Effect of Iatrogenic Blood-Contaminated Equine CSF on *Sarcocystis neurona* Western Blot Reactivity and CSF Indices

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Small amounts of blood contamination in the cerebrospinal fluid (CSF) of a horse that has only been exposed to *Sarcocystis neurona* (serum Western blot positive) can result in *S. neurona* immunoreactivity in the CSF. CSF albumin and IgG concentrations remained normal with moderate blood contamination. The albumin quotient remained normal with marked blood contamination. The IgG index increased (out of the normal range) with less blood contamination. Authors’ addresses: Dept. of Clinical Studies, New Bolton Center, School of Veterinary Medicine, University of Pennsylvania, 382 West Street Rd., Kennett Square, PA 19348-1692 (Miller, Sweeney, and Russell) and Equine Biodiagnostics, Inc., A165 ASTeCC Bldg., University of Kentucky, Lexington, KY 40506-0286 (Sheetz and Morrow). © 1998 AAEP.

1. Introduction
When presented with a cerebrospinal fluid (CSF) sample grossly contaminated with blood, clinicians usually do not submit it for equine protozoal myeloencephalitis (EPM) testing. However, even a clear sample often will have microscopic evidence of hemorrhage (red blood cells). It would help clinicians to know how much blood can contaminate the CSF without altering the Western blot results for EPM antibodies. The objective of our study was to determine the volume of blood required to convert Western blot negative CSF to Western blot positive CSF in horses whose serum tested either negative or strongly, moderately, or weakly positive by Western blot analysis. A second objective was to determine how the CSF indices (albumin quotient and IgG index) were affected by blood contamination.

2. Materials and Methods
Equine CSF that was negative for Western blot *Sarcocystis neurona* antibody was pooled for serial dilutions with blood. Heparinized blood was obtained from 12 horses. Three horses were placed in each of four groups according to whether their serum tested negative, weakly positive, moderately positive, or strongly positive by Western blot analysis. Six serial tenfold dilutions (10^{-1} to 10^{-6}) of heparinized blood in CSF were prepared. Red blood cell (RBC) counts were determined by using a hemocytometer. The Western blot *S. neurona* antibody test and CSF indices were determined on each dilution of blood in CSF. The Western blot *S. neurona* reactivity was reported as negative, weakly positive, moderately positive, or strongly positive. CSF and serum albumin were measured spectrophotometrically by...
using a Brom Cresol Green-based colorimetric albumin reagent. CSF and serum IgG concentrations were measured by using radial immunodiffusion. CSF albumin and IgG concentrations were considered normal if they were <56.0 mg/dl and <10 mg/dl, respectively. Albumin quotients and IgG indices were calculated by using standard equations previously reported and normal values of <2.4 and <0.27, respectively.

A subjective assessment of the contaminated CSF samples was performed by an individual blinded to the dilution. The five categories were as follows: red, blood tinged, pink, cloudy, and clear.

3. Results
The RBC count for the most dilute sample that was still weakly positive was considered the cutoff point. For the strong samples, the cutoff points were 7050, 85.8, and 11 RBC/µl. For the moderate samples, the cutoff points were 6325, 73.7, and 8.25 RBC/µl. For the weak samples, the cutoff points were 700,000, 75,000, and 685 RBC/µl. All CSF dilutions with negative blood tested negative.

Mean albumin levels in the six serial tenfold dilutions were 209.9, 63.1, 39.4, 36.6, 35.9, and 35.1 mg/dl, respectively. Mean CSF IgG concentrations for the six tenfold dilutions were 129.6, 15.5, 6.2, 5.4, 5.0, and 4.6 mg/dl, respectively. Mean CSF albumin and IgG concentrations became elevated at a mean RBC of 72,946/µl. Albumin quotients were normal in all except the highest dilution (10⁻¹), which had a mean RBC of 835,603. The IgG index in 7/12 horses was first elevated at a mean RBC of 72,946/µl and in 5/12 horses at a mean RBC of 7621/µl.

All first dilutions (10⁻¹) were characterized as red; the second dilutions (10⁻²) were characterized as blood tinged, and the third dilutions (10⁻³) were characterized as cloudy. The remaining dilutions were clear.

4. Discussion
Recent studies have reported that blood contamination of CSF had no significant effects on various CSF indices; these studies only measured the small amounts of blood normally encountered during ordinary sampling techniques. Other studies have indicated the usefulness of the CSF indices in determining iatrogenic blood contamination or blood-brain barrier compromise.

A CSF albumin concentration of >56.0 mg/dl and an albumin quotient of >2.0 are thought to indicate blood-brain barrier compromise. The results of our study have shown that a considerable degree of iatrogenic RBC contamination can occur before the CSF albumin concentration or the albumin quotient becomes abnormal. During CSF sampling, it is not uncommon to get up to 500 RBC iatrogenically from which a normal albumin quotient could be obtained. Thus, the CSF albumin concentration and the albumin quotient cannot be used to rule out iatrogenic blood contamination. A marked blood contamination was required to increase the CSF IgG concentration. Our study showed that with blood contamination alone, the IgG index may be increased in the face of a normal albumin quotient.

Even with a large amount of blood contamination (a mean of 781 RBC/µl), the sample can appear clear, as stated in other reports of CSF evaluation. Thus, a subjective evaluation of CSF by color does not have a good negative predictive value for blood contamination, unless the CSF is grossly blood tinged or red.

Lastly, we found that depending on the serum sample, any amount of whole blood contamination has the potential to cause some S. neurona Western blot reactivity. The absolute CSF RBC count cannot be used as a predictable cutoff for a false-positive test for S. neurona antibodies. Obtaining a CSF sample free of any blood is difficult; thus, if a CSF sample is determined to be weakly positive for S. neurona antibodies, the results should be interpreted cautiously and only in light of clinical findings.

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References and Footnotes

Equine Biodiagnostics, Inc., Lexington, KY 40506.