cause of disease
  - Remove parasites with a protocidal drug
- Test to determine level of inflammation
  - C-reactive protein ELISA
  - Modulate the immune response with levamisole
Our theory is that S. Neurona induces IL6 mediated neuroinflammation that is sensitive to levamisole.

We’re still up in the air on a few details...
...but more drug is not better
CRP is an acute phase protein
Production is stimulated by IL6 cytokine
Normal 7.4 µ/ml; High is over 10 µ/ml
Not specific to *S. neurona*
IL6 is a pro-inflammatory cytokine
IL6 works in the periphery
IL6 crosses into the brain (receptor mediated)

IL6 is inversely related to the neurotransmitter serotonin

Neuroinflammation
Inflammatory Encephalitis

PHARMACOLOGY AND EXPERIMENTAL THERAPEUTICS VOL 327:316-323
Serotonin is released from the pre-synaptic neuron where it crosses the synaptic cleft and attaches to the post-synaptic receptors.

Serotonin is recycled in the cleft by SERT, serotonin transporter protein.

The presence of serotonin is necessary for expression of IL6, low levels induce and higher levels decrease IL6.

Net effect is high IL6 is pro-inflammatory
We looked the other way, at the other half of the equation. Ellison 2010
Aminorex binds SERT, the transporter protein, releasing serotonin to the post synaptic neuron and an increase in serotonin which turns off IL6.
* In order to determine the effects of levamisole on parasites, in the horse, one must look at aminorex, *not* levamisole.
Not so fast, my friends…

Serotonin acts as a negative feedback molecule on autoreceptors located on the pre-synaptic neuron. They decrease the production of serotonin when it is too high.

Levamisole is an indirect serotonergic agonist in the horse, its effects are immediate, within hours.
Our tools that help with the diagnosis and treatment of EPM

- SAG 1, 5, 6 ELISA
  - Multiple testing
- CRP ELISA
  - Measures C-reactive protein that is increased in response to IL6 inflammation
  - Multiple testing
- Decoquinate/levamisole - EPM
- Levamisole - neuroinflammation
Seeking FDA Approval

- INAD 012092
  - decoquinate/levamisole tablets
  - Patent protected
- INAD 012219
  - levamisole tablets for horses
- Field efficacy trials are ongoing
  - Contact us to enroll an animal into our field trials
Do not go where the path may lead,
go instead where there is no path and leave a trail.

Ralph Waldo Emerson
EPM 2011

Many groups of scientists have researched *Sarcocystis neurona* and EPM. Each group has added new knowledge to form a big picture of infections and the relationship to disease. Until recently, the pieces didn’t seem to fit into one picture and that leaves different groups of scientists with polarizing ideas.

We are exploring the antigenicity, pathogenicity, and molecular traits of *Sarcocystis neurona* in horses. We also explore the horse’s reactions to infections beyond antigens. Our results give us new insights into past research. Hindsight, as you know, is 20-20.

**About Hosts**

Realizing and proving that the opossum was a definitive host for *S. neurona* was a big step forward in EPM research, attributed to Clara Fenger, University of Kentucky. Researchers could focus on sporocysts, which are infective to horses. Sporocysts used in challenge experiments were important in order to determine the intermediate host(s) and complete Koch’s postulates for EPM. For example, nude or immunodeficient mice (ID mice) are susceptible to *S. neurona*. The lab strain isolated from horse with clinical EPM and the sporocysts from feral opossums, when compared, were 99.8% the same genetically. One important gene of *S. neurona* was merely 0.2% different than the same gene in *S. falcatula*. Once the genetic similarity was determined, intermediate host susceptibility took center stage. Surprisingly, the intermediate host specificity for *S. falcatula* includes budgies, *not* immunodeficient mice. The biological difference, the ability to infect one host and not another, is a founding principle in pathogenic protozoal identification. Intermediate host specificity highlighted important biological differences between the two closely related parasites. The idea that *S. neurona* was identical to *S. falcatula* was wrong. Twenty years later, the molecular differences between *S. neurona* and *S. falcatula* remain complicated, but distinct. Antigenic differences are complicated and confound our understanding of EPM.

**Papers in Review**


Equine protozoal myeloencephalitis was recognized in horses in the 50’s and S. neurona was isolated in 1991. The cultured S. neurona enabled Clara Fenger, then a student at the University of Kentucky, and her associates to compare the in vitro material to sporocysts obtained from wildlife. Fenger’s group used molecular markers of Sarcocystidae to suggest a definitive host would be in the dog family. (It was later discovered that S. neurona does infect dogs–there are ongoing investigations to determine if S. canis could be S. neurona). These markers were used to identify S. neurona in feces and intestinal digest of wildlife specimens. These authors concluded that the opossum was involved in EPM. Aflutter with excitement over her new and important data, Dr. Fenger visited another research group. Perhaps a little too optimistic about the character of the average scientist, she neglected to procure a confidentiality agreement. Sharing lunch and ideas, she unwittingly revealed her findings which were immediately put to use by her colleagues, allowing them to review their work in light of hers and giving them the distinction of suggesting that S. neurona cycled between opossums and birds. The incident left scars and is probably partly responsible for the independence maintained by scientists involved in EPM research.

The race was on to experimentally induce EPM from opossum oocysts. Fenger and her co-workers used wild caught opossum oocysts to infect horses and induce clinical signs of EPM. The infections didn’t result in organisms in the CNS. They failed to complete Koch’s postulates because they failed to re-isolate S. neurona from the CNS of an experimentally infected horse. They described ataxia in the challenged horses and inflammatory lesions that did not include the organism. Likewise, S. falcata (Florida) failed to induce EPM. There were no antibodies detected in the S. falcata experiments when the samples were tested by the Western Blot (EBI, KY). The field was open to those that could identify sporocysts as S. neurona, produce sufficient numbers for challenge experiments and isolate the organism from the CNS of a horse. This task remains unfulfilled to this day. What followed was gathering data about the intermediate host range of S. neurona and the realization that the opossum harbors more than S. rileyi, S. neurona, and S. falcata as was thought in 1997.

Dr. Fenger and her group identified an outbreak of EPM in 12 of 21 horses on a farm. She found “EPM may develop as an epizootic. Fenger reported subtle clinical signs that were originally considered unimportant that ultimately progressed to obvious neurologic signs.” She co-patented the use of pyrimethamine and trimethoprim-sulfamethoxazole for the treatment of EPM. Also, she reported “adverse effects associated with EPM treatment (pyrimethamine and trimethoprim-sulfamethoxazole) included worsening of neurologic signs, anemia, abortion, and leukopenic and febrile episodes.”
From these papers we found the roots of some long held ideas. The body of work contributed by CK Fenger and her associates (1994-1997) identified the opossum as the definitive host of S. neurona, a cornerstone in this field. They were able to recognize that subtle signs are important in horses with EPM. They recognized that disease is not isolated to an individual horse when sufficient infectious material is present. It is expected that farms with one case of EPM will have others. Most important, in hind sight, is realizing there are CNS lesions associated with clinical signs but no parasites.

Despite recognizing and reporting the initial subtlety and ubiquity of EPM, there is a long standing belief by some that only a few horses are susceptible to infections, presumably due to some defect of their immune systems that allow them to succumb to disease. Sharon Witonsky, in conjunction with Pathogenes used our research model to show that the parasite itself can manipulate the equine immune system. The parasite uses strain specific down regulation measured by proliferation responses. Our research showed that any horse is susceptible to infection using pathogenic strains. Most horses have mild infections that can resolve. Our work also shows that the level of challenge for a horse is low, in the thousands, not millions of organisms, as used in most studies. We also observed that infected horses have a statistically significant rise in titer the longer the infection continues. The higher the titer, the longer the infection. It is beneficial for the diagnosis of EPM to show that a horse has a two to four fold rise in titer in conjunction with signs consistent with EPM.

Fenger's group presented the proof that opossums are definitive hosts for S. neurona and they clearly believed that S. neurona and S. falcatula were synonymous. They conducted an experimental challenge in horses using oocysts derived from opossums fed sparrows, hosts of S. falcatula. We now know S. neurona and S. falcatula are not the same molecularly or biologically. Foals in their study immuno-converted on immunoblots post challenge and demonstrated clinical signs consistent with EPM—this observation was the opposite of the University of Florida S. falcatula (Florida) infection challenge in which horses did not seroconvert, again based on Western blot (EBI, KY). In 2010 we determined that S. falcatula (Florida) displays a surface antigen that is genetically identical to a surface antigen of S. neurona.

Experimental infections of these horses with oocysts were, at best, a mixed oocyst population challenge because wild caught opossum oocysts (these oocysts could have S. neurona or S. falcatula) were added to the challenge dose derived from sparrows. There is no definitive evidence that S. neurona was present in this challenge study, they reported immuno conversion on immunoblots, this in contrast to the UF S. falcatula study and indicates S. neurona may have been present . They did prove, by bird challenge (budgie), that S. falcatula comprised some, if not all, of the oocyst population that they used. They demonstrated inflammation and saw clinical signs of ataxia.

In hindsight, we know that some S. falcatula strains could be confused with S. neurona on immunoblots. These confounding S. falcatula strains may infect horses, induce antibodies, and share surface antigens that are almost identical to one phenotype of S. neurona. Fenger’s work illustrates that some S. falcatula strains induce ataxia in horses, but S. falcatula doesn’t cross into the central nervous system. An alternate view is that they introduced S. neurona from the wild caught opossums using a mixed oocyst challenge. A challenge with a small number of S. neurona oocysts could also support their results and based on the work of others is the most likely scenario.

Repeated exposure of horses in an environment contaminated with S. neurona is highly likely. Dr. Fenger’s work supports the idea that it would take very few oocysts to infect horses, ataxia would be seen clinically, and
no organisms would be present in the central nervous system, although there would be lesions consistent with inflammation. It is apparent that treatment for organisms that do not enter the CNS would differ from those that do and that the pathology of the neural tissue inflammation is crucial to our understanding of EPM in the horse.
We have been working with *Sarcocystis neurona* in the laboratory for 12 years. Of course, EPM was a concern in my ambulatory equine practice for many years before I went back to the bench. At that time I thought *S. neurona* caused infections and disease (aren’t they the same thing?) and Western blot could help me with a diagnosis and treatment.

The following definitions have such specific meanings it’s probably good to jot them down.

EPM has three definitions depending on who you are talking to: EPM for government validation “Gold Standard”: Equine protozoal myeloencephalitis is a disease defined by the center of veterinary biologics (CVB) as *Sarcocystis neurona* in the central nervous system of an equine displaying ante mortem neurological signs attributed to lesions in one or more regions of the central nervous system. For a diagnosis, the organism must be isolated from the CNS. Isolation of the pathogen is post mortem evidence that the horse had EPM. Less stringency is used by clinicians at university equine hospitals. It is EPM for referral clinics “Parasites or PCR”: Diagnosticians include postmortem confirmation of *S. neurona* infection by microscopic identification, immunohistochemistry, culture, or polymerase chain reaction (PCR). Examples of referral clinics are university equine hospitals. Also included are histopathological lesions consistent with EPM, the tissues don’t have *S. neurona*, but specific inflammatory lesions that are similar to those found in classical disease are used for the diagnosis. This criteria is used in all the Ohio model infections and many experiments that examine antibodies in “diseased” animals. Then there are the rest of us, EPM for practicing veterinarians or “clinical EPM”: Suspect EPM horses are surviving animals that have neurologic signs consistent with asymmetric or multifocal central nervous system lesions or both for which other likely differential diagnoses were excluded. Ancillary diagnostics used by practicing veterinarians can include serum testing, CSF testing, and response to treatment. These horses survive.

We all know that the etiologic agent of EPM is *Sarcocystis neurona*. Only antigen type I or II are involved in EPM, this was reported by Wendte (2010). It is the major evidence that gives the SAG 1, 5, 6 ELISA credibility to indentify infections, determine drug resistance, and response to treatment. What is important is that a diagnostic test for EPM isn’t available. To be a diagnostic test the test must be licensed by CVB. Licensing a diagnostic is a long and complicated procedure that can not be achieved for EPM, mainly because-in our opinion-EPM’s signs result from inflammation and not the actual parasite. A diagnostic can be licensed to identify antibody in a sample, but it isn’t economically viable. Antibody tests (that detect antibody to *S. neurona*) do not have to be licensed. These tests are “validated”. Validation is a strict set repeat tests between and in laboratories.

An antibody test can discern infection, infection is sarcocystosis caused by *Sarcocystis*. Infections with *S. neurona*, that results in antibody production, may lead to EPM—the presence of clinical signs are the key factor. How often sarcocystosis leads to EPM is interesting. Remember protozoal myeloencephalitis is restricted to disease and must have parasites in the CNS to be called EPM. There are 14 cases of EPM for every 10,000 horses. This is the prevalence, the number of animals with the disease. There are 7,200,000 horses in the United States. The incidence is the number of animals that are inflicted during a specific period of time.
Sarcocystosis is defined as a visceral infection that causes a measurable immune response in horses. Equine sarcocystosis is caused by *S. fayeri*, *S. bertrami*, *S. neurona*, and some strains of *S. falcatula* (Florida, but not Cornell). Ohio State University was key in documenting sarcocystosis during some of their infection work. Equine sarcocystosis is pretty common. Equine sarcocystosis produces antibodies that are measured by tests such as IFAT, SAG 2, 3-4 ELISA, and Western blot.

Response to treatment is a marked improvement after specific treatment for EPM as assessed by a veterinarian. In some cases, a reduction in the presence of antibodies is included in a response to treatment. This is on label claims for triazine drugs.

The identification and development of the surface antigens, specifically SnSAG 1, was the topic of my PhD thesis at UF. Dan Howe at UK also worked on SAG’s, he developed a different set of molecular markers that were important to Western blots. SAG’s are surface antigens. The SAG’s, 1, 5, and 6 are three unique, mutually exclusive, immunodominant surface proteins of *S. neurona*. The SAG’s distinguish the antigen type (phenotype) of the organism and are unique to the strains that display each protein. The phenotype markers SnSAG 1, SnSAG 5, and SnSAG 6 are intraspecies markers for *S. neurona*. The SnSAG’s 1, 5, and 6 are not antigenically cross-reactive. This can be a bit confusing, similar size SAG’s look identical on Western blot tests, and that led some people to think SAG’s are cross-reactive. But intraspecies markers are not cross-reactive.

Antigens can look similar because they are indistinguishable using some tests. And, to complicate things, organisms have common, or shared, antigens. These shared SAG’s are surface antigens that are common to all organisms in the genus *Sarcocystis*. Shared SAG’s include SnSAG 2, 3, and 4. Shared SAG’s are inclusive of the genotyping SAG’s, do not distinguish phenotype, and are antigenically cross-reactive between species, phenotypes, and strains.

*Sarcocystis falcatula* have SAG’s as well. Some are unique. It is the common, or shared, SAG’s that get confused between tests. The shared SAG’s are interspecies markers because they have highly similar nucleic acid sequences and are antigenically identical, close enough so a horse can’t tell them apart. That makes them cross-reactive.

Phenotype denotes visible traits. *Sarcocystis neurona* has distinguishing traits, the ones we use are molecular and can be identified by antibody reaction. Phenotype is defined as antigen type and is represented by one of the mutually exclusive SAG’s that classify *S. neurona* strains into one of three groups: SnSAG1, SnSAG5, or SnSAG6. Phenotypes are antigenically unique and indicate some virulence and drug resistance factors that occur between strains.

A finer marker is genotype and it is defined as the nucleic acid sequence that further sub-divides phenotypes of *S. neurona* into strains. Sequence level analysis of five SAG’s and nine microsatellite (MS) markers identify 12 antigen types and 33 MS types yielding a total of 35 genotypes. (The genotype designations were originally numbered for their appearance in Sea Otter deaths.) SnSAG 1 strains can be either genotypes II or VI, SnSAG 5 strains can be genotypes I, III, or V, and SnSAG 6 are genotype, IV. The majority of *S. neurona* strains are classified as Ag type I or II. *All equine infections are either Ag type I or II.*

SAG 1, 5, 6 ELISA testing uses recombinant proteins representing the phenotypes of *S. neurona* (SAG’s 1, 5, and 6) and determines the antigen type of the infecting organism in horses, dogs and cats. Sea mammals can be
tested as well. Mixed infections-this is common—are detected by our test. Opossums have mixed infections. Opossums shed a mixture of oocysts so it’s no surprise that horses get several infections at the same time. The limits of the SAG ELISA are to phenotype. A 2-4 fold increase in titer 3-4 weeks apart indicates active infection. That would be sarcocystosis. The presence of antibody does not guarantee parasites are in the CNS (and as proven by the Ohio experiments, clinical signs make no guarantees either).

Other IgG tests include SAG 2-3-4 ELISA, IFAT, Western Blot. These commercial tests detect sarcocystis infections in horses. The limits of these tests are to the genus Sarcocystis. A 2-4 fold increase in titer can indicate active infection with S. neurona or S. falcatula, or possibly release of antigen due to cyst degradation (S. fayeri). No phenotype determination or identification of mixed infections are determined using these tests. Active infection is not distinguished from cyst degradation. Drug resistance can’t be determined using these tests.

Anti/protozoal drug susceptibility is the susceptibility of S. neurona strains to the killing effects of antiprotozoal drugs measured by in vitro and in vivo assays. There are differences in the efficacy of antiprotozoal drugs that are demonstrated by dose and phenotype.

![S. neurona Phenotype Distribution in 9 Horse sera. The results can influence successful treatment strategy.](image-url)
Hindsight, they say, is 20-20. Re-viewing the EPM literature can result in a paradigm shift that changes ones way of thinking about the disease. The universally accepted paradigm of EPM can change by reviewing the literature. A shift in our opinions about EPM and it’s treatment resulted in the vast majority of treated horses improving in days. The purpose of this section is to introduce some commonly held beliefs and illustrate an alternative interpretation of the peer reviewed data. A cursory understanding of some molecular biology techniques and a willingness to review published data is all it takes to form your own opinions. Understanding these topics is the key to unlock the mysteries of EPM.

Many statements are true, however it is the relevance between the true statements upon which we build our understanding, a true/true and related statement.

There are five concepts that are readily accepted about EPM—so accepted that they are urban myths. The five myths that deserve scrutiny are: 1) The prevalence of *S. neurona* is high but disease is low; 2) Many strains of *S. neurona* cause disease; 3) *Sarcocystis neurona* is neurotropic; 4) Horses have a genetic predisposition for EPM and 5) Disease is due to parasites in the central nervous system.

The purpose of the following topics is to show the true/true, unrelated ideas that are pervasive (and unhelpful) when confronted with a horse with signs of EPM. Changing the perspective on these five issues makes EPM not only comprehensible, but highly treatable.

For example, most horses (when tested by Western blot tests) have antibodies detected in the serum (true)—these are interspecies tests. *Sarcozystis neurona* can be isolated from horses with EPM, with difficulty—post mortem—thus it is one etiologic agent of EPM (true). The majority of horses that have signs consistent with EPM have antibodies (when tested by Western blot tests) to *S. neurona* (true). Lesions most often associated with EPM are inflammatory—serum antibody tests don’t detect inflammation.

The subjective Western blot test detects both non-specific and specific antigens to *S. neurona* therefore it is expected that any group of horses would have antibodies to *S. neurona*. Why do only a few of these horses succumb to EPM? Why don’t all horses with antibodies and clinical signs have lesions with parasites in the CNS? Why do some horses without antibodies respond to treatment? The logical conclusion is while the set horses that have *S. neurona* antibodies detected by Western blot are numerous, this test doesn’t define the set of horses with EPM. The point isn’t that the Western blot is deficient as a diagnostic test for EPM but rather, why isn’t it a good test?

A most obvious answer is that EPM (disease) is more than infection by *S. neurona*. Can an antibody test be useful to manage EPM cases?

The name *Sarcozystis neurona* implies that it is neurotropic—meaning that the organism readily enters the CNS where it causes damage to these sensitive tissues. Does the rare occurrence of disease indicate that there are a few, particularly rare strains that are responsible for disease? Or is it the horse, some horses are genetically pre-disposed to get EPM due to some deficiency of the immune system? And, once in the CNS, do
the parasites cause career and sometimes life-threatening lesions that renders treatment useless? Is it possible that there are two diseases? Or, a syndrome, two aspects of one disease? How does the answer to these questions change our treatment approach?

*Sarocystis neurona* myeloencephalitis is rare. Protozoal myeloencephalitis by its name indicates inflammation is significant to the pathogenesis of disease. Why is the inflammatory component of the disease ignored in the treatment plan? Inflammation (a histological diagnosis) is always mentioned in papers that examine tissues from horses with EPM. In fact, EPM is so linked to inflammation, that a diagnosis “consistent with EPM” is based on histological evidence of inflammation in the absence of parasites. If one were to make a distinction between cases with parasites actually detected in the CNS (the definition of EPM) versus inflammatory encephalitis, then we propose subsets of horses would be identified that require different treatments. Two subsets of EPM suspect animals may be those with protozoal infections and those with inflammatory disease.

An effort to identify *S. neurona*-induced inflammatory encephalitis versus other causes of inflammatory encephalitis requires that all disease causing *S. neurona* strains are identifiable and that strains or species of *Sarcocystis* that don’t cause disease are excluded from the analysis. This distinction can’t be made using interspecies antigens (antigens that are shared among *Sarcocystis* species). If there are non-pathogenic species of *Sarcocystis* (produce no disease) that induce antibody production, interspecies antibody detection has no value.

The idea that *S. neurona* is neurotropic, that means it has a predilection to enter the CNS, has its roots in the name of the strain associated with EPM. And with that mind set, it makes sense to treat protozoa with drugs that enter the CNS. *Sarcocystis* reportedly can enter the CNS of their intermediate hosts. If there is nothing special about *S. neurona*’s proclivities, then what is the role of inflammation in EPM? Inflammatory lesions are far more common when compared to protozoa-associated lesions in the EPM literature. What if inflammation is the primary mediator of the signs of disease?

The inflammatory response to pathogens elicit tissue injury. There are several interconnecting mechanisms of inflammation, the most familiar may be the potent mediators of inflammation that are derivatives of arachidonic acid. One familiar principal pathway of arachidonic acid metabolism is the cyclooxygenase (COX) pathway that responds well to steroids and NSAID’s. A lack of response by an EPM horse to steroids and NSAID’s indicates that an alternative inflammatory path is involved in the pathogenesis of disease. Acute inflammation and chronic inflammation may respond differently to treatment. Chronic inflammation can indicate the inflammatory response is out of proportion to the threat it is faced with or is directed against inappropriate targets. In the first case, the result can be more damage to the body than the agent itself would have produced. The clear research focus on the pathogen rather than treating obvious acute and chronic inflammation present in cases of EPM has denied many horses useful life.

Treating inflammation without a clear understanding of the inflammatory mechanisms involved in the underlying pathology of disease is as unproductive as treating EPM with anti-protozoal drugs has been. Adding NSAID’s to FDA approved anti-protozoal drugs is not effective. Understanding the role of inflammation in EPM can not be under-emphasized.
Major points: 1. The relationship between antibody to *S. neurona* and EPM requires specific antigen tests. 2. Recognizing inflammation as a significant part of EPM pathology that causes signs of disease is key to successful treatment and management of the EPM horse.

3. Reconciling long held beliefs can change the EPM paradigm.
EPM is a clinical diagnosis

Equine protozoal myeloencephalitis (EPM) is a devastating disease that has confused researchers and veterinarians for over 20 years. Most importantly, EPM is a clinical diagnosis made by a veterinarian, there are no diagnostic tests to definitively diagnose disease. The most common diagnosis for neurologic disease in horses in the United States is EPM—it’s an epidemic! Less than 17,000 horses are cured and relapses are common. The disease costs many millions of dollars to the horse industry—and that was before the release of Marquis. The average cost of Marquis is $3000.00, but many horse owners report the costs are far higher.

So what makes us think that we have solved the EPM problem? Remember the scene in the movie City Slickers where Billy Crystal’s character, Mitch, is alone with Curly, played by Jack Palance? Curly is giving Mitch some life advice.

Curly: “Do you know what the secret of like is?” (holds up one finger) “This.”

Mitch: “Your finger?”

Curly: “One thing. Just one thing. You stick to that and the rest don’t mean s***.”

Mitch: “But, what is the ‘one thing’?”

Curly: That’s what you have to find out.

One thing about EPM

We set out to understand EPM. Once we understood the role and types of S. neurona antibodies in horses, we understood a great deal about EPM. Antibodies are made by the body in response to foreign substances. The merozoite stage of S. neurona produces highly specific foreign antigens, called SAG’s that are unique enough to be used like fingerprints. These fingerprint antigens are SAG 1, SAG 5, and SAG 6. Each merozoite only has one of the 3 SAG’s (they are mutually exclusive) but mixed infections are very common. The presence and levels of antibodies induced by the SAG’s are detected by SAG 1, 5, 6 ELISA. A rise in titer, generally a 2 to 4 fold increase, indicates active infection. A decrease in an antibody titer indicates that the parasite is gone and this can be measured after appropriate therapy. Even an active S. neurona infection is not EPM because EPM is a clinical diagnosis, there have to be signs associated with infection. However, an S. neurona infection can induce clinical signs that are consistent with a clinical diagnosis of EPM. It isn’t just semantics.

The ACVIM consensus statement (J. Vet Intern Med 2002; 16:618-621) states that “a clinical diagnosis of EPM is currently best established in horses that have neurological abnormalities consistent with EPM and that have a positive immunoblot test on an uncontaminated CSF sample, in which lameness or other causes of neurological disease can be excluded...Finally, a favorable response to treatment, especially when subsequently followed by a relapse of similar clinical signs, is also supportive of a diagnosis of EPM in the living horse.” They are saying to rule out everything else as a diagnosis for the horse with neurological signs, identify antibody to S. neurona (they indicated a CSF sample had to be used but they didn’t have the specific antigens SAG 1, 5, or 6 to evaluate back then—our tests don’t require CSF), and then a response to treatment—with subsequent relapse. Again, our work with decoquinate/levamisole has clarified that a relapse is perhaps a
misinterpretation of the disease. The use of decoquinate/levamisole revealed an inflammatory component to the disease that needs to be treated. So far, horses with an inflammatory encephalitis need longer treatment—3 weeks or more in some cases, but with levamisole at a dose that targets the mechanism of disease.

Our one thing—we check the serum titers on horses suspected of having EPM. We recognize that the EPM horse has antibodies to SAG 1, 5, or 6 and the levels of antibody increase over time. That means the horse will change from negative to positive on the Peptide ELISA. A horse with signs of EPM due to S. neurona will show a rise in titer—and we determine the titer. A higher titer indicates how long the horse has had disease, not the severity of the exposure.

**Five Myths that defined EPM**

Veterinarians have been long told that EPM is a prevalent disease that is hard to diagnose. Some horses have a genetic predisposition to infection from one of many strains. The organism, S. neurona is neurotropic, that means it has a predilection for the central nervous system of the horse. These are five myths that have confounded the understanding of this disease.

The prevalence of EPM is unknown. The fact is that we don’t really know what the exposure, infection rate, or disease due to S. neurona is in the equine population. The answer to the question of prevalence of infection and determining active disease requires species specific antigens that discriminate between sarcocystis sp. that cause disease and those that don’t. All active S. neurona infections don’t result in EPM. The belief that EPM is prevalent came from Western Blot data and that test relies on interspecies antigens to detect antibodies. Also, confusion between EPM and active S. neurona infections adds to the myth. Recently it was shown that the morbidity of EPM is 0.88% of the equine population and that value hasn’t changed in many years (Frank Andrews, LSU). Horses have antibodies to S. neurona commonly, but don’t show clinical signs in every infection. Many horses resolve S. neurona infections without showing signs. Horses are re-exposed and each challenge increases the chances of clinical signs that are due to an inflammatory response. Infections don’t always progress to EPM (parasites in the CNS). Based on S. neurona specific SAG 1, 5, and 6, EPM is an uncommon disease and S. neurona infections that don’t progress to EPM can cause clinical signs. There is more to EPM than S. neurona infection.

A second myth is that EPM is hard to diagnose. Most veterinarians can identify a horse with clinical signs consistent with EPM. Most of these horses, as well as asymptomatic horses, have antibodies when non-species specific tests are used—so these tests aren’t helpful. Horses with clinical signs of EPM, that have increasing antibodies to SAG 1, 5, or 6, and those that respond to effective treatment can confirm the diagnosis of EPM.

Some veterinarians believe that horses that get EPM are predisposed to get the infection. I don’t believe it. I base my view on two things. First, I can infect any horse with my model of infection. Any ages, breeds, or sex of horse, and I use the same challenge for all of them, acquire EPM using my model. I ran a study for a company was looking at a microchip that would detect genes activated in infections. Their premise was that certain equine genes are turned on (up-regulated) by disease and these are putative disease specific markers. The hypothesis was false for EPM, (we used 13 horses of various backgrounds). The up-regulated genes were just the ones you’d expect: IgG, cytokines, and genes that regulate inflammation. There were no selective markers to differentiate horses with EPM. The hypothesis wasn’t even true for acute versus chronic disease, the “blinded” gene examiners were unable to determine which horses were challenged and which horses were controls when
presented with the genetic data alone. In another study, we found a non-specific marker for EPM that was consistent, and that was serum amyloid A. Unfortunately serum amyloid A is a marker that is present in induced inflammation, it’s lack of specificity gives it little use to support a diagnosis of EPM.

Another myth is that there are many strains of *S. neurona*. It’s true, but it’s not important that there are many strains of this organism. Identifying the strain isn’t what is essential to those of us treating EPM, it’s the phenotype. There are only three phenotypes of *S. neurona* and they are important to a discussion of EPM. Antibody responses in horses can be distinguished by the phenotype of the infection. This is very important because some phenotypes can resolve quickly or spontaneously, some are resistant to Marquis, diclazuril, or pyrimethamine-sulfa drugs, and not all of the phenotypes can cross into the CNS and cause what researchers call EPM—organisms that are multiplying in the CNS. Strains are defined by many genotypes, the only level of distinction possible serologically is the phenotype.

**One thing leads to another**

The confusion about this disease started when Western Blot was used to identify antibodies and not disease (but seropositive animals were identified as diseased). It made sense that if *S. neurona* invades the CNS, after all it was uniquely neurotropic, infections would truly be difficult to treat. If the organism is in the CNS we expect drugs to take a long time to eliminate disease and we accept, and expect, poor efficacy based on this grim assumption. These assumptions were wrong. Antibody tests showed us which horses to treat. We also discovered that a second disease is found in the horse with EPM. This disorder is inflammatory encephalitis that is not responsive to antiPROTOZOAL DRUGS. Inflammatory encephalitis responds to a course of immune modulating anti-inflammatory drugs. The basis we use to determine the presence of a lingering inflammatory encephalitis is the lack of antibody post-treatment—*but clinical signs remain or return.*

We know this because we study infections using our EPM inducing model. We also study field cases that are naturally induced. We found decoquinate/levamisole works 97% of the time to alleviate the signs of *S. neurona* infections and EPM cases. Ninety-three percent of the horses respond to the antiPROTOZOAL drug, and an additional 4% of the horses responded to the immune modulating component levamisole. We recognized diffuse inflammatory brain disease, secondary to *S. neurona* infections, that responds to immune modulating drugs. And finally, our single minded approach using specific antibody markers led us to discover SAG 6. The antigen SAG 6 explains the difference between other test results and our Peptide ELISA results.

Equine protozoal myeloencephalitis is a commonly diagnosed, uncommon disease that is recognized by clinical exam and antibodies. Most horses are exposed to opossum feces and *S. neurona* infected horses produce antibodies identified by one or more of the three phenotypes, SAG 1, 5, or 6. *Sarcocystis neurona* infected horses, and horses with EPM, respond to decoquinate/levamisole. Response to treatment can identify the presence of inflammatory encephalitis. And inflammatory encephalitis can be managed successfully. We quickly realized that EPM is more than *S. neurona* infections and we are beginning to understand the mechanism of inflammatory disease that can be part of EPM. Clinical exam is crucial to recognizing and evaluating the horse with EPM. The clinical exam after treatment is just as important. Clinical management of EPM takes understanding the disease process and monitoring responses to drug therapy.
27,003 horses have EPM

Posted on June 15, 2011 by Administrator

How many horses have EPM?

One thing we’d like to know: how many horses in the United States have EPM? Most authors start out telling us how important and serious EPM is and then they tell us the seroprevalence, how many horses have antibodies to S. neurona. And then they say that EPM occurs in less than 1% of horses. That’s rare.

Published studies determine the prevalence of EPM on seroprevalence, the presence of antibody determined by Western blot. That number is between 30% to (more than) 60% and is regional. Seroprevalence has little to do with actual cases of EPM. Horses with antibodies to S. neurona are horses that can succumb to EPM. An infection is a prerequisite for disease and antibodies result from infections. Seroprevalence can tell us how many horses are at risk to get protozoal myeloencephalitis due to S. neurona. The number of horses with EPM, based on antibody (tested by Western blot) is high.

Even if we knew how many horses had a definitive diagnosis of EPM, that won’t help get us to the true incidence of disease. A definitive diagnosis is defined as an animal from which the organism was cultured from the central nervous system. The number of horses seen at referral clinics can give us an idea of true disease. If our defined population is the group of horses diagnosed at a referral clinic for their ataxia (or suspect EPM)—we’d expect the number of horses that have EPM to predominate and therefore skew the data to an increase in the number of horses with the disease. Other lesser causes of neurological disease (viral infections, trauma, wobbler syndrome) are included. It is difficult to isolate the organism, thus some of these horses are diagnosed with EPM based on histological lesions that consist of inflammation.

The comprehensive NAHMS study conducted in 1998 and published in 2001—the only government document, tells us that this serious disease is rare. For this study the authors surveyed veterinarians and horse facilities.

An estimate of the EPM horses in the United States

We expect to approximate the number of horses that contract EPM annually and guess it will be between “less than 1%” and the number predicted by Western blot seroprevalence. This is the morbidity. The current United States horse population is considered to be 7, 200,000. That means that the number of horses with EPM is less than 72,000 if we believe that disease is less than 1%.

The NAHMS reports that a veterinary diagnosis (with or without the support of diagnostics) was made in 14 cases out of 10,000 horses in 1998. That is a rate of 0.0014 or 0.14%. This rate means that only 10,080 horses have EPM. Intuitively, that is low. The NAHMS number is less than 72,000 and is the low estimate of 10,080.

I spoke with Frank Andrews, current (and past) president of the EPM Society. Dr. Andrews thought 50,000 would be a high number. He presented the Proportional Morbidity Data at the EPM meeting recently. The rate “which is PMR, proportional morbidity rate, is 0.88% and roughly correlates to incidence (the number of new cases presented to Universities over 17 years).” He agrees that the actual incidence of disease is low, but exposure is high in some areas. He also says that the PMR has not changed in the 17 years included in the study.
but pointed out that there was a spike in cases from 1995-1998. The reasons for the spike seen in morbidity data for EPM were the development and use of the Western Blot and an increase in cases sent to universities due to drug efficacy trials, not an outbreak of disease. That means there are potentially 63,360 cases.

**Narrowing it down**

The increase due to drug study trials and hopes of a diagnostic test gave a falsely elevated rate for the years 1995-1998. We removed these years from the data set—14 years for analysis. Andrews says the rate was unchanged over all the years and removing the spike should render the data to more accurately reflect the true rate over time. The adjusted rate is more realistic and is 0.65%. That would still yield the high value for morbidity because these cases are from referral centers. It’s high because this is the rate determined from cases of ataxic horses that were seen at referral university clinics, other causes of ataxia are not sent for a diagnosis. But are there any other factors we should consider?

Our data from the Peptide ELISA shows that only the animals with a titer against SnSAG 1 and SnSAG 5 should be considered for animals that are susceptible to EPM. Data based on the Ohio State model that showed that SnSAG 5 can’t experimentally induce disease (disease is defined as parasites isolated from the CNS of a horse). The number of isolates from horses with SnSAG 5 strains and seroprevalence of SnSAG 5 antibodies in pure and mixed infections will yield how many animals can get EPM from this strain. Our phenotype data shows that 6.6% of the animals with a presumptive diagnosis of EPM by clinical examination are infected with the SAG 5 phenotype. The Ohio studies indicate this strain will produce signs in response to inflammation.

Observations in the field support our findings. In the published field EPM cases for which the organism was isolated, only 7% displayed the SAG 5 phenotype, and there was evidence of mixed infections based on some of the published reports. The SnSAG 5 infections often resolve without treatment, based on the OSU studies.

Potentially all the SnSAG 1 infected animals are at risk for EPM.

The PRM report, adjusted for the spike, estimates the rate of EPM at 43,992. That is 61 horses in 10,000. We averaged the high and the low estimates numbers to approximate the number of horses that have EPM and it is 27,003.
One step forward

In a recent paper we summarized data from a small group of horses, the initial set that were diagnosed with EPM and then got treatment. The paper tells you that *decoquinate and levamisole reduce the clinical signs and serum SAG 1, 5, 6 antibodies in Sarcocystis neurona infected horses*. It’s a little disappointing that the editors snipped out one whole *years* worth of work. The reason was that “the message to the audience was lost”. The target audience, I’m told, doesn’t want to read about *how* and *where* we developed our tools and obtained our data...only the results.

Slashed from the manuscript was that twelve antigen types are described for *S. neurona*, yet only two phenotypes (SnSAG 1 and SnSAG 5) represent the majority that are associated with disease. Population geneticists discovered the highly lethal SnSAG 6 *S. neurona* (lethal in sea otters). We found a novel *S. falcatula* gene, antigenically indistinct from SnSAG 6, in our DNA stash—we cloned it into an expression vector. We determined the presence of antibodies against the SAG 6 phenotype (infections) in equine sarcocystosis. We used antibodies to disease-associated SAG phenotypes of *Sarcocystis neurona* in our indirect ELISA, using sera from horses with a presumptive diagnosis of sarcocystosis, to begin our study. Plenty of horses have antibodies to the *S. neurona* SAG 6 phenotype. We found normal horses have antibodies—they clear the infections without getting disease. It’s important to realize where SnSAG 6 (the gene) came from and what it means when we detect antibodies to SAG 6 in horses.

Lost in translation

Some veterinarians believe that when there are signs of EPM—there are parasites in neural tissues. And horses that have signs for a long time have scaring in the neural tissues therefore nothing can be changed.

Even veterinarians that read the published literature missed the point that inflammation can be a BIG part of the problem, even after the parasites are eliminated. The dead end cycle of *S. neurona* in the horse—from the oocyst found in the environment to the brain tissues of the horse—has been discussed. We aren’t the first to say it, it was mentioned by Solfay (Ohio Transport Model) that “Immune-mediated pathology rather than parasite mediated pathology was considered as one source of signs observed in oocyst challenge experiments because parasites were not demonstated in these studies”, that was in 2002.

We’ve seen the results of inflammation (causes severe signs in horses) using SnSAG 1 strains in our challenge infections. In some cases, we saw large inflammatory responses and ataxia despite treatment with triazine drugs *before challenge*. Our work indicates that it is important to identify the phenotype of the infection
because some phenotypes could be lethal, or more inflammatory to the horse. We can correlate the treatment results with phenotype and may eventually understand the role of each molecule in the inflammatory process.

The opossum (*Didelphis virginiana*) is the source of infectious oocysts for horses.[i] Highly similar DNA sequences for 18s rRNA genes amplified from opossum sporocysts and *S. neurona* merozoites led researchers to suggest that the opossum was likely the definitive host of *S. neurona*. This prompted other researchers to suggest that *S. neurona* and *S. falcatula* were synonymous. The idea was based on greater than 99% homogeneity between a 742-bp segment of the 18S rRNA gene from *S. falcatula* and *S. neurona* (SnSAG1) amplified by PCR from oocysts that were collected from opossums.[ii] Additional studies that failed to induce clinical EPM in horses (that were challenged with an authenticated population of *S. falcatula* sporocysts) led these researchers to suggest that the organisms were not synonymous. Bioassay was a significant parameter discerning species differences.[iii] The inability of *S. falcatula* to infect horses in their studies was based on the absence of clinical signs of EPM and *S. neurona* immunoblots that failed to detect antibodies in serum and CSF post-challenge (evidence there was no infection). Confirmed infections of horses by using sporocysts have been elusive and the UF studies were based on oocyst challenge. Further work done by Heskett and MacKay concluded that sporocyst infection challenge may not reliably result in CNS infection.[iv]

Serum samples obtained from a Missouri horse with confirmed clinical EPM (SnSAG 5 strain) bound antigens of *S. falcatula* when *S. falcatula* blotted antigens were probed with the serum.[v] These data also indicated that there are differences in immunoblot patterns between two *S. falcatula* strains when this serum and specific rabbit sera were used. The ability to detect *S. falcatula* antibodies in serum/CSF may depend not only on the species of *Sarcocystis* but also the strains used in the antigen preparation. Possibly antigen selection is responsible for the failure to detect seroconversion in the University of Florida challenge studies.

An advance in genotyping has allowed better analysis of relatedness between *S. neurona* strains. Based on limited available genetic data, sequence-level analysis of five surface antigens (Ag) genes (SnSAG 1, 3, 4, 5, and 6) and nine microsatellite (MS) markers identified 12 Ag types and 33 MS types among *S. neurona*-infected samples based on the allele combinations detected at each locus.[vi] The resolution of strains of *S. neurona* is achieved using genetic sequence analysis. The distinction of phenotype is achieved by detecting specific antibodies against recombinant proteins SnSAG 1, 5, and 6 because these surface antigens are mutually exclusive genes of *S. neurona*. *Sarcocystis neurona* SnSAG 1 and 5 primers are *S. neurona* specific while SnSAG 2-3-4-6 are amplified by *S. neurona* and *S. falcatula* DNA. [vii] Due to the highly conserved orthologs to SAG 2, 3, 4 in the closely related *S. neurona* and *S. falcatula* antibody detection of these phenotypes would not be useful in diagnostic tests due to cross-reactions. [xvi]

Our story

One goal of our studies is to clarify the ability of horses to develop antibodies against *S. falcatula*—as demonstrated in the Missouri isolate, but not demonstrated in the UF *S. falcatula* challenge studies. Two *S. falcatula* isolates were subjected to genetic analysis using the SnSAG primers described by Wendte.[v] Lung tissues from birds that were infected with opossum oocysts described as *S. falcatula* that were obtained from the Florida horse infection challenge were placed in vitro culture.* An additional strain of *S. falcatula* (Cornell) was characterized at the same time. DNA samples from the cultured merozoites were analyzed by high
resolution genetic typing. We report the results of the genetic sequence analysis that identified a novel gene, SfSAG 6, from the Florida isolate but not present in the Cornell isolate. The gene was named SfSAG 6 due to the high similarity to SnSAG 6 of *S. neurona*. The recombinant SfSAG 6 protein was assayed by ELISA to determine the presence of antibody in equine serum from horses with clinical EPM.

We detected antibodies against *Sarcocystis* SAG 6 organisms; however it is not possible to distinguish SnSAG 6 and SfSAG 6 organisms with antibody tests, the sequence for both genes are highly similar (greater than 95%) and cross reactions are anticipated. It is unclear the role *S. falcatula* plays in clinical EPM. Prior experimental evidence showed that authenticated *S. falcatula* oocysts derived from opossum feces didn’t infect horses and antibodies against *S. neurona* were not produced. It is not surprising that challenge studies failed to show antibodies induced in *S. falcatula* studies because the sera was screened against antigens of *S. neurona*. Alternatively, the oocyst model didn’t induce sarcocystosis that resulted in antibody production – make this assumption and some of the other serum tests make sense. Clinical EPM, with isolation of the organism from the CNS, was not reproducible in other oocyst models despite evidence that merozoites infect visceral organs of the horse. Heskett and MacKay concluded that challenging horses via oocysts may not reliably result in CNS infections. Merozoites enter the CNS of horses and produce clinical signs of EPM in the Trojan horse – merozoite model. The methods of induction of disease or strain differences are possible factors in differences between oocyst and merozoite induced infection studies.

It is possible that *S. neurona* SAG 6 can infect horses while *S. falcatula* cannot. It is equally possible that *S. falcatula* can infect horses. *Sarcocystis falcatula* can infect leukocytes of horses, at least SfSAG 6 strains can.

We detected phenotypes of *S. neurona* that infect horses by specific ELISA tests using recombinant proteins. Antibodies against SnSAG 1, 5, and 6 were detected in normal horses indicating that *S. neurona* infections that resolve are common. It is interesting that only 4% of normal horses have antibodies against SnSAG 1 strains which dominate the isolations published from field infections. We detected the presence of *S. falcatula* SAG 6 antibodies in normal and diseased horses. This was not surprising because *S. falcatula* specific antibodies were detected by Marsh in a report that identified *S. neurona* SAG 5 phenotype merozoites from the CNS tissues of a Missouri horse. Studies by Wendte indicate the majority of disease in animals is due to SnSAG 1 or SnSAG 5 phenotypes, and only these phenotypes have been recovered from the CNS of diseased horses.

*Sarcocystis neurona* SAG 6 serum antibodies were detected in 3.5% of clinically ill horses (as a single infection) undergoing treatment for EPM indicating that *S. neurona* SAG 6 phenotypes can cause disease. Undoubtedly, the results of SAG 6 testing can be due to cross-reaction between SnSAG 6 and SfSAG 6 if *S. falcatula* causes disease in horses. Twenty-six percent of the sera from animals that did not have clinical signs of EPM did have SAG 6 antibodies. The difference between horses with and without disease may be due to species, *S. neurona* eliciting disease while *S. falcatula* does not (as was previously reported). However, *S. falcatula* reactive antibodies were detected in at least one natural case of EPM—the Missouri horse and SAG 6 antibodies are detected in horses when SfSAG 6 recombinant proteins are used for detection. Infections with SAG 6 phenotypes in horses with EPM are rare, it will be interesting to determine if the infections are less virulent in horses.

The use of bioassay (in budgies or immune deficient mice) or genetic sequence analysis is required to distinguish *S. neurona* from *S. falcatula* and it’s possible that horses can discern the difference between these organisms. Both *S. neurona* and *S. falcatula* are identical in antibody tests using antigens SAG 2 and 3, (SAG 4
is variable in some *S. falcataula* strains, we didn’t show these data—we’ll share this information if you are interested**) therefore these diagnostic antigens are not specific for *S. neurona*. Horses with antibody to SAG 6 and clinical signs of disease responded to treatment indicating that SAG 6 strains of *S. neurona* are important in clinical EPM. However, other *Sarcocystis* that do not induce disease would be detected by tests that are not specific for *S. neurona* and phenotype profiling would be important to discern the difference.

*A kind gift of Ellis Greiner

**an aberrant SnSAG 4 gene was previously reported. Ours was a presumed SfSAG 4 pseudogene...but could be a clue to some strain variability in sarcocysts.
What makes *S. neurona* different?

Posted on June 13, 2011 by Administrator

**Pioneers**

A physicist, John P. McAlpin, put forth a different view of the Big Bang Theory which involved strings and membranes, he even devised new math to explain his novel ideas. His presentation to his peers fell flat. He said, “Ultimately, if your ideas aren’t fashionable or agreed upon, if you believe what you are doing is right, persist. There is a conventional wisdom in the field and people are very slow to adopt new ideas. Frankly, many people have built their careers on the status quo and they don’t want a new idea coming along and rocking the boat.”

Clinicians can detect the subtle signs that are present a few days after *S. neurona* infects a horse. We, and others that studied experimentally induced infections, saw early signs consistently appear *before* ataxia and lameness manifested. It is hard to change the conventional opinion (a horse with EPM shows moderate ataxia) to recognizing that behavior changes and very subtle gait problems are indicative of infections.

**Tools we developed**

The tools that help us with basic research are first, a model to *cause* EPM. The model lets us design tests and investigate treatments. We develop antibodies that we use for controls and markers. Our set of recombinant antigens were designed and produced in the laboratory. Then we tested vaccines and treatments. We are developing more antigens, tests, vaccines, and treatments to combat EPM. Our current antigens can be used as vaccines.

**What’s in a name?**

The name *S. neurona* is the *basis* for misunderstanding this organism and the disease that it induces, equine protozoal myeloencephalitis. "Neurona" implies that this parasite is neurotropic, or certainly found in the central nervous system. This is an organism with a predilection for the central nervous system. We disagree for the following reasons.

There are a lot of common interactions between hosts and *Sarcocystis* species. When sarcocysts were identified in *intermediate hosts* a logical search could root out the identity of the definitive host. Sorting out the relationships between hosts is much more difficult when only a part of the life cycles are known. *Sarcocystis neurona* was identified as the etiologic agent of protozoal disease in a horse with EPM. The organism was named *S. neurona* due to the notorious neurological disease associated with the merozoite. It’s identity had been elusive for so long. Dr. J. P. Dubey had selected the name awaiting the final isolation of the organism. But the name is misleading if merozoites of other *Sarcocystis* can cause encephalitis. *Sarcocystis neurona* has often been labeled aberrant and different. Promiscuous even. And neurotropic.

Does *S. neurona* follow or break the rules? When does *S. neurona* behave *just like you’d expect other sarcocysts to behave*? A most important question: are other *Sarcocystis* neurotropic? Neurotrophism is the idea that *S. neurona* has a predilection for the central nervous system. Is there another reason why *S. neurona*
ends up in the CNS of horses? Does *S. neurona* end up in the CNS in the majority or minority of infections? To determine if *S. neurona* is truly different, we looked at general sarcocystis traits.

**Its Compliated!**

*Sarcocystis* use definitive hosts that include carnivores, including dogs, cats, mammalian wildlife, birds, reptiles and even humans. Common among definitive hosts is predation. Predators eat muscle tissue that can contain parasites, and that is how they are infected. The specific host varies with the species of *Sarcocystis*, most species are highly specific for their hosts. Dogs, for example, are definitive hosts for *S. cruzi* (*S. bovicanis*), *S. tenella* (*S. ovicanis*), *S. arieticanis* and four more species. There are other canines that are definitive hosts for *S. cruzi* that include wolves, coyotes, raccoons, foxes and hyenas. Wolves, raccoons and jackals are also definitive hosts for *S. miescheriana*. The rule is that hosts can harbor numerous, highly specific sarcocystis.

The opossum harbors several species of sarcocystis, there’s nothing different about the definitive hosts ability to be involved with multiple species. And the species of sarcocystis that infect dogs can infect other carnivores. A sarcocyst probably wouldn’t be expected to change host preference from a dog to a bird, or a cat to a reptile. That’s because parasites uniquely adapt to their hosts over long periods of time. It would take something special to allow an ability to infect new hosts, like a genetic mutation. We read that *S.neurona* does that, infects feline, canines, equines, avian, and marine species. We suspect that there is confusion in identifying the organism rather than the organism being so cosmopolitan.

An intermediate host is usually prey. Prey are infected, eaten by predators, and the cycle begins. The intermediate hosts scenario is as complicated as that of the definitive hosts. Some species have several names. *Sarcocystis bertrami* and *S. equicanis* are the same species. Intermediate hosts are cattle, sheep, goats, buffalo, pigs, horses, poultry, birds, dogs, cats, rodents, rabbits and wildlife. Complicating the whole picture is that it is possible, and often the case, that an animal is both intermediate host for some species and definitive host for others. Then add multiple infections...

**The relationships of *S. neurona* and hosts**

Cats are the definitive hosts for *S. hirsuta* (*S. bovifelis*), *S. porcifelis*, *S. gigantea* (*S. ovifelis*), *S. muris*, and eight more species. Don’t forgot to count *S. neurona*. Cats were shown to be definitive hosts of *S. neurona*, rarely and only experimentally—cats are intermediate hosts. Opossums are considered the definitive hosts for *S. neurona* (and the close cousin, *S. falcatula*).

*Sarcocystis neurona* can be found in an unusually large number of intermediate hosts including domestic cats, armadillos, skunks, raccoons, sea otters and probably other species such as birds. Besides horses, symptomatic infections have also been reported in a pony, cats, a lynx, mink, raccoons, skunks, sea otters, harbor seals and a monkey. And the jury is still out if birds may act as vectors/ transport hosts for *S. neurona*.

**The simple story**

We need to take another page from the physicists and that is to try and simplify our ideas about *S. neurona*. Instead of looking at how unusual they seem and how aberrant their behavior in hosts, what can we find that is common to their genus? Most animals infected with Sarcocystis spp. are asymptomatic, and the parasites are
seen mainly as an incidental finding at necropsy. However, clinical cases are occasionally reported, particularly in the intermediate host. Muscle wasting can occur. *Sarcocystis neurona* has caused rare cases of neurologic disease in a pony, domestic cats, a lynx, raccoons, skunks, mink, sea otters, harbor seals and a monkey. But these findings don’t make *S. neurona* the exception.

Muscle inflammation caused by *S. fayeri* is rare but reported in horses with infections. The symptoms in naturally infected horses include myositis, muscle atrophy, weight loss and decreased appetite. Fever, anorexia, mild depression, mild anemia and wasting can be seen in infected ponies and horses.

Are horses unique or are neurological signs seen in other infected animals as well? Neurological signs have been seen in cattle, sheep, dogs and cats. In cattle, *Sarcocystis spp.* can cause acute disease in calves, eosinophilic myositis, abortions and neurologic disease. Signs seen in heavy infections include fever, anorexia, wasting, decreased milk production, diarrhea, muscle spasms, weakness, hyper-excitability, pneumonia, hemorrhages, anemia, icterus, prostration and death. Rare cases of necrotic encephalitis have also been reported in cattle.

*Sarcocystis tenella* is thought to be the most pathogenic of the *Sarcocystis* species in sheep. It can cause anorexia, fever, decreased weight gain, anemia and death in lambs, and has been associated with abortions in ewes. Neurologic signs including encephalomyelitis, muscle weakness, hind limb paresis and ataxia have been seen in naturally infected sheep.

A *Sarcocystis* species, *S. canis*, has been linked to encephalitis, hepatitis and generalized coccidiosis in young dogs. The neurological signs included depression, generalized weakness, recumbency, nystagmus and seizures. Meningoencephalitis or encephalitis is associated with infections.

*Sarcocystis* in horses, cows, sheep, dogs, birds and cats can cause encephalitis. It isn’t *unusual behavior* for *sarcocystis*, in its intermediate host, to occasionally cause neurological signs. This outcome is likely based on dose, health status of the animal, and probably the level of stress. Or it could be based on virulence of a strain. What’s important is that the evidence shows that *S. neurona* behaves in intermediate hosts and horses just as others of its genus do.
EPM: Fact or Myth?

HORSES HAVE A GENETIC PREDISPOSITION TO SARCOCYSTIS NEURONA INFECTIONS

Is there scientific evidence to justify the common belief that there is a genetic bias in some horses that disposes them to an increased susceptibility to Sarcocystis neurona infections and subsequent disease? If such a bias toward infection existed I’d expect to see other opportunistic protozoal infections in these animals. So why is this idea prevalent?

We don’t know the prevalence of Sarcocystis neurona infection (visceral infection with antibody production) or disease (CNS involvement with ataxia) in horses. The prevalence of EPM was discussed in EPM Facts or Myths: Prevalence is high, disease is low. If data from referral clinics at universities is an indication then the prevalence has not changed in 20 years and is close to 1% of the population.

The host’s genetic makeup is not questioned in diseases that are common because all most of the exposed animals get disease. Protozoa such as Toxoplasma have used models that indicate that some hosts are biased toward these infections. Are infections in horses common and how does infection relate to disease? Western blot serology data reports high exposure rates in horses despite the relatively low cases of EPM that are diagnosed. The survey results that used the Western blot are the root of the genetic bias theory since many horses have antibodies but few horses have disease.

Therefore we strive to understand the exception—the presence of disease. Are there some horses that are genetically unable to ward off disease? Or what is different about the S. neurona-horse relationship that explains this discord, infection versus disease rates? It is worthy of discussion. We propose that serological surveys based on unique antigens that identify clonal strains of S. neurona indicate that virulent parasite strains are rare but infections to less virulent strains are common. Further, our new serological data may indicate that protective immunity is induced by infections by the less virulent strains. The acquired immunity may be antigen specific.

Strong evidence links the opossum and S. neurona to EPM in the horse. Opossums harbor S. neurona (the data is based on oocysts that can infect immunodeficient mice and not budgies) 18.1% of the time. More often, opossums carry S. falcatula (47.7% of the time, it infects budgies, not mice). The other sarcocystis that can be confused with S. neurona (species that will be detected by immunoblot and also infect immunodeficient mice) include S. kirkpatricki (present in 66% of examined opossums) and S. speeri (18.1%). A logical conclusion is
that the presence of opossums provide an oocyst rich environment and the horse should test seropositive on immunoblots. And this is what we see. A good percentage of these seropositive horses would not develop EPM (see EPM Facts or Myths: How many strains of S. neurona infect horses?). Still, 18 % of the horse population that contact opossum feces in the environment should get infections caused by S. neurona and therefore EPM. Is that the case? Do all horses that get S. neurona infections (and produce antibodies) get EPM? No, they don’t. We find that all exposed horses do not get disease. We eliminated false positive test results (common with the immunoblot) by using S. neurona specific antigens. We found that horses are commonly exposed to the three clonal lines of neurona distinguished by unique antigen type and that the distributions of antibodies to these antigens differ between normal horses and those with a presumptive diagnosis of EPM.

A presentation by Saville (authors include Reed, Granstrom, Andrews, and Morley) at the 1997 AAEP states that EPM is the most common neurologic disease seen at Ohio State University Veterinary Teaching Hospital. The high prevalence of disease led these clinicians, as well as others (Dubey, Davis, Giles, Rivas), to suggest that S. neurona has an affinity for the CNS of horses. But this may not be true (see EPM Facts or Myths: Is S. neurona neurotropic in the horse?).

What can our understanding about acquired immunity reveal if strain virulence rather than neurotrophism is responsible for infections? Researchers believe that neurologic disease is common (at least in Ohio). Saville followed acquired immunity of 6 horses, biweekly, with serum and CSF taps. These horses joined forty-four seropositive horses, residents on a farm from 1-10 years. The high seroprevalence indicated to the authors that factors for infections were common in this environment. Perhaps exposure was due to S. falcatula. Oocysts were not collected and evaluated for the presence of S. neurona despite the tumult stirred up at the time by the University of Florida’s work.

The results of this study concluded that none of the horses developed EPM, but neither had any of the residents on the farm. None of the six animals developed CSF antibodies to S. neurona. You will read later that a killed vaccine can induce thecal antibody production (antibodies present in the CNS), that vaccination with a single S. neurona protein (SnSAG1) induces antibody production in the CNS, and experimental challenge with just a few organisms, likewise, induces antibody production in the CNS. One would expect natural exposure to do the same. It is difficult to support the assumption that S. neurona was present on this farm and the Saville group’s conclusion that there was evidence of protection against EPM due to re-exposure.

Certainly the presence of S. falcatula, found the majority of time in opossum feces, is plausible on this farm. The absence of neurological disease and cross-reactivity of S. falcatula on western blot is likely and supports the idea that S. falcatula should be considered a factor in evaluating their results. The Saville group did note chronic elevated IgG index, unrelated to neurological disease, which was unexplained. They considered that repeat CSF sampling may have induced inflammatory changes in the subarachnoid and dura. This is a concern in CSF taps.
done in horses. A final point was that disease may be slow to manifest, perhaps greater than a year, the observation period for this study. This idea is better supported by the presence of acute cases of EPM in horses that travel from the US to Europe and develop EPM a year to years later in the absence of opossums and S. neurona.

At the same time this work was being presented, the aforementioned killed merozoite vaccine was conditionally licensed. Concerns that vaccination would corrupt the western blot test results prompted researchers to examine the immune response to vaccination. The vaccine induced IgG antibodies in the serum and, sometimes, the CSF. Additionally, intradermal skin test reactions stimulated cellular immunity attributed to vaccination. Histology was necessary to detect the immune reaction because the reactions were microscopic. The authors concluded that inflammation to dermal cells (used to grow the merozoites for vaccine preparation) was a factor in the results. “The placebo-vaccinated horses had microscopic lesions similar to the horses receiving the S. neurona vaccine, which suggests that the placebo vaccinated horses may have been immunized against these media components when vaccinated with the placebo vaccine containing the media and adjuvant.” Is this convincing evidence of the production of cellular immunity? Not to me. An exciting side note is that these results may indicate a method to determine autoimmune reactions that can mimic EPM that follow vaccination using vaccines that contain equine dermal cells.

The ability of the immunodeficient or nude mouse, to develop disease furthered our knowledge about S. neurona infections. Mice are not subject to S. neurona infections, defective immune systems made them susceptible. In addition to demonstrating neuroinvasion which was a requirement for a model for experimental infections with S. neurona, the mice also served as a biological tool for isolation of S. neurona from infected tissues. This quickly became a method to identify S. neurona. Other sarcocystis that are found in the opossum, to which these mice are susceptible were identified but well controlled studies could reconcile this issue.

Immuno-suppression was often used to isolate the asexual stage of S. neurona from the CNS of horses. Immuno-suppression, however, did not induce EPM in horses challenged with S. falcatula. These data added to the perception that defective immunity was important in EPM. And strengthened the view S. falcatula is not a factor in EPM. The horse could be immuno-compromised if it was stressed, diseased, old, or perhaps had a genetic defect. Arabian foals with severe combined immunodeficiency (SCID) lack specific B- and T-cell responses. Screening for a mutation in the DNA-dependent kinase catalytic subunit can be used to identify these foals. These foals have been used to examine adaptive immune responses to various infectious diseases. When a SCID foal was given S. neurona sporocysts, the foal did get a parasitemia (parasites in the blood) -- however, this foal did not get neurological disease. The same results were seen in SCID mice. Despite a low level parasitemia that was demonstrated in some mice, there was no neurological disease and the mice remained healthy. If the infected mice were given anti-interferon gamma antibody (to deplete any interferon gamma) the mice succumbed to neurologic disease. This is good evidence that interferon gamma is critical to the resistance of the host to S. neurona.
In another study using SCID foals, the researchers demonstrated that infected SCID horses developed a parasitemia and were unable to terminate the parasitemia or clear *S. neurona* from visceral tissues, but the infection did not result in clinical signs of neurologic disease. They also challenged immunocompetent horses, offspring of the same Arabian horses that were heterozygous for SCID that had produced the SCID foals. These immuno-competent horses rapidly controlled parasitemia and infection of visceral tissues but experienced neuroinvasion and clinical signs of neurologic disease. They concluded that a requirement for adaptive responses may be important for neuroinvasion or neurovirulence.

Humoral immunity, antibody, was not critical for protection against experimental infections with *S. neurona* in B-cell-deficient mice. These mice did not seroconvert when challenged, and they did not get protozoal myeloencephalitis. Transient infections were seen in the lung, liver, and spleen, but the infections resolved by day 60. In another experiment, it was shown that normal mice produce IgG when challenged but cell-mediated immunity was thought to play a more critical role in protection against infection. When immunodeficient (CD8) mice were challenged, parasites were found in the brain and thus it was suggested that CD8 cells are critical for immunity.

Interferon gamma, known to be important in protective immunity in other protozoal infections was also examined. Interferon gamma knock-out mice (they can’t produce interferon gamma) were unable to clear infections. Lymphocyte stimulation assays (LPA) detect innate and adaptive cellular immune responses by *in vitro* stimulation of lymphocytes. Researchers found that using LSA assays that employ SnSAG1 as an antigen, responses were significantly higher in EPM-negative than EPM-positive horses. Gamma interferon production was detectable by 24 hour after culture in response to SnSAG1 in all EPM-negative horses. There was still no detectable gammaIFN production in EPM-positive horses after 72 hr in culture. They concluded that the parasite was able to induce an immuno-suppression toward parasite-derived antigens as parasite-specific responses are decreased. This work indicated that it is the parasite, not the host's genetics that direct the pathogenesis of EPM in the horse. Alternatively, the suppressed-reaction horses already had EPM; it was possible that there is underlying pathology to account for the results.

The next body of work looked at LPA assays, again in natural cases of EPM and they found that the lymphocyte reactions from these naturally infected horses had significantly lower proliferation responses. This work corroborated the prior group’s work using more markers of cellular immunity. Additionally, it was noted that horses with natural cases of EPM had significant differences in a non-specific stimulation test- responses to nonspecific mitogens phorbol myristate acetate (PMA) and ionomycin (I). The PMA/I test response was decreased when compared to uninfected horses. The same work was repeated in experimentally induced EPM. The experimental model allowed researchers to compare pre- and post-infections in the same horse, eliminating the possibility of underlying pathology. They found infected horses had significantly decreased proliferation responses to PMA/I as soon as 2 days post infection. Surprisingly, the level of immune suppression seen at 2 days was consistent with the
severity of EPM seen in the horses at 60 days. This test is used currently to assist veterinarians in assessing difficult cases of EPM.

The studies using experimental infections used over 75 horses with various ages, breeds, and sexes with no detectable genetic bias to infection. The lymphocyte stimulation assays indicate that the parasite quickly subverts the equine immune system to allow neurological disease. Because SnSAG1 was shown to induce gammaIFN production in EPM-negative horses the recombinant protein was used to vaccinate horses prior to challenge with the expectation that cellular as well as humoral immunity could be stimulated. The results of this work confirmed that vaccination with rSnSAG-1 produced antibodies in horses that neutralized merozoites when tested by *in vitro* culture and significantly reduced clinical signs demonstrated by *in vivo* challenge. Four of five vaccinated horses did not develop EPM while all of the non-vaccinated horses displayed signs of EPM including ataxia.

Finally, when experimentally challenged horses are examined for genetic markers that increase after challenge, we can identify what the immune system is doing. It is important to use experimentally challenged horses because naturally infected animals are too undefined. The parameters of the ability to respond to infections, prior experience with *sarcocystis*, time of onset and duration, dose, and parasite virulence are all controlled in experimental challenge studies validating the work. We now have molecular genetic evidence that the pathways and the production of cytokines are stimulated in infections. The genes that produce IgG are stimulated. Interferon gamma genes and the genes for their regulation are stimulated. The MHC II gene family that encodes MHC molecules that play an important role in the immune system and autoimmunity, chiefly the macrophages and B cells, are stimulated.

When taken together, the above research indicates that if a genetic predisposition to succumb to EPM exists it is the presence of adaptive immunity in the host, rather than a genetic phenotype that allows infections. The evidence to date strongly points to the ability to produce interferon gamma in the first days of infection are critical to resistance to disease. Our work using a large number of immunocompetent, non-Arabian horses indicates the majority (>90%) will succumb to EPM if challenged. If an interferon gamma deficient horse exists, we presume it is rare. Thus, the adaptive immune background of the horse, the prior experience with *S. neurona*, may be more important than the host’s genetics. Other known parameters important in the outcome of equine infections include the dose of parasites and as yet un-described parasite virulence factors.

ii Isolation of a third species of Sarcocystis in immunodeficient mice fed feces from opossums (Didelphis virginiana) and its differentiation from Sarcocystis falcata and Sarcocystis neurona. Dubey JP, Speer CA, Lindsay DS.

iii Isolation in immunodeficient mice of Sarcocystis neurona from opossum (Didelphis virginiana) faeces, and its differentiation from Sarcocystis falcata. Dubey JP, Lindsay DS.

iv Sarcocystis speeri N. sp. (Protozoa: Sarcocystidae) from the opossum (Didelphis virginiana). Dubey JP, Lindsay DS.

v Prevalence of sarcocystis species sporocysts in wild-caught opossums (Didelphis virginiana). Dubey JP.

vi Relationships among Sarcocystis species transmitted by New World opossums (Didelphis spp.). Rosenthal BM, Lindsay DS, Dubey JP.

vii Prevalence of Sarcocystis neurona sporocysts in opossums (Didelphis virginiana) from rural Mississippi. Dubey JP, Black SS, Rickard LG, Rosenthal BM, Lindsay DS, Shen SK, Kwok OC, Hurst G, Rashmir-Raven A.

viii Prevalence of Sarcocystis species sporocysts in Northern Virginia opossums (Didelphis virginiana). Elsheikha HM, Murphy AJ, Mansfield LS.

ix Concurrent presence of Sarcocystis neurona sporocysts, Besnoitia darlingi tissue cysts, and Sarcocystis inghami sarcocysts in naturally infected opossums (Didelphis virginiana). Elsheikha HM, Fitzgerald SD, Rosenthal BM, Mansfield LS.

x Sarcocystis falcata from passerine and psittacine birds: synonymy with Sarcocystis neurona, agent of equine protozoal myeloencephalitis. Dame JB, MacKay RJ, Yowell CA, Cutler TJ, Marsh A, Greiner EC.

xi Multiple DNA markers differentiate Sarcocystis neurona and Sarcocystis falcata. Tanhauser SM, Yowell CA, Cutler TJ, Greiner EC, MacKay RJ, Dame JB.


xvi Characteristics of a recent isolate of Sarcocystis neurona (SN7) from a horse and loss of pathogenicity of isolates SN6 and SN7 by passages in cell culture. Marsh AE, Barr BC, Lakritz J, Nordhausen R, Madigan JE, Conrad PA.

xvii Characterization of Sarcocystis neurona isolate from a thoroughbred with equine protozoal myeloencephalitis. Bowman DD, Cummings JF, Davis SW, deLahunta A, Dubey JP, Suter MM, Rowland PH, Conner DL.

xviii Isolation in immunodeficient mice of Sarcocystis neurona from opossum (Didelphis virginiana) faeces, and its differentiation from Sarcocystis falcata. Dubey JP, Lindsay DS.

Sarcocystis neurona: parasitemia in a severe combined immunodeficient (SCID) horse fed sporocysts.
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Depletion of natural killer cells does not result in neurologic disease due to Sarcocystis neurona in mice with severe combined immunodeficiency.
Sellon DC, Knowles DP, Greiner EC, Long MT, Hines MT, Hochstatter T, Hasel KM, Ueti M, Gillis K, Dame JB.
Humoral immunity is not critical for protection against experimental infection with Sarcocystis neurona in B-cell-deficient mice.
Witonsky SG, Gogal RM Jr, Duncan RB Jr, Norton H, Ward D, Yang J, Lindsay DS.
Prevention of meningoencephalomyelitis due to Sarcocystis neurona infection in mice is mediated by CD8 cells.
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Migration and development of Sarcocystis neurona in tissues of interferon gamma knockout mice fed sporocysts from a naturally infected opossum.
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IN VITRO SUPPRESSED IMMUNE RESPONSE IN HORSES EXPERIMENTALLY INFECTED WITH SARCOCYSTIS NEURONA.
Horses experimentally infected with Sarcocystis neurona develop altered immune responses in vitro.

1 www.ivis.org/proceedings/AAEP/1997/Saville2.pdf
2 see allergic encephalitis at www.pathogenes.com
3 see LPA www.pathogenes.com
Equine protozoal myeloencephalitis is a devastating disease to horses and their owners. A link between *Sarcocystis neurona* infections and severe ataxia directed a hunt for *neurona* in the central nervous system of horses that were ataxic. We’ve discussed this before and indicated how the name causes confusion.

Horses with EPM can get better, even horses with long standing ataxia can improve—but not with antiprotozoal therapy. Ataxia isn’t pathopneumonic for EPM because EPM presents other signs than ataxia and horses are ataxic due to other conditions. Prodromal signs of EPM are often recalled once the diagnosis is made. Our work doesn’t support the view that parasites rummage around in the CNS and cause irreparable damage. Our work, and the work of others, supports the idea that inflammation is key to chronic, relapsing, or progressive signs of disease.

Our induced infection model showed the early signs that always precede ataxia—these cases were not recognized by veterinarians that evaluate the disease. Early disease was unrecognized because researchers evaluated cases during the chronic or end stages of disease. Chronic cases are 30 days or more after exposure to *S. neurona*.

We oppose the view that a horse with EPM has organisms in the CNS and lingering signs are due to neurological scaring. Some horses have parasites in the CNS, they are a small minority of EPM horses. It’s possible that there is no permanent damage and treatment based on rehabilitation is worthwhile once inflammation is controlled.

**Background.** At the 2000 AAEP annual meeting Barbara Daft and her co-workers presented data from spinal cord examinations obtained from horses with neurological signs (28%), race track catastrophe (50%), and undefined (22%). They were able to identify *S. neurona* in 12 of the 234 horses, a mere 5%, one would expect the organism to be detected in 65 animals—the ones with the neurological signs. They looked hard. The “CNS examination consisted of microscopic examination of 20 spinal cord and brain stem cross sections followed by gross examination by transversely slicing brain and cord every 3 to 5 mm and selecting additional areas for microscopic examination.” (We can’t tell from the paper, but those listening to the oral presentation indicated that some normal horses (no clinical signs) harbored *S. neurona* organisms in the CNS (Personal communication, JB). Only 8 horses (3%) had lesions without parasites, and the lesions were consistent with EPM. This is pretty good evidence that in the majority of EPM-suspect cases (horses with neurological disease) the organism isn’t found in the CNS. Where are the merozoites? Why do horses present with multi-focal, diffuse signs of disease? The above paper indicates that parasites aren’t scattered throughout the spinal cord.

**Our induced infection model.** Horses were selected based on normal neurological exams, normal physical exams, normal levels of serum vitamin E, normal cervical spinal (neck) radiographs, no serological evidence of EPM, EHV-1, or other infections. We used a panel of board certified neurologists (that are skilled in diagnosing EPM) to examine our horses without any knowledge of infection, treatment, or therapies—they were blinded, or masked, to the study. And we induced clinical disease. Our panel of veterinarians examined the animals over 4 months. This is our source of data from 75 acute cases. We reviewed the literature to relate our signs with histopathological data from other studies in other animals.
We read the work done by Edith Box. She studied *S. falcatula* in its normal hosts, the opossum and the budgie. *Sarcocystis neurona* isn’t special it’s just another *Sarcocystis* that behaves like a *Sarcocystis*. [This topic is discussed in the following link: www.pathogenes.com/blog/2011/06/13/what-makes-s-neurona-different/]

Oocysts, obtained from opossum feces, are ingested by the intermediate host, the budgie, sporozoites infected the (birds) viscera via a parasitemia that originated in the intestine. The most sensitive organ to infection was the lung. Other susceptible tissues included the liver, spleen, brain, kidney, and then skeletal muscles.

*Sarcocystis neurona* doesn’t infect equine muscle tissues— it hasn’t been found in equine muscles yet (we discussed some issues related to this topic in [http://pathogenes.com/blog/2011/04/08/active-chronic-active-and-chronic-epm-infections/](http://pathogenes.com/blog/2011/04/08/active-chronic-active-and-chronic-epm-infections/)). The absence of proof of *S. neurona* in equine muscle is suggestive that *S. neurona* isn’t in horse muscles, it isn’t proof of absence. No major effort to find *S. neurona* in equine muscles has been published (although we believe one group did feed diseased horse muscles to opossums resulting in fat opossums). Importantly, Dr. Box found:

“A range of doses of oocysts (500,000 to 400) produced infectious merozoites that were found in the liver at 2 days after ingestion, while 40-60 oocysts used for infection did not invade the liver in 2 days.” The horse is an abnormal host and perhaps rapid infection of the liver only occurs in monumental infections.

“At 2 days the oocyst dose was correlated with infections but no correlation was noted with older infections indicating that the later meronts were derived from earlier merogony rather than dispersion of sporozoites.” Here is an important point, indicating that continued infection is due to replicating parasites and not the original infection.

The merozoite population goes quickly from the gut to the liver and lung, the lung supports more prolific growth. Hematogenous spread is the most likely scenario for the distribution of sporozoites and merozoites. Encephalitis was seen in these birds. In this study, and others, *S. falcatula* is hepatotropic. The merozoite burdens were unrelated to overall intensity of infection. There was an inverse relationship between presence of merozoites in the brain and the lung that was unexplained, however the conclusion was that cerebral merogony did not originate from pulmonary meronts. ([J. Parasitol Vol. 75 No 2 April 1989](http://journals.asm.org/content/journal/jp/75/2/1311.full)).

*Infection day 0-3 in the horse* On day 0-14 we gave our horses *S. neurona*. We infected their leukocytes and reintroduced them by IV injection. We used 6,000 organisms for a total challenge dose of 84,000 organisms.

We avoided the gut phase of the infection (oocysts) in our model. Information from infections stemming from ingested oocysts in the equine gut is available from E. Elitsur out of W. J. Saville’s lab (Ohio State). They used a transport stress model to infect the horses and used 250 million oocysts, each oocysts contains 4 sporozoites—1000 million organisms.

Extrapolating from Box’s work, we’d expect to see some organisms in the liver. Six ponies were used in the Ohio State study and tissues were examined on day 1, 2, 3, 5, 7, and 9. *Sarcocystis neurona* merozoites were detected by bioassay in susceptible mice and cell culture—if the organism was there, they were likely to find it. Tissues from lymph nodes, liver, lung, brain, and spinal cord were assayed. They did find organisms and that proved their isolation techniques worked. In later studies, they used the same methods but failed to recover the organism in the central nervous tissues from symptomatic horses. This is strong evidence the organisms weren’t there. In this case, it is negative evidence, not absence of evidence.
By day 3 the Elitsur group found organisms. They found them in the mesenteric lymph nodes, on day 1. The organisms were present in the mesenteric lymph nodes, liver, and lung by day 2. We induced a parasitemia and we could isolate the organism back from the blood by leukocyte culture on days 1, 2, and 3. Our horses were clinically normal on all three days and had clinically undetectable infections. It’s evident from the Elitsur group that merozoites are present in the equine viscera before day 3 of infection from oocysts.

**Infection days 4-6** The ponies at Ohio State still had organisms detected in the mesenteric lymph nodes and liver on day 5. It is day 6 when a few of our horses begin to drool and drop feed. The drooling and dropping feed generally precedes other signs and will progress to mild lip paresis, decreased tongue tone and often a droopy lower lip in a few more days. A rare observation was an abnormal tail carriage. The dose of merozoites was 36,000 merozoites, we thought that the merozoites would need days to replicate in the horse. Of course, laboratory testing of serum from this horse isn’t useful, antibody won’t be measurable.

I’d like to digress here a moment and let you know that intuitively we’d expect the merozoite model to be days ahead of the oocyst model in clinical signs and pathology. The expected lag between the oocyst model and the merozoite infection is the time required for the oocysts to excyst and replicate in the viscera, we expected 4-6 days as we observed in *Eimeria* studies in chickens. The merozoites should have a head start. We were wrong. When we use chicken infections as model, *Eimeria* merozoites and oocysts, the oocysts go through several cycles, the first replication is the sporozoite to merozoite division—about 6 hours. Unexpectedly, we saw a lag with the merozoites and patent infections were seen in the gut at day 6 just when we saw the patent infections from oocysts. Merozoites didn’t shorten the disease in chickens. The Ohio State ponies had merozoites present by day 1, from then on the models are equivalent—except for the phenotype that was used for the infections. We stuck with phenotype SAG 1 while Ohio State used mixed phenotypes.

**Infection days 7-9** The early infections, 7-9 days, in field cases are not diagnosed. There will be some dropping feed but most horses appear normal. Available laboratory IgG tests do not help a diagnosis at this early stage, however results from lymphocyte proliferation assays developed by Dr. Sharon Witonsky (Virginia Tech.) have been linked to active, early disease and measurable changes are present. This was discussed in [http://pathogenes.com/blog/2011/10/02/double-trouble/](http://pathogenes.com/blog/2011/10/02/double-trouble/)

By day 7 the Ohio State ponies have histological lesions in the brain and spinal cord that are consistent with EPM. They didn’t find the organism. If it was there, they would have found it. They reported clinical signs in their other experiments with this model. What’s causing the signs if it isn’t direct neurological damage from the parasite? There are differences between the phenotypes of *S. neurona* and one of these differences is pathogenicity—but in the face of 1000 MILLION bugs that get to the lymph nodes, liver, and lung why aren’t they found in the brain with the lesions? Logically, there is a different mechanism causing the lesions, the swelling, the pathology, and the clinical signs.

**Infection day 10-14** Ohio State is out of ponies. They did do other experiments and killed animals looking for those with merozoites in the brain tissues at later and later days after challenge. But they never found them. We did an earlier study and demonstrated merozoites from infections that used 100 to 100,000 organisms—compared to Ohio State this is a small challenge. We recovered the parasites from the CNS by culture, but it wasn’t easy. We used highly specific antibodies to capture organisms on beads from large amounts of tissue digested into slurries and then cultured those beads. And our infections were later than 2 weeks. When the duration of infection is ten to 14 days, the clinical signs in most horses reveal mild behavior changes, dropping
feed, and mild stumbling. Occasionally, rarely, we found a horse that was clearly lame or ataxic at this stage. These signs are not remarkable enough to suspect EPM. But importantly, changes are now notable in the lab data. The majority of the infected horses developed antibodies to phenotype antigen SAG 1, we used a SAG 1 strain. To fully appreciate the EPM test result one would need to know the antibody level prior to infection, data most often missing to make an astute diagnosis in a field case. A single point test and clinical exam would not point to a diagnosis of EPM—one would call the blacksmith or the dentist.

Day 15 The disease has progressed; the owner or handler will notice a difference in the horse. Behavior changes as well as gait abnormalities are present. From now on the rate of finding the actual parasite in the CNS is less than 10% in known infections. The rate of parasite recovery is 0% in the Ohio State model. In field infections, which are referred to an academic institution the rate of parasite recovery will be less than 5%. If the pathology was due to the presence of parasites, organisms shouldn’t be so hard to find by those that seriously look for them.

A single point laboratory test along with the veterinary exam is useful information, but two tests that show a change in titer are most valuable. If a base titer has not been determined a test followed by another in two weeks will be informative. Horses that received anti/protozoal therapy will not test positive despite disease. Work with both models, at Ohio State and ours, clearly showed a delay in the production of antibodies if the horses are medicated prior to infection. Medication prior to infection does not prevent clinical signs, another indicator that it isn’t the presence of CNS parasites but some other factor causing multi-focal inflammation that manifests as clinical disease (Prophylactic administration of ponazuril reduces clinical signs and delays seroconversion in horses challenged with Sarcocystis neurona. J Parasitol. 2006 Jun;92(3):637-43).

Infection day 17 Ataxia is now a clear Grade I in our horses. Both the clinical exam and the antibody titer would indicate EPM should be on a differential diagnosis. If a rise in titer, 2 to 4 fold, is appreciated (because a background titer was known) a confident diagnosis can be made. At this point, initiation of treatment with anti/protozoal drugs to which the organism is sensitive will elicit an improvement that is noticed in a week to 10 days.

Infection day 20 In our overwhelming infections, the trend is for the horse to get worse, now a Grade II. We define a Grade II as a neurological ataxia easily detected and exaggerated by backing, turning, loin pressure or neck extension. The horse can be examined on an incline, this is difficult for the ataxic horse. By day 25 the infected horse is clearly a Grade II and with stress or exercise, is a Grade III. This horse cannot be ridden and should not be exercised; free movement in a small paddock with non-aggressive mates is acceptable. Stall confinement is not an advantage. The horse will begin to show wear on the hind toes from dragging the feet. Squared off toes are more pronounced if the ground is hard and the horse is not stall confined. There will often be evidence of trauma to the pasterns from hitting or interfering. The horse will have a wide base stance. The horse will lay down more than a normal horse. Head trauma is not uncommon, mostly scrapes or mild swelling. Corneal ulcers can be present. Corneal ulcers may be a result of an abnormal ability to blink or due to direct trauma.

In smaller dose infections that are typical of field cases, the horse effectively rebuffs the infection eliminating the parasites. The very mild signs resolve, perhaps unnoticed, and the horse is once again normal. Antibodies are detected by ELISA testing. The next infection exacerbates the cycle and antibody levels rise. Without clinical signs this horse does not have EPM (because EPM is a clinical diagnosis). It may or may not have sarcocystiasis.
This horse is exposed and has detectable antibodies. As many as 80% of horses in the United States have antibody.

On average, the antibody titers range from 16-800 in the Peptide ELISA–the titer can be 3200. There is no correlation between antibody titer and signs or eventual severity of disease. There is a statistically significant correlation that, as the disease progresses, the titer rises, so the correlation is with duration of infection. Those animals that receive anti-protozoal therapy prior to the challenge continue to show a delayed antibody response and are sero-negative by all serological antibody tests.

*Infection day 30* The horses continue to get worse, often quite ataxic. These animals have chronic EPM. The organisms continue to exist in the horse because antibodies are produced. When we eliminate parasites with decoquinate antibody titers drop, it takes 8 weeks to see a drop to <2. When effectively treated at this stage the animals make a quick and full recovery. A negative predictor is seizure activity. Horses with seizures don’t do as well and require more aggressive anti-inflammatory treatment. Many veterinarians reported seizure activity in suspected cases of EPM and we found this surprising. In our induced disease model we did see seizures, but it was uncommon. In our model studies the horses that eventually seized showed minimal signs prior to the acute onset of Grade IV disease. In our studies we used CSF taps at 30 day intervals, it is possible that mild inflammation due to the taps exacerbate the seizure activity. Statistically, we did not find that data from CSF taps improved the diagnosis of EPM or predicted the outcome of treatment. The infected horses have multifocal, diffuse signs when examined by clinicians. When horses “relapse” they typically show the same signs that were initially present indicating a site specific lesion (unrelated to the presence of parasites). These horses can be treated and be put back to useful work.
Our interpretation of the SAG ELISA can be a bit subjective so we go to great lengths to explain what we mean. First, our objective evaluation is made by the presence or absence of antibodies. We have a set cut-off value determined by OD for each well of the test so it is an objective evaluation of positive/negative for that well. Every horse serum is tested on each antigen (SAG 1, 5, and 6) at 4 to 8 dilutions. The titer is the reciprocal of the last dilution that has a positive well. Some sera are selected for the strip test. We randomly select sera for the strip test, these data are for USDA and validation of our antibody detection test.

The level of antibodies that are detected by ELISA are compared to known infections. And then we compare the results to a known positive sera. We know these “positive control” samples have antibody because we vaccinated the horses. One horse has a titer at 40,000 so we dilute that serum to a standard range. Sera from this horse was stored and aliquots have been used as a positive control to which all the tests are compared for over 6 years—that’s thousands of tests. This sera is used to standardize all of our test reagents, buffers, antigens, and secondary antibodies so they are exactly the same in each test that we run.

The subjectivity of the test interpretation is based on the nature of EPM. We are well aware that normal horses are exposed to S. neurona and they can have antibody. They don’t necessarily have clinical signs of disease and therefore, by definition, don’t have EPM. Disease is rare but antibodies aren’t.

And we know that a horse can have an infection, resolve the parasitic part of the disease and be left with immune mediated disease that looks just like EPM. Treating the immune mediated part of the EPM-like disease with anti/protozoal drugs is like waiting until the cows come home. You’ll have an empty barn.

We know that some strains of S. neurona are resistant to ponazuril, diclazuril, and pyrimethamine/sulfadiazine. The choice of drug, dose, and duration are important in deciding what drugs to use for EPM. And that is why it is so important to know which antigenic strain causes the signs that you see. We know that, for the strains we have tested in the lab, at very miniscule amounts, Oroquin-10 is effective at killing 100% of the protozoa that causes EPM. Oroquin-10 has the added benefit of treating the immune mediated part of the disease.

Most importantly, we know that not all horses have EPM. Sometimes this diagnosis is made as a last resort, after every other cause of ataxia/lameness/signs was ruled out. Protozoal myeloencephalitis can be overdiagnosed. Occasionally, we hear that Oroquin-10 didn’t work but some other drug (that does not treat protozoa, like Bactrim for example) fixed the horse right up. Well, perhaps it’s wise to consider that the horse didn’t have EPM. In all these cases (that were brought to our attention) the animals were not tested for S. neurona—so before you treat, you need to test. Or EPM will remain a mystery.

It is the nature of research studies to have unknowns. We try to control for these unknowns—for that we use statistics. Take, for example, the absolute number of unique SAG’s that a Sarcocystis can display. No one knows the number for sure, and I doubt if we ever will. We can make a guess based on the size and genetic sequence of the S. neurona genome, the behavior and the population dynamics of the organism, and what has been detected. By these methods we think we’ve identified one more antigen type in horses than anyone else
has found (SnSAG 6) and we test for it, and interpret it. Any undiscovered antigen type would be awfully rare in horses, a statistical anomaly—even if someone was looking for it. But it could be out there. Statistics controls for these “Black Swan” events (The Black Swan: The Impact of the Highly Improbable by Nassim Nicholas Taleb) but no clinician has the time to gather the data needed for this type of analysis. So here is our take on your serum test result and how we base our recommendations. And sometimes, we just say treat and look for a treatment response, based on some small aspect of the history that makes sense to us. In these cases, re-testing close to treatment is informative—diagnostic even. Generally, here is what we are thinking:

**No Antibody detected in serum:** Antibodies are present by day 17 after infections. Testing early in disease may result in negative titers. Testing 2 to 4 weeks apart will show a rise in titer and confirm infection. If clinical signs are present without serum antibodies either early infection or inflammatory encephalitis may exist (or another disease condition). Response to Oroquin-10, indicated by an improvement in clinical signs and a four-fold rise in titer in 10 days indicates active infection. Response to treatment with no increase in antibody indicates inflammatory encephalitis. No response to treatment indicates another diagnosis is appropriate and we believe that no anti/protozoal drug will make a difference.

**Antibody present in serum** A positive response to Oroquin-10 is indicated by improvement in clinical signs and a reduction in antibody titer (<8 on all SAG’s). The antibodies will increase during treatment and then fall over 4-6 weeks, it is appropriate to re-test 5-6 weeks following treatment to confirm elimination of organisms. We hear so often that the horses respond within the 10 day treatment period, but there have been a handful that did respond after a few weeks. This is rare. If you don’t see an improvement very quickly then we are pessimistic about helping.

**Re-exposure to S. neurona** Re-exposure to S. neurona can be a concern. Antibody titers will increase upon re-exposure. This response can be monitored by using the S. neurona antibody test kit after effective treatment. The prevention therapy indicated may help. We see these cases, after 4 or 5 months in which continued monitoring showed no antibody and then all of a sudden, before clinical signs, there is a spike in antibody. Everyone of these cases we identified have opted for prevention.

**Inflammatory encephalitis** Clinical signs of EPM can be due to inflammation associated with S. neurona. Clinical signs can also be present with other microbial or viral infections and vaccine reactions. We have identified 6 cases of successful treatment and immediate regression associated with vaccination. Levamisole treatment alleviates the clinical signs. We find the immune modulation protocol helpful with post-S. neurona associated inflammation.

Werthy was put on Oroquin-10 and completed the treatment successfully. Dianne saw no change after treatment. We hear this in 1 of 15 cases, the rest of the animals respond well keeping our successful treatment of EPM at 93%. We believe that this means that 7% of EPM horses (ataxic horses with significant antibodies) have inflammatory issues. In order to be evaluated statistically, we need your pre and post-treatment serum samples. We also need the feedback to questions that are on our ELISA submission form. If we don’t get the post-treatment serum we don’t have all the feedback we need to keep our data set complete.

This is what Dianne said: *I contacted you after completion of the Oroquin when I did not notice any improvement. I decided to do the levamisole anyway even though you said that it may not help. He is 1 week into the levamisole treatment and now is steadily improving. Here’s hoping for a recovery. Dianne*
There are several things we like about Dianne’s recent contact with us. First, she filled out the survey message completely and that gives us a lot of useable information. We didn’t like to hear that our treatment failed to resolve Werthy’s clinical signs …but it’s such important information for us. Just think, if ten years ago people were on a list somewhere and said that their horse had been diagnosed, tested, and then the outcome of various treatments were tabulated and discussed. Someone would have figured out that EPM was a complex condition that may need multiple therapies. The part that inflammation plays in the clinical signs of EPM would have been addressed. And how much of a difference it could have made— to so many animals. It took a long time and a lot of horse-owner frustration with the status quo to get things noticed. Insanity. Doing the same thing over and over and expecting different results.

It’s not that inflammation was overlooked. It just wasn’t discussed on the level that it should have been. We’ve mentioned the data gathered from the Ohio transport model studies. Inflammation was documented and mentioned. And what about Dr. Thomas R. Bello, in his 2008 paper he discussed addressing immunity in cases of EPM. He didn’t have the advantage we did, a defined induction model, but he certainly recognized the immune system as an important part of disease.

If you wonder why large companies don’t jump on discoveries that might result in tweaking a drug formulation, I can give you 100,000 reasons. The application fee to label levamisole HCl for horses is $93,000. That alone is a deal breaker for a large company to move forward with a label change. Add the costs of the studies to even do the safety and effectiveness...it’s astronomical. Estimates to license a drug for horses are between 2-8 million dollars. Fortunately (or not) we are a tiny company taking one drug through FDA using the MUMS program.

Under a MUMS designation, if you have no FDA licensed drugs, they waive fees to help out. That means after our drug is licensed we will no longer be able to partake in MUMS benefits. Even if we discovered a method to cure that pocket pet of some incurable disease MUMS wouldn’t be available for us. And, if we did discover a different formulation—paste versus top dress pellet or tablet versus syrup based oral compounds it becomes a brand new submission worthy of huge fees. Changing the label as to dose—it is a brand new submission. Everything starts again. Without MUMS the cost to say “use twice as much drug” would cost millions of dollars. And more animal research. It is absolutely critical to get it right the first time because economics dictate it will be the only time. And this is where your feedback helps. We think we have it right. We need to be sure.

We are working with FDA to license Oroquin-10. Part of that process is the requirement to determine exactly what effects each active ingredient will have on the disease process. Our FDA project reviewers determined that we need to look at EPM in 24 induced cases, dividing the animals up into treatment groups and measuring the specific effects of each active drug in our treatment. The most obvious goal that comes to our mind is showing that decoquinate kills the protozoa, we’d strive to show we couldn’t isolate protozoa from the groups that got decoquinate and were able to show parasites isolated from the other treatment groups. And that gives us pause because the Ohio researchers clearly showed us how hard isolating the organism could be—they failed. Why won’t we?

We have specific tests for antibody…and we can show that antibody declines after 6 weeks of effective therapy…but will levamisole have an effect on antibody production? No one knows. If levamisole and decoquinate drop antibody titer, even for different reasons, there would be no clinical distinction between the two active ingredients. If you know the answer to this, please call.
And how do you test for inflammation? We know that levamisole should be the ingredient that is effective against improper immune responses, why we’d bet the farm on it. Oh. We already did bet the farm on it. If we had a test for *S. neurona*-associated immune disease, that we could guarantee, we’d sure offer it to you as a diagnostic and save you some of that Oroquin-10 money! Dianne is for that one.

We do have a great assay, we can detect EPM due to *S. neurona* in acute and chronic infections—thank Dr. Sharon Witonsky for that one. But before you start sending in all those tubes of blood to be helpful, let us first say that we have to culture the lymphocytes from the blood. The lymphocytes are evaluated for suppression of responses to certain molecules (mitogens). That means that the blood has to arrive within 24 hours so the lymphocytes are viable and want to respond to mitogens in the test tube. They have to be able to respond (we control for that) so we can measure a lack of response. We measure those lymphocytes that are *recalcitrant*. I’m not sure I’d bet the whole farm on levamisole showing a spectacular change, but FDA won’t require spectacular, only a 95% confidence based on a statistical binary outcome of success/failure. If anyone has a spectacular idea to objectively measure (no exams here—that’s subjective) the direct of levamisole on inflammation we’re all ears.

I had high hopes of the promising TGF-beta test. It’s an ELISA and we like those types of tests. But TGF-beta is tricky. It’s one of those molecules that *up-regulates* in the periphery and paradoxically, *down regulates* in the CNS. We don’t like to bet the farm on paradoxes. And TGF-beta is non-specific. Non-specific paradoxes aside, we can test the TGF-beta levels on your serum samples that are sent for ELISA just to get an idea if this assay will be fruitful. What we are interested in are those animals that don’t respond to Oroquin-10, but respond to levamisole. We’d also like serum from any animals that don’t get any protozoal treatment, but respond positively to levamisole, or animals that re-lapse and respond to levamisole. You get it…we want samples from levamisole responsive horses with a minimum of confounding factors.
Like the classical circular reference, “Which came first, the chicken or the egg?”, (a parameter is required to calculate the parameter itself) is present in regulatory medicine. The context is our goal to obtain a fully licensed drug to treat EPM. The approval process sets the timeline (5 years) to fully license the product. The requirements for a *conditionally* approved FDA license for a product are *safety* and a *reasonable expectation of efficacy*. In order to show that the drug is *effective* it must be used in a certain number of target animals (that are afflicted with the treatable condition), and this implies that there is an approved formulation. Approval, in this context, means that the product is made in an FDA approved facility from sanctioned ingredients with enough back story on the drug to deem worthiness. The Food and Drug Administration won’t put their seal of approval on a product unless a certain standard is met—that means they “set the bar” and all products that follow the first licensed drug have to meet, and usually exceed, that standard. When it is a first product presented to treat a specific condition, the bar can be pretty low. Obviously, our product isn’t the first licensed product. We have the opportunity to be the best.

*You have to break eggs to make omelets*

An educated guess about what to put into the formulation is key. It would be difficult to move several products through the system—and certainly conditionally licensing multiple products is cost-prohibitive. Once the application to FDA is sent the die is cast. A quick review of licensed drugs indicates that there is a shortened path available to “tweak” a formulation and each of these improvements built upon formerly licensed products. Even these corrections require a lot of data and paperwork. We’d prefer to hit the target first time.

The opportunity to test treatments and tweak the formulation is available using an “investigational” drug classification. An investigational-use product ensures that several parameters are met such as 1) the production facility has been inspected, 2) the raw materials are kosher, and 3) the analytical methods are in place and evaluated. In order to get these steps in the books, it is necessary actually produce serial batches of product. Three “pilot” batches are required. Investigational use drugs are then used in two kinds of studies, controlled challenge studies (if these types of studies are available) and target animal field use studies. When the currently licensed EPM products were brought to market, there were no laboratory controlled challenge studies used to test EPM drugs. A huge factor was that horses had to *get EPM* in the untreated (control) challenged animals, and that didn’t happen with the oocyst challenge models.

Of course, the investigational use label allows the product to be put into the capable hands of practicing veterinarian treating the condition. From my quick review of public documents for licensed drugs, the efficacy (investigational use) study is generally done in veterinary teaching hospitals or large referral clinics. One reason why the investigational study to show efficacy is done in these types of facilities is that there is a lower teaching threshold for the veterinarian concerning the paperwork that is involved. FDA requires one-on-one instruction to the veterinarians conducting the investigational use study. Veterinarians are *notorious* for skipping those lines where it says “sign here” and we’d like all cases to be evaluated in the same manner. It may take a lot of travel to properly instruct vets to fill out a form and conduct the exact same exams. It isn’t *impossible* to put an investigational drug into the hands of the field veterinarians, it is just a less used path.
There are two reasons to take the road less traveled EPM. First, EPM has a low morbidity in horses. (1) Field veterinarians don’t refer cases or, as the morbidity paper concludes, there isn’t much disease out there—a traditional multi-center study would take years as opposed to a broader study. The low numbers of EPM horses seen in clinics could result from the prohibitive cost of referral/treatment to the horse owner. The investigational use drug is restricted to veterinarians selected to participate in the study and any afflicted horse will have to be near one of the participating field study veterinarians. And that is the second reason to take an alternate path. Our goal is to make the drug available where it is needed. We offer a CE course to instruct veterinarians about EPM and bring them up to speed on the current literature. The veterinarians that complete the course will be trained so they can participate in our studies.

Investigational studies can help determine effectiveness. It is doubtful that drugs, once on the market, will go back through the licensing process to correct the dose to one that is more effective, that can be done through the professional channels like peer reviewed literature. Folks publish papers and talk about the drugs, this promotes off label use, but is worth it to get the treatment right. The re-licensing process would constitute a new license, and cost 5-10 million dollars.

After the investigational study is concluded gathering enough data to satisfy FDA’s “reasonable expectation of efficacy” a safety study is appropriate. The safety study requires 32 animals. Safety study horses are given higher doses of product and for a longer duration than would be intended for use by the veterinarian in the treatment of disease (if veterinarians follow the FDA approved label). Generally, the doses examined in the safety studies are one, two, and five times the dose and three times the duration—based on the final label use. These studies are done early on and can also serve as a titration for efficacy if intuition about the initial formulation is in doubt. You can see how this is important when you look at a drug like Marquis. The doses recommended by those that were involved in the efficacy trials are now beyond the highest of those used in the safety studies. Not that one would expect Marquis to be toxic to the horse at 7X or even 6-8 months of treatment—just toxic to the bank account.

That isn’t the case with pyrimethamine because this drug (ReBalance) was shown to be toxic at a 2X dose in the study documentation. The safety study also showed that anything beyond the one month treatment period was toxic. Some still recommend these higher doses to treat EPM.

The safety study is a large milestone because that allows FDA to issue a conditional license. The conditional license allows the drug to be used as it was intended, by veterinarians that diagnose the condition under field conditions. The field trial can also be used to support safety under field conditions. This conditional period is used to complete any target animal trials, either field or laboratory controlled, and also to get all that paperwork into FDA. Paperwork is just the nature of regulatory medicine. They have rules and they are rule followers. The good part is that they are animal lovers, horse owners, and some, former equine practitioners. We found FDA team members to be sympathetic and helpful. At the end of this road our drug will be fully licensed.

To review, to get the fully licensed drug, a conditional license is needed. And to get a conditional license a reasonable expectation of efficacy and a safety study is require. And to get the safety study an investigational use designation is needed (for field effectiveness and safety). In order to get the investigational use designation, an effective formulation must be anticipated and produced in a licensed facility. That leads us back to the chicken/egg conundrum: What came first: the effective drug or the drug with an expectation of efficacy?
Research is the process of answering a question. As the idea is brought forward, the scientist designs the experiment in such a way as to answer the specific question posed. Also, there is a process of checking out all the expected and unexpected variables that can influence the results that could be unintended consequences. Most try to eliminate the confounding variables. It is important to ensure that the intended question is actually what is tested—and that can be quite tricky. Remember the oocyst challenged horses that were used in a study to determine, or show, that *Sarcocystis* sp. oocysts caused EPM in horses by Fenger way back in 1997? (2) The authors set out to show that *Sarcocystis* sp. oocysts from opossums cause EPM. Therefore, the question is “Do oocysts from opossums cause EPM?”

They reported clinical signs of EPM, no isolation of *S. neurona* (which is the gold standard—and very difficult), but they did report signs of EPM (except one, that was a confirmed wobbler at post). The authors went to great lengths to show us, and we believe them, that they used *S. falcataula* in their studies. Oocysts were derived from budgies and *S. falcataula* (but not *neurona*) can infect budgies. The biological assay using budgies to identify opossum oocysts as *S. falcataula* stands the test of time. Back then, the authors thought that *S. falcataula* and *S. neurona* were identical and set out to show that *Sarcocystis* sp. oocysts from opossums cause EPM. In this context it is highly appropriate that they would show the oocysts used in the studies infected budgies, unfortunately *S. falcataula* doesn’t cause EPM (we think). Most believe that *S. falcataula* oocysts can’t cause EPM in horses (UF researchers put this issue to bed after conducting several studies) (3) therefore the work by Fenger et. al. could be interpreted differently. The confounding factor is that the feral opossums could have harbored *S. neurona*. Or some other *Sarcocystis* sp. in the opossum feces could cause EPM...or even that there is a strain of *S. falcataula* that can cause EPM. We now know that *S. falcataula* SAG 6 antibodies can be confounding. They got the results they expected.

Undoubtedly histopathological lesions were documented and lesions were a direct result of oocysts from opossums...but not the statement that *S. neurona* was the culprit because they never showed us that *S. neurona* was in their challenge inoculum. They actually went to great lengths to prove the opposite. Had they isolated *S. neurona* from the CNS of these horses they would be on firmer ground. And if you follow our thoughts, maybe what they saw was host derived immune mediated disease and not parasite associated pathology.

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One way to make sure we ask the right question is to test our drug in horses early on. We tested the drug in our personal horses for safety. And then we determined a dose from the experience of veterinarians over 30 years. And then we conducted a field study, using veterinarians who see disease, using the dose that we determined was effective. It has taken 6 months to obtain what we think is reasonable expectation of efficacy. You can read about the answer to our question “Is Oroquin-10 effective at treating horses with clinical signs of EPM in horses?” in the *International Journal of Applied Research in Veterinary Medicine*, Vol. 10, issue 1, 2012. At the end of this preliminary target animal field study we report that Decoquinate Combined with Levamisole Reduce the Clinical Signs and Serum SAG 1, 5, 6 Antibodies in Horses with Suspected Equine Protozoal
Myeloencephalitis. Some critics saw this as putting the cart before the horse. They just don’t understand the process or think we may fall into conducting myopic studies. We learned from their mistakes. We wanted to ask the right questions, prepare for any unforeseen circumstances, and in the end, break some eggs.

1. Are cases of Equine Protozoal Myeloencephalitis (EPM) in North America on the rise? Agricola Odoi, BVM, MSc, PhD, Sharon Witonsky, DVM, PhD, DACVIM, and Frank Andrews, DVM, MS, DACVIM. San Antonio: s.n., 2011. Equine Protozoal Myeloencephalitis SIG ACVIM conference.


The Characters

The recent proceedings from the EPM Special Information Group at the 2012 ACVIM meeting contained an abstract of interest. The abstract, presented by Amy Johnson (of New Bolton, who reported that strains lacking SnSAG 1 predominate in the mid-Atlantic region—a finding with which those of you with horses in the mid-Atlantic region, can take umbrage) and Jennifer Morrow (Equine Diagnostic Solutions—she heads up the lab running the 2, 4/3 ELISA) discussed that serum: CSF titer ratios may provide the most accurate means of anti-mortem diagnosis of equine protozoal myeloencephalitis.

These two compared the IFAT and the surface antigen 2, 4/3 ELISA using stored serum/CSF samples from clinical patients seen at New Bolton Center. The data set used 9 confirmed cases—the horses had neurologic deficits and post-mortem lesions consistent with EPM—we’ll have to ask if that was the presence of parasites or just inflammatory lesions. If these were the same data set used in Dr. Johnsons 2010 paper, the horses didn’t definitively have EPM because “immunohistochemistry or PCR testing (or both), for S. neurona and other neurologic diseases was utilized at the discretion of the pathologists to confirm a diagnosis but were not performed in every case (Johnson, J Vet Intern Med 2010;24:1184-1189). We know how important distinguishing between the presence of parasites and the presence of inflammation may be in pre-mortem clinical management.

Negative cases were comprised of 28 animals with various clinical signs and post-mortem lesions consistent with another disease. The authors also used sample sets (serum and CSF) from six suspect cases. These animals were suspect EPM because they improved after treatment, and other diseases were excluded from the differential diagnosis. Another 14 cases that had various signs and a strong presumptive diagnosis of another disease were included and they comprised another negative group. It’s crucial to understand the groups of horses, the cohorts, under investigation in any study. In this study there were 15 animals with EPM—9 with signs/histological lesions (we don’t know if parasites were present) and six with a response to treatment. Upfront, we wholeheartedly condone the response to treatment as a supporting diagnostic for EPM. The ideal drug would elicit a response in a few days.

The Plot

This study was titled Comparison of an indirect fluorescent antibody test and surface antigen ELISA’s for antemortem diagnosis of Equine protozoal myeloencephalitis in naturally occurring cases. We must object to any IgG test proposed for the diagnosis of EPM because IgG tests alone can’t identify or characterize the disease equine protozoal myeloencephalitis. Only the infectious part of the disease is diagnosed by IgG testing. Our view is that EPM is a condition that is comprised of infectious protozoa and the inflammatory responses of the host that manifest as clinical signs and the manifestation of clinical signs can be after the infectious event. Coupled with response to treatment, specific peptide ELISA tests (SAG 1, 5, 6) can be a part of a diagnostic plan.

The Sting
Johnson and Morrow report the highest accuracy with these tests was when the ELISA or the IFAT were used with paired serum and CSF samples. They said “the use of IFAT serum results alone, SAG 2, 4/3 ELISA serum results alone, and SAG 2, 4/3 ELISA CSF results alone decreased overall accuracy of diagnosis. The more accurate testing methods should be utilized whenever possible.” They propose using CSF testing along with their antibody tests.

The Prequel

The use of an antibody index was investigated and reported 10 years ago. The antibody index was a test with contamination as the raison d'ètre. Blood contamination of CSF occurs during collection and confounds the results. Martin Furr investigated antigen-specific antibody occurrence in the CSF after peripheral injection of ovalbumin (J Vet Intern Med 2002:16:588-592 Antigen-specific antibodies in cerebrospinal fluid after intramuscular injection of ovalbumin in horses). He also investigated the possibility if there was antibody production, the time over which antigen-specific antibodies could be detected. He found a low but positive CSF titer after peripheral injection with a non-replicating protein antigen that “has dramatic implications for the evaluation of CSF antibody testing to confirm CNS disease, such as EPM.” He defined the principle of the C-value as the antigen-specific antibody ratio to the total IgG in CSF is equal to the corollary ration in the serum. Values of greater than 1 imply local, intrathecal production of antibody. His study found C-values of greater than 1 in horses that were peripherally vaccinated supporting that C-values of >1 do not conclusively prove CNS infection.

Dr. Furr suggested that intrathecal antibody production (IT) assessed by antibody index was a better measure of antibody production because the C-value appears to overestimate IT production so he added the caveat that specific studies to test this hypothesis must be performed to conclusively determine this fact. An important conclusion from his study was that passive movement of antibody across the blood-brain barrier can be a source of antigen-specific antibody in the CNS. The thoughts at the time were that if specific antibody titers were used (recombinant SAG’s, for example) the data would be more meaningful. We observed similar phenomena using rSAG1 vaccination in previously naïve horses. We reported that vaccination with rSAG 1 resulted in detectable antibody against S. neurona SAG 1 in the CSF of the vaccinates by rSAG1 ELISA. It is perhaps why horses vaccinated with rSAG 1 did not show signs of EPM when challenged. The unvaccinated challenged horses all succumbed to EPM. The site of protective antibody production induced by vaccination may be, in part, IT antibody. We don’t dismiss the possibility that peripheral antibody is protective, we were able to show that after vaccination or challenge neutralizing antibody was present against the same phenotype of S. neurona—neutralizing antibodies were not present to heterologous organisms (SAG 5 with a SAG 1 challenge or rSAG 1 vaccination). However, after challenge with SnSAG 1 organisms, animals showed neutralizing antibodies against all phenotypes. These observations don’t argue against inflammation as a major contributor to clinical signs of EPM.

Based on our studies with S. neurona challenged horses (SAG 1 strain), CSF test results did not enhance the diagnosis of EPM. The diagnosis was made by clinical exam and serum antibody against rSAG 1 ELISA. The sera and CSF from our experiments were also tested by EBI’s diagnostic at the time, Western Blot. The CSF Western Blot did not enhance the detection of diseased horses. We ran duplicate samples every 30 days from pre-challenge to 120 days post challenge in 24 horses—there was no evidence that CSF testing was of value over the serum diagnostic. We don’t recommend CSF testing for these reasons.
The paper Clinical diagnosis of Equine Protozoal Myeloencephalitis (EPM)* and the review by Frank Andrews** (of the paper Sensitivity and specificity of Western blot testing of cerebrospinal fluid and serum for diagnosis of equine protozoal myeloencephalitis in horses with and without neurologic abnormalities*** both conclude the western blot was slightly better when testing CSF; however, serum testing yields similar results and may be preferred due to the risk of CSF sampling (Andrews) and “the IgG index provides limited diagnostic information regarding diagnosis of EPM”. The previously reviewed ACVIM consensus statement for EPM does not recommend routine use of CSF. Again, we believe it is time for the ACVIM to make a new statement regarding EPM diagnosis. More appropriate would be a statement regarding diagnosis of S. neurona infections and a discussion of the inflammatory issues—different aspects of the same disease. But don’t expect their opinion on the information gleaned from CSF testing to change.

** Frank Andrews. Equine Medical Review published by Bayer, Veterinary Learning Systems, Yardley, PA
*** B. M. Daft, B. C. Barr. JAVMA 221(7):1007-1013, 2002

Our Arsenal

Our weapons for the war on S. neurona/EPM include the Peptide SAG 1, 5, 6 ELISA’s, a specific antibody test panel for all the strains of neurona. We include the SnSAG 6 strain and this strain may be missed by other tests. We also are bringing an inflammatory test to the fight—once validated, it may shed light on what have previously been very confusing cases—those cases which are due to inflammation. These cases present with clinical signs, perhaps long after the organism is eliminated. And, most important, response to treatment as a diagnostic aid. The authors Johnson and Morrow used the historical response to treatment in 40% of their presumptive positive cases in their study. We use response to treatment daily to help you with your EPM horses and guide future treatment decisions. Commercial tests will always vie for your diagnostic dollar. The best test is the one that helps you determine a course of therapy that alleviates disease in your patient. Try several and determine what works best for you.
Protozoa are complicated

Equine protozoal encephalomyelitis is a confusing disease because we want to view it like other diseases that we understand, simpler diseases like bacteria and virus. Bacterial infections, once they are identified using antibody tests, respond to the right antibiotic. If we know that if the particular bacteria is resistant to a specific antibiotic, we isolate the organism, test the sensitivity and select a more appropriate treatment. We can’t isolate protozoa from a live horse and not very easily from a dead one— which eliminates in vitro sensitivity testing in deciding which drug may be the right choice.

A viral infection will run its course. There are some antiviral therapies and we try those depending on the etiologic agent. Once viral infections are identified we treat the symptoms until the immune system eliminates the infection. Pathogenic protozoa, successful ones, manipulate the immune system and remain in the host until an opportunity (stress, perhaps) arises. Identifying the early signs of protozoal infections and isn’t easy in the horse. The horse may be an aberrant host and much of the time the horse will eliminate the infection. And that’s another reason equine protozoal myeloencephalitis is so darn frustrating. When will the horse get better without treatment and when will subtle infections become big problems? The key point is that when there are clinical signs, no matter how slight, the horse isn’t winning the battle and we need a strategy that will eliminate the infection. We’d also like to build protective immunity.

Pathogenic protozoa are far more complicated than bacterial or viral pathogens on many levels, one of which is the array of proteins (antigens) that they have in their arsenal to confuse the hosts protective immune responses. We can recognize S. neurona infections by detecting specific antibodies (we use SAG 1, 5, and 6) because these are produced against the antigens that are unique only to Sarcocystis neurona. Antibodies to these proteins indicate infection, old or new; current infection is detected by serial titers that are rising. But protozoa use these antigens to their advantage. These antigens disguise early infecting merozoites and thereby manipulate the immune system of the host.

Ideally, the protective immunity the host acquires against protozoa is cellular immunity (CD4, Th1, CD8, and interferon gamma). Until the cellular immune system is properly acquainted with the pathogen, the protozoa can win the infection battle. By waving the antigen flag the host’s immune system kicks in and responds to the infection as if it were a bacteria or virus—and that maybe where the host is duped. Maybe the B cell response tunes down the hosts cellular response just long enough for the parasite to switch the system to one that is not favorable to clear these intracellular organisms and disease ensues. We think the unfavorable collection of turned on leukocytes are CD4, Th2, and cytokines IL4 and these are elicited by the presence of the parasites.

Dr. Sharon Witonsky (Virginia Tech) discovered that in as few as 72 hours the T lymphocytes are turned off in a very specific way. We can measure that in natural and experimental cases of EPM. We ran a blinded experiment to determine if this response could be predictable and useful to identify infected horses.

What surprised us is that not only did she identify animals that were infected...but she had the correct order in the severity of clinical signs (disease) that the horses ultimately displayed. The more suppression the
lymphocytes showed on the assay, the more severe the disease many weeks later. And this prediction was made at 72 hours. Of course we didn’t know that the order was correct until months into the experiment. And we found something else that was interesting, and that is the suppression of the lymphocytes was specific to protein displayed on the parasites. When we used a SAG 5 organism for the proliferation assay the suppression was not observed and no prediction as to outcome could be made— if we challenged the horses with a SAG 1 strain, that is what we did. We didn’t determine the mechanism of the response but no doubt determining the suppression mechanism will help us identify early infections in the horse and perhaps even the outcome of the infection. It may be that the initial antigen that the parasite so blatantly displays dupes the immune system into turning off cellular immunity that would protect the host against infection. Also interesting is that the cellular immunity may actually cause an inflammatory response that results in swelling and changes in the spinal cord tissues that look like EPM—ataxic horses.

Can’t see the forest for the trees?

It’s all very confusing, to us as well as the horse owner. But as we analyze our data from our drug trial we are finding some patterns. It is certainly true that we only record a few facts about each horse but the questions we ask are the ones we have identified as important. The answers you provide enabled us to build a decision tree—a technique that is used to generate rules about the data and perform generalizations and predictions.

The Decision Tree

In order to generate a set of rules we constructed a decision tree for EPM suspect horses to help you manage your case of EPM. This was done top-down and we partitioned the data into subsets that contained instances that had similar values.

Response to treatment as a diagnostic aid

Just a side note here: If you want to use the ACVIM position statement protocol for confirming an EPM diagnosis by detecting the presence of antibodies to S. neurona (they say CSF and western blot, but we’ve found that since this statement was issued, stats tell us we only need serum when using the Peptide ELISA) and a response to treatment here is the ten day plan. We know that in S. neurona infections respond to decoquinate/levamisole and that the response is detected clinically in 3-5 days. A rational approach would be to pull blood and send it for the Peptide ELISA titer and institute therapy.
Predicting the outcome

Horses that are treated and re-tested give us a pool of similar animals to follow and we record the outcome of the treatment. A decline in antibody level, when accompanied by a rapid reduction of clinical signs of disease, is a positive indication that the disease is gone. While our data set is small (200 so far) it means that the predictions are speculative. But as we record more data on each horse then there is a better chance that we can get more insight into the underlying function or relationship between the outcomes. We show our decision tree (above) and based on what you see clinically as well as the treatment you choose, you can see how similar animals responded (by percentage) and perhaps use a logical approach to your next steps.

There are a few horses with “relapse episodes” that are associated with vaccination (products that contain equine dermal cells). Sometimes this association is not with vaccination but deworming and small strongyle infections. While the inciting cause may have been *S. neurona*, the inflammatory trigger remains unidentified. All of the horses in this relapsing group have had several episodes of EPM. They were treated multiple times over several years showing mild to moderate responses to ponazuril, diclazuril, or both. There is no documented change in their clinical signs with the addition of sulfa/pyrimethamine to the treatment protocol.

We can begin to examine this group of horses for the lymphocyte suppression that we saw in natural and experimental cases of EPM. When we identify the population of lymphocytes that are *stimulated* during these episodes and identify the triggers that stimulate this lymphocyte population we may understand the mechanism of the disease process and then we may know how to begin treating this syndrome. For now, we believe that levamisole (and when needed DMSO and steroids) are rational approaches for these horses.
We were intrigued by a recent paper (F. P. da Cruz), he studies *Plasmodium berghei*. *Plasmodium* is an apicomplexan parasite that is similar to *Sarcocystis neurona*. *Plasmodium* causes malaria. While there are many differences between *Plasmodium* and *Sarcocystis*, there are enough similarities to get excited when something intersects with our work.

Another parasite, *Toxoplasma gondii*, is similar enough to *S. neurona* so that comparisons are made. For instance, Dan Howe indicated that stage-specific expression of the *Sarcocystis* SAG's may be important for the parasite to progress through its developmental stages and complete its life cycle successfully because that is how it works in Toxo. The *S. neurona* merozoite (the stage that is found in horses) surface is covered by multiple, related proteins which are similar to the family of surface antigens of *T. gondii* called SAG/SAG-related sequences. While you can make predictions on how similar members of a family may behave, we all know from experience you have to be cautious in making too close a prediction on any one member. And that is why the experiments are important. The expression of these SAG proteins in *Toxo* and *Neospora* are life-cycle stage specific and seem necessary for parasite transmission and persistence of infection.

Dr. Howe reported that the SnSAG merozoite surface antigens of *S. neurona* are expressed differentially during the bradyzoite and sporozoite life cycle stages. He found that SnSAG 2, 4, and 5 were either absent, or expression was greatly reduced, in bradyzoites. That makes infections hard to diagnose. If no antigen is expressed, the animal can’t make an immune response that is detected by testing. If the animal can’t detect the infection then there won’t be a protective response either. Dan Howe found that the antigens common to all *Sarcocystis*, SAG 2, 3, 4 are expressed in sporozoites—the stage that is in the gut of the horse. The pathogenic protozoa move on to new stages in order to create disease. Thus, when the horse ingests sporocysts and the sporozoites are released—these common proteins are available for an immune response and are detected by non-specific tests. As the parasite moves into the next stages and into the blood of the horse, there is down-regulation of the SAG 5. However, the presence of SAG 1, 5, and 6 are the specific triggers for an immune response we detect in our specific Peptide ELISA. We know that antigens SAG 1, 5, and 6 are expressed on merozoites because sick horses have antibodies to these antigens. It may be that stages that are unexpected exist in horses.

What we found most exciting was that the drug screen targeted at Plasmodium liver stages identified a potent multistage anti-malarial drug. *Plasmodium* parasites undergo a clinically silent and obligatory developmental phase in the host’s liver cells before they are able to infect erythrocytes and cause malaria symptoms. Can *Sarcocystis neurona* do the same thing? Decoquinate emerged as the strongest inhibitor of Plasmodium liver stages, both in the lab and in live animals! The important finding is that decoquinate kills the parasites replicative blood stages and is active against developing gametocytes (the sexual stages of the parasite). The mechanism of action is by selectively and specifically inhibiting the parasite’s mitochondria. The apicomplexan parasitic protozoa have different mitochondria than animals and that is why the drug decoquinate has no action on the animal.

We find this important because the Plasmodium mechanism elucidated by de Cruz may be why decoquinate is so effective in cases of EPM. Oral administration of decoquinate effectively prevents the appearance of disease...
in Plasmodium. Soon, decoquinate may take the lead in treating malaria. It concerns us because some researchers believed that decoquinate would not have an action on multiple stages of Sarcocystis neurona. They didn’t doubt that we could eliminate gut infections or that we could prevent disease but they took a step back in agreeing with us that we’d kill the disease-causing stages found in the horse. We induced an infection in a filly and measured one million parasites in her peripheral blood. And then we administered our treatment of decoquinate. We analyzed her blood by culture and PCR. The masked samples were sent to a parasitology lab at NIH. They detected parasites on treatment day 4 but not treatment day 5 or beyond. Decoquinate effectively removed the parasites by day 5 of treatment.

We are moving forward with our prevention program. We can use drugs to prevent EPM in horses. The method requires that the horse is exposed to S. neurona and requires that the horse has an opportunity to use the immunity that it develops—the horse has to be in a place with virulent oocysts. We already know how much drug it takes to prevent disease in the face of our challenge model. We are now determining if it as effective in the field as it is in our experiments. If you have an interest in this step in EPM research, you can contact us through the web page. The program is called VAXIN and will be administered by veterinarians. It will require horses that have been effectively treated for EPM using our treatment. The use of static drugs for prevention of S. neurona infections remain to be shown, however it was shown by Martin Furr that trazines did not prevent infections. For our study we need proof of exposure (a SAG 1, 5, or 6 titer ≥ and are at continued risk for EPM—relapse that is not due to inflammation or other animals on the farm that are exposed. This step complements our testing and treating strategy.
Rehabilitation
Posted on January 1, 2013 by Administrator

We are often asked what can the post-treatment EPM horse be asked to do. Exercise is an important consideration. Each case is different. It is important to make sure the horse is ready for increased work—pay lots of attention to exercise intolerance.

When we were conducting one particular study with challenged horses, we determined disease by observing subtle signs. We were disappointed when our masked, board certified examiner didn’t appreciate the disease. We started lounging the horses and found they could only tolerate about three minutes of work before they would stumble and sometimes, fall. We exercised them daily and increased the work by 2 minutes every few days. It was difficult for them to navigate a small area so we used a very large round pen. Free lounge was the easiest for them. Our observer returned in 4 weeks, she noticed a 2 to 3 grade deficit in the horses. What we learned was the exercise made them worse. We have used exercise as part of our infection model ever since.

If signs are subtle in a suspect EPM horse, a weeks worth of daily lounge can help with exacerbating the signs of disease. And that goes for the recovering horse. Turning the horse out for free exercise, so long as it won’t injure itself, is valuable exercise. It is important to begin work slowly. I usually recommend the horse is hand walked, up and down hills. This is good if they can handle an incline. Those that can’t handle a hill, or if there are no hills, begin hand walking in straight lines. When the horse can jog in hand down an incline without error, or jog in a line and then halt without knuckling over behind, it’s time to have a neurological exam and determine the fitness for riding. A horse that is undergoing or has undergone EPM treatment should be evaluated in hand and under saddle by a veterinarian. Using a pony and building the horse up is another way to rehabilitate the recovering EPM animal.

When the horse can tolerate a couple of minutes of lounge in both directions (use a watch) it is time to increase the work. Increase only a minute in each direction every 3 days. As the work increases to 10 minutes each direction and no errors, it’s time to evaluate the suitability for riding. A horse that is handling the work will play once it is turned loose, unless it’s demeanor suggests that play is undignified.

If the horse shows fatigue or if reoccurring signs are apparent, back up the time by several minutes and start again. A day or two off should be enough time to determine if there was too much work or if disease is back. If the animal isn’t back to normal after a brief rest then it is time to run a CRP test and determine the presence of inflammation. I don’t suggest work until the initial 10 day treatment has finished. It is possible for horses with grade IV deficits to get back to full use, it takes slow consistent work. We have a list of owners that have or are rehabilitating animals that had grade IV disease that are happy to share their programs.