Prevalence of Sarcocystis neurona and Neospora Hughesi in horses from Kentucky based on presence of serum antibodies to parasite surface antigen

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Review of Literature: Origin

- EPM is fairly common in North America
  - 60% of horses in the U.S. have been exposed to S. Neurona
  - However only 1% of horses may develop EPM (Ellison et al. 2007)
Review of Literature: Symptoms

Symptoms include:

- Gait abnormalities
- Ataxia
- Mentation
- Soreness
- Lethargy

Carr et al. 2014
Review of Literature: Effect on the Body

- Horses diagnosed with EPM based on the presence of *S. Neurona* antibodies in the serum. (bodily fluids, blood)

- Horses with EPM had lower proliferation responses.
  (Norton et al. 2006)
The Parasite

- Parasite enters and travels to the brain or spinal cord.

- Nervous system of horse is effected (Dubey et al 2001)

- Horses come in contact with the parasite through contaminated feed or water (Mackay et al 2012)
Review of literature: DNA markers: *S. Neurona*

- Random-amplified polymorphic DNA techniques were used to enlarge DNA.

- Isolates of *S. Neurona* were taken from the infected horse. (Tanhauser, 1999)

- DNA sequence analysis of polymerase chain reaction (PCR) products.

- This was then used to design PCR primers to amplify specific *Sarcocystis* DNA products (Murphy et al, 2005)
The parasite: Neospora Hughesi

- Known to be another parasite that causes EPM in horses.
- Now being identified in horses across the United States. (Horses in 24 states tested positive for antibodies)
- Tested serums and CSF of horses to see if they came into contact with this parasite. (Yowell, 2010)
Hosts of EPM Parasite:
The Opossum: Raccoon

- The opossum is the most common host.
- Both schizonts and merozoits carried by the opossum. (Dubey et al., 2001; Granstrom et al. 1998)
- Stages of S. neurona may be found in many parts of the raccoons body: (Stanek et al.2002)
  - Blood
  - Intestines
  - tissues
How do you test for EPM?

- Histopathology of the horse:
  - Tissue samples mounted on microscopic slides

- Immunohistochemistry-an assay that shows specific antigens in tissues by the use of markers that are either fluorescent dyes or enzymes

- PCR- copying DNA strands

- Immunohistochemical findings- schizonts, merozoites found.

(Mullaney et al. 2005)  
(Jones. et al. 2002)
Diagnosing EPM

- Veterinarians test for EPM by conducting a neurological examination.

Tests:
Western blot test, ELISAS (Enzyme linked Immunosorbent assays), CSF (cerebral spinal fluid) (Granstrom et al, 2000; Rossano et al, 2003)
Hypothesis

H0 – Horses that come into contact with the parasites S. neurona or N. Hughesi are less likely to form antigens of this parasite.

H1 - If horses come into contact with the parasite S. Neurona or N. Hughesi, they are more likely to form antibodies against the antigens of this parasite.
Methods

• Sera was taken from 133 horses living in the pastures of University of Kentucky’s farm.

• ELISAS conducted on both parasites S. neurona and Neospora Hughesi
Procedure

- Each sera was put into individual tubes. The tubes were then mixed with antigens of the parasites S. neurona and N. Hughesi.

- This was to determine the Seroprevalence of antibodies that were against the parasites surface antigens.
Procedure

Solutions used:

- For washing well plates, antibody incubation solution, as well as substrate used in plates: Block solution (PBS, normal goat serum (second antibody), dry milk, and tween)
- Stop reagent: Diluted sulfuric acid
- Recombinant protein was then diluted: left incubated over night.
Procedure

• After ELISA protocol was completed well plates were checked for blue coloring.

• Sulfuric Acid then added to turn the color yellow so that the ELISA reader could calculate the dilution of each well.
Seroprevalence of \textit{Sarcocystis neurona} and \textit{Neospora hughesi} in University of Kentucky Horses

- \textit{S}\textsubscript{n}: 99.2\%
- \textit{N}\textsubscript{h}: 3.8\%
MY Mentor: Dr. Howe

- Gluck Equine Research Center
  Department of Veterinary Science
  University of Kentucky

- *Sarcocystis neurona* Genome Sequencing Project

- Examination of SnSAG Gene Family in Sarcocystis neurona

- Enzyme linked Immunosorbent Assay (ELISAs) Based on Sarcocystis neurona Surface Antigens (SnSAGs)


(cont.)


