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Brief Communications

West Nile virus infection in reindeer (*Rangifer tarandus*)

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Abstract. West Nile virus (WNV) infection in 4 reindeer (*Rangifer tarandus*) resulted in lymphohistiocytic encephalomyelitis within the medulla oblongata and cervical spinal cord. Immunohistochemistry revealed WNV antigen within neurons and among mononuclear cell infiltrates. These represent the first known cases of clinical WNV infection in Cervidae. Clinical signs and lesions were similar to those described in horses. Nucleotide sequence of a 768-bp region of the WNV E-glycoprotein gene revealed 1 nucleotide mutation, which resulted in a single amino acid substitution from a serine to a glycine (position 227 of E-glycoprotein) when compared with the prototype WNV-NY99 strain (isolated from Bronx zoo flamingo 382-99).

West Nile virus (WNV) is a member of the Flaviviridae family (genus *Flavivirus*), isolated originally from the blood of a febrile human in Uganda in 1937.[15] Mosquito vectors transmit the virus among reservoir bird populations, and susceptible mammalian species are infected incidentally. Ideal conditions for the propagation of WNV transmission include 1) a viremic host bird, 2) active ornithophilic mosquito vectors, and 3) large numbers of amplifying avian host species.[13] Infection of incidental hosts can result in a variety of host–pathogen responses ranging from subclinical to severe neurological disease with encephalitis and death.[7] West Nile virus is 1 of the most geographically widespread flaviviruses and is related to Japanese encephalitis virus, Kunjin virus, and St. Louis encephalitis virus. The virus was first detected in North America in the summer of 1999 in the New York City metropolitan area, during an epizootic involving birds, horses, and humans. Each summer and fall since 1999, the geographic range of WNV in North America has continued to expand westward.[12] The WNV isolate responsible for the initial epizootic in North America is genetically similar to an isolate recovered from the brain of a dead goose in Israel in 1998.[9]

In outbreaks since the mid-1990s, several epidemiological trends for WNV infections have been observed that include 1) increased frequency of occurrence in humans and horses, 2) apparent increase in severe clinical disease in humans, and 3) high avian death rates accompanying the human outbreaks, especially in the United States and Israel.[12] The range of susceptible host species has also increased to include nu-

merous wildlife species.[10] The purpose of this report is to describe WNV infection with concurrent neurological disease in reindeer and to describe the lesions associated with WNV infection in reindeer.

Reindeer 1, 2, and 3 were housed with 17 other reindeer at the National Animal Disease Center (NADC), Ames, Iowa. The reindeer had originated from a farm in southern Michigan and had been shipped as a group to Ames in May 2002 for use as noninfected controls in tuberculosis diagnosis research. Upon arrival, reindeer had been dewormed with ivermectin[a] and vaccinated with a multivalent clostridial bacterin.[b] Reindeer 4 was a 5-year-old male, 1 of several reindeer in a wildlife park in eastern Nebraska.

In September 2002, 4 months after arrival, over a 7-day period, reindeer 1, 2, and 3 exhibited similar clinical signs before death. All were adult males, ranging in age from 2 to 4 years. Reindeer 1 and 2 were found in lateral recumbency, unable to rise to sternal recumbency, even with assistance. Both animals were febrile (40 C) and exhibited paddling of all 4 limbs. Both animals were euthanized by IV sodium pentobarbital. Reindeer 1 had a history of diarrhea of 1–2 weeks duration before death. Reindeer 3 was first observed separated from the remainder of the herd, depressed, head tilt to the left, flaccid tongue, dysphagic with difficulty in drinking, and febrile (40 C). Treatment consisted of dexamethasone (20 mg), flunixin meglumine (1.1 mg/kg), and IV fluids. Within 12 hours, the animal's condition had progressed to lateral recumbency and paddling similar to that observed in the first 2 reindeer, at which time the deer was euthanized as described above. Reindeer 4 was clinically normal and grazing the previous day but was found dead the next morning.

All reindeer were in good physical condition. Normal-appearing forage was present in the forestomachs of all animals. There were no gross lesions in any of the reindeer. Brain was collected from reindeer 1 and immersed in neutral 10% buffered formalin. The following tissues were collected for histopathology from reindeer 2, 3, and 4: tongue, tonsil, salivary gland, eye, brain, myocardium, lung, cervical and
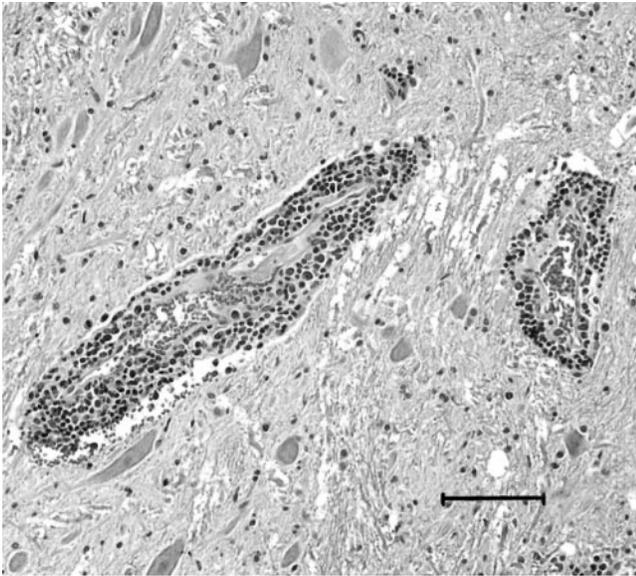
Figure 1. Photomicrograph of section of brain from reindeer 3. Note perivascular infiltrates of mononuclear inflammatory cells. Bar = 150 μm. HE.
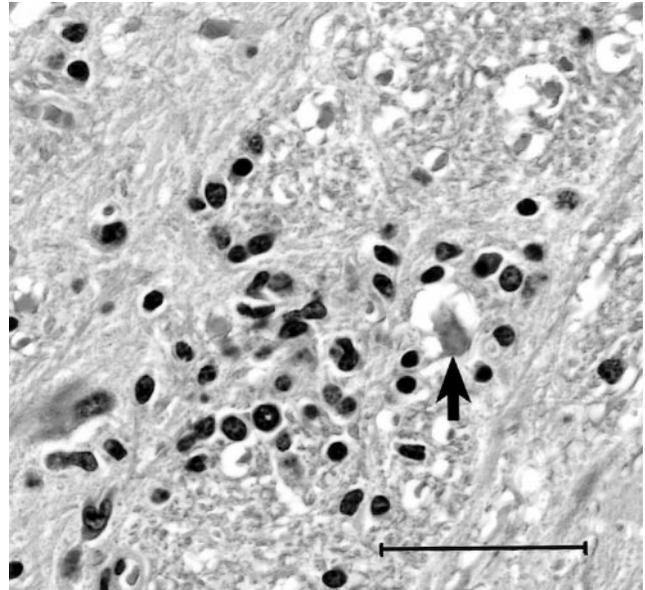

Figure 2. Photomicrograph of section of brain from reindeer 3. Note shrunken degenerate neuron surrounded by mononuclear inflammatory cells. Bar = 50 μm. HE.

thoracic spinal cord, abomasum, rumen, small intestine, mesenteric lymph node, spleen, liver, adrenal gland, kidney, colon, urinary bladder, and skeletal muscle. Tissue specimens were immersed in 10% neutral buffered formalin, processed routinely, embedded in paraffin, sectioned at 4 μm, and stained with hematoxylin and eosin (HE). Microscopic lesions in nervous tissue from all 4 reindeer were classified as mild to moderate and confined primarily to the medulla oblongata, cerebellum, and cervical spinal cord. In sections of medulla oblongata, cerebellum, and spinal cord, blood vessels in the pia mater and scattered blood vessels in the neuropil were surrounded by variable numbers of mononuclear cells (Fig. 1) and occasional neutrophils. Infiltrates of small numbers of mononuclear cells and neutrophils were present in the neuropil adjacent to vessels. Scattered neurons were hypereosinophilic with a granular appearance or had undergone necrosis with neuronophagia present (Fig. 2). Multiple small foci of gliosis and microgliosis were also noted. Sections of kidney from reindeer 2, 3, and 4 were characterized by mild multifocal lymphoplasmacytic interstitial nephritis, and small numbers of proteinaceous and mineralized casts were present within collecting ducts. No significant microscopic lesions were seen in other tissue specimens examined from any of the reindeer.

Formalin-fixed, paraffin-embedded sections of brain, spinal cord, and other tissues were mounted on poly-L-lysine–coated slides for immunohistochemical (IHC) labeling of WNV antigen using anti-WNV primary antibody.[c] An isotype-matched mouse antibody was substituted in the procedure to serve as a negative control. Brain from a WNV-infected bird was used as positive control tissue. Immunohistochemistry identified WNV antigen in the cytoplasm of few neurons in the medulla oblongata and cervical spinal cord. Positive immunohistochemical reactivity was also present in foci of mononuclear cell infiltrates within the medulla

oblongata and spinal cord (Fig. 3). The intensity of staining varied from section to section but was typically mild. Interestingly, positive immunohistochemical staining for WNV antigen was not seen in other tissues.

Samples of brain from reindeer 2 and 3 were also collected and submitted for bacteriologic isolation. Samples of brainstem and cerebellum were submitted for molecular diagnosis of WNV infection by reverse transcriptase–PCR (RT-PCR) using selected primers that amplified a portion of the highly
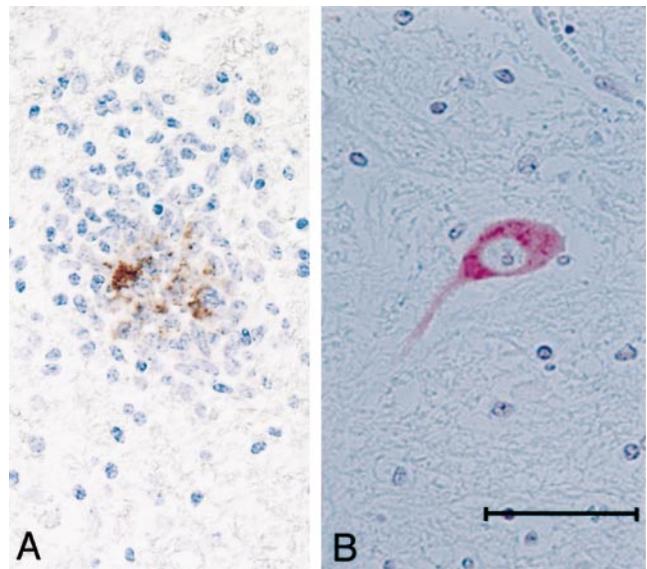

Figure 3. Photomicrograph of section of brain from reindeer 3, A, and 4, B. Note amorphous immunohistochemical staining within focus of inflammatory cells, A, as well as intraneuronal staining, B. Bar = 50 μm. Immunoperoxidase method, hematoxylin counterstain, A, alkaline phosphatase method, hematoxylin counterstain, B.

conserved E region of the genome of the WNV identified as NY99.[8] No bacterial pathogens were isolated; however, brain specimens were positive for WNV by RT-PCR. Viral RNA was extracted from cerebrospinal fluid (CSF) collected from reindeer 3 and analyzed by RT-PCR using primers EDL/E-U1476[3] and EDL/E-L2538[3] (specific for the WNV E-glycoprotein gene). A second round of seminested PCR was done using primers EDL/E-U1476[3] and EDL/E-L2244.[3] A 768-bp fragment generated using seminested PCR was sequenced and shown to differ by 1 bp from the sequence of prototype NY99. This difference results in 1 amino acid substitution from a serine to a glycine (position 227 of E-glycoprotein) when compared with the prototype WNV-NY99 strain (isolated from Bronx zoo flamingo 382-99).[9]

Blood was collected from all NADC reindeer approximately 3 months before the death of reindeer 1, 2, and 3; from reindeer 2 and 3 immediately before euthanasia; and from the remaining NADC reindeer approximately 3 days after the death of reindeer 1, 2, and 3. Blood was also collected from 10 captive white-tailed deer (*Odocoileus virginianus*) in a nearby pasture at approximately the same time points that blood was collected from the reindeer. Antibody titers to WNV were determined using the plaque-reduction neutralization test (PRNT) as described previously.[11] Plaque reduction of ≥90% was recorded as positive. A PRNT titer of ≥1:10 was considered significant. Blood samples collected from all NADC reindeer including reindeer 1, 2, and 3 approximately 3 months before euthanasia were negative (1:10 serum dilution showing plaque reduction of <90%) for antibodies to WNV. Antibody titers to WNV in sera from reindeer 2 and 3 just before euthanasia were ≥1:100 (1:100 serum dilution showing ≥90% plaque reduction). Of the remaining reindeer that did not demonstrate clinical signs of WNV infection, 9 of 17 (53%) also developed antibody titers of ≥1:100 and 6 of 10 (60%) captive white-tailed deer sampled from a nearby pasture showed similar seroconversion from negative to positive (≥1:100) during a similar time period.

Cerebrospinal fluid was collected from reindeer 3 at the time of necropsy. Whole blood was collected in potassium ethylenediaminetetraacetic acid for complete blood counts. Results were compared with reference intervals that were determined previously for this group of reindeer at NADC. Examination of blood from reindeer 2 and 3 revealed leukocytosis with a mature neutrophilia and lymphopenia. Elevated fibrinogen was noted in reindeer 2. Examination of CSF from reindeer 3 revealed a lymphocytic pleocytosis composed primarily of small- and medium-sized lymphocytes.

The criteria for a clinical diagnosis of encephalitis caused by WNV in horses must include 1 or more of the following clinical signs: ataxia (stumbling, staggering, wobbly gait, or uncoordination), inability to stand, multiple limb paralysis, or death.[11] Clinical signs must also be accompanied by 1 or more of the following diagnostic criteria: isolation of WNV from tissue, blood, or CSF; 4-fold or greater change in antibody titer to WNV in paired sera; or an elevated titer to WNV in a single serum sample.[11] Probable cases are defined as those where clinical signs are accompanied by the detection of WNV antigen in tissue by IHC.[11] According to these

definitions, 2 reindeer in this report are confirmed WNV infections, and 2 reindeer are probable WNV infections.

To the authors' knowledge, this represents the first description of WNV infection in reindeer. The clinical signs seen in reindeer infected with WNV in this report, included fever, depression, head tilt, and recumbency, similar to clinical signs seen in horses infected with WNV.[5,11] Moreover, these cases were observed in September–October 2002, a season similar to the peak in clinical equine cases reported in the eastern United States in 2000.[5,11] This similarity is likely the result of both vector activity and a similar incubation period for reindeer and horses.

Microscopic lesions in affected reindeer are similar to those seen in naturally infected horses[5] and in experimentally infected hamsters (*Mesocricetus auratus*).[18] Lesions in horses are described as mild to moderate lymphocytic or histiocytic encephalomyelitis with focal gliosis, perivascular cuffing of mononuclear cells, neuronal degeneration, neuronophagia, and petechial hemorrhages.[5] Lesions in horses are primarily confined to the lower brain stem and spinal cord. Degenerative and necrotic changes to neurons have been attributed to apoptosis.[16,18] Lesions generally are not seen in other organs.

With the exception of viruses that induce hemorrhagic disease, clinical signs from arboviral infections are rare in Cervidae, although serologic evidence of exposure to western equine encephalitis virus, California encephalitis virus, Venezuelan equine encephalitis virus, and St. Louis encephalitis virus has been reported.[17] As this report demonstrates, the mammalian host range for WNV is not completely defined. Encephalitis caused by naturally occurring WNV infection has been reported in horses[5,6] and man.[4] Experimental WNV infection results in encephalitis in mice and rhesus monkeys (*Macaca mulatta*).[15] However, mammals such as dogs, rabbits, guinea pigs (*Covia porceius*), and hedgehogs (*Erinaceus europaeus*) do not develop encephalitis after experimental inoculation with WNV.[2,15] Experimental infection of sheep with WNV does not result in clinical signs; however, neonatal death and hydranencephaly may be seen in lambs of ewes infected experimentally before conception or during gestation.[1]

Diagnosis of natural WNV encephalitis in reindeer 2 and 3 is on the basis of seroconversion and the presence of WNV-specific nucleic acid in the brain and CSF. Reverse transcriptase–PCR amplification was positive with brain tissue and CSF using different primer sets targeting the E-glycoprotein gene. Nucleotide and deduced amino acid sequence revealed 1 nucleotide mutation within the amplification product of 768 bp, which resulted in an amino acid substitution from a serine to a glycine (position 227 of E-glycoprotein) when compared with the prototype WNV-NY99 strain (isolated from Bronx zoo flamingo 382-99).[9] All other North American WNV isolates have a serine residue at position 227 of the E-glycoprotein, and mutations at this position have been found previously in 2 WNV isolates from India with substitutions from serine to asparagine (Culex WNV isolate G22886; GenBank AF196524) and serine to threonine (human WNV isolate; GenBank AF196524).[14] Whether mutation within this region of the E-glycoprotein is a result of an immune evasion mechanism or the adapta-

tion of the WNV to the reindeer host is not known at present. A phylogenetic tree based on the E-gene nucleic acid sequence data from prototype WNV lineage I and II viruses and known North American WNV isolates was constructed. The WNV reindeer isolate grouped within WNV lineage I together with North American and Israeli WNV isolates (data not shown).

Although not specific for WNV infection, leukogram changes in affected reindeer were consistent with a stress leukogram and suggestive of a viral infection. This is in contrast to dogs infected experimentally with WNV and horses naturally infected with WNV where, in the latter species, no detectable changes in hemogram or leukogram are seen.[2,5]

As the geographic range of WNV expands, prevention and control measures become increasingly important. Current recommendations for the prevention of WNV infection in horses include reducing sources of standing water essential for mosquito breeding and decreasing exposure to mosquito bites.[11] Although a killed virus vaccine is commercially available and licensed for use in horses, it is not currently licensed for use in reindeer. However, recommendations regarding the elimination of mosquito-breeding sites and reducing exposure to mosquito bites are warranted for reindeer.

Reindeer may be more susceptible than other species of Cervidae to WNV because adjacent or near the pastures, where 3 of the 4 reindeer in the current report were kept, 30 elk (*Cervus elaphus*) and 40 white-tailed deer were located. No clinical signs suggestive of WNV infection were seen in either elk or white-tailed deer during this period, although a subgroup ($n = 10$) of these white-tailed deer showed a similar seroconversion to WNV as that seen in the reindeer. Additionally, sheep, bison (*Bison bison*), horses, and cattle located near the reindeer pasture did not show clinical signs suggestive of WNV infection.

Sources and manufacturers

a. Ivomec, Merial Limited, Iselin, NJ.
b. Ultrabac-7, Pfizer Animal Health, Exton, PA.
c. West Nile immune ascitic fluid (VR-1267AF), American Type Culture Collection, Manassas, VA.

References

1. Barnard BJH, Voges SF: 1986, Flaviviruses in South Africa: pathogenecity for sheep. Onderstepoort J Vet Res 53:235–238.
2. Blackburn NK, Reyers F, Berry WL, Shepherd AJ: 1989, Susceptibility of dogs to West Nile virus: a survey and pathogenecity trial. J Comp Path 100:59–66.
3. Briese T, Rambaut A, Pathmajeyan M, et al.: 2002, Phylogenetic analysis of a human isolate from the 2000 Israel West Nile virus epidemic. Emerg Infect Dis 8:528–531.
4. Briese T, Xi-Yu J, Huang C, et al.: 1999, Identification of a Kunjin/West Nile-like flavivirus in brains of patients with New York encephalitis. Lancet 354:1261–1262.
5. Cantile C, Di Guardo G, Eleni C, Arispici M: 2000, Clinical and neuropathological features of West Nile virus equine encephalomyelitis in Italy. Equine Vet J 32:31–35.
6. Cantile C, Piero FD, Di Gurardo G, Arispici M: 2001, Pathologic and immunohistochemical findings in naturally occurring West Nile virus infection in horses. Vet Pathol 38:414–421.
7. Garmendia AE, Van Kruiningen HJ, French RA: 2001, The West Nile virus: its recent emergence in North America. Microb Infect 3:223–229.
8. Johnson DJ, Ostlund EN, Pedersen DD, Schmitt BJ: 2001, Detection of North American West Nile virus in animal tissue by a reverse transcription-nested polymerase chain reaction assay. Emerg Infect Dis 4:739–741.
9. Lanciotti RS, Roehrig JT, Deubel V, et al.: 1999, Origin of the West Nile virus responsible for an outbreak of encephalitis in the northeastern United States. Science 286:2333–2337.
10. Malakoff D: 2003, Researchers scramble to track virus' impact on wildlife. Science 299:1176.
11. Ostlund EN, Crom RL, Pedersen DD, et al.: 2001, Equine West Nile encephalitis, United States. Emerg Infect Dis 4:665–669.
12. Petersen LR, Roehrig JT: 2001, West Nile virus: a reemerging global pathogen. Emerg Infect Dis 4:611–614.
13. Rappole JH, Derrickson SR, Hubalek Z: 2000, Migratory birds and spread of West Nile virus in the Western hemisphere. Emerg Infect Dis 6:319–328.
14. Scherret JH, Poidinger M, Mackenzie JS, et al.: 2001, The relationships between West Nile and Kunjin viruses. Emerg Infect Dis. 7:697–705.
15. Smithburn KC, Hughes TP, Burke AW, Pault JH: 1940, A neurotropic virus isolated from the blood of a native Uganda. Am J Trop Med Hyg 20:471–492.
16. Swayne DE, Beck JR, Smith CS, et al.: 2001, Fatal encephalitis and myocarditis in young domestic geese (*Anser anser domesticus*) caused by West Nile virus. Emerg Infect Dis 4:751–753.
17. Trainer DO: 1973, Wildlife as monitors of disease. Am J Public Health 63:201–203.
18. Xiao S-Y, Guzman H, Zhang H, et al.: 2001, West Nile virus infection in the golden hamster (*Mesocricetus auratus*): a model for west nile encephalitis. Emerg Infect Dis 4:714–721.