West Nile virus encephalomyelitis in eight horses

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West Nile virus was first isolated in the United States in 19991 and caused human, avian, and equine illness and death. The virus was first isolated from a febrile woman in Uganda in 19372,3 and West Nile virus encephalomyelitis has been reported in humans in Africa, Europe, the Middle East, west and central Asia, and Oceania.1 Equine encephalomyelitis caused by the virus has been reported in Egypt,4 Morocco,5 Portugal,6 France,7 and Italy.8 The most recent epizootics occurred in Morocco in 1996, Italy in 1998, the northeastern United States in 1999, and France in 2000.1 Identification of West Nile virus in the United States in 1999 represented the first occurrence of this virus in the western hemisphere.

West Nile virus is an arbovirus in the Japanese encephalitis antigenic complex of the genus Flavivirus, family Flaviridae.3 The virus has been isolated from 43 mosquito species, primarily in the genus Culex. Virus isolation from other hematophagous arthropods such as ticks has also been reported, but the role of ticks in transmission has not been documented. The virus has been isolated from a large number of avian species (56 in the United States alone)9 and mammalian species including lemurs, chimpanzees, rodents, bats, camels, mules, donkeys, horses, goats, cattle, water buffalo, sheep, pigs, rabbits, and dogs, as well as amphibians.3

Results of most research studies suggest that birds are the only animals in which a viremia develops that is sufficient to infect mosquitoes and propagate the infective cycle.3 A transient, low-level viremia has been documented in naturally and experimentally infected horses and donkeys.4,11 The level of viremia is thought to be insufficient to infect mosquitoes.3 Madagascan lemurs may be one of the few mammals that develop a viremia sufficient to infect feeding mosquitoes.13 Because of these findings, it is highly unlikely that a horse infected with West Nile virus would transmit the virus to humans or other horses in typical circumstances.

West Nile virus encephalomyelitis was diagnosed in 8 horses with neurologic signs hospitalized at the George D. Widener Large Animal Hospital at New Bolton Center, University of Pennsylvania in the fall of 1999 and 2000. The diagnosis was made via serologic testing in 5 horses and via serologic testing and reverse transcriptase polymerase chain reaction (RT-PCR) testing performed on brain tissue in 3 horses. The purpose of this report was to describe findings in the 8 affected horses.

Signalment and Clinical Findings

The initial horse was referred on Sep 29, 1999, and the other 7 horses were referred during a 13-day period between Sep 29 and Oct 10, 2000. All horses were from a narrow geographic region that comprised portions of New York and New Jersey. Five horses were from the Elmont and Long Island regions of New York, and 3 horses were from New Jersey. Two horses lived on the same farm, and 2 were from the same racing facility. Affected horses included 2 geldings, 2 stallions, and 4 mares. Three of the mares were pregnant (duration of gestation, 120 to 150 days). Two fetuses were confirmed viable by use of reproductive examinations throughout the period of hospitalization, whereas 1 pregnant mare was euthanatized shortly after referral. Seven horses were Thoroughbreds; 1 was a Quarter Horse. Horses ranged from 2 to 18 years old.

Although reported historical findings were variable, several similarities among horses were apparent. Initial complaints included low-grade fever (38.4 to 39.4 °C [101.2 to 103.0 °F]; n = 4), acute onset of ataxia in all 4 limbs (3), marked hypermetria (2), and recumbency (1). Median duration of fever prior to initial evaluation was 30 hours (range, 12 to 60 hours); median duration of neurologic signs prior to initial evaluation was 12 hours (range, 6 to 36 hours). Two horses initially had unilateral forelimb lameness that progressed to bilateral lameness and ataxia, whereas 1 horse had signs of unilateral radial nerve paralysis.

Physical examination at our hospital revealed findings that were similar to historical complaints; additionally, 1 horse had anisocoria and slow pupillary light responses, and the recumbent horse had seizure activity. Three horses had head tremors and lip twitch-
ing. Six of the 8 horses had hypersensitivity to touch and sound. One horse was somnolent at referral and periodically fell to its knees; 4 other horses also fell to their knees during the initial period of hospitalization.

Several differential diagnoses were considered, depending on clinical signs at referral, and included infection with alphaviruses (western equine encephalomyelitis [WEE], eastern equine encephalomyelitis [EEE], and Venezuelan equine encephalomyelitis viruses) or equine herpesvirus 1 (EHV-1), rabies, equine protozoal myelitis (EPM), leukenoencephalocasia (fumonisins B1 toxicity), encephalopathy associated with hepatic, intestinal, or renal diseases, and myelopathy caused by cervical stenosis (considered in 1 horse).

**Laboratory Findings**

To rule out renal or hepatic causes of encephalopathy, routine serum biochemical analyses were performed; results for all horses were within reference ranges. Serum ammonia and bilirubin (total and indirect) concentrations and sorbitol dehydrogenase activity were within reference ranges in samples obtained from the horse evaluated in 1999. Results of CBC and serum fibrinogen concentrations were within reference ranges in all horses.

Serum antibody titers for EEE, WEE, equine infectious anemia, and St. Louis encephalitis were evaluated in 1 horse, and results were negative. Four of 8 horses tested were seronegative for EHV-1. Four horses were tested for antibodies against *Sarcocystis neurona* by use of western blot analysis of CSF collected from the lumbosacral space; 2 horses had positive results, 1 had a weak positive result, and 1 had negative results. One horse was seropositive by use of western blot analysis of serum; a CSF sample was not obtained from this horse. The other 3 horses were not tested for antibodies against *S. neurona* or EHV-1 in blood or CSF because of the high suspicion of West Nile virus infection. Cytologic evaluation of CSF revealed no abnormalities in 3 horses, whereas 1 horse had a low leukocyte count but high total protein concentration (151 mg/dl) and xanthochromia. Because neurologic abnormalities (unsteadiness, violent reaction to touch) made handling difficult, CSF was not obtained from the other 4 horses.

**Diagnosis of West Nile Virus Infection**

In 7 of 8 horses, 3 serologic tests were used to confirm diagnosis of West Nile virus infection: the indirect fluorescent antibody (IFA) test, an IgM-capture ELISA, and the plaque reduction virus-neutralizing antibody test. Five horses had positive results for all 3 tests. The only test available in 1999 was the plaque reduction virus-neutralizing antibody test; horse 1 was seronegative by use of this test. Horse 2 was euthanatized soon after referral to our hospital; serologic evaluation revealed positive results for the IFA test and IgM-capture ELISA, whereas the plaque reduction virus-neutralizing antibody test yielded negative results, possibly because the horse was euthanatized prior to developing virus-neutralizing antibodies. In this horse, virus isolation from brain tissue was successful. At referral, horse 5 was seronegative via IgM-capture ELISA, IFA results were considered suspicious-positive, and results of the plaque reduction virus-neutralizing antibody test were positive. A serum sample obtained from horse 5 seven days after referral yielded positive results via the IFA and plaque reduction virus-neutralizing antibody tests, although results of the IgM-capture ELISA remained negative. Results of RT-PCR testing of brain tissue were positive for West Nile virus in all 3 euthanatized horses, and virus isolation from brain tissue was successful in 2 of 3 euthanatized horses. Results of fluorescent antibody testing for rabies virus in brain tissue of all 3 horses were negative.

**Treatment and Outcome**

Treatment for all horses was supportive and varied according to the individual needs of each horse. Seven horses received dimethyl sulfoxide (1.0 gm/kg [0.45 gm/lb] of body weight, IV, q 24 h for 3 days) as well as the nonsteroidal anti-inflammatory drugs flunixin meglumine (1.1 mg/kg [0.5 mg/lb], IV, q 12 h) or phenylbutazone (2.2 mg/kg [1.0 mg/lb], IV, q 12 h). Two horses were also treated with dexamethasone (0.03 to 0.05 mg/kg [0.014 to 0.023 mg/lb], IV, q 1 to 3 days). Four horses were treated with trimethoprim-sulfamethoxazole (30 mg/kg [13.6 mg/lb], PO, q 12 h) and pyrimethamine (1.0 mg/kg [0.45 mg/lb], PO, q 24 h), pending results of tests for EPM. Isotonic polyionic fluids were administered IV to 6 horses. Parenteral nutrition was implemented in 2 horses in which somnolence precluded normal eating. One horse was monitored and confined to a stall but not otherwise treated. Each horse was initially handled according to a protocol established at the hospital for management of suspected rabies or alphavirus infections, which included isolating the horse and wearing face shields and gloves when collecting blood or CSF and gowns and gloves when handling the horse. When positive serologic results for West Nile virus were obtained or it became obvious that the horse did not have rabies or alphavirus infection, the isolation protocol was not used; however, clinicians continued to wear gloves whenever blood was collected from these horses.

Horse 2 had radial nerve paralysis and was euthanatized 12 hours after referral because of rapid progression to recumbency with difficulty swallowing and a concern for rabies as a likely differential diagnosis. Despite heavy sedation and treatment, horse 4 continued to have seizures. Initially, anesthesia was required to allow removal of the horse from a trailer; ketamine hydrochloride (2.2 mg/kg [1.0 mg/lb], IV), diazepam (0.02 mg/kg [0.01 mg/lb], IV), and xylazine hydrochloride (1.0 mg/kg, IV) were administered, followed by thiopental (8.3 mg/kg [3.87 mg/lb], IV). The horse required further sedation with xylazine (1.1 mg/kg, IV), diazepam (0.02 mg/kg, IV), and ketamine (1.0 mg/kg, IV) during the first night of hospitalization. Attempts at controlling seizures with phenobarbital (12 mg/kg [5.45 mg/lb], IV) were not successful, and the horse was euthanatized on day 4 of hospitalization. Horse 6 developed central blindness within the first 24 hours of hospitalization and was euthanatized because of recumbency and seizure activity after nearly 2 weeks of hospitalization. Five horses were somno-
lent at some time during hospitalization, and 5 horses had episodes of falling to their knees. Three other horses developed muscle fasciculations that were most pronounced in the neck and triceps region. Typical duration of neurologic signs in horses that survived was difficult to assess. Typically, muscle fasciculations, hypersensitivity to touch and sound, and episodes of somnolence or falling to the knees had resolved completely or were substantially improved within 4.5 days (range, 2 to 7 days) after referral. Median duration of hospitalization for the horses that survived was 9.5 days (range, 5 to 15 days). Of the 5 surviving horses, 2 did not have observable neurologic deficits at the time of discharge (5 and 15 days after referral). Three horses had hypermetria, mild ataxia, or both at the time of discharge (9, 10, and 11 days after referral).

Clinical follow-up was available on all surviving horses. Horse 1 returned to race training within 3 months and did not have neurologic deficits at that time. Horse 3 returned to race training in 6 weeks, with no residual neurologic deficits. Horses 3 and 7 remained pregnant and do not have neurologic deficits. Horse 8 returned to race training in 3 months, without detectable neurologic deficits. The present serologic status of these horses is not known.

Postmortem examinations were performed on the 3 horses that were euthanatized; there were no gross lesions within the brains. Tissues were fixed in neutral-buffered 10% formalin, processed routinely, and stained with H&E; selected sections were stained by use of an immunohistochemical technique to detect West Nile virus epitopes by use of a mouse monoclonal antibody. Microscopic examination revealed multifocal moderate to severe perivascular lymphohptic rhombencephalitis with perivascular hemorrhage, neutrophils, and multifocal microgliosis. The spinal cord was examined in horse 6 only; gross lesions were not evident, although microscopic changes similar to those seen in the brain were detected. To reduce exposure of personnel to infected horses at postmortem, other tissues were not examined. West Nile virus antigen was identified within the cytoplasm of a few neurons, moderate numbers of fibers, glial cells, and a few neutrophils.

**Discussion**

The clinical signs and neuropathologic findings we observed share similarities with those reported in other epizootics. In addition, the horses reported here had clinical signs that have been less commonly reported in naturally occurring infections. Common clinical findings in horses in previous epizootics included some degree of hind limb ataxia and forelimb or hind limb hypermetria. Monoparesis, paraparesis, and tetraparesis that progress to recumbency have been reported, as well as somnolence, falling headfirst into bodies of water, muscle tremors, diminished pupillary light reflex, muscle rigidity, and seizure activity. One horse had signs of facial nerve paralysis. A biphasic fever has been reported in horses experimentally infected with West Nile virus. Fever has not been a consistent finding in all equine outbreaks, although it is almost always detected in humans clinically affected with the virus.

In the horses reported here, clinical signs that were consistent with those seen in other epizootics included somnolence, muscle tremors, ataxia, various degrees of monoparesis, paraparesis, or quadriparesis, hypermetria, and seizure activity. Four of the 8 horses were febrile. A less commonly reported sign that we also observed was blindness (3 horses) that appeared to be central in nature. Ophthalmologic examination was not performed because of lack of patient compliance. Severe hyperesthesia and sensitivity to sound (7 horses) were also observed. The most hyperesthetic horses required sedation to conduct basic diagnostic and therapeutic procedures such as IV catheter placement and CSF collection from the lumbosacral space. These horses generally were poorly responsive to conventional doses of sedatives and therefore required repeated doses.

The IFA test was developed as a screening test for exposure to West Nile virus, and samples with positive results are further evaluated by use of IgM-capture ELISA and the plaque reduction virus-neutralizing antibody test. The IgM-capture ELISA also yields positive results for other flaviviruses, including St. Louis encephalitis virus and Japanese encephalitis virus. However, this test is of use in identifying horses or other animals exposed to West Nile virus. Each IgM-capture ELISA is species-specific, so the equine test is not used to test serum from other species. In theory, the IgM-capture ELISA may identify horses that have recently been exposed to a virus and are beginning to mount an IgM response (10 to 12 days after infection); antibodies may then be detectable in serum for a few days or weeks. The plaque reduction virus-neutralizing antibody test is more specific for detection of antibodies against a specific flavivirus such as West Nile virus. The virus-neutralizing antibodies are often not detectable until at least 3 weeks after infection but may remain detectable in serum for several months or longer.

Three of 8 horses died, which is similar to the 39% mortality rate for horses with West Nile virus infection in the United States in 1999 and 2000. As in previous reports, the horses that were euthanatized at our clinic were those that became recumbent. Generally, clinical signs worsened or improved several days after referral, although 1 horse did not become recumbent until 2 weeks after referral, which was similar to a foal that developed severe neurologic signs 10 days after improvement of ataxia, central blindness, and fever. There has also been a report of horses dying spontaneously after becoming recumbent; this was not seen at our clinic.

The horses reported here had prominent rhombencephalic lesions and corresponding clinical signs. Horses involved in an epizootic in Italy had mild rhombencephalitis and prominent multifocal spinal cord lesions. In the Italian outbreak, consciousness and mental clarity of the horses generally were not affected, and ataxia and recumbency were the main clinical signs. These differences may be related to the virulence of different West Nile virus isolates.

The number of northeastern states with West Nile virus infections in horses and humans has increased...
since the virus was first reported in the United States in 1999.6 Because of migratory patterns of affected birds and the ability of the virus to overwinter, it is likely that the infection will be reported in even more states in the future.

Unlike the disease in humans, in which clinical signs of infection are most often detected in the elderly, we found that young horses were affected (median age, 6 years). Humans who die from this disease are most often in their midsixties or older, although fatal age, 6 years). Humans who die from this disease are most often in their midsixties or older, although fatal signs of infection are most often detected in the elder-

References