Sarcocystis neurona Antibodies in Equine Cerebrospinal Fluid

William V. Bernard, DVM, Dipl. ACVIM

The immunoblot testing for antibodies to Sarcocystis neurona has improved the antemortem evaluation of the neurologic equine. As with those for other infectious diseases, antibody tests are an adjunct to diagnosis and rarely can be considered a definitive test. The Western blot testing for cerebrospinal fluid antibodies to S. neurona should be considered a useful diagnostic test in the evaluation of equine neurologic disease. However, the use of the Western blot alone or the use of the Western blot in clinically normal individuals may lead to a false-positive diagnosis and inappropriate therapy. Author’s address: Rood and Riddle Equine Hospital, P.O. Box 12070, Lexington, KY 40580. © 1998 AAEP.

1. Introduction
Equine protozoal myeloencephalitis (EPM) is a neurologic disease that can affect the central nervous system (CNS), including the spinal cord, brainstem or brain. These regions may be affected individually (focally) or in combination (multifocally). Therefore, clinical signs can be quite variable dependent upon the location of the organism within the central nervous system. The development of a test that detects antibody to the protozoan parasite was made possible subsequent to culture of the organism in 1991.1 A positive serum antibody test indicates exposure to Sarcocystis neurona. Equine exposure to S. neurona is common; positive serum Western blots have been reported in >30% of those horses tested in certain geographic areas of the country.2,3 A positive cerebrospinal fluid (CSF) Western blot (WB) indicates the presence of antibody, specific for S. neurona antigen, in cerebrospinal fluid. Theoretically, this should indicate the presence of the antigen in nervous tissue. A histopathologic evaluation of CSF positive clinically neurologic patients identified a positive predictive value of 85% and a negative predictive value of 92%.4 This paper presents information regarding the CSF in clinically normal horses. The presence of CSF antibodies in clinically normal horses suggests that a cautious interpretation of a positive CSF S. neurona WB may be warranted.

2. Materials and Methods
The records and results from 943 horses in which a cerebrospinal fluid analysis was performed were reviewed and tabulated. The CSF collection occurred between the years of 1993 and 1997. Signs of neurologic disease were based upon a complete neurologic examination. The neurologic examination included an examination of the cranial nerves and musculoskeletal system, and an observation of the gait of the horse when it walked in a straight line and when it circled to the right and left. Horses were separated into two groups based on their clinical signs. Group 1 consisted of those horses with clinical signs of neurologic disease, a gait abnormality, or a client complaint of a performance problem. Group 2 consisted of those horses in which CSF was evaluated as a screening for S. neurona antibodies. Group 2 horses had no evidence of neurologic disease and no client complaint of a clinical problem. Cerebrospinal fluid was evaluated for antibodies to S. neurona by means of WB; S.
neurona antigen was evaluated by means of polymerase chain reaction (PCR); and a red blood cell count was evaluated by the use of a hemocytometer. Cerebrospinal fluid was noted as being either S. neurona WB positive, PCR positive, or both. Red blood cell counts were used to determine the presence of blood contamination of the CSF. Cerebrospinal fluid with >10 red cells/µl was considered to be blood contaminated and was excluded if WB positive.

3. Results

Results of 943 CSF samples (Table 1) found that 31% of horses screened for EPM (neurologically normal) were WB positive. Furthermore, 29% of horses with a client complaint, neurologic condition, or gait abnormality were WB CSF positive. Of the 943 CSF samples evaluated in this review, 19 (1.9%) were PCR positive.

4. Discussion

It is apparent from the results provided in Table 1 that a large number of clinically normal horses are CSF S. neurona WB positive. Possible explanations for the presence of antibodies in the CSF of clinically normal horses include blood contamination of the CSF at the time of collection; blood–brain barrier compromise, with leakage of antibodies across the blood–brain barrier; persistence of antibody response subsequent to removal of the antigen; antibody crossing a normal intact blood–brain barrier; and cross-reactivity of the WB test with an antigen other than S. neurona.

Blood contamination of cerebrospinal fluid at the time of collection is a very plausible explanation for the high rate of positive S. neurona WB positive horses. The vast majority of CSF WB positive horses are serum positive. The amount of blood (WB positive) contamination that results in conversion of a negative to a positive CSF has not been determined. In this paper a number of <10 red blood cells/µl was chosen as an acceptable amount of blood contamination. Studies to determine the acceptable level of CSF blood contamination and the best method of determining blood contamination are needed. The albumin quotient (AQ) has been suggested as a method of determining the presence of blood contamination or blood–brain barrier compromise. The AQ is the ratio of CSF albumin concentration to serum albumin concentration. Initial studies suggested that an albumin quotient of greater than 2.0 indicated blood contamination. A subsequent study found a similar value for the AQ ratio of equine CSF. The reliability of the AQ in determining blood contamination has to be confirmed, as it is not uncommon for a blood-contaminated sample (discolored CSF) to have a normal AQ ratio. A recent evaluation of total albumin content appears promising in the evaluation of CSF blood contamination.

In normal, healthy individuals, intrathecal (CSF) antibody is a representation (microfiltrate) of peripheral immunoglobulin. The high seroprevalence of S. neurona would suggest that if the immunoblot sensitivity is high, then S. neurona antibody may be found routinely in normal CSF. Therefore, a second explanation for the presence of S. neurona antibody in clinically normal horses is that CSF antibody is a representation of extrathecal (peripheral) antibody. A third and fourth explanation for the presence of S. neurona specific antigen in the CSF of clinically normal horses are the absence of clinical signs with the presence of organisms and a persistence of antibody response subsequent to antigen removal. Is it not possible that the number of protozoa that may be present in the CNS may not be adequate enough to produce clinical signs? Is it not possible that the location of the organism or the induced inflammatory response is not sufficient to produce clinical signs? Histopathologic confirmation of EPM often lacks identification of the organism in the presence of a characteristic inflammatory response. It is possible that the antibody response could persist in the absence of clinical signs of CNS disease. Antibody can be identified in the CSF of humans months to years after clinical recovery from infectious CNS disease.

References


Table 1. CSF WB Antibody to S. neurona in Clinically Normal and Abnormal Horses

<table>
<thead>
<tr>
<th>CSF WB Standing</th>
<th>Clinically Normal</th>
<th>Clinically Abnormal</th>
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<tbody>
<tr>
<td>Positive (%)a</td>
<td>79 (31)</td>
<td>205 (30)</td>
</tr>
<tr>
<td>Negative (%)</td>
<td>175 (69)</td>
<td>484 (70)</td>
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<tr>
<td>Total</td>
<td>254</td>
<td>689</td>
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aThe CSF was red blood cell count negative.