EPM II

CE COURSE 0007692 FL 3 HOURS

THE UNDERSTANDING EPM: DISEASE, DIAGNOSIS AND TREATMENT.

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COURSE GOALS AND OBJECTIVES

- After completion of this course you will:
  - have a general understanding of pathogenesis of *Sarcocystis* infection in horses;
  - recognize differences between equine *Sarcocystis* infections due to equine protozoal myeloencephalitis and equine muscular sarcocystosis;
  - have a broad understanding of testing and formats;
  - review current treatments.

It is good to understand the points next to this symbol

BASICS OF SARCOCYSTOSIS

Sarcocystis are parasitic protozoa that may cause disease in horses. Infection with *Sarcocystis* is called sarcocystosis and is usually confined to the gut and muscle.

Infection results in circulating antibodies against parasite antigens. Antibodies are often against the immuno-dominant antigens that are displayed on the surface of the parasite.

SARCOCYSTIS FAYERI OR *S. NEURONA* CAUSE SARCOCYSTOSIS IN HORSES

- Horses are <u>natural</u> hosts for *S. fayeri.*
  - *S. fayeri* forms sarcocysts. *S. fayeri* sarcocystosis is called **equine muscular sarcocystosis, (EMS)**.
  - *S. fayeri* can cause clinical neuromuscular signs in horses. *S. fayeri* releases **toxins** from degrading sarcocysts. **Antitoxin may be measured in serum**.
- Horses are <u>aberrant</u> hosts for *S. neurona.*
  - *S. neurona* is not known to form sarcocysts in horses (current data).
  - *S. neurona* is the predominate agent of EPM. *S. neurona* causes inflammation that may persist after parasites are eliminated.

ASEXUAL STAGES OF *S. NEURONA* IN THE HORSE ARE NOT TRANSMITTED TO OTHER ANIMALS. THESE STAGES DISPLAY STAGE RELATED ANTIGENS

- Schizonts and merozoites, the asexual stages of *S. neurona*, can be found in the CNS of horses with sarcocystosis. **This is rare**.
- Infection induces antibodies against some antigens displayed by *S. neurona*. Some antigens are *transiently* available for immune stimulation. The stage related expression of antigens impact diagnostic testing.

- *60-80% of horses have detectable antibody but do not develop EPM. Parasites are eliminated in most S. neurona infections.*

SARCOCYSTOSIS

- Antibodies are present for 2 to 8 months after parasites are eliminated. The length of time antibodies can be detected depends on the immune background of the animal.

- EPM means sarcocystosis, the organism is actively present. A horse may have antibodies long after the parasite has been eliminated. Detecting antibody doesn't mean there are parasites present!

- All commercial diagnostic tests measure antibody, each uses different antigens! There are no "EPM" diagnostic tests. 

ANTIBODY TESTING

There are no USDA licensed "EPM" diagnostic tests! Antibodies persist after parasites are eliminated precluding their use as diagnostic for active infection.

Antibodies do not detect disease-EPM is also a disease syndrome with an inflammatory component.

Antibodies against *S. neurona* along with clinical signs are supportive of a diagnosis of EPM. A seronegative sample supports that signs are less likely due to EPM.

IS DISEASE DUE TO INFECTION OR INFLAMMATION?

Parasites elicit innate immune reactions that are pro-inflammatory.

- Serial testing can determine if the protozoa are present by observing an increase in titer, a four fold increase, after treatment

- Anti-protozoals don't treat inflammation. Clinical signs can be due to dysregulation of inflammation. Dysregulated immunity presents with clinical signs and may be misdiagnosed as a relapse when no parasites are present!

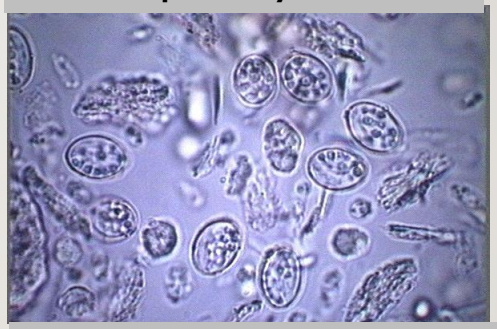
http://pathogenes.com/w/defining-epm-sarcocystosis/

Sarcocystis are usually named from the stages of the lifecycle of the parasites within the hosts they infect: the intermediate host that harbors the **sarcocyst** and the definitive host that sheds **sporocysts**.

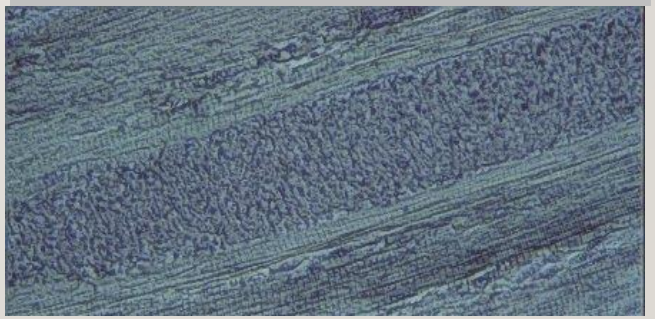
A combination of the two hosts used to complete the life cycle of the parasite is the name: example: *S. equicanis* cycles between horses and dogs. S. fayeri is another name for *S. equicanis*.

It is an aberration that "neurona" isn't called S. dasypusdidelphis or *S. dasypus!*

Sporocysts



Sarcocyst



EXCEPTION TO THE RULE:

Sarcocystis neurona was named for the asexual stage of an undefined protozoa found in a horse with sarcocystosis in 1991 (Dubey):

Unfortunately, naming a parasite to infer that it has a predilection for or only inhabits the CNS changes diagnosis and expectations for treatment!

An anti-protozoal is not expected to treat associated inflammation. It is long recognized that inflammation is the hallmark of EPM but no treatment or treatment protocol addresses INFLAMMATION!

EQUINE INFECTIONS

- *Sarcocystis* have a fecal-oral cycle in the intermediate or aberrant host.
- *S. fayeri* is a common infection in horses. *S. neurona* is an uncommon infection in horses.
- *S. fayeri* sarcocysts are not associated with inflammation while *S. neurona* is associated with CNS inflammation-but isn't required to inhabit the CNS to elicit inflammation.
- *S. fayeri* sarcocyst toxin (sarcocystine) is associated with inflammation in horses.

http://pathogenes.com/w/sarcocystis-fayeri/

EQUINE INFECTIONS VS EPM
SEMANTICS HAVE TREATMENT IMPLICATIONS

S. neurona is expected to be in the CNS of horses with EPM. Organisms don't need to be in the CNS for sarcocystosis-infection and elimination resulting in antibody and inflammation . Inflammatory responses induced by *S. neurona* (sarcocystosis) are responsible for the clinical signs of EPM.

It was experimentally shown that leukocytes and resulting inflammatory responses (cytokines) are required for clinical signs of EPM.

SARCOCYSTIS NEURONA

(PREVIEW: **S**URFACE ANTI**G**ENS ARE NUMBERED AND CALLED SAG'S!)

The opossum (*Didelphis virginiana*) is the definitive host for *S. neurona.* Several intermediate hosts support the life cycle of *S. neurona,* armadillos (*Dasypus novemcinctus*) and raccoons (*Procyon lotor*).

Experimentally, *S. falcatula* does not cause EPM (Cutler). *S. falcatula* shares some surface antigens (SAG 4 and SAG 6) with *S neurona*! It is because *S. falcatula* doesn't infect horses that the shared antigen can be used to detect serotype SAG 6 *S. neurona* in horses!

AN ABNORMAL NEUROLOGICAL EXAM IS THE HALLMARK OF EPM.

EPM is diagnosed by physical and neurological examination. The diagnosis is supported by ancillary diagnostic tests to support etiology.

Horses with idiopathic encephalomyelitis or polyneuritis equi show clinical signs due to undetermined etiologies-often identified as EPM.

Innate immune responses stimulate proinflammatory cytokines that can be autoreactive against peripheral nerves and result in signs that look like EPM.

OVERVIEW OF SARCOCYSTOSIS

Horses ingest *Sarcocystis* **sporocysts.** Resulting visceral infections stimulate immune responses (antibody and inflammatory cytokines). Most horses control and eliminate infections. Antibodies are produced and inflammation can persist after infection. *S. fayeri* forms sarcocysts in muscles and establishes persistent infections. *S. neurona* does not form sarcocysts and is usually eliminated by the immune system.

Antibodies can persist after infection, or remain if there is chronic environmental exposure to sporocysts or *S. fayeri* sarcocystine from sarcocysts, bradyzoites in sarcocysts may stimulate antibody production.

Cytokines stimulated by innate responses can become unregulated.
Dysregulation of inflammatory responses can look like clinical sarcocystosis.

OVERVIEW OF SARCOCYSTOSIS

- *S. neurona* are most likely transported to the CNS from the gut or the viscera to the CNS by leukocytes.
- The genus and species may be crucial to invasion of organisms into the CNS. *S. fayeri* doesn't enter the CNS.
- Inflammatory cytokines cross the blood/CSF barrier readily.

REVIEW

- *Sarcocystis neurona*, agent of EPM, is an common infection but rarely results in disease.
- *Sarcocystis fayeri*, agent of EMS, is a common infection and results in sarcocystosis and release of toxins from cysts.
- Clinical signs of sarcocystosis are similar for disease due to both organisms.
- Dysregulation of immunity can result in neuromuscular disease and is unrelated to active infections.

ACVIM CONSENSUS 2002

- The consensus opinion was intended to serve as an aid to equine clinicians attempting to establish a diagnosis of EPM in horses presented for evaluation of neurological disease.

- The ACVIM 2002 consensus opinion is outdated!
- Veterinary Parasitology 2015. Dubey, JP et al. An update on Sarcocystis neurona infections in animals and equine protozoal myeloencephalitis (EPM)

TESTING RELIES ON DETECTING ANTIGENS

- "EPM" tests detect antibodies against *S. neurona.*
- "EPM" tests do not detect inflammation.
- Tests are "validated" on characterized sera based on the laboratory criteria that run the tests. It is important for the veterinarian to understand how samples are selected for validation of each testing laboratory.

DIAGNOSTIC TESTS TO SUPPORT A DIAGNOSIS OF EPM

Antibody detection is based on antigens associated with *S. neurona*. Serum or CSF can be used in tests, some labs strongly suggest CSF to strengthen the interpretation of a positive result.

All antibody tests use *S. neurona*^{SAG1} strains! 

All antibody tests rely on antigens expressed by *S. neurona* during the in-animal phase of infection. Because some stages of *Sarcocystis* downplay the expression of antigens during infection antibodies won't be produced and detected on some tests. Test format and selection of antigens matters for testing.

2002 CONSENSUS OPINION

- "The reluctance to perform a spinal tap due to risk, cost or inexperience is understandable and although not the preferred approach, a positive serum IgG test in the presence of neurologic signs and history compatible with EPM, supports a diagnosis of EPM." ACVIM 2002, Cornell 2011

- Some tests: IFAT, WB, and SAG 2, 4/3 strongly suggest CSF testing to strengthen "probability" that antibody relates to EPM.

INCLUSIVE TESTS

Inclusive tests use antigens **common** to all *Sarcocystis* as diagnostic antigens. Cross-reactivity of antibodies against **common** antigens are an issue. Some labs dilute out cross-reactive *S. fayeri* antibodies and don't detect most *S. fayeri* infections. S. fayeri can cause neuromuscular disease and these infections will be overlooked.

IFAT, Western Blot, SAG 2, 4/3 ELISA are inclusive tests.

Labs that run these tests strongly advise using CSF to increase diagnosis of the presence of organisms in the CNS

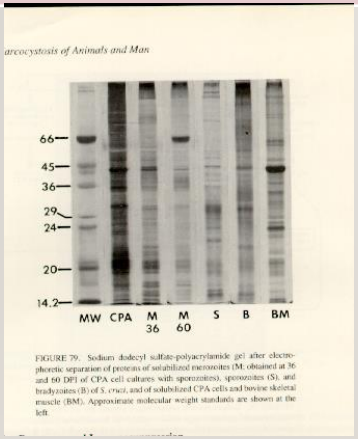
EXCLUSIVE TESTS

Exclusive tests use antigens SAG 1 and 5 that are only displayed on *S. neurona.* SAG 6 and *S. fayeri* antitoxin are not species specific antigens. The are considered exclusive; host specificity makes these antigens exclusive for test purposes.

S. falcatula shares the antigen SAG 6, but S. falcatula *doesn't infect horses* and *S. fayeri* is the only *antitoxin-producing sarcocyst* found in horses. The down side of relying on exclusive antigens is the remote possibility that a rare, undiscovered strain exists and displays an alternate, mutually exclusive SAG antigen. NIH researchers involved in Apicomplexan population genetics state "it is unlikely that any new disease causing strains of *S. neurona* will be found".

ASSAY FORMATS

Test	Format
Western blot (WB)	Parasite proteins are separated by molecular weight into reactive "bands".
IFAT	*S. neurona* SAG 1 strains are immobilized on slides.
ELISA	Plates are coated with recombinant proteins and are highly specific to antigens used for the test. Can be inclusive (SAG 4, 2/3) or exclusive (SAG 1, 5, 6, S. fayeri).



This is an example of a western blot taken from *Sarcocystis of Animals and Man*. The blot shows the difference in protein bands from several stages of parasite growth. The M 36 lane represents the proteins seen at 36 days of culture while the M 60 lane shows the proteins after 24 more days of culture. This exemplifies the difficulty of producing homogenous antigens for WB testing.

ANTIGEN SELECTED IN DIAGNOSTIC TESTS

Test	Antigens	Type
WB Non-reducing conditions enhance diagnosis	Inclusive	Semi-quantative; subjective interpretation of various "bands" that vary with the testing laboratory. Reduced gels provide linear epitopes while non-reduced gels provide conformational epitopes.
IFAT	Inclusive	Subjective interpretation of indirect detection of surface immunofluorescence based on conformational epitopes. Antigen detection varies with fixation method, strain and stage of organism.
ELISA 2, 4/3	Inclusive	Quantative, detects SAG's 2, 4/3 shared by *Sarcocystis,* are not species specific. Peptide 4/3 does not occur in nature, the synthesized peptides show linear epitopes.
ELISA 1, 5, 6, *S. fayeri*	Exclusive	Quantative, detects SAG's 1and 5 unique to pathogenic *S. neurona*, serotype specific; SAG 6 and SF antitoxin are host specific. Antigens use conformational epitopes

Antigens have epitopes to which antibodies bind. Each epitope is 3-6 amino acids belonging to the protein (usually 250 or so amino acids). Linear epitopes are sequential, while conformational epitopes (naturally occurring) are from amino acids separated in sequence but brought together by the proteins structure.

SOURCE OF ANTIGENS USED IN TESTS

Test	Antigen
IFAT	SnSAG1 S. neurona (UCD1)
EBI WB	SnSAG1 S. neurona (Sn2)
Neogen WB	SnSAG1 S. neurona (Sn2)
The rSAG1 ELISA	SnSAG1 SnPath1 (genetically identical to UCD 1)
rSAG 5 ELISA	Synthetic DNA based on published sequences for SnSAG5 (horse)
rSAG 6ELSA	Synthetic DNA based on published sequences for SnSAG6
SAG2, 4/3 ELISA	SnSAG1 (SN3)

All tests use SAG 1 phenotype *S. neurona!* To detect other serotypes the phenotype antigens must be present (SAG 5 and SAG 6 ELISA) or antigens common to all *Sarcocystis* must be used, this decreases the specificity of inclusive tests.

TEST VALIDATION FOR *S. NEURONA* ANTIBODIES VARY WIDELY

True positives (TP) are from horses with parasites confirmed in the CNS at post-mortem exam.

In papers that validate tests "EPM" often means that inflammatory lesions were observed on histopathology but no parasites were confirmed in the CNS of horses.

Antibodies don't detect inflammatory lesions! Be sure and read the primary literature carefully to determine the "validation" procedure for each test.

SUMMARY FOR TEST VALIDATION

Test	Validation samples obtained from:
WB (Granstrom 1993)	Several hundred necropsies, field cases
WB (Rossano 2000)	Six (6) true positive EPM horses
IFAT (UC Davis)	Eight (8) true positive EPM horses
SAG 1 (Ellison 2001)	Six (6) true positive EPM horses, 50 vaccinated horses
SAG 5, 6 (Ellison 2010) *S. Fayeri* (Ellison 2016)	10 horses vaccinated with each recombinant antigen
SAG 2, 4/3 (Howe 2013)	44 "EPM" by inflammatory lesions

Researchers validate tests using the most pure "true negative" and "positive" samples that represent the antigen used in the detection method. However, using disease free animals from an endemic area for the "true negatives" lowers the specificity of tests.

RECOMMENDED DIAGNOSTIC SAMPLE

Test	Recommended for best interpretation
WB (Granstrom 1993)	Serum and uncontaminated (RBC) CSF
WB (Rossano 2000)	Serum and uncontaminated (RBC) CSF
IFAT	Serum and uncontaminated (RBC) CSF
SAG 1 (Ellison 2001)	Serum
SAG 5, 6 (Ellison 2010) *S. fayeri* (2016)	Serum
SAG 2, 4/3 (Howe 2013)	Serum and uncontaminated (RBC) CSF, AI or C-value to determine contamination of CSF. Serum:CSF titer ratio <100. "Serum titer alone is poor indicator of disease."

INTERPRETATION OF RESULTS

- The presence of antibody indicates prior or current infection.
- Antibody positive and clinical signs *may* indicate sarcocystosis in an animal.
- A negative result, except in acute onset or a treated horse, indicates no infection.

The test indicates a positive when antibodies are detected at a specific level defined by the test validation protocol. Some laboratories relate results to a specific number of horses with lesions in the CNS. Some laboratories measure antibodies and do not relate the antibody to active disease.

Update!

Pathogenes can provide predictive values for your state

THE SIGNIFICANCE OF THE TEST RESULTS

Test	Interpretation
WB	Negative, suspect, weak positive, positive.*
WB (Rossano)	Negative, suspect, weak positive, positive.*
IFAT	Based on mathematical modeling/simulation. Cross reactivity with non-pathogenic *S. fayeri*
ELISA SAG 1, 5, 6 or *S. fayeri*	Titer $\geq$8 is a positive test.
ELISA 2, 4/3	Serum: CSF <100 indicates antibody in CNS, serum titer $\geq$ 4000 "correlates better with EPM" Cross-reacts with $SF_{Cornell}$ SAG 4 but not SF_{Fla1} SAG 4

*ACIVM comment specific to WB: "In situations in which test sensitivity and specificity are determined from samples that had neurological disease (suspect EPM) incidence of disease is high leading to skewed results…diagnostic efficiency depends on positive predictive value.

TEST RESULTS ARE MORE MEANINGFUL WHEN DISEASE PROBABILITY IS HIGH. TESTS VALIDATED WITH TRUE NEGATIVE SERA INSTEAD OF NEGATIVE SAMPLES FROM AREAS OF ENDEMIC DISEASE WILL OVERESTIMATE SPECIFICITY.

SURFACE ANTIGENS OF *S. NEURONA*

- There are six immunodominant SAG's: 1-6
- Mutually exclusive expression of SAG 1, 5, 6 allows species serotyping
- Stage related expression SAG's 2-5 has been reported.

SAG 1	SAG 5	SAG 6
• Genotype II • Genotype VI	• Genotype I • Genotype III • Genotype V	• Genotype IV

SAG 1 AND 5 STRAINS **DOMINATE** ANIMAL DISEASE CAUSED BY *S. NEURONA*

- SAG 1 strains used for antigen blots do not distinguish SAG 1, SAG 5 and 6 strains. Western blots must rely on detection of common antigens.

- There are two SAG 1 (serotype) genotypes. They differ based on 30 molecular markers, but display identical SAG 1 proteins. Horses do not differentiate genotype, just serotype, SAG 1

- Strain SN3, SAG1 genotype, displays a SAG 4 protein that is common to *S. falcatula*, a source of cross-reactivity in tests that use SAG 4*!*

TESTS THAT DETECT SAG 4 OF *FALCATULA*

- False positive tests for sarcocystosis may be due to SAG 4 reactivity! Seropositive SAG 4 animals usually don't have sarcocystosis!
- Western blot tests rely on an antigen profiles to determine results, the profiles for TRUE EPM horses do not have pathognomonic profiles!
- The presence or absence of SAG *genes* and selective display of proteins are clinically relevant issues!

AN ACADEMIC UNDERSTANDING OF SAG EXPRESSION

- *S. neurona* shows stage related expression of SAG's 2, 3, 4, 5.
- Antigens must be displayed to elicit an immune response.
- Stages that reside in the brain don't always express *S. neurona* SAG's 2,3,4,5 and possibly SAG 1, confounding the reliance on CSF antibody testing to determine the presence of parasites!
- Antiprotozoal treatment can affect the display of certain SAG's by inhibiting the growth of some stages! The animals would be false negative when tested.

SAG EXPRESSION IS STAGE RELATED

- Stage related expression (Gautam and Grigg) in SAG 2, 3, 4, and 5.
- Strains SN6 and SN7 (Dubey) showed protein differences with long term culture.
- **The journeyman's understanding of SAG expression: Changes in antigen expression can affect interpretation of test results.**

SAG 1, 5, 6 ELISA'S TEST EXCLUSIVE ANTIGENS

- SAG 1, 5, and 6 are specific to *S. neurona* and represent all the known genotypes.

- It was statistically shown that testing CSF didn't increase diagnostic value using the SAG 1 ELISA in a blinded, controlled challenge study. The study showed:

Antibody levels increase with duration of infections.

A four-fold increase in titer indicates active infection.

S. FALCATULA COMPLICATES TESTING

- *S. falcatula* did not cause disease in horses when the strain *S. falcatula* (FLA1) was used in challenge studies.
- Marsh showed that horses with EPM have antibodies against *S. falcatula* tested by immunoblot! That means these horses did get infections with SF or there are cross-reactive antibodies.
- *S. falcatula* SAG 6 and *S. neurona* SAG 6 do cross react on non-specific tests. *IF* horses can't be infected *THEN* the antibody is due to *S neurona* SAG 6 infections.

EXAMPLE: ANTIBODY CONFOUNDS THE DETECTION OF DISEASE

The SAG 4 protein varies with strains of *Sarcocystis*. SAG 4 is the same in two biologically different *S. falcatula* strains (Marsh, Grigg):

	SnSAG 6	$SF_{Cornell}$SAG4	SF_{Fla1}SAG4	Horse infection
S. neurona	+	+	-	+
S. Falcatula Cornell	-	+	-	-
S. Falcatula FLA1	+	-	+	-

CSF testing may help differentiate infections when this cross reactive antigen, SAG 4, is used. SAG 4 variability is not an issue when specific *S. neurona* antigens are used.

INFECTION AND INFLAMMATION = EPM

Inflammation manifests as an abnormal neurological examination. Parasite mediated neuroinflammation may persist after the parasites have been eliminated.

Non-parasite mediated neuroinflammation is present after protozoal treatment or after resolution of infections. Associated autoimmune disease can result in, or from neuroinflammation.

THE ROLE OF C -REACTIVE PROTEIN (CRP) IN CLINICAL DISEASE

- CRP is an acute phase inflammatory protein induced by the cytokine IL6 (appears with infections, non-specific). CRP is an enzyme that releases IL6 receptors into the circulation; CRP can stimulate IL6 production in a positive feedback loop when the pathway is dysregulated.

- The soluble IL6/IL6 receptor can cross the blood brain barrier. IL6/IL6 receptor complex is pro-inflammatory in the CNS. IL6 inflammation does not require parasites in the CNS!

SERUM CRP TEST

The test format is an ELISA and equine specific CRP levels are reported in µg/ml from the serum. Validation for CRP levels are published in infections with protozoa, bacteria, virus. To relate the levels of CRP associated with EPM (n=2720):

	Total	*S neurona* seropositive	S neurona seronegative
Clinical signs	1532	582	950
CRP elevated	652	226 (39%)	426 (45%)

Clinical signs were observed in 90% of the animals tested for serum CRP levels and forty–three percent of the horses with clinical signs showed an elevated CRP (n=652). Thirty-nine percent of seropositive horses with a presumptive diagnosis of EPM (antibody and clinical signs) had an elevated CRP. Seronegative horses had an elevated CRP in 45% of the cases.

INTERPRETATION OF CRP

A value of >15 µg/ml is a positive result that indicates inflammation that was stimulated by innate immunity due to parasites, bacteria or virus.

A value of > 29 µg/ml is associated with autoimmune mediated inflammation.

- Treating the infection will result in a drop in serum CRP. CRP may be used to monitor effective therapy for immune mediated disease in some cases.

WHAT DOES CRP MEAN?

- The evidence suggests 45% of horses with clinical signs don't have active protozoal infections!
- Anti-protozoal therapy is expensive and ineffective in these cases.
- Treating the inflammation can be effective.
- Stage related expression of SAG's won't alter CRP

SOMETIMES ANTIBODY TESTS ARE CONFOUNDED!

Anti-protozoal treatments may affect antibody production and detection (Furr: "ponazuril decreases antibody production but not clinical signs").

Erroneous conclusions are drawn when samples that have treatment-induced alteration of antibodies are used to evaluate tests.

Duration of infection: Antibodies are produced in 14-17 days after infection. If the infection in a naïve animal is within this period they will be negative. An experienced animal will show an amnestic response sooner than an naïve animal.

JOHNSON, BURTON, SWEENEY (J VET INTERN MED 2010;24:1184–1189) INCORRECTLY STATE "STRAINS THAT LACK SAG 1 PREDOMINATE IN THE MID-ATLANTIC REGION"

Validation sera/CSF in the Johnson study may yield results that show SAG 1 expressing strains of *S. neurona* are not prevalent in the mid-Atlantic region!

- Confirmed positive = non-surviving horses (post-treatment) with inflammation in CNS tissues.
- Suspect positive = surviving horses with neurologic signs w/o other cause and response to treatment.
- Confirmed negative = non-surviving horses with other CNS disease or no CNS lesions.

CONCLUSIONS ABOUT TESTING

- Testing serum for antibodies to *S. neurona* can be valuable to manage the EPM horse.
- Diagnostic tests with different formats can not be directly compared.
- An understanding of the test, its validation, and interpretation of the results are important to clinical application of testing.
- A negative test is useful and rules out EPM unless the animal has been exposed to antiprotozoal drugs or the infection is between 14-17 days. Some drugs may alter antigen expression.

TREATMENT OF EPM

- The first generation treatment for EPM was sulfonamides and pyrimethamine.
- Second generation treatments are the trizine antiprotozoal agents.
- Third generation treatments are combination treatments that treat protozoal infections and the inflammation associated with them.

The Freedom Of Information Summary is the source of information for all studies associated with licensing. They are available on the web!

JAVMA Vol 242, No 4, 2013 Dirikolu, Johnson, Foreman, Tobin

NADA STUDY	Rebalance	Marquis Clinical Field Study 1	Marquis Clinical Field Study 2	Protazil
Study Number	141-240	141-188	141-188	141-268
Design	Demonstrate the safety and effectiveness for the treatment of Equine Protozoal Myeloencephalitis (EPM).			
Controls	No	N/A	N/A	N/A
Animals Enrolled	97 Horses	113 Horses	12 Horses	214 Horses
Total Acceptable Cases	26 Horses	47 Horses	7 Horses	42 Horses
Breed	Any	Any	Any	Any
Age	9 months – 32 years	2 – 30 years	2 – 19 years	9.6 months – 30 years
Sex	F/MC	F/MC	F/MC	F/MC
Previously Treated	NA	Greater than 3 months	Any treatment accepted	NA
CSF (Enrollment)	Yes	Yes	optional	Yes
Gait Score (Enrollment)	≥1	≥2	≥2	≥ 2
Gait Score (Success)		≥ 1 @ 90 days	≥ 1 end treatment (28 days)	≥ 1
Interim Analysis	NA	Yes	NA	NA
Video Review	yes	Video: 18 of 24 (75%)	NA	Video: 10 of 24 (42%)
Treated @ Dosage	1X	47 to 5 mg/kg	7 to 5 mg/kg	68 to 1 mg/kg
Evaluated @ Dosage	1X	1X	1X	1X
Results (Improved)	1X-16 of 26 (61.5%) 1X-14 of 26 (53.8%)Gait and WB success duration >180	5 mg/kg–28 of 47 (60%)	5 mg/kg-7 of 7 (100%)	1 mg/kg–28 of 42 (67%)

OROGIN AND NEUROQUEL

- **Orogin (INAD 012092)**
  - For the treatment of EPM due to *S. neurona* in horses
  - Undergoing safety and effectiveness studies
- **NeuroQuel (INAD 012219)**
  - For the treatment of residual or recurrent clinical signs associated with *S. neurona* infections

THERAPEUTIC AGENTS FOR EPM

Drug	FDA approval	Action	Failure due to:
ReBalance	INAD 141-240	pyrimidine synthesis	Ineffective dose in CNS
Marquis	INAD 141-188	pyrimidine synthesis	Re-growth of parasites after treatment
Protazil	INAD 141-268	pyrimidine synthesis	Re-growth of parasites after treatment
Orogin	INAD 012-092*	parasite mitochondria and IL6 inflammation	ND

* Currently undergoing license process under MUMS

COMBINING DRUGS

- There is synergism between toltrazuril and trimethoprim or pyrimethamine.
- It is not advised to use trimethoprim with pyrimethamine.
- Orogin is the only treatment designed to treat inflammation associated with disease.

- Combining levamisole with static-acting antiprotozoals may be unwise due to the effect of cholinergic agonists on the protozoa.

RELAPSE WITH SULFADIAZINE/PYRIMETHAMINE..

- "..is most likely caused by the failure of maintenance of coccidiocidal concentrations of the standard treatment drugs in the CSF as a result of either lack of ability of these agents to pass through the blood-brain barrier or the short elimination half-lives of these agents in horses."

- 2 out of 3 horses relapse with standard treatment. (MacKay 1992)

ADVERSE REACTIONS WITH SULFADIAZINE/PYRIMETHAMINE

- Anemia, neutropenia, thrombocytopenia, leukopenia, diarrhea, urticaria.
- Teratogenic, neonatal disorders, abortion.
- Affects breeding performance of stallions.
- JAVMA VOL 242 FEB 15 2013

EFFECTIVENESS AND RELAPSE WITH PROTAZIL*

- 67% effectiveness, 5-17% relapse
- Prevent relapse with Protazil (diclazuril) by dosing 7 mg/kg (7X). (MacKay 2008)
- Re-growth of parasites when drug is removed. (Lindsay Dubey 2000).
- Side effects rare

JAVMA VOL 242 FEB 15 2013

EFFECTIVENESS AND RELAPSE WITH MARQUIS*

- Effectiveness 62%, 10% relapse^
- Rob MacKay (2008) recommends 35 mg/kg/day for four days (7X) and treatment duration should be extended to 2 months.
- FDA approved dose is 5 mg/kg/day for 28. Higher dose FDA approved in 2015.
- *JAVMA VOL 242 FEB 15 2013 ^MacKay 2008

POST- TRIAZINE TREATMENT

- CSF Western blots were positive in 90% (Diclazuril) treated horses after 6 to 12 months.
- CSF Western blots were positive in 75% of (Toltrazuril) treated horses after 6 to 12 months.
- Does CSF presence of antibody detected by immunoblot indicate active infection? NO!

OROGIN

- MUMS INAD 012092
- Intern J App Res Vet Med 2012
- Decoquinate/levamisole
- Purpose is to treat inflammation and protozoa

EPM IS A MANAGEABLE DISEASE!

EPM can be managed effectively. Management requires an understanding of the underlying pathology and what tests and treatments are appropriate.

3 CE HOURS

- Please call 352-591-3221 for clarification of any topic in this presentation. References are available on request.
- Complete the quiz and email your comments to sellison@pathogenes.com
- Qualified veterinarians will receive a certificate of completion upon review of the email.

QUIZ

- 1. True or False?

Clinical signs of EPM can be due to infection, inflammation, or both. Residual or recurrent signs after antiprotozoal therapy may be due to unresolved inflammation.

- 2. True or False?

The incongruity between *S. neurona* infection and the occurrence of clinical signs may be due to tests which detect cross-reactive antibodies.

QUIZ

- 3. True or False?

Inflammation can be induced by *S. neurona* and remain after protozoa are eliminated. These clinical signs can give the impression the horse has relapsed.

QUIZ

- 4. True or False?

Not all strains of *Sarcocystis* can enter the central nervous system and infect CNS tissues.

Extra credit: Which phenotype infections account for the majority of animal disease by *S. neurona*.

QUIZ

- 5. True or False?

- The IFAT and 2, 4/3 ELISA strongly suggest testing CSF. For a better prediction of infection in the CNS a serum: CSF ratio that is <100 is suggested as positive.

QUIZ

- 6. True or False?

C-reactive protein is present when inflammation due to infection is present. This test won't distinguish the etiology of infection but may be an indicator of treatment success.