

**Schedule and Proceedings for Equine Protozoal Myeloencephalitis SIG: The Present  
and the Future: Diagnostics, Treatment & More**  
May 31<sup>st</sup> 8 am – 12 pm

- I. ABSTRACTS (8-10 AM) (15 minutes each, with 5 minute question period)
1. 8:00 Seroprevalence in US based on samples submitted to EBI (Morrow)
  2. 8:20 Persistence of titers in horses (and incidence in India) (Brown)
  3. 8:40 SAG-1 strains (expression, implications on diagnostics) (Gaji)
  4. 9:00 Total and *Sarcocystis neurona*-specific Igg and specific index (C) in horses challenged with *Sarcocystis neurona* sporocysts (MacKay)
  5. 9:20 Effect of intermittent ponazuril on experimental *Sarcocystis neurona* infection in horses (MacKay)
  6. 9:40 Treatments: NTZ efficacy in vitro (Duda)

Break

- II. ROUND TABLE DISCUSSIONS (10:30-12:00 Noon) (Tentative)
1. TREATMENT (10:30-10:50):
    - A) Possible mechanisms/explanations for non-responders and those that relapse
    - B) Alternative treatments/protocols and experiences
  2. DIAGNOSIS: What do we need for a good diagnostic test? (10:50-11:15)
    - A) Current diagnostic tests available, brief description of tests
      1. EBI/IDEXX WB (Morrow)
      2. Antech SAG-1 (Ellison)
      3. Neogen (MacKay)
      4. MSU WB (Schott)
      5. IFAT Davis (Pusterla)
    - B) Other approaches to diagnostics (other antigen or antibody specific tests, cytokines/chemokines, gene expression, immune function)
  3. STATUS OF CURRENT MODELS (11:15-11:40) (Saville/Reed, Ellison)
    - A) What models are there: current applications
    - B) Information derived from models
  4. TOPICS FOR FUTURE SIGS (11:40- 12:00)

**SEROPREVALENCE OF *SARCOCYSTIS NEURONA* IGG IN UNITED STATES SUBMISSIONS TO EQUINE BIODIAGNOSTICS (EBI):A RETROSPECTIVE ANALYSIS OF 2000-2005.** JK Morrow<sup>1</sup>, SM Reed<sup>2</sup> and DE Granstrom<sup>3</sup>; <sup>1</sup>Equine Biodiagnostics/IDEXX, Lexington, KY; <sup>2</sup>the Ohio State University College of Veterinary Medicine, Columbus, OH; <sup>3</sup>USDA ANRI, Baltimore, MD.

Few studies have reported the seroprevalence rates of IgG against *Sarcocystis neurona* in horses residing in the United States. Submissions to clinical laboratories are inherently biased because testing is being performed due to suspicion of disease and caution must be exercised when reporting data from such databases. Historically, EBI has been reluctant to generate individual state prevalence figures, knowing that these numbers were likely an overestimate of those in the general equine population. However, as a result of discussions with referring veterinarians about the prevailing misperception that all horses test positive for *S. neurona* IgG, we have recently determined the individual state seroprevalences for sera submitted to EBI from 2000-2005.

When reviewing our data, it is important to keep in mind: (1) the results of all known infection study samples were excluded (2) results were counted in the state of the referring veterinarian (3) geographic regions within a given state were not distinguished (4) states in which submissions were less than 100 were excluded: AK, HI, ID, ND, NV, RI.

Nationwide, with submissions from all 50 states, the overall seroprevalence was 58% (~28,000 sera testing positive out of ~50,000 tested by the EBI western blot (WB) from 2000-2005). Moreover, the individual rates were fairly consistent year to year. Excluding those states with less than 100 submissions in the 6 year review period, the state with the highest averaged seroprevalence was Arkansas at 85%, while Arizona showed the lowest at 31%. As might be expected, the prevalence was lower in western states (AZ, CA, CO, ID, MT, ND, NM, NV, SD, UT and WY) and the far northeast (ME, NH and VT).

Most of the published seroprevalence reports (for CA, FL, KY, MI, MO, MT, OH, OK, OR, PA and WY) were based on relatively small study size and on a random population sampling rather than clinical cases. With the exception of Oklahoma, the prevalences from the EBI database analysis were higher than those reported in these studies.

While a positive serum WB result supports a diagnosis of EPM in a horse exhibiting clinical signs, there is strong consensus that a negative result, with few exceptions, rules out that diagnosis. Although >70% of sera tested positive, 10-20% tested negative in the states of AL, AR, GA, IA, IN, KS, LA, MI, MO, MS, NC, OK, TN, WI and WV. The rate of samples testing negative ranged from 22-42% in states of moderate seroprevalence (CT, DE, FL, KY, MA, MD, MN, NE, NJ, NY, OH, OR, PA, SC, TX and VA). Our data demonstrates that many horses test WB negative in our laboratory and emphasizes that the WB remains a useful tool in diagnosing *S. neurona* EPM.

**ANTIBODIES TO *SARCOCYSTIS NEURONA* ARE PRESENT IN HORSES FOR MANY YEARS FOLLOWING THE HORSES' REMOVAL FROM ENDEMIC AREAS.** Christopher M Brown, Jennifer K Morrow, Carla L Carleton. Department of Clinical Studies, Ontario Veterinary College, University of Guelph, Ontario, N1G 2W1, Canada (Brown), Equine Biodiagnostics/IDEXX, 1501, Bull Lea Road, Lexington, KY 40511 (Morrow), and Department of Large Animal Clinical Sciences, College of Veterinary Medicine, Michigan State University, East Lansing, MI 48824 (Carleton).

Using a standard western blot assay to detect IgG antibodies to *S. neurona*, we examined the sera of 228 Thoroughbred horses living on 4 farms in India. Eighty-six of these horses were of North American origin (average age 13.6 years) and had lived in India for between 1 and 13 years (average 5.5 years). There were 124 Indian-born horses (average age 8.3 years), plus 9 horses originally from Ireland, 9 from England, and 2 from France. Thirty-six (42%) of the horses of North American origin were positive for antibodies to *S. neurona*, compared to only 1 (0.8%) of the Indian-born horses. All horses native to Ireland and England were negative, and the 2 horses originally from France were positive. Of the 36 positive horses of North American origin 22 had been in India for between 1 and 5 years, and 14 had been in India for between 6 and 9 years. We conclude that antibodies to *S. neurona* can be detected in the serum of horses of North American origin for many years after they have been removed from an area endemic for *S. neurona*. This could be due to a very long half-life of such antibodies, or reflect chronic infection with the parasite and ongoing antibody production, or both.

**THE MAJOR MEROZOITE SURFACE ANTIGEN SNSAG1 IS NOT EXPRESSED BY MULTIPLE STRAINS OF *SARCOCYSTIS NEURONA*** R.Y. Gaji<sup>1</sup>, B.A. Patil<sup>1</sup>, D. Lindsay<sup>2</sup>, J.P. Dubey<sup>3</sup>, A. Marsh<sup>4</sup>, W.J. Saville<sup>4</sup>, D. Granstrom<sup>3</sup>, and D.K. Howe<sup>1</sup> <sup>1</sup>Department of Veterinary Science, University of Kentucky, Lexington, KY <sup>2</sup>Department of Biomedical Sciences and Pathobiology, Virginia-Maryland College of Veterinary Medicine, Virginia Tech, Blacksburg, VA. <sup>3</sup>USDA, ARS, BARC, Beltsville, MD. <sup>4</sup>Department of Veterinary Preventive Medicine, The Ohio State University, Columbus, OH.

Merozoites of *Sarcocystis neurona* express a gene family of related surface antigens that have been designated SnSAGs. These parasite surface antigens are abundant and immunogenic and are therefore excellent candidates for development of EPM diagnostics and/or vaccines. Prior research has demonstrated that the major surface antigen SnSAG1 is absent from an EPM isolate of *S. neurona* (SN-MU1). We have examined the database of parasite gene sequences generated by the *Sarcocystis* expressed sequence tag (EST) sequencing project and found that a second EPM isolate, SN4, lacked mRNA transcripts for the SnSAG1 gene. In contrast, numerous other *S. neurona* genes (e.g., SnSAG2) were highly represented and very well conserved in the collection of SN4 strain ESTs, thus verifying that SN4 is a *bona fide* strain of *S. neurona*. In order to examine the possibility that other SnSAG1-minus strains exist, an assortment of *S. neurona* isolates were examined by western blot using monospecific polyclonal antiserum against SnSAG1. A total of 13 *S. neurona* strains were tested, 9 of which were isolated from cases of EPM. Importantly, 5 of the strains were found to be SnSAG1-minus. In addition to the previously mentioned SN-MU1 and SN4 strain, the EPM isolates SN6 and SN7 and the other isolate SNSO-1 were negative for SnSAG1. Collectively, these data suggest that SnSAG1-minus strains of *S. neurona* may be relatively common, which has significant implications for the development of accurate serologic tests and prospective vaccines. Furthermore, these results are consistent with other evidence that suggests there may be appreciable heterogeneity among different isolates of *S. neurona*.

**TOTAL AND *SARCOCYSTIS NEURONA*-SPECIFIC IGG AND SPECIFIC INDEX (C) IN HORSES CHALLENGED WITH *SARCOCYSTIS. NEURONA* SPOROCYSTS.** RJ. MacKay, KA. Heskett. University of Florida, Gainesville FL.

Current diagnosis of equine protozoal myeloencephalitis (EPM) depends on the detection by immunoblot of *Sarcocystis neurona* IgG in blood and cerebrospinal fluid (CSF). Specific antibody produced in the CNS is thought to indicate CNS infection with *S. neurona*. Because the immunoblot cannot identify the source of antibody (i.e., CNS vs. extraneural), this test has low positive predictive value for diagnosis of EPM. The purpose of this study was to develop a specific CSF coefficient (*C*) for detection of *S. neurona* IgG of CNS origin.

For calculation of *C*, total and specific IgG in blood and CSF samples was first determined in samples from horses that had been challenged experimentally with *S. neurona* sporocysts. Serum total IgG was measured by standard radial immunodiffusion technique whereas CSF total IgG was quantified in a sandwich ELISA developed by the authors. Affinity-purified sheep anti-horse IgG-heavy and light chains was used as coating antibody, horse reference serum (Bethyl Laboratories) as standard, and whole-molecule horseradish peroxidase-conjugated rabbit anti-horse IgG as detecting antibody. A direct ELISA was developed for the detection of *S. neurona* IgG (hereafter referred to as *S. neurona* titer). A stock solution of solubilized *S. neurona*, prepared from the WSU-1 isolate, was used as the coating antigen; serum from a horse that had been repeatedly inoculated with this antigen preparation was used for generation of a standard curve, and horseradish peroxidase-labeled rabbit anti-horse IgG was the detecting antibody. CSF coefficients (*C*) were calculated by the following formula:

$$C = \frac{S \text{ neurona IgG}_{\text{CSF}}}{S \text{ neurona IgG}_{\text{serum}}} \times \frac{\text{Total IgG}_{\text{serum}}}{\text{Total IgG}_{\text{CSF}}}$$

Samples were from horses in 4 different experimental groups: *Group 1* - horses given  $5 \times 10^5$  *S. neurona* sporocysts and no treatment; *Group 2* - horses given sporocysts and ponazuril (20 mg/kg) every 7 days; *Group 3* - horses given sporocysts and ponazuril every 14 days; *Group 4* - no sporocysts or treatment. Blood and CSF was collected prior to sporocyst challenge and 101 - 157 days after challenge.

Total serum IgG (mean  $\pm$  SEM) before challenge was  $3258 \pm 174$  mg/dl and  $18.5 \pm 2.4$  mg/dl in blood and CSF, respectively. Significant effect of challenge or treatment on total IgG in serum or CSF was not found. There were ( $\geq 10$ -fold) increases in *S. neurona* titer (specific IgG) in blood (median value increased from 2154 to 30,066 U/ml in groups 1-3;  $N = 14$ ) and CSF ( $\leq 7.8$  to 162 U/ml) of all challenged groups; titer increases were not seen in blood (starting and ending median values for group 1 of 1920 and 1678 U/ml;  $N = 1$ ) or CSF ( $\leq 7.8$  to ( $\leq 7.8$  U/ml). In both blood and CSF, the increases in median titer values for each challenged group were significantly higher than those for the unchallenged group. In contrast to the results for *S. neurona* titer, effect of sporocyst challenge or treatment group on specific index *C* was not found in either experiment. Immunoblots were performed on the same samples and results were classified according to a 2-level (positive, negative) system. In both blood and CSF samples, *S. neurona* titers and *C* values were significantly higher in the samples classified as immunoblot-positive than they were for those classified as negative.

Experimental infection of horses with *S. neurona* sporocysts caused significant increase in serum and CSF *S. neurona* titers but significant increases in total IgG and specific index *C* were not found. These results could be interpreted to mean *either* that specific index is insufficiently sensitive to detect CNS production of IgG during experimental infection *or* that experimental infection does not result in production by the CNS of specific IgG.

**EFFECT OF INTERMITTENT PONAZURIL ON EXPERIMENTAL *SARCOCYSTIS NEURONA* INFECTION OF HORSES.** RJ. MacKay, ST Tanhauser, KD Gillis, IG Mayhew, TJ Kennedy. University of Florida, Gainesville, FL (MacKay, Tanhauser, Gillis), University of Edinburgh, Roslin, Midlothian, Scotland (Mayhew), Bayer Animal Health, Merriam, KS (Kennedy).

There is high prevalence of *S. neurona* infection in US horses. A small proportion of infected horses develop equine protozoal myeloencephalitis (EPM). There is interest in using EPM treatments to prevent CNS invasion in *S. neurona*-infected horses.

This study evaluated effect of intermittent ponazuril administration on immunoconversion against *S. neurona* in horses. Horses immunoblot-negative for *S. neurona* IgG (5/group) were given  $5 \times 10^5$  *S. neurona* sporocysts by nasogastric tube, then given ponazuril at 20 mg/kg every 7 or 14 d for 84 d. Control horses (5/group) were either not treated or not challenged. There was significant ( $P < 0.05$ ) effect of treatment on immunoconversion (detected by immunoblot) in CSF but not in serum. All challenged horses became serum-positive within 56 d. Only 2/5 horses treated every 7 d converted in CSF; all other *S. neurona*-challenged horses became CSF-positive within 84 d. Magnitude of the anti-17-kD antibody response in CSF ("relative quantity CSF") was significantly reduced by 7-d but not by 14-d treatments. Consistent neurologic or other clinical abnormalities were not seen in any group. Likewise, histologic examination of CNS did not reveal either protozoa or consistent inflammatory changes.

Although ponazuril treatment every 7 d reduced immunoconversion in CSF, this effect was not absolute. Protocols using ponazuril may have application to prevention of EPM.

#### **THE EFFICACY OF NITAZOXANIDE *IN VITRO* ON MEROZOITES OF *SARCOCYSTIS NEURONA*.**

Duda, L. \*, Lindsay D. S. \*\*, Liotta, J. L. \*, Bowman, D. D. \*. \*Department of Microbiology and Immunology, College of Veterinary Medicine, Cornell University, Ithaca, NY, and \*\*Department of Biomedical Sciences and Pathobiology, Virginia-Maryland College of Veterinary Medicine, Virginia Tech, Blacksburg, VA. [This work was performed as an honors science project by L. Duda of Central Valley High School, Montgomery, NY, in the class of Joseph Francalossi]

Equine protozoal myeloencephalitis (EPM), one of the most debilitating neurological diseases in horses, is caused by the protozoan parasite *Sarcocystis neurona*. Currently, several products are routinely used in the treatment of EPM. This study was conducted to verify previous work showing the efficacy of the active ingredient, nitazoxanide (NTZ), in one product Navigator® against the parasite *in vitro*. Assays were performed in T-25 flasks containing cultures of bovine turbinate (BT) cells. To the flasks were added a known volume of schizonts of *S. neurona*. In the flasks, three concentrations of NTZ were used to inoculate the flasks; each drug concentration was used to treat four flasks of infected BT cells. Four control flasks were prepared, each containing DMSO and RPMI with 2% fetal bovine serum and 100 UG of streptomycin. At a concentration of 5 µg nitazoxanide/ml, most of the parasites were killed. However, at this concentration, a good portion of the host cells, approximately 90%, also died. At 1.0 µg NTZ/ml, 76.2% of the parasites were killed and the monolayer of host cells remained relatively intact. Treatment of infected cells with 0.1 µg NTZ/ml of culture fluid did not control the parasite's multiplication, and > 90% of the host cells were destroyed by the parasite. Thus, comparatively, NTZ at 1.0 mg was the most successful treatment with minimal damage to host cells. This work verifies the data that is present on the label of the product and the preliminary work performed on the *in vitro* efficacy of this compound against the EPM agent, *Sarcocystis neurona*.