

**Proceedings from the AAVP  
(American Association of veterinary parasitologists)  
47<sup>th</sup> Annual meeting in Nashville TN.**

**#15 Evaluation of in vitro and in vivo cell mediated immune response of horses immunized with a killed sarcocystis neurona vaccine.**

*Presented by A. Marsh and sponsored by Fort Dodge Animal Health , Iowa*

A commercially available killed S.Neurona vaccine was conditional licensed by the US dept of Ag. The purpose of this study was to evaluate CMI response in horses immunized with this vaccine in comparison to horses receiving a placebo. 13 horses seronegative to s.neurona by immunoblot were divided into the vaccinated group (9 horses) and one placebo group (4 horses); one seropositive horse was placed into the placebo group (1 horse). Animals were given two doses of s.neurona vaccine or a placebo intramuscularly, three weeks apart. The CMI responses following each vaccination were measured by lymphocyte blastogenesis assay with parasite antigen. In addition, an intradermal antigen skin test to detect delayed (cellular) reactions to s.neurona antigen was performed following the 2nd vaccination. Results from peripheral blood lymphocyte blastogenesis assay indicate that vaccinated horses are successfully primed for CMI response to the s neurona antigen following the first vaccination as compared to pre vaccination statue. This difference was not detected in the placebo controls. The induction of specific CMI response by vaccination with s.neurona vaccine was supported by the results of skin testing. Skin reactivity was evident in 7/9 of the s.neurona vaccinates evaluated. Microscopic evaluation of skin biopsy samples were taken from a subset of the two groups to compare microscopic features of cellular infiltrates with physical characteristics at 72 hours.

**#18 Prevention by ponazuril of CNS infection in S.neurona-challenged mice.**

*Presented by RF Franklin, RJ Mackay, Uof FL, and Bayer Animal health, Merriam KS*

EPM is a neurologic disease of horses caused by Sarcocystis neurona. There is interest in using EPM treatments as prophylaxis. The objective of this study was to evaluate the efficacy of ponazuril for PREVENTION of CNS infection in s. neurona-challenged interferon knockout mice.

{this report goes on and on and I will condense it to the best of my ability-make a long story short}

Two dosages were used with these mice 20mg and 200mg and it didn't seem to make all that much of difference. The mice used were given  $5 \times 10^6$  to the third power, And they were dosed on day 1,3,7,10 or 14. Control groups were given no sporocysts and/or no drugs. Mice were killed at onset of neurologic signs around 30days post-challenge. In

contrast to negative control mice, untreated s.neurona-challenged mice lost weight by 18 d and showed neurologic signs after 22 days. There was a significant ( $P<0.05$ ) effect of ponazuril on weight gain. Only mice given 200mg on days 7 or 14 remained free of neurologic signs. Treatment did reduce mortality especially at 200mg 7d after challenge. This effect was not significant. Although schizonts were seen histologically in the CNS of all mice, there was significant reduction of schizont numbers by 200mg given on day 7. Ponazuril provides some dose-dependent protection of mice against s. neurona challenge and this effect was most apparent on day 7, at a stage when the organism is undergoing extraneural shizogony. **Stages found earlier (invading sporozites) or LATE (once within the CNS) were resistant to ponazuril. (I FOUND THIS PART VERY INTERESTING, COULD EXPLAIN WHY MARQUIS IS NOT THE MAGIC BULLET)**

**"Quote" Because single dose therapy did not provide complete protection in mice, this experiment does not provide rationale for a protocol of intermittent dosing for the prevention of EPM.**

### **#22 Equine model for sarcocystis neurona infection**

*S. Ellison, T. Kenned, K. Brown, Pathogenes Inc and Bayer Corp, Animal Health.*

Experimentally infecting horses with sarcocystis neurona may enhance our understanding of the pathogenesis of infection in natural disease thereby improving the diagnosis and assisting researchers in developing rational strategies for the treatment and prevention of EPM in the horse. The development of an experimental model of sarcocystis neurona encephalitis in a horse is described. S.neurona was isolated from the spinal cord of an ataxic horse and placed in continuous culture. Cultured s.neurona merozoites derived from the ataxic horse were used to induce infection in another horse with resultant encephalitis and ataxia. After re-isolation and invitro culture, the dose of merozoites necessary to induce clinical signs of EPM and recovery of the organism from the CNS tissues was titrated in three horses. Vertical transmission to the fetus was accomplished in one horse. Our experimental model of infection induced clinical disease defined by ataxia, grossly visible spinal cord lesions, organisms demonstrated by immunohistochemistry and isolation of merozoites from CSF and cervical spinal tissues To the best of our knowledge, this is the first report of histologically confirmed experimental infection of the horse with S. neurona.

### **#31 Pyrantel tartrate alters sarcocystis neurona sporocyst infection in gamma interferon knock out mice.**

*EA Fruttlin (student presentation), MG Rossano, RA Vrable, AJ Murphy, JP Baneene, HC Schott, J Patterson, and LS Mansfield, MSU*

Sarcocystis neurona causes EPM. Horses are infected by ingesting sporocysts shed in opossum feces Pyrantel tartrate, the active compound of strongid-C a daily anthelmintic feed supplement, is lethal to s.neurona merozoites in cell culture at concentrations greater

than 0.0025 M ( $8.91 \times 10^{-4}$  g/ml). Here, we used a gamma interferon knock out mouse model to test the hypothesis that pyrantel tartrate kills the sporozoite stage of *S. neurona*. 15 mice were divided into 3 groups. All mice were treated individually by gastric intubation for 6 days. Mice in group 1 were given 0.2 ml of water on days 1 and 2, 1000 viable *S. neurona* sporocysts in 0.2 ml water on day 3 and 0.2 ml of water on days 4-6. Mice in Group 2 were given 0.2 ml of pyrantel tartrate suspension ( $1.782 \times 10^{-4}$  g per 0.2 ml water) on days 1 and 2, 1000 viable *S. neurona* sporocysts in 0.2 ml of pyrantel tartrate suspension on day 3 and 0.2 ml of the pyrantel tartrate suspension on days 4-6. Mice in group 3 were given 0.2 ml of pyrantel tartrate suspension on all 6 days and were infected with *S. neurona* sporocysts. Mice were observed daily for adverse clinical signs and euthanized at an approved humane endpoint or, if healthy, 2 weeks after the last sick mouse died. Brain, liver, spleen, lung and kidney samples were taken from each mouse for histopathology, and brain samples for cell culture. Mean survival times were compared between groups using the student's t-test. Mice in Group 2 lived significantly longer than mice in Group 1. All animals in Group 1 had histopathologic evidence of encephalitis, granulomatous pneumonia, and protozoa in the brain and lungs. 4 of 5 animals in Group 2 had granulomatous pneumonia, 1/5 had mild meningitis, 4/5 had no brain lesions and 5/5 had protozoa in the lungs. The remaining Group 2 mouse had no significant histological lesions in any organ. Merozoites were cultured from the brain of all mice in Group 1 and Group 2. All mice in Group 3 showed no signs of illness, no histological changes and no growth in cell cultures. The results of this study support the hypothesis that pyrantel tartrate affects *S. neurona* sporozoite infection in gamma interferon knock out mice when given orally at the time of infection and have prompted further studies to investigate the possibility of using Strongid-C as a preventative of EPM in horses. The work was supported by Pfizer grant 71-2954