After prolonged treatment in horses for equine protozoal myeloencephalitis (EPM), cerebrospinal fluid antibodies to Sarcocystis neurona may persist. The albumin quotient did not change and the IgG index decreased in horses with EPM after repeated spinal taps 2–3 months apart. A decrease in IgG index, a measure of intrathecal antibody production, may indicate a favorable response to treatment for EPM. Authors’ address: Dept. of Large Animal Clinical Sciences, College of Veterinary Medicine, The University of Tennessee, P.O. Box 1071, Knoxville, TN 37901-1071. © 1997 AAEP.

1. Introduction

The cerebrospinal fluid (CSF) indices, albumin quotient (AQ), and IgG index are indicators of blood–brain barrier permeability and intrathecal antibody (IgG) production, respectively. The indices are calculated from measurements made on consequent serum and CSF samples and aid in the diagnosis of neurologic disease; they are also used as an allied test with the immunoblot test (Western blot) in the diagnosis of equine protozoal myeloencephalitis (EPM). Thus, the diagnosis of EPM is made by the presence of Sarcocystis neurona (SN), or perhaps Sarcocystis falcataula, antibodies in CSF and a normal AQ and a normal to increased IgG index.

A spinal tap is usually performed during the initial examination to make a diagnosis and then subsequent spinal taps are performed at approximately 2- to 3-month intervals to evaluate response to treatment and to determine when to discontinue treatment (when CSF is negative for SN antibodies). The practice of repeated CSF taps in clinical patients is becoming commonplace in equine practice and has been reported to increase the CSF indices, because of dural trauma from the spinal needle and the introduction of foreign antigens into the CSF. Recently, a study in clinically normal horses showed a significant increase in CSF indices after repeated (bi-weekly) spinal taps for 1 year. However, there are no reports on the effects of repeated spinal taps on CSF indices in horses with EPM.

The purpose of this study was to measure consequent CSF and serum albumin and IgG concentrations and calculate the CSF indices in horses with EPM after repeated CSF taps. This study will help determine the effects of repeated CSF taps on the CSF indices, which may have importance in the diagnosis of EPM and interpreting follow-up Western blot testing for EPM.
2. Materials and Methods
Cerebrospinal fluid was obtained from the lumbosacral space (L/S) of 12 horses presented to The University of Tennessee Veterinary Teaching Hospital with clinical signs consistent with EPM. In order to be included in this study, the CSF from the horses had to be positive for SN antibodies as measured by Western blot testing and have at least one subsequent spinal tap. All horses in this study were treated with trimethoprim sulfadiazine 15 mg/kg PO q 12 h or sulfadiazine 20 mg/kg PO q 24 h and pyrimethamine 1.0 mg/kg PO q 12 h until the Western blot test was negative for SN antibodies, or 30 days past the last spinal tap, or the horses are currently on treatment.

Serum and CSF albumin concentrations were measured by using a wet chemistry analyzer. The technique for measuring the CSF albumin concentration was adapted to measure the lower concentration of albumin in CSF. Serum and CSF IgG concentrations were measured by using radial immunodiffusion (RID) as previously described. 

From the measurements of consequent serum and CSF albumin and IgG concentrations, the CSF indices were measured by using the following formula:

$$\text{AQ} = \frac{\text{CSF albumin (mg/dl)}}{\text{serum albumin (mg/dl)}} \times 100,$$

$$\text{IgG index} = \frac{\text{CSF IgG (mg/dl)/serum IgG (mg/dl)}}{\text{(CSF albumin/serum albumin)}}$$

CSF and serum values obtained were compared by using an analysis of variance for repeated measures. Significance was determined at a level of $p < 0.05$.

3. Results
The horses in this study had an average of three L/S CSF taps (range 2–8) done over an average of 132 days (range 60–210 days). One horse had eight CSF taps in a 210-day period, two of which contained frank blood. Two other horses had frank blood in the CSF obtained. Follow-up spinal taps were done approximately 2 weeks later in horses in which frank blood was seen in CSF. Values obtained from spinal taps containing frank blood were not included in this study.

In this study, 10/12 horses were positive and 1/12 horse was suspect positive on Western blot for SN antibodies in the initial CSF sample. One horse was negative on CSF Western blot test for SN antibodies on the initial CSF sample but converted to positive in 30 days. The CSF Western blot test was negative in one horse after 60 days, and two horses were negative on Western blot after 90 days of treatment. Of the remaining horses, 9/12 were Western blot positive on the last spinal tap. Treatment was discontinued 30 days after the last positive spinal tap in 5/9 horses, and 4/9 horses are still being treated.

The mean ± SD albumin quotient in the horses was 1.55 ± 0.5 (range 0.69–2.26) in the initial spinal tap and 1.58 ± 0.6 (range 1.0–2.91) in the last spinal tap. However, the AQ increased in 3/12 horses during the study.

The mean ± SD IgG index in the horses was significantly decreased from 0.534 ± 0.205 in the initial spinal tap to 0.333 ± 0.238 in the last spinal tap. The decrease in the IgG index was due to a significant decrease in CSF IgG concentration in the horses. However, the IgG index increased in 3/12 during this study.

4. Discussion
Horses in this study had an average of three spinal taps performed over an average period of 132 days while on EPM treatment. One horse had eight spinal taps done in 210 days. The Western blot test was negative for SN antibodies in only 3/12 horses after 90 days of treatment. The Western blot was positive for SN antibodies in 5/12 horses after the final spinal tap and these horses were treated for 30 days and removed from treatment. The other horses in the study (4/12) remained Western blot positive for SN antibodies after the final spinal tap and are still being treated. It would appear from these data that horses with EPM require prolonged treatment to clear antibodies from CSF, because only 3/12 horses were antibody negative after a mean treatment length of 132 days. Antibodies in the CSF to SN may be associated with persistent infection. However, persistent antibodies in the CSF to SN may be derived from the plasma, because antibodies (IgG) are primarily derived from the plasma in healthy animals. This could explain the presence of persistent CSF antibodies, because more than 53% of normal horses and all of the horses in this study had serum antibodies to SN.

There was no change in AQ and a significant decrease in the IgG index after repeated spinal taps in horses in this study. Three horses showed a mild increase in AQ and two horses showed a mild increase in IgG index in subsequent spinal taps. This is in contrast to a previous study that showed an increase in AQ and IgG index after repeated spinal taps. However, in that previous study, spinal taps were performed every 2 weeks for 1 year, which could have caused inflammation and the introduction of foreign antigens that did not resolve before subsequent spinal taps. In contrast, horses in this study had adequate time to decrease inflammation and process and eliminate foreign antigens in the 2 to 3 months between spinal taps. The significant decrease in IgG index in the horses in this study may be due to the treatment for EPM. Appropriate EPM treatment may have led to the observed decrease in IgG index, an indicator of intrathecal antibodies. This decrease may be due to the elimi-
nation of the organism from the central nervous system.

In conclusion, prolonged treatment is needed to decrease or clear antibodies to SN from the CSF in horses with EPM. Some residual antibodies to SN in the CSF may be derived from the plasma even after the organism has been eliminated from the central nervous system. It appears that repeated spinal taps performed 2 to 3 months apart do not result in an increase in the CSF indices. Also, the IgG index decreased in horses during EPM treatment and may be helpful as an indicator of effective treatment if measurements are done on serial CSF samples.

References

Equine Biodiagnostics, Inc., Lexington, KY 40506.