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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Abstract

Sera from 133 horses living in Kentucky were taken and enzyme linked Immunosorbent assays (ELISA's) were taken. The Sera was tested for major antibodies to surface antigens of the parasites Sarcocystis neurona, and Neospora Hughesi. 99.2% of horses were positive for antibodies against S. neurona, however only 3.8% were positive for antibodies against N. Hughesi. As shown from this data horses in Kentucky are not as exposed to N. Hughesi, as they are to S. neurona. Therefore as shown in this experiment the data shows that Sarcocystis is much more common then Neospora.

Introduction

Equine Protozoal Myeloencephalitis (EPM) is one of the most dangerous neurological diseases that can impact a horse's health. It is a deadly disease that is most common in North America. This Neurological syndrome is a major threat to horse owners and horse enthusiasts throughout the United States. The most common origin of EPM is caused by a parasite known as Sarcocystis neurona, however it wasn't till very recently that another parasite was also discovered. This parasite is known as Neospora Hughesi. Statistics show

that 50% of horse are infected with S. neurona, and the exposure rate to Neospora is 10-25% (Mackay, 2011) There are several factors in which these parasites can affect a horse's health, both mentally and physically. These factors serve to make EPM one of the most severe neurological diseases that a horse can contract.

Sarcocystis neurona is the most common causative agent. Many experiments have revolved around the study of this parasite. Protozoa can be found in the central nervous system, and can affect both the brain, and spinal chord. This may lead to inflammatory cell response, or neuronal destruction (damage of the neurons). The parasites that causes EPM has multiple life stages, for example merozoits are most commonly found in the cytoplasm of neurons. A foal that was diagnosed with severe EPM was thoroughly examined and it was shown that several life stages of Sarcocystis neurona were found in the spine, and CNS (Ellison et al. 2007). There are many ways in which a horse can be tested for EPM. As shown in Veterinarian Parasitology, immune responses in horses naturally infected with EPM were tested. It was shown that some horses diagnosed with the neurological disease tested positive for other neurological diseases as well. Based on the presence of S. neurona antibodies in the serum (yang et al. 2006). In a similar experiment 22 infected horses were used, as well as a control group of horses from

Florida and Virginia. Their blood was extracted and stored overnight. Later the blood was examined, using a Western blot test. Traces of S. neurona antibodies were found, which showed the horses came into contact with the parasite (Andrews et al. 2007).

An Enzyme Linked Immunosorbent Assay is one of the most common ways to test for EPM. Cerebral Spinal fluid or Sera may be used from a range of horses. The sera or CSF is then placed into well plates where a base solution is made, later it is shown which samples have developed antibodies to antigens against the parasite. Another common experiment is a Western Blot or immunoblot test. This is a widely used analytical technique used to detect specific proteins in a sample of tissue homogenate or extract. In a specific experiment 23 serums and CSF samples representing each of the four-immunoblot patterns were selected from 220 horses that presented neurological signs of EPM. Horses were later examined for inhibitory effects on the infectivity of S. neurona by an in vitro neutralization assay. (Zhao et al. 2013) (Veterinarian parasitology, volume 183.) It was shown that there was a high correlation between immunoblot band pattern and neutralizing activity. Two proteins SnSAG14 and SnSAG16 were shown, it suggested that these are surface proteins and may be useful components if the horse was infected (ting et al. 2004).

After extensive research by scientists throughout North America, it was found that *S. neurona* was not the only cause of EPM. Another Protozoal parasite had been found. This parasite was known as Neospora Hughesi. N. Hughesi is a type of protozoa pathogen, and the life cycle of this parasite is similar to *S. Neurona* life cycle. The parasite enters the horse's body and causes complications with the CNS. Similar to S. neurona horses come in contact with this parasite through contamination (Yeargan et al. 2010). An example of an experiment conducted relating to N. Hughesi was a recombinant NhSAG1 ELISA. This was a sensitive and specific ASSAY for detecting antibodies against this parasite in equine serum Several previous studies have examined the Seroprevalence of Neospora antibodies in horses throughout the United States (Dubey 1999). This parasite had been known, but wasn't being researched until recently. However Sarcocystis neurona still remains the most common parasite.

Hypothesis:

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.

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

Materials and Methods (Procedure)

Sera was taken from 133 horses living in the pastures of University of Kentucky's farm. Each were put into individual tubes. The tubes were then mixed with antigens of the parasites S. neurona and N. Hughesi. To determine the Seroprevalence, of antibodies that were against the parasites surface antigens. Enzyme Linked Immunosorbent Assays (ELISA) was then conducted on the samples. This was done to show specific antibodies to antigens of the parasites Sarcocystis Neurona and Neospora Hughesi. To Conduct ELISAS (enzyme linked Immunosorbent assays) tubes were then each labeled with the name of the foal or horse being used, so the sera could be matched accordingly. This was done after a data sheet was made to layout where the controls would be along with the rest of the sera's.

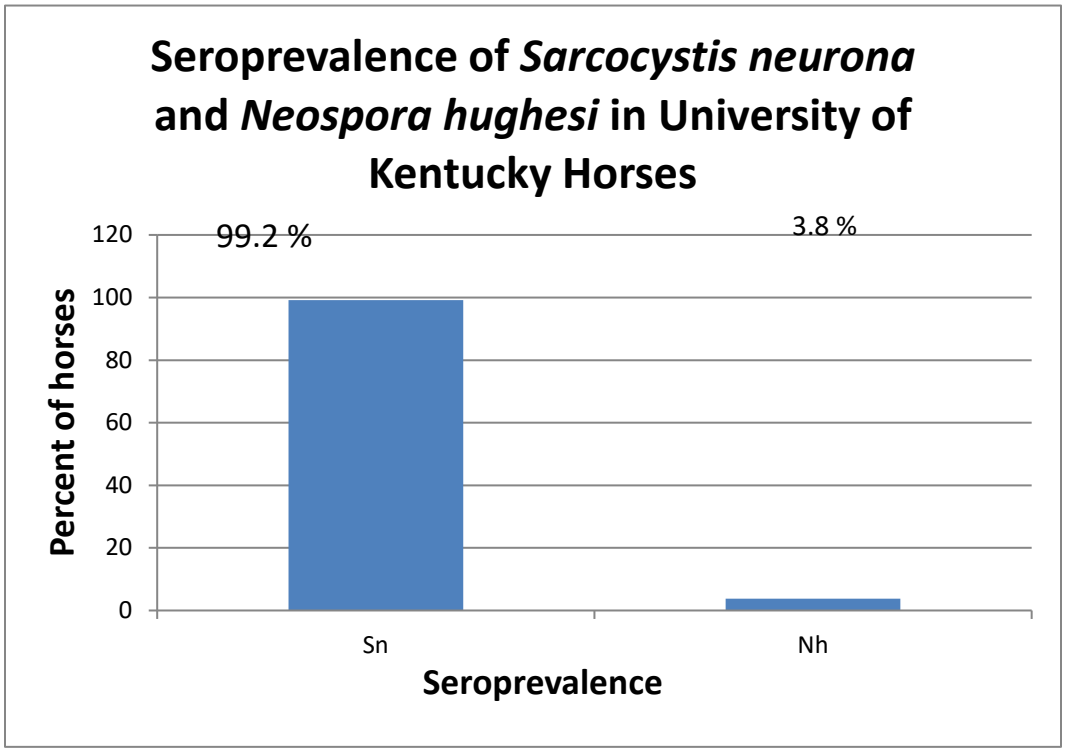
ELISA protocol includes Block solution, consisting of PBS, normal goat serum, dry milk, and tween. Wash solution was used for cleaning well plates (5 times each), Antibody incubation solution, as well as substrate used in plates consisting of TMB at room temperature. Lastly a stop reagent was used, which consisted of diluted sulfuric acid. The

recombinant protein was then diluted; the antigen was then put into a binding micro liter plate, and incubated over night. After it was rinsed three times with PBS tween. Wells then were blocked for one and a half hours, and then rinsed with ELISA wash solution. After the horse serum was diluted in diluent solution to desired amount (1:250, 1:500). The plate was then covered and incubated for an hour (at 37 degrees), and then washed five times. Goat anti horse antibody was used after the second antibody was diluted. This was then incubated for a few minutes, after the well plates were taken out, and then checked for blue coloring. After sulfuric acid was added to turn the blue color to a yellow color. It is important after each wash to slap the plate faced down on paper towel in order to reduce the amount of background antibody binding, and after secondary antibody will be added to not let the wells dry out, as this would cause the HRP (Human Research Planning) enzyme activity to be reduced.

Results and Discussion

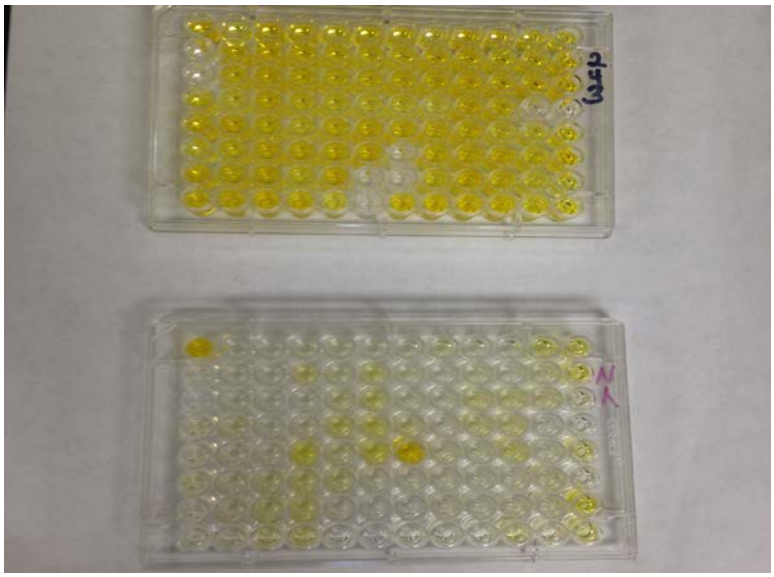
As shown in the Diagram below, 99.2% of horses were positive for Sarcocystis Neurona however only 3.8% of the 133 samples of horse sera had antibodies against the parasite N. Hughesi. This shows that there are less cases of N. Hughesi then there are S. Neurona. S. Neurona has been around longer then this newly found parasite has been therefore

there are more horses that have developed antibodies after coming into contact with this parasite. Neospora is not as common in North America region therefore the horses are less prone to coming into contact with it, thus developing antibodies to the antigens of this parasite.



As shown in these diagrams all of the Sera samples were mixed with the antigens of the parasites N. Hughesi and S. Neurona. They then were put in individual plastic test tubes and labeled with different numbers. They were each put on a chart so that they could be laid out accordingly. The ELISA for rNHp29 had a dilution of 1:40. Controls were

needed; the control was NP pool with a dilution of 1:250, foal 1, and a blank test tube with no antigen. After the ELISA was conducted the plate was scanned in the computer. Samples that turned a darker yellow in the wells were samples that had more antigens to antibodies then the lighter colored wells. The numbers show the amount of absorbance based on the dilution of the sera's. Both controls remained zero.



Discussion

The chance of a horse fully recovering after being diagnosed with EPM is less then 40%. It has been shown that horses are natural intermediate hosts for these parasites, both *S.* neurona and N. Hughesi. EPM is however not contagious among horses. It is very important to diagnose EPM quickly, as this will improve chances of survival for the horse.

However the symptomology of EPM will worsen over time, if not quickly treated. Horses with severe cases of EPM often do not survive. There are number of ways EPM can be treated if caught in its early stages. Most treatments however, are very expensive. It is common that a blood cell analysis is taken before treatment, to ensure that it is in fact EPM that the horse has. The medication Diclazuril can be used, and helps to improve the condition of horses infected with EPM's by as much as 75%. The use of oral paste Ponazuril has also been used as a preventative agent. Ponazuril acts against the parasite, and many of its stages. It travels through the blood stream and even triggers the central nervous system to kill the parasite. Another remedy that may be used in the treatment against EPM is supportive therapy. This is a treatment that is designed to improve, strengthen, or maintain a horse's health (Jones et al. 2002). An additional side effect of EPM is brain and spinal phenylbutazone chord inflammation. Some anti-inflammatory medications have been used, such as flunixin meglumine, or. These are all possible treatments that can be used in the fight against EPM.

Through extensive study, it has been revealed that between 50-70% of horses in the country have been exposed to *S. Neurona*. The disease Equine Protozoal Myeloencephalitis poses a significant problem to horses throughout the United States. It is important that research into new drugs and therapies continue. The parasites involved

in causing this dangerous neurological disease can destroy the physical and mental health of horses. This can be devastating to both the horse, and human partner alike. Though most people no longer rely on horses to provide the basic necessities of agricultural life, the unique bond between horses and humans remains as strong as ever. For those that benefit directly from this relationship, it is imperative that this deadly disease is brought under control as quickly as possible.

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