Detection of *Sarcocystis neurona* Antibodies in the Serum and CSF of Foals

A. Grimsley Cook, DVM, MS; V. Buechner-Maxwell, DVM, MS; L. L. Donaldson, DVM, PhD; J. K. Morrow, PhD; N. A. Parker, DVM, MS; Francois E. Elvinger, DVM, PhD

Maternal antibodies to *Sarcocystis neurona* are passively transferred from a seropositive mare to her foal in colostrum and can remain detectable by Western blot for up to 9 months of age, although most foals are seronegative by 6 months of age. Antibodies are detectable in CSF of neonatal foals from several days to age up to at least 3 months of age. Author’s address: Dept of Large Animal Clinical Sciences, Virginia Maryland College of Veterinary Medicine, Duck Pond Drive Phase II, Blacksburg, VA 24061. © 2001 AAEP.

1. Introduction

Equine protozoal myeloencephalitis (EPM), caused by the protozoal organism *Sarcocystis neurona*, is the most commonly diagnosed equine neurologic disease in the United States.1 EPM is diagnosed by detecting antibodies to *S. neurona* in CSF with the Western blot (WB). Exposure is determined by WB analysis of serum.2

Evaluation of results of serologic tests in young horses for the presence of *S. neurona* specific antibodies may be complicated by several factors, including the possibility of in utero exposure and the potential for the passive transfer of *S. neurona* specific maternal antibodies. Transplacental infection with protozoa is possible,3 although it has not been specifically reported with *S. neurona*. Specific antibodies should be present in the presuckle serum if exposed to the organism after 180 days.4 One goal of this study was to examine the potential for in utero exposure by testing for detectable presuckle serum concentrations to *S. neurona*.

A mare that is seropositive for EPM, and thus has circulating antibodies to *S. neurona*, would presumably pass these antibodies to her foal in the colostrum. Another goal of this study was to determine how frequently foals obtain detectable antibodies to *S. neurona* by passive transfer. Serial sampling of seropositive foals determines approximately when maternal antibodies are metabolized, rendering foals seronegative by WB. A final goal of this study was to determine time to seronegative status.

It has been established that neonatal foals have a more permeable blood–CSF barrier than adult horses, which can result in higher concentrations of immunoglobulins in the foal's CSF.5,6 Maternal antibodies to *S. neurona*, if ingested in colostrum, may pass into the CSF, resulting in a false positive WB. A second study was undertaken to determine if antibodies to *S. neurona* are present in the CSF of neonatal foals, and if so, to determine if they are detectable at several months of age.
2. Materials and Methods

Serum Antibody Study

Thirty-four pregnant mares (33 seropositive, 1 seronegative) were identified for the first study. Foalings were attended, and foals did not have access to other sources of colostrum. Samples collected immediately after birth included presuckle foal serum samples, colostrum, and mare serum samples. Postsuckle serum samples from the foals were then collected at 24 hours after birth and at monthly intervals thereafter until at least 7–9 months of age.

WB was performed on sera from mares and foals for the presence of *S. neurona* specific antibodies, as described. Radial immunodiffusion was performed at 24 hours to quantify passive transfer of maternal immunoglobulins (IgG), as described.

The LIFETEST procedure of the SAS system was used to calculate Kaplan–Meier estimates of the mean and standard error of time to seroconversion, including censored observations. Pearson’s correlation analysis was used to test the 24-hour serum IgG concentrations for correlation with time to seroconversion.

CSF Antibody Study

CSF was collected aseptically from the atlanto-occipital (AO) space of 15 healthy TB foals between the ages of 2 and 8 days. Foals were anesthetized with xylazine and propofol and placed in lateral recumbency. Blood samples were obtained from the mare and foal at this time.

Western blot was performed on the foal CSF/serum and mare serum. Radial immunoglobulin was used to quantify the amount of immunoglobulin in CSF and serum. Serum and CSF albumin concentrations were determined spectrophotometrically. Cytology and chemistries of the CSF were also performed. CSF indices were calculated.

At least 7 days after the initial AO tap, a second CSF and serum sample was collected from 5 of the 13 foals previously found CSF positive. Ages ranged from 13 to 41 days. A third CSF and serum collection was performed 49 days later on the same 5 foals at which time their ages ranged from 62–90 days.

The FREQ procedure of the SAS system was used to perform an exact calculation of Pearson’s chi square to show association between the grades of serum and CSF immunoreactivity in the neonatal foal.

3. Results

Colostral Transfer of Antibodies

All of the foals included in this study were negative by WB analysis prior to ingestion of colostrum. Passive transfer of *S. neurona* antibodies from seropositive mares occurred in 100% of the foals. The single foal from a seronegative mare was seronegative post colostrum.

Thirty-one of 33 seropositive foals became seronegative by 9 months of age (range 1–9 months), with a mean time to seronegativity of 4.2 months (SE = 0.39 months). The one foal from the only seronegative mare remained seronegative throughout the 9-month sampling period.

Radial immunodiffusion of 24-hour samples showed adequate passive transfer in all of the foals (mean: 1593 ± 338; range: 842–2159 mg/dl IgG). Serum IgG concentrations at 24 hours were not correlated with time to seronegativity (r = 0.099; p = 0.59).

Detectable Antibodies in the CSF

In the 2nd study, 13 of the 15 mares were WB seropositive. The corresponding 13 foals were also seropositive when sampled at 2–7 days of age. Twelve of the 13 seropositive foals were CSF WB positive. There was a significant association between WB reactivity in the serum and the CSF (P = 0.0005). The 2 foals from seronegative mares were serum and CSF WB negative.

Cytologic evaluation of the CSF samples indicated that fluid was obtained without microscopic evidence of peripheral blood contamination (mean RBC 8 ± 11 mm$^{-3}$; median 3 mm$^{-3}$). The quantity of CSF IgG was consistently elevated as compared to established adult reference ranges (CSF IgG: mean 25.6 ± 10.8 mg/dl; median 22.4 mg/dl), while CSF albumin levels were within reference range (CSF albumin: mean 51.5 ± 15 mg/dl; median 51.5 mg/dl).

The 5 foals that were retested were serum and CSF WB positive at the second sampling. At the third sampling, ⅔ foals were CSF negative and ¼ was seronegative. The quantity of CSF IgG was considerably decreased in all samples (range: 8–14 mg/dl).

4. Discussion

Due to the recent growing concern over EPM, horses are frequently screened for exposure to *S. neurona* by performing a WB on serum. In the young horse, several factors can complicate interpretation of a positive WB result, including the potential for in utero exposure to *S. neurona*, passive transfer of maternal antibodies to *S. neurona*, and persistence of these maternal antibodies. No previous studies have been published that characterize these factors.

In this study, it was shown that seropositive mares do transfer antibodies against *S. neurona* to their foals in colostrum. Antibodies to *S. neurona* were detected in all of the 33 foals born to seropositive mares post-colostrum ingestion (100%). The high rate of occurrence in this study suggests that passive transfer of *S. neurona* antibodies occurs frequently in the population at large. In contrast antibodies were not detected in any of the foals prior to ingestion of colostrum.

Kinetic studies have been performed to evaluate the decay or the metabolism rate of other specific
equine maternal antibodies. Antibodies to *S. neurona* cannot be reliably quantitated by current testing methods, preventing determination of a decay rate. The mean time to seronegative conversion was 4.2 months, with a range from 1 month to 9 months. Analysis between total serum IgG at 24 hours and time to seronegative conversion showed no correlation \((r = 0.099; \ p = 0.59)\). This may suggest that antibody metabolism occurs at different rates in individual foals. Alternatively, it may indicate that a higher concentration of *S. neurona* antibodies is passed by some mares, which does not correlate with the total IgG concentration at 24 hours.

While the seroprevalence to *S. neurona* appears to increase with age, the clinical disease of EPM appears to be a greater risk to young horses.\(^9\)–\(^11\) With the potential presence of maternal antibodies, it is difficult to interpret a seropositive test in a young horse. In this study, the majority of foals (87%) were seronegative by 6 months of age, which suggests that young horses cannot be accurately screened by serum testing before at least 6 months of age, as maternal antibodies may be detected.

It has been speculated that the presence of an enhanced intracellular tubular transport system in foals and other neonates allows for enhanced transport of proteins and protein fractions across the blood-CSF barrier.\(^12\) In the normal neonatal foal, CSF protein concentration is significantly higher at birth to several weeks of age.\(^13\) The second portion of this study demonstrates that foals cannot be reliably tested for EPM by CSF evaluation, as antibodies to *S. neurona* can be found in the CSF of seropositive neonatal foals from seropositive mares. The WB cannot distinguish between maternal antibodies and endogenous antibodies to *S. neurona*. Therefore, a positive CSF WB in a young foal may not support a diagnosis of EPM but may indicate that the foal ingested antibody-rich colostrum. The second and third CSF samplings demonstrate that antibodies to *S. neurona* can persist in the CSF for at least several months.

These studies demonstrate that age of a foal is an important consideration when testing for EPM. Maternal antibodies to *S. neurona* are passively transferred from a seropositive mare to her foal in colostrum and can remain detectable by WB for up to 9 months of age, although most foals are seronegative by 6 months of age. Antibodies are detectable in CSF of neonatal foals from several days of age up to at least 3 months of age.

**Acknowledgments**

This study was supported by grants from the AAEP Foundation, Inc., Virginia Tech Clinical Research Fund, and the Virginia Veterinary Medical Association. The authors thank Dr. Wendell Cooper of the Middleburg Agricultural Research Center, Virginia Tech, and Dr. Ann Dunnington of the Dept. of Animal Science, Virginia Tech for the use of their foals in this study.

**References and Footnotes**


*Equine Biodiagnostics Inc, Lexington, KY

VMRD, Pullman, WA

ver. 7.01, SAS Institute, Cary, NC