Equine Protozoal Myeloencephalitis–
A Comparison of Western Blot Results for Serum and Cerebral Spinal Fluid With Postmortem Findings in Normal and Neurologic Horses

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When testing spinal fluid, the Western blot (WB) detected with high accuracy horses with neurologic disease due to Sarcocystis neurona. The test was useful in ruling out the disease in normal and neurologic horses. However, spinal fluid WB had many false positives in identifying pathologically negative animals, for both normal and neurologic horses. The WB should be interpreted cautiously and used only as one aid among others in the diagnosis of neurologic disease. Authors addresses: California Animal Health and Food Safety Laboratory System, University of California, Davis, CA 95616 (Daft, Barr, Ardans, Read, Kinde, Johnson, Woods, Anderson, Hietala, and Adaska); University of California, Davis, School of Veterinary Medicine, Davis, CA 95616 (Hird); California Horse Racing Board, Suite 300, 1010 Hurley Way, Sacramento, CA 95825 (Bell, Cornish, and Hurley); 811 W. Olive Ave, Redlands, CA 92373 (St. Leger). (c) 2000 AAEP.

1. Introduction
The western blot (WB) for S. neurona performed on serum and spinal fluid (CSF) is widely used for antemortem diagnosis of neurologic disease and for screening normal horses. Because CSF collection is difficult, the WB is often performed on the more readily available serum sample only. However, seroprevalence to S. neurona is high in normal horses and studies to evaluate the blood test have not been reported.

There are difficulties with interpretation of positive CSF WB results. The test detects minute amounts of antibody to S. neurona so that slight contamination of the CSF with blood can result in false positive readings in seropositive horses. In addition, breach of blood-brain barrier and cross reactivity with other protozoa may give false positive results. The purpose of this work was to reassess the value of the WB for both serum and CSF and to evaluate the usefulness of the WB in normal horses.

2. Materials and Methods
This work compares postmortem findings in the central nervous system (CNS) of 234 horses, submitted between 1996 to 1999, with WB results for serum and CSF. Twenty-eight percent of these horses had a history of neurologic signs, about 50% were race horses euthanized for catastrophic musculo-skeletal
injuries, and the remainder were euthanized for other medical conditions. Blood and CSF were collected by veterinarians at euthanasia or by laboratory personnel within 3.5 h of death. All WB testing was done by one laboratory (Equine Biodiagnostics, Inc., University of Kentucky) which reported results as Negative, Positive with Low Reactivity, Positive, and Positive with High Reactivity. In this report, positive WB results were combined for analysis (except as stated otherwise).

For the CSF WB Positive group, only horses with a CSF red cell count of < 100/μL were examined postmortem. CNS examination consisted of microscopic examination of 20 spinal cord and brain stem cross sections followed by gross examination by transversely slicing brain and cord every 3 to 5 mm and selecting additional areas for microscopic examination. Based on pathologic findings, horses were classified as EPM-Negative, EPM-Positive (S. neurona demonstrated in lesions by immunostaining) and EPM-Suspect (EPM-compatible lesions present but S. neurona not demonstrated). EPM-Positive and EPM-Suspect horses were combined for analysis.

3. Results
The CSF-Positive group (n = 112) had a mean red cell count of 15.1/μL (range, 0 to 89). Postmortem results of this group classified 12 horses EPM-Positive, 8 EPM-Suspect and 92 EPM-Negative. All horses diagnosed EPM-Positive were either CSF-Positive or Positive with High Reactivity (no Positive with Low Reactivity results). In the CSF-Negative group (n = 122) were 3 EPM-Suspect horses and 119 were EPM-Negative. For all horses combined, the CSF WB gave a sensitivity of 87% and a specificity of 56%. When the results for normal (n = 169) and neurologic horses (n = 65) were analyzed separately, the sensitivity was similar for both groups, and specificity was 44% for neurologic and 60% for normal horses. For normal and neurologic horses, predictive value for CSF-Negative horses was 99 and 92% whereas predictive value for CSF-Positive horses was 10 and 32%, respectively.

When CSF-Positive with Low Reactivity results were combined with CSF-Negative results, specificity improved to 72 and 85% for neurologic and normal horses respectively though this also resulted in approximately 10% loss of sensitivity. Seronegative results correctly predicted EPM-Negative status for 97% of animals. Results for post-mortem sampled horses did not differ significantly from those for horses sampled at the time of death.

4. Discussion
Results of this work showed that the WB for serum and CSF is useful in ruling out the disease in normal and neurologic horses. Similarly, the CSF WB accurately identified horses with clinical signs due to S. neurona. These results were in agreement with other researchers. However, the CSF WB frequently had false positive results for both neurologic and normal horses. Findings also suggest that CSF WB Positive with Low Reactivity results are better interpreted as negative or at best inconclusive. It is recommended that veterinarians take a cautious approach to the interpretation of the WB and to educate horse owners regarding the test’s limitations. This may reduce client concern, and unnecessary treatment of horses suffering from neurologic disease other than EPM.

References