West Nile Virus encephalomyelitis in horses: 46 cases (2001)

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Objective—To determine signalment, clinical findings, results of diagnostic testing, outcome, and postmortem findings in horses with West Nile virus (WNV) encephalomyelitis.

Design—Retrospective study.

Animals—46 horses with WNV encephalomyelitis.

Procedure—Clinical data were extracted from medical records of affected horses.

Results—On the basis of clinical signs and results of serologic testing, WNV encephalomyelitis was diagnosed in 46 of 56 horses with CNS signs. Significantly more males than females were affected. Increased rectal temperature, weakness or ataxia, and muscle fasciculations were the most common clinical signs. Paresis was more common than ataxia, although both could be asymmetrical and multifocal. Supportive treatment included anti-inflammatory medications, fluids, antimicrobials, and slinging of recumbent horses. Results of the IgM capture ELISA and the plaque reduction neutralization test provided a diagnosis in 43 horses, and only results of the plaque reduction neutralization test were positive in 3 horses. Mortality rate was 30%, and 71% of recumbent horses were euthanatized. One horse that had received 2 vaccinations for WNV developed the disease and was euthanatized. Follow-up communications with 19 owners revealed that most horses had residual deficits at 1 month after release from the hospital; abnormalities were resolved in all but 2 horses by 12 months after release.

Conclusions and Clinical Relevance—Our findings were similar to those of previous WNV outbreaks in horses but provided additional clinical details from monitored hospitalized horses. Diagnostic testing is essential to diagnosis, treatment is supportive, and recovery rate of discharged ambulatory horses is < 100%. (*J Am Vet Med Assoc* 2003;222:1241–1247)

West Nile virus (WNV), an arthropod-borne virus introduced to the New World in 1999,[1,2] causes CNS disease in birds, horses, and humans.[3,4] This virus was first isolated in 1937 from the West Nile province of Uganda and is classified as a member of the genus Flavivirus (family Flaviviridae).[5,6] West Nile virus is transmitted by multiple mosquito species to birds, horses, and humans.[4] Horses are considered dead-end hosts, as are humans, but birds develop substantial viremia and are considered a major reservoir host.[4,7,8]

West Nile virus was an Old World virus of Europe, Asia, Africa, and the South Pacific when it was discovered as the cause of encephalitis and death in birds, humans, and horses of the New York City area.[9,10] By the year 2000, WNV had spread beyond New York into 7 northeastern states, and approximately 60 horses were reported to have WNV encephalomyelitis.[11,12] In the late summer and fall of 2001, WNV became endemic in Florida.[13] By December 2001, 738 horses were considered by the Animal Health and Plant Inspection Service to have confirmed WNV encephalomyelitis in 20 states.[13] Of the 738, 550 were Florida and southern Georgia horses, representing the majority of this outbreak.

A case definition for WNV encephalomyelitis was established in 2001 based on testing of serum from clinically affected horses by both the **IgM capture ELISA (MAC-ELISA)** and **plaque reduction neutralization tests (PRNTs)** developed and performed by the **National Veterinary Services Laboratory (NVSL)**.[14,a] A confirmed case was defined as a horse with 1 or more clinical signs that must include ataxia, inability to stand, multiple-limb paralysis, or death. Confirmatory testing must include either viral isolation from tissue, blood, or CSF; an associated 4-fold or greater change in PRNT antibody titer; or concomitant single positive results with the **MAC-ELISA** test and the PRNT test (≥ 1:10). A probable case of WNV encephalomyelitis was defined as a horse that was clinically affected for < 21 days and had a positive MAC-ELISA result and a negative PRNT result; the horse had to be from a county where WNV was currently confirmed in either a mosquito, bird, human, or horse.[14] Given the generalized clinical signs provided in the case definition, which do not distinguish WNV encephalomyelitis from other neurologic diseases in horses, we hypothesized that there were antemortem clinical features and clinicopathologic characteristics that could retrospectively be

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used to diagnose WNV encephalomyelitis. The purpose of the study reported here was to determine the signalment, clinical findings, results of diagnostic testing, case outcome, and postmortem findings for horses with WNV encephalomyelitis observed in a veterinary teaching hospital.

Criteria for Selection of Cases

Medical records for 56 horses suspected of being affected were examined. The horses were admitted to the Alec & Louis Courtelis Equine Teaching Hospital at the University of Florida in Gainesville, Fla, and tested for antibodies against WNV during the year 2001. Records of horses tested solely for surveillance were excluded. Criteria for diagnosis of WNV encephalomyelitis and inclusion in the study included positive results of MAC-ELISA and PRNT or positive results of either a single MAC-ELISA or PRNT test in a nonvaccinated horse combined with appropriate history and clinical findings.

Procedures

Signalment information obtained from the records included breed, sex, and age. Number of days to referral after onset of clinical signs, initial complaint, number of days of hospitalization, and WNV vaccination[a] status were also recorded. High body temperature was defined as rectal temperature > 38.3°C (100.9°F), and the number of days this occurred was recorded. Any appetite abnormality was also recorded.

A full neurologic evaluation was performed on all horses, and neurologic findings were extracted from the records. Level of consciousness was assigned 1 of 5 grades (**Appendix 1**).[15] Signs of spinal cord damage (eg, paresis and ataxia) were assigned a grade from 0 (normal gait, normal spinal reflexes) to 4 (**Appendix 2**).[16]

Clinicopathologic data—Additional data obtained from the medical records included results of CBC and serum biochemical profile, if performed. The MAC-ELISA and PRNT were performed by the NVSL[b] on all horses.[14,17] The MAC-ELISA was performed at a serum dilution of 1:400, and the PRNT was performed at serum dilutions of 1:10 and 1:100. Identical diagnostic tests were not performed on all horses; tests included the **hemagglutination inhibition (HI)** assay for WNV,[c] **eastern equine encephalitis (EEE) viral neutralization (VN)** test,[b] **western equine encephalitis (WEE)** VN test,[b] **EEE IgM capture test (EEE-MAC)**, EEE HI assay,[c] WEE HI assay,[c] equine herpesvirus-1 testing performed either via the indirect fluorescent antibody test[c] or VN,[b] and **equine protozoal myelitis (EPM)** western blot testing performed on serum and CSF.[d] Records were also evaluated for any diagnostic imaging, which included radiography of the appendicular skeleton, thorax, or abdomen, and ultrasound.

Treatment—All medications, including amount, frequency, and duration, were recorded. Supportive care in the form of IV administration of fluids and parenteral nutrition was extracted.

Postmortem analysis—A complete postmortem examination was performed on certain euthanatized horses. For horses with rapidly progressing neurologic signs, postmortem rabies testing of half of the brain was performed.[e] The remainder of the fresh brain was tested for WNV[e] by use of **virus isolation (VI)** and real-time **polymerase chain reaction (PCR)**[f] assay. **Immunohistochemistry (IHC)** with a monoclonal antibody[g] was performed on fixed tissue examined by a pathologist as described.[18]

Outcome—After analysis of all records of horses investigated for WNV encephalomyelitis, final outcome was determined on the basis of clinical signs, diagnostic testing, and other findings. Telephone calls to owners or trainers regarding the status of horses since discharge were made after 6 months.

Statistical analyses—Median, range, and mean ± SD were determined for various extracted data. Where applicable, the *z*-test for comparing rates and proportions was used to analyze differences in clinical findings.[19] A confidence interval of 95% was selected for the difference of proportions, and values of $P \leq 0.05$ were considered significant.

Results

Signalment and history—Records of 56 horses evaluated because of suspicion of WNV encephalomyelitis were retrieved. Ten of 56 horses were not considered to have WNV encephalomyelitis on the basis of clinical findings and antemortem testing. Eight of these 10 horses had negative results of the MAC-ELISA and the PRNT, and 2 had positive results of the PRNT at dilution of 1:10 and 1:100. The other horse, with positive results at 1:100, had received 2 WNV vaccine[a] injections, was lame in the right forelimb, and had osteochondritis dissecans of the right shoulder joint. The other PRNT-positive horse was not vaccinated and had high blood ammonia concentration. The horse was discharged without further diagnostic tests; it was euthanatized on the farm, and a necropsy was not performed. The remaining 8 horses were seronegative via both tests and did not have clinical signs consistent with WNV encephalomyelitis. These 10 horses were not included in the rest of the analysis.

West Nile virus encephalomyelitis was diagnosed in 46 horses on the basis of clinical signs and results of diagnostic testing. Among the 46 horses, 18 had received 1 injection of WNV vaccine,[a] and 1 had received 2 injections. In this population, 43 of 46 horses had positive results of MAC-ELISA, and 46 of 46 had positive results for neutralizing antibody via the PRNT. On the basis of the established case criteria,[14] 43 of 46 horses were considered confirmed WNV encephalomyelitis, and 3 of 46 were not considered confirmed but had consistent clinical signs and history. Two of the 3 horses that had negative results of the MAC-ELISA and positive results of the PRNT had not received any vaccine. The third horse also had negative results of the MAC-ELISA and positive results of the PRNT and had received 1 vaccine injection 1 week prior to development of clinical signs. In these 3 horses, WNV encephalomyelitis was diagnosed on the basis of clinical signs, indication of exposure to WNV (positive results of PRNT), duration between onset of clinical

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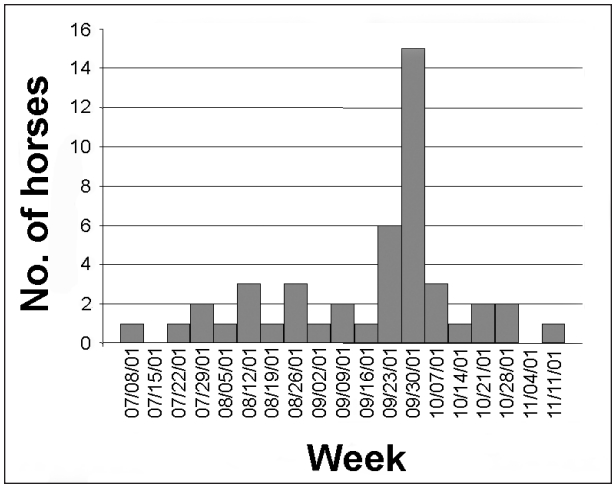

Figure 1—Weekly distribution of horses admitted to the College of Veterinary Medicine, University of Florida, because of West Nile virus encephalomyelitis during 2001.

signs and vaccination, and incomplete initial vaccination series.

The first horse with WNV encephalomyelitis was evaluated initially at the teaching hospital on July 12, 2001 (**Fig 1**), and the peak period for admission was mid-September to mid-October. Median number of days between onset of clinical signs and referral was 3 days (range, 1 to 14 days), and median number of days of hospitalization was 7 days (range, 1 to 41 days).

Among the 46 affected horses, 29 were male (26 castrated and 3 sexually intact), and 17 were female, and this difference in sex distribution was significant (P = 0.028). Forty-one percent of the horses were Quarter Horses, and 20% were Thoroughbreds. Other breeds were Arabian (11%), mixed breed (9%), Tennessee Walking Horse (7%), Morgan (4%), Standardbred (2%), Paso Fino (2%), and ponies (4%). Four horses were < 1 year old, 17 were 1 to 5 years old, 18 were 6 to 10 years old, 6 were 11 to 15 years old, and 1 was > 15 years old. Eighty-five percent of the horses were admitted for suspected neurologic abnormalities. Other complaints included febrile illness consisting of high rectal temperature, anorexia, and signs of depression (n = 3); lameness (3); and colic (3). All of the horses evaluated because of a non-neurologic complaint were examined and found to have neurologic signs at the time of admission.

Clinical evaluation—Weakness was asymmetric and multifocal; among 43 horses with weakness, 6 had grade-1 weakness, 13 had grade 2, 13 had grade 3, and 11 had grade 4. Sudden collapse and knuckling were usually associated with forelimb weakness (**Table 1**). Among the 33 horses with ataxia, 6 had grade 1 ataxia, 9 had grade 2, 7 had grade 3, and 11 had grade 4. Ataxia and weakness were intermittent in certain horses. A sudden worsening of weakness or ataxia for short periods occurred in 9 horses. This was accompanied by a mild increase in rectal temperature (≥ 38.3°C). Because of the unstable gait of affected horses, placing deficits were not uniformly tested; however, 5 horses did have placing deficits.

Table 1—Clinical signs detected in horses with West Nile virus (WNV) encephalomyelitis

Clinical sign	No. affected/No. tested (%)
Fever	30/46 (65)
Anorexia	26/46 (57)
Weakness or ataxia	46/46 (100)
Asymmetrical	18/46 (39)
Forelimbs only	7/46 (15)
Hind limbs only	12/46 (26)
Forelimbs and hind limbs	27/46 (59)
Dogsitting	4/46 (9)
Recumbent	14/46 (30)
Weakness	43/46 (94)
Ataxia	33/46 (72)
Dysmetria	18/22 (82)
Abnormal mentation	31/46 (67)
Fasciculations	28/46 (61)
Face or neck only	12/46 (26)
Entire body	16/46 (35)
Cranial nerve (CN) deficits	20/46 (44)
CN VII paresis	13/46 (28)
CN XII paresis	9/46 (20)
Lack of menace reflex	3/46 (7)
Seizure	2/46 (4)
Dysphagia	1/46 (2)
Head tilt	1/46 (2)
Teeth grinding	9/46 (20)
Pytalism	3/46 (7)

No abnormality of mentation (grade 1) was detected in 15 of 46 horses. Most horses had signs consistent with grade 2 (12/46) or 3 (15/46). Four horses could be aroused only with difficulty. Among the horses with grade-3 mentation, 4 had aggression. Sixteen of the horses with grade-3 and grade-4 mentation had sudden periods of cataplexy or narcolepsy. Compulsive behavior characterized by circling or stall walking was also observed in 4 horses, and 2 horses had seizures. Cranial nerve deficits were symmetrical or asymmetrical in certain horses.

The proportion of horses with concomitant high rectal temperature and spinal cord deficits was 61%, with concomitant high rectal temperature and change in mentation was 65%, and with concomitant fasciculations and spinal cord deficits was 54%. A combination of any 3 of the most common abnormalities was detected in < 30% of the horses.

Clinicopathologic data—At the time of initial evaluation, 11 horses were lymphopenic (mean ± SD, 1,158 ± 298 cells/µL [reference range, 1,500 to 5,000 cells/µL]). None of these horses had received corticosteroids before testing. Hyperbilirubinemia was detected in 33 of 46 horses (4.66 ± 2.26 mg/dL [reference range, 0.3 to 2.5 mg/dL]). Eight horses had high creatinine concentrations (2.4 ± 0.5 mg/dL [reference range, 1.0 to 1.9 mg/dL]). The serum globulin concentrations were high in 12 of 46 horses (4.6 ± 0.3 mg/dL [reference range, 2.1 to 4.0 mg/dL]). Electrolyte abnormalities consisted of hypokalemia (2.6 ± 0.35 mEq/L [reference range, 3.3 to 5.2 mEq/L]), hypochloremia (88.1 ± 7.2 mEq/L [reference range, 95 to 106 mEq/L]), and hyponatremia (126 ± 3.6 mEq/dL [reference range, 137 to 146 mEq/L]). Analysis of CSF was performed on 35 of the 46 horses, and 27 of 35 had abnormal CSF[20] with mononuclear pleocytosis, high protein concentration, or both.

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Ancillary diagnostic testing—The WNV HI test was performed on 37 of 46 horses, and all results were positive at a dilution > 1:20. One of 29 horses tested had positive results of the EEE-MAC. Three of 36 horses tested had positive results of the EEE-HI, and all 36 had positive results of the WEE-HI. Seven horses had a 4-fold difference between the EEE-HI and WEE-HI titers in which the EEE-HI was greater than the WEE-HI. Eleven horses were tested via VN for EEE and WEE, and all had positive results at either 1:10 or 1:100. Seventeen of 22 horses tested positive by use of the equine herpesvirus-1 VN test at dilution > 1:4. Nine of 9 horses tested positive by use of the equine herpesvirus-1 IFA test at dilution > 1:100. Paired serum testing was not performed on any horse. Serum or CSF was tested for antibodies against the agent of EPM in 43 of 46 horses; 23 of 43 tested positive on CSF, and 20 tested negative on either serum or CSF.

Diagnostic imaging—Cervical radiography was performed in 2 of 46 horses, and both had abnormalities consistent with cervical stenotic myelopathy. In 3 horses, results of skull radiography were normal. Two horses had normal results of thoracic radiography. Upper airway endoscopy was performed on 1 horse to rule out temporohyoid osteoarthropathy, and results were within normal limits. Abdominal ultrasound was performed on 2 horses, and no abnormalities were detected. Sonographic evaluation of tendons was performed in 2 horses, and results were consistent with septic tenosynovitis in both horses.

Treatment—Flunixin meglumine (0.5 to 1.0 mg/kg [0.22 to 0.45 mg/lb], IV, q 12 h) was administered to 39 of 46 horses for a median of 7 days (range, 0 to 26 days). Dimethylsulfoxide (1 g/kg [0.45 gm/lb], IV, as a 10% solution, q 24 h) was given to 43 of 46 horses for a median of 3 days (range, 0 to 8 days). Dexamethasone (0.05 to 0.10 mg/kg [0.022 to 0.045 mg/lb], IV, q 24 h) was administered to 12 of 46 horses for a median of 4 days (range, 1 to 11 days). Mannitol (0.5 to 1.0 g/kg, IV, q 24 h) was administered to 2 horses for 3 days. Phenylbutazone (1.1 to 2.2 mg/kg [0.5 to 1.0 mg/lb], PO, q 24 h or q 12 h) was administered to 7 horses between 1 and 19 days. Antimicrobial treatment included trimethoprim-sulfamethoxazole (30 mg/kg [13.6 mg/lb], PO, q 24 h) and pyrimethamine (1 to 2 mg/kg [0.45 to 0.90 mg/lb], PO, q 24 h); this was administered to 34 of 46 horses for a median of 5.5 days in the hospital, and 30 of 34 horses were discharged on this medication pending the results of the WNV testing. In addition, 2 horses received potassium penicillin (22,000 IU/kg [10,000 IU/lb], IV, q 6 h), gentamycin sulfate (6.6 mg/kg [3 mg/lb], IV, q 24 h), and metronidazole (15 mg/kg [6.8 mg/lb], PO, q 24 h). Other drugs administered included omeprazole (2 to 4 mg/kg [0.90 to 1.8 mg/lb], PO, q 24 h) given to 5 horses and ranitidine (6.6 mg/kg PO, q 8 h) given to 2 horses. Twenty-four of 46 horses were administered IV fluids (20 to 40 mL/kg [9 to 18 mL/lb], IV) for a range of 1 to 11 days. Two horses received 1 gal of mineral oil. Sedation and tranquilization included acepromazine (7 horses; 0.02 mg/kg [0.009 mg/lb], IV, or 0.05 mg/kg [0.023 mg/lb], IM), xylazine (15 horses; 0.5 to 1.0 mg/kg, IV), butorphanol tartrate (5 horses; 0.01 to 0.02 mg/kg [0.0045 to 0.0090 mg/lb], IV or IM), and dormosedan (5 horses; 0.02 to 0.04 mg/kg [0.009 to 0.018 mg/lb], IV or IM). Fourteen horses received xylazine and ketamine (2.2 mg/kg, IV) before CSF collection procedures. One horse received pain relief through an epidural catheter.

Outcome—Eleven of 46 (24%) horses were euthanatized in the hospital, and 3 were euthanatized 1 to 6 months after discharge for cervical stenotic myelopathy, septic tenosynovitis, and possible renal failure, respectively; thus, overall mortality rate within 6 months of hospital admission was 30%. All horses that were euthanatized had positive results of the MAC-ELISA and PRNT. Mortality rate in recumbent horses was 10 of 14; 8 horses were euthanatized within 7 days of hospitalization, and horses that died from sequelae were euthanatized 2 weeks after onset of clinical signs (**Table 2**). Almost half of the horses (20/46) developed sequelae, which resulted in the deaths of 4 horses.

Histologic examination was performed on 12 euthanatized horses; 11 of 12 had encephalomyelitis, and 1 had myelitis only. Horses with clinical signs that were greater in the hind limbs had more severe inflammation in the thoracic or lumbar spinal cord than in the cervical spinal cord, compared with horses that had clinical signs that were greater in the forelimbs. Inflammation was detected in the cortical gray matter in 7 horses, either as a single focus or as multiple foci. Inflammatory cells were detected in the basal ganglia in 4 horses and in the thalamic nuclei in 4 horses. Three horses had inflammatory foci in the midbrain, 2 had foci in the pons, and 6 had foci in the medulla oblongata. The 2 horses with decreased tongue retraction had inflammatory foci in the medulla oblongata. Overall, inflammatory foci were detected in only the gray matter in 2 horses and predominantly in gray matter in the remaining 10 horses. Perivascular cuffs of lymphocytes with fewer macrophages and occasional neutrophils commonly extended into the neuropil.

A rabies protocol for diagnostic testing was performed on 8 of 12 brains, and all results were negative. West Nile virus was isolated from 2 of 8 brains by use

Table 2—Sequelae associated with WNV encephalomyelitis in 46 horses

Sequelae	No. of horses	Outcome	Cause of death
Septic tenosynovitis	2	Euthanatized	Yes
Staphylococcal cellulitis	1	Euthanatized	Yes
Enterocolitis	2	2 discharged, 1 euthanatized	Yes
Abrasions or cuts	6	3 euthanatized	Yes*
Facial or ventral edema	3	1 euthanatized, 2 discharged	No
Muscle atrophy	1	Euthanatized	No
Corneal ulcers	3	Discharged	No
Injection abscess	2	Discharged	NA
Large colon impaction	1	Discharged	NA
Subcutaneous emphysema	1	Discharged	NA

*Includes the 3 horses euthanatized because of septic tenosynovitis and cellulitis. NA = Not applicable. Certain horses had more than 1 sequela.

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of viral culture and detected by use of PCR assay in those same brains. Portions of brain and spinal cord were tested by use of IHC, and 8 of 12 horses had positive results. Two of the 4 horses with negative results had moderate to severe postmortem autolysis. The remaining 2 horses were euthanatized at 14 and 41 days after admission. The horse euthanatized at 14 days had no residual neurologic deficits and died from sequelae.

Of the 32 surviving horses, 19 owners or trainers of the horses could be contacted to assess the horse's condition. Sixteen of these owners believed that the horses had recovered from WNV encephalomyelitis by 6 months after discharge. Three horses were clinically normal at discharge, 5 were clinically normal within 1 month, 5 were clinically normal by 3 months, and the remaining 4 were clinically normal by 6 months. Persistent but reversible problems consisted of weakness, ataxia, or both; behavioral changes; muscle tremors after exertion; and knuckling and falling in the forelimbs. Twelve horses had returned to their normal performance level at the time of the follow-up call. Four horses that recovered had some other problem that precluded their use. Two owners stated that their horses never recovered completely from WNV encephalomyelitis. One horse was having trouble using its hind limbs, and the other horse continued to be weak and would stumble and fall often when ridden.

Discussion

Fifty-six horses from Georgia and Florida were admitted to the University of Florida from July to December 2001 for suspicion of WNV encephalomyelitis, representing a large number of WNV investigations performed in a secondary or tertiary setting. More importantly, this represents a large number of horses observed closely on a daily basis by large animal internists. Differences in duration of observation and hospitalization status likely account for the variations in clinical signs that exist among other outbreaks.[10,14,21-25] Data from horses from other WNV enzootics, such as those that occurred in Italy, France, Morocco,[21-24] and the United States in 1999 and 2000,[14,25] have been published, but most of that information was obtained from field cases or limited numbers of hospitalized horses.

Of the 46 horses with WNV encephalomyelitis, 43 horses had antibodies against WNV by use of MAC-ELISA and PRNT testing. The other 3 horses all had increased rectal temperature, signs of spinal cord abnormalities, and muscle fasciculations. The specificity and sensitivity of the MAC-ELISA are unknown, but there likely are horses that fail to produce an IgM antibody concentration that surpasses the cutoff value for this test. All 3 horses had positive results for neutralizing antibody, which is indicative of exposure to WNV. In limited testing, little detection of IgM at the cutoff concentration has been detected in vaccinated horses.[26] Furthermore, a single vaccine injection results in limited detection of neutralizing antibody with the PRNT, but 2 injections result in substantial increases of neutralizing antibody, as detected by the PRNT.[26] The results of testing in these 3 horses were likely a reflection of exposure, not response to vaccination.

Peak prevalence of referral was in September and October, consistent with WNV activity in the United States during 1999 and 2000.[10,12] Mean age of the affected horses in our study was lower than the age range of 12 to 14 years previously reported.[10,21-26] On a national basis, disease and mortality rate are reported to be greater in older horses.[26] The younger age in our population likely reflected a bias in the horses that may be referred. The mortality rate reported in our study of hospitalized horses was 30%, and the overall mortality rate for affected horses in the United States in 2001 was 38%.[14,25] Our lower mortality rate is probably not important and may reflect the influence of age on survivability.

Although the most common initial complaints consisted of neurologic abnormalities, others reasons for referral included colic, lameness, anorexia, and increased rectal temperature. In humans, the syndrome called West Nile fever includes fever, joint ache, rash, and gastrointestinal tract signs; horses likely also have systemic lesions and fever.[27] Increased rectal temperature, signs of depression, and anorexia were not as commonly described in other WNV encephalomyelitis enzootics.[15,16,24] In horses evaluated during the year-2000 US epizootic, fever was detected in 23% of horses, whereas fever was detected in > 60% of our patients.[14] Several reasons for this difference may exist. Our horses were in a climate-controlled environment and receiving anti-inflammatory treatment. Because of these conditions, rectal temperature > 38.3°C was considered abnormal. For affected horses in the French epizootic in 1996, rectal temperature ≥ 38.5°C (101.3ºF) was used as the criterion for fever and yielded results consistent with our findings, in which 62% of horses were considered febrile.[24] This mildly increased rectal temperature may have been encountered in field cases of other studies but not considered important, because horses were recumbent and affected in late summer. Horses may only be febrile for a short period, likely early in the disease, and fever may be associated with viremia. Early recognition of the disease in 2001 may have resulted in more frequent finding of this clinical sign. During our clinical assessment of horses suspected of having the disease, increased rectal temperature was critical in differentiating viral encephalitis from other diseases such as EPM and cervicospinal myelopathy. Recrudescence of increased rectal temperature was predictive for resumption of neurologic signs that necessitated prolonged treatment.

Flaviviruses cause polioencephalomyelitis (inflammation of the gray matter of the brain and spinal cord) with lesions that increase in number from the diencephalon through the hindbrain and frequently increase in severity caudally through the spinal cord.[3,18,22,28,29] Horses in our study had signs consistent with diffuse CNS disease, primarily consisting of changes in mentation, signs consistent with spinal cord abnormalities, and defects in cranial nerves of the hindbrain. In our study, > 60% of the horses had abnormal behavior, varying from decreased alertness to signs of delirium and somnolence; a smaller percentage had agitation, aggressive behavior, or both. The histo-

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logic changes within the brains, which included inflammatory foci and detectable virus in the thalamus, medulla, and pons, were consistent with changes in behavior. Although the thalamus integrates all sensory input to higher centers, lesions within the midbrain and rostral pons may affect the reticular formation, which besides the forebrain regulates consciousness.[21] The reticular formation projects to the thalamus, which in turn sends diffuse projections to the entire cortex.[30] This formation also may travel directly to the base of the forebrain, which is the source of cholinergic stimulation to the entire cerebral cortex. Disturbances of the reticular formation and the midbrain may induce behavior changes ranging from severe aggression to somnolence and even coma, which was consistent with our observations.

West Nile virus-induced motor deficits are multifocal, asymmetric, and primarily characterized by weakness and ataxia. Spinal deficits have been consistently described in > 90% of affected horses.[7,14,21-25] These 2 clinical signs are likely a reflection of brain and spinal cord disease through direct infection of the spinal cord, interruption of motor tracts in the hindbrain, and loss of fine motor control through infection of the large nuclei of the thalamus and the basal ganglia. Ataxia can be attributable to interruption of general proprioception. Although ataxia was commonly detected and could be profound, most of the horses in our study had difficulty standing primarily because of profound weakness. This is different from the horses described during the year-2000 US enzootic in which ataxia was detected more commonly than weakness. This difference may have been due to differences in numbers of affected horses, stage of disease, and degree of recumbency that prevented assessment of weakness.[14] Most of the horses that were euthanatized in our study had histologic lesions predominantly in the gray matter with inflammatory foci within the midbrain and hindbrain and increasing severity caudally through the spinal cord. Lower motor neuron disease characterized by weakness would thus be a common clinical sign associated with these spinal cord lesions.

Involuntary skin and muscle fasciculations, tremors, and hyperesthesia are extremely common in affected horses. Fasciculations were described in 40% of horses in the year-2000 report and in 61% of horses in our study. The fasciculations were of short duration and responded dramatically to anti-inflammatory medication; differences in frequency of this finding may have been attributable to time of examination and medications administered prior to referral. Compared with the consistent finding of spinal cord signs, fasciculation, while easily detected, should not be the primary diagnostic criterion in horses with WNV encephalomyelitis. The pathogenesis of this abnormality likely includes loss of fine motor control, which is regulated mainly by the basal ganglia.[30] Movement disorders are detected with flavivirus infection in a long-term Parkinson-like syndrome in rats[31] and experimental infection in monkeys.[32]

Histologic lesions within the pons and medulla oblongata can explain clinical deficits of cranial nerves VII, XII, and IX. Unilateral and bilateral facial nerve paralysis was the most common abnormality. Paresis of the tongue was the next most common clinical sign. Dysphagia was not common, although it has been reported.[24]

In addition to testing for exposure to WNV, serum samples were submitted for measuring EEE, WEE, and equine herpesvirus titers. Single-sample testing and differences among tests used by different laboratories limited the usefulness of serologic testing for these encephalitides. The horse with positive results of MAC-ELISA for EEE could have been concurrently exposed to EEE virus, but clinical signs were more consistent with WNV infection. Detection of 4-fold or greater differences between EEE and WEE titers in a single serum sample is typically used to indicate recent exposure to EEE virus rather than vaccination. Several horses that had clinical signs consistent with WNV infection had a 4-fold difference between HI titers for EEE and WEE; therefore, either these horses had concurrent exposure to EEE virus or this criterion is of limited use to detect recent exposure to EEE virus.

Antibodies against the agent of EPM were detected in CSF of most of the horses in our study, indicating possible concomitant infection. However, all horses with positive results of MAC-ELISA that were euthanatized for WNV encephalomyelitis did not have any changes consistent with EPM. Because the remaining horses were only treated for a short period and most recovered, EPM was unlikely in those horses. Other possibilities that might explain the detection of antibodies against the agent of EPM in the CSF of the horses in our study include breakdown in the blood-brain barrier because of meningoencephalitis, blood contamination of the CSF sample during collection, and oversensitivity of the western blot assay for diagnosis of EPM.

Although there was limited antigen detected by use of IHC, this method was more reliable than virus isolation for confirmation of WNV infection at postmortem. Because virus isolation was performed after rabies testing, limited samples of brain that actually contained virus were likely examined. The use of the real-time PCR assay was also not any more sensitive than viral culture. This may be a sampling issue also, because the same tissues that were used for viral culture were used for PCR assay. The IHC assay was not successful at confirming the presence of virus in all horses. A diagnosis was still made in horses with negative results of IHC on the basis of clinical signs, results of CSF analysis, and serologic results for WNV.

Treatment was directed at alleviating CNS inflammation, treating possible protozoal disease, and providing supportive fluid and nutritional care. Because weakness and ataxia were intermittent in some horses, we recommend that provision of assistance to stand through slinging be used to fully assess the degree of paresis in recumbent horses. Fluid therapy is a critical component of supportive therapy because of the use of nonsteroidal anti-inflammatory drugs with concurrent dehydration or azotemia. Protection from traumatic injury will also reduce additional expense of therapy and mortality rate in affected horses.

Although variations in clinical disease exist between enzootics, the case definition should also

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include horses with spinal cord deficits and either fever or fasciculations. A positive finding via the IgM capture alone reliably confirmed WNV encephalomyelitis for vaccinated horses and nonvaccinated horses; thus, many horses in which WNV encephalomyelitis would only be considered probable by the 2001 definition could likely be considered to have a confirmed diagnosis.

[a]West Nile virus vaccine, Fort Dodge Laboratories, Fort Dodge, Iowa.
[b]Animal and Plant Health Inspection Service, National Veterinary Services Laboratory, USDA, Ames, Iowa.
[c]Kissimmee State Diagnostic Laboratory, Florida Department of Agriculture and Consumer Services, Kissimee, Fla.
[d]Neogen Corp, Lexington, Ky.
[e]Florida Department of Health Laboratory, Starke, Fla.
[f]TaqMan, Applied Biosystems, Foster City, Calif.
[g]BioReliance Corp, Rockville, Md.

Appendix 1

Grades of abnormality in mentation in horses evaluated for West Nile virus (WNV) encephalomyelitis

Grade	Description
1	Always alert and responsive to environment.
2	Occasional periods of decreased alertness and response to the environment.
3	Signs of depression or delirium; although capable of responding to stimuli, response frequently inappropriate.
4	Semicomatose; responsive only to noxious, auditory, or visual stimuli.
5	Comatose and unresponsive to repeated noxious stimuli.

Appendix 2

Grades of ataxia or weakness attributable to spinal cord damage in horses with WNV encephalomyelitis

Grade	Description
1	Clinical signs barely detectable at a walk.
2	Clinical signs detectable at a walk and exaggerated by backing, turning, swaying, loin pressure, or neck extension.
3	Clinical signs detectable at rest and easily at a walk with a tendency to buckle or fall with backing, turning, loin pressure, or neck extension.
4	Stumbling, tripping, falling spontaneously, or recumbent and unable to rise.

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