Findings in cerebrospinal fluids of horses infected with West Nile virus: 30 cases (2001)

Heather L. Wamsley, DVM; A. Rick Alleman, DVM, PhD, DACVP, DABVP; Michael B. Porter, DVM, PhD; Maureen T. Long, DVM, PhD, DACVIM

Objective—To evaluate CSF in horses with confirmed West Nile virus encephalomyelitis.

Design—Retrospective study.

Animals—30 horses.

Procedure—Results of CSF analyses from horses with acute neurologic signs attributed to West Nile virus infection that was confirmed by immunoglobulin M antibody capture ELISA were reviewed and analyzed.

Results—Among 30 CSF samples, findings in 8 (27%) were within reference ranges and in 22 (73%) were abnormal. Among the 22 abnormal samples, mononuclear pleocytosis was found in 16 (73%) and high protein concentration with nucleated cell count within reference range was found in 6 (27%) samples. A predominance of lymphocytes was found in 11 of 16 samples with mononuclear pleocytosis, and a predominance of large mononuclear cells was found in 5 of 16 samples. Sensitivities of analyses of CSF obtained from the lumbosacral and atlanto-occipital regions of the spinal cord were 89 and 50%, respectively.

Conclusions and Clinical Relevance—Results suggest that in horses with acute onset of neurologic signs caused by West Nile virus encephalomyelitis, findings in the CSF are likely to be abnormal, mononuclear pleocytosis with lymphocytic predominance may be most commonly observed, and CSF collected from the lumbosacral region may be abnormal more commonly than CSF collected from the atlanto-occipital region. (*J Am Vet Med Assoc* 2002;221:1303–1305)

West Nile virus (WNV) is an Old World flavivirus that is maintained in endemic regions by a bird-mosquito cycle.[1,2] Since it was initially reported in the Western Hemisphere in 1999,[3,4] WNV has been isolated from Canada to Florida.[2] The continued isolation of WNV during 2000 and 2001 throughout eastern North America[2,5,6] and the observation that WNV can overwinter in temperate regions[7] suggest that it is likely endemic and may continue to expand its geographic range. During outbreaks of infection with WNV in mammals, clinical illness is most frequently reported in humans and horses.[1,8,9] Histologically, infection in horses results in lesions in the CNS that extend from the basal nuclei to the sacral spinal cord and are characterized by mild to severe, multifocal, nonsuppurative polioencephalomyelitis[1,9] with infiltration of the perivascular regions by T lymphocytes and macrophages.[9]

Analysis of CSF is routinely performed as an ancillary test to aid in the diagnosis of neurologic disease with clinical signs referable to the CNS. Cerebrospinal fluid is an ultrafiltrate of plasma produced predominantly by the ventricular choroid plexuses. It generally flows over the cerebral hemispheres and then caudally, surrounding the spinal cord. This directional flow may result in distinct differences between samples collected from the atlanto-occipital and lumbosacral sites of the same animal, particularly an animal with spinal cord disease. Although results of CSF analyses are rarely specific and may be within reference ranges even during advanced diseases of the CNS, findings can potentially provide information rapidly to guide therapeutic decisions, determine future diagnostic tests, and aid in prognostication.[10-13]

Previous reports of WNV encephalomyelitis in horses have included minimal information about results of CSF analyses.[1,9,14-23] The purpose of the study reported here was to determine results of CSF analyses from 30 horses with acute neurologic signs attributed to confirmed WNV infection. We hypothesized that among these horses, results of CSF analysis would differ between sites of collection.

Criteria for Selection of Cases

Medical records were reviewed for horses evaluated between July and November 2001 at the College of Veterinary Medicine, University of Florida, with acute onset of neurologic signs attributed to WNV infection that was confirmed by immunoglobulin M antibody capture ELISA.[a]

Procedures

Medical records were reviewed for signalment, method of sample collection, method of sample processing, and results of analyses. A single CSF sample had been collected at the atlanto-occipital or lumbosacral site by use of routine methods from each horse and processed immediately.

CSF analyses—Results of gross inspection to determine color and turbidity were recorded. Direct cell counts of undiluted CSF were performed with a hemacytometer. Protein concentration was quantitated with an automated,[b] colorimetric method and pyrogallol reagent.[c] Two Wright-Giemsa-stained,[d,e] cytocentrifuged[f] preparations of each CSF sample were microscopically examined and differential cell counts were

From the Departments of Physiological Sciences (Wamsley, Alleman) and Large Animal Clinical Sciences (Porter, Long), College of Veterinary Medicine, University of Florida, Gainesville, FL 32610.

The authors thank Tina Conrad, LaVonne Gray-Williams, Maxine Lampert-Sacher, Lane Pritchard, and Freida Sessions for technical assistance.

Address correspondence to Dr. Wamsley.

performed. Results of CSF analyses were classified as abnormal if the nucleated cell count was > 8 cells/μL, if the protein concentration was > 61 mg/dL (atlanto-occipital) or > 77 mg/dL (lumbosacral), or if both the nucleated cell count and protein concentration were high. Results were classified as mononuclear pleocytosis if the nucleated cell count was > 8 cells/μL and the sum of the percentages of cells that were small lymphocytes and large mononuclear cells was > 90%. Mononuclear pleocytosis was classified as mononuclear with lymphocytic predominance if lymphocytes constituted ≥ 60% of the nucleated cell population and large mononuclear cells constituted < 40%, or as mononuclear with a predominance of large mononuclear cells if large mononuclear cells constituted > 40% of the nucleated cell population and lymphocytes constituted < 60%. Results were reviewed to assess the degree of blood contamination.

Statistical analyses—Results were entered into a database[g] and range, median, and interquartile range were calculated. Sensitivity of CSF analysis was calculated for samples collected from the atlanto-occipital site and samples collected from the lumbosacral site. A 1-sided Fisher exact test was used to compare the frequency of samples with abnormal results collected from the atlanto-occipital site with those collected from the lumbosacral site. A Kruskal-Wallis 1-way ANOVA with collection site as the fixed effect was used for comparison of the nucleated cell count, differential cell count, and protein concentration.[h] Values of $P < 0.05$ were considered significant.

Results

Records of 30 horses evaluated between July and November 2001 met criteria for inclusion in the study. No substantial differences in patient signalment, method of sample collection, or method of sample processing were detected between samples collected from the atlanto-occipital site and those collected from the lumbosacral site. Furthermore, there was no substantial blood contamination in any of the samples.

Results of CSF analyses—Among the 30 CSF samples, results were within reference ranges in 8 (27%) and abnormal in 22 (73%) samples. Among the 22 abnormal CSF results, mononuclear pleocytosis was found in 16 (73%) and high protein concentration with nucleated cell count within reference range was found in 6 (27%). Lymphocytic predominance was found in 11 of 16 samples with mononuclear pleocytosis; predominance of large mononuclear cells was found in 5 of 16 samples. Mild iatrogenic blood contamination was observed in 24 of 30 (80%) CSF samples. Mild xanthochromia was observed in 6 of 30 (20%) CSF samples. Among the 22 abnormal samples, the protein concentration ranged from 64 to 316 mg/dL (median, 100 mg/dL; interquartile range, 85 to 125 mg/dL), the nucleated cell count ranged from 0 to 882 cells/μL (median, 14 cells/μL; interquartile range, 8 to 62 cells/μL), and the erythrocyte count ranged from 0 to 2,850 RBC/μL (median, 107 RBC/μL; interquartile range, 6 to 376 RBC/μL).

Results by collection site—Among 30 CSF samples, 12 (40%) were collected from the atlanto-occipital site and 18 (60%) from the lumbosacral site. Six of 12 samples from the atlanto-occipital site and 16 of 18 lumbosacral samples were abnormal; this difference was significant ($P = 0.027$). Among 16 samples that were abnormal because of pleocytosis, 5 were collected from the atlanto-occipital site and 11 from the lumbosacral site. Among 6 samples that were abnormal because of high protein concentration but with a nucleated cell count within reference range, 1 was collected from the atlanto-occipital site and 5 were collected from the lumbosacral site. Among the 12 samples collected from the atlanto-occipital site, the range, median, and interquartile range of the protein concentration were 36 to 104, 61, and 51 to 83 mg/dL, respectively. Among the 18 samples collected from the lumbosacral site, the range, median, and interquartile range of the protein concentration were 52 to 316, 110, and 85 to 132 mg/dL, respectively. The difference in protein concentration between collection sites was significant ($P < 0.001$), whereas differences in nucleated cell count and differential cell count between collection sites were not significant.

Discussion

Results of our study suggest that in horses with acute onset of neurologic signs attributable to WNV encephalomyelitis, findings in the CSF are likely to be abnormal, mononuclear pleocytosis with lymphocytic predominance may be most commonly observed, and results of analysis of CSF collected from the lumbosacral site may be abnormal more commonly than those of samples collected from the atlanto-occipital site.

As is frequently found with other CNS diseases, the results of CSF analysis in affected horses may be within reference ranges and the abnormalities may not be specific for a given disease. The abnormalities detected in this study had substantial overlap with those associated with other diseases that should be considered as differential diagnoses,[1] such as other viral encephalitides (rabies; equine herpesvirus encephalomyelopathy; eastern, western, and Venezuelan equine encephalomyelitis), equine protozoal myeloencephalitis, verminous meningoencephalomyelitis, cervical stenotic myelopathy, metabolic encephalopathy, hypocalcemia, botulism, and toxicoses such as tremorgenic toxicosis and fumonisin B1 toxicosis.[19] Further ancillary tests such as imaging and serologic tests are often necessary to aid in diagnosis.

Many diseases of the CNS, especially those that are infectious, are commonly associated with mononuclear pleocytosis, high protein concentration, or both. Acute eastern and Venezuelan equine encephalomyelitis and equine herpesvirus encephalomyelopathy are unique in that the former 2 diseases are typically associated with neutrophilic pleocytosis, and the latter disease is typically associated with marked xanthochromia and high protein concentration. Results of our study suggest that CSF findings associated with WNV infection are distinctive in that a marked neutrophilic component was not found in any CSF sample, and that mild xanthochromia was an uncommon finding.

Given the caudal flow of CSF and the anatomic

distribution of the lesions caused by WNV infection, which can extend from the basal nuclei to the sacral spinal cord, it is not surprising that results of lumbosacral CSF analysis were more frequently abnormal than those of atlanto-occipital CSF analysis. In healthy horses, the difference in atlanto-occipital and lumbosacral protein concentration collected from the same horse is 20 to 25 mg/dL.[24,25] Although CSF was not collected from both sites of any horse in our study, the magnitude of the difference between atlanto-occipital and lumbosacral protein concentrations within the study population was twice that seen in healthy horses; this difference was significant and likely the result of the spinal cord damage caused by WNV. As reported,[9] WNV infection causes a nonsuppurative polioencephalomyelitis characterized by infiltration predominantly by T lymphocytes and to a lesser degree by macrophages. This is consistent with our most common CSF finding of mononuclear pleocytosis that was predominantly lymphocytic.

[a]Maureen T. Long, Department of Large Animal Clinical Sciences, College of Veterinary Medicine, University of Florida, Gainesville, Fla.

[b]Hitachi 911 Automatic Analyzer, Boehringer Mannheim Corp, Indianapolis, Ind.

[c]Microprotein-PR, Sigma Diagnostics Inc, St Louis, Mo.

[d]Harleco Wright's stain, EM Science, Gibbstown, NJ.

[e]Harleco Giemsa stain, EM Science, Gibbstown, NJ.

[f]Cytospin2, Shandon Inc, Pittsburgh, Pa.

[g]Excel, Microsoft Corp, Seattle, Wash.

[h]NCSS 2001, NCSS Statistical Software, Kaysville, Utah.

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