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Natural West Nile virus infection in a captive juvenile Arctic wolf (*Canis lupus*)

Isabelle Lanthier[1], Michel Hébert, Donald Tremblay, Josée Harel, André D. Dallaire, Christiane Girard

Abstract. A case of West Nile virus (WNV) infection in a captive 4-month-old Arctic wolf (*Canis lupus*) is described. The animal had vomiting, anorexia, and ataxia before death. Histopathology revealed multifocal severe renal lymphoplasmacytic vasculitis, mostly affecting small arterioles, with fibrinoid degeneration of some vessel walls. Many small foci of gliosis were detected in the cerebral cortex. West Nile virus was demonstrated in the kidneys and cerebrum by immunohistochemistry and polymerase chain reaction. The described renal changes represent a novel pathological finding of WNV infection.

West Nile virus (WNV), a flavivirus transmitted by mosquito bite, was reported for the first time in North America in 1999.[3–5] Outbreaks have affected horses and wild indigenous and captive exotic birds mostly. Other mammals, like cats, squirrels, rabbits, and chipmunks, are known to be infected occasionally.[3,4] In 2002, the infection was detected in a captive seal at a US aquarium and for the first time in a reptile, an alligator, in Florida. West Nile virus–infected birds were detected for the first time in the province of Québec (Canada) during the summer of 2002. The pathological, immunohistochemical, and polymerase chain reaction (PCR) findings of a natural WNV infection in a captive juvenile Arctic wolf are described.

A wolf pup was born in February 2002 and kept in captivity with its mother in Southern Québec. The pup was vaccinated twice against canine distemper virus (CDV), canine hepatitis virus (CHV), and canine coronavirus (CC). At 4 months, the animal had nasal and ocular discharge, vomiting, anorexia, and lethargy. It was isolated and received supportive treatment for 4 days, with some improvement. The clinical signs progressed, the pup developed ataxia, and died 24 hours later. The animal was sent to the Diagnostic Service of the Faculté de médecine vétérinarie, Université de Montréal, St.-Hyacinthe, Québec, Canada, for necropsy.

The wolf was in poor body condition. There was abundant bilateral grayish mucoid exudate at the nasal turbinates. The liver was yellow, friable, and mildly enlarged. Small and large intestinal content was largely uncoagulated blood. The intestinal serosa appeared mildly granular. Tissue samples were fixed in 10% buffered formalin and embedded in paraffin. Sections were cut at 3 μm and stained with hematoxylin–eosin–saffron (HES).

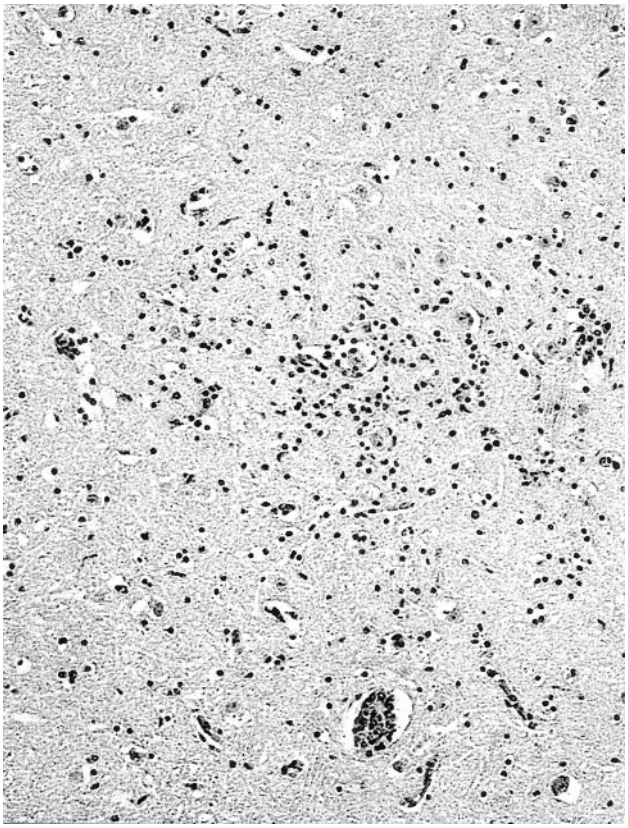

Figure 1. Cerebral cortex of an Arctic wolf with foci of gliosis and mild infiltration of the Virchow–Robin spaces by lymphocytes. HES, 200×.

Fresh samples of ileum, liver, and nasal turbinates were submitted for aerobic culture and *Leptospira* spp. isolation. Cultures of *Escherichia coli* and *Staphylococcus* spp. developed from the nasal turbinates. No parasites or ova were detected in feces by flotation. Direct fluorescent antibody examination of sections of small intestine was negative for CC and canine par-

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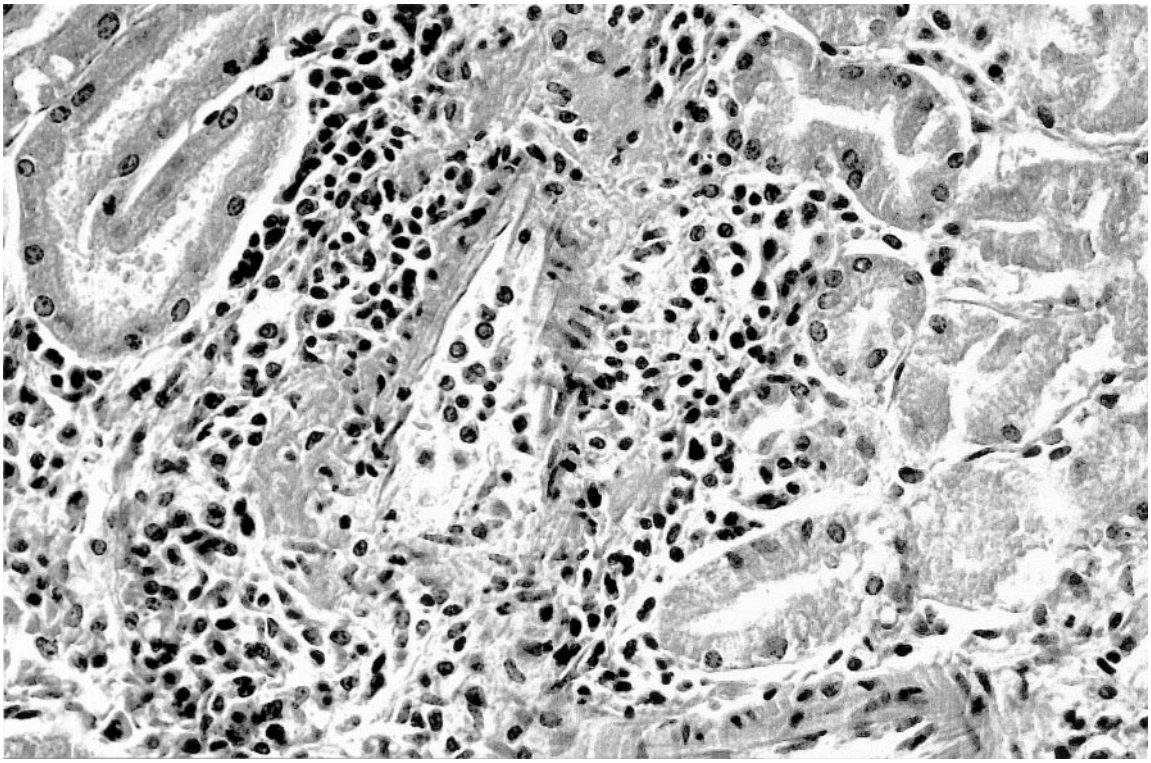

Figure 2. Kidney of an Arctic wolf pup with severe lymphoplasmacytic arteritis and fibrinoid degeneration of the vessel wall. HES, 400×.

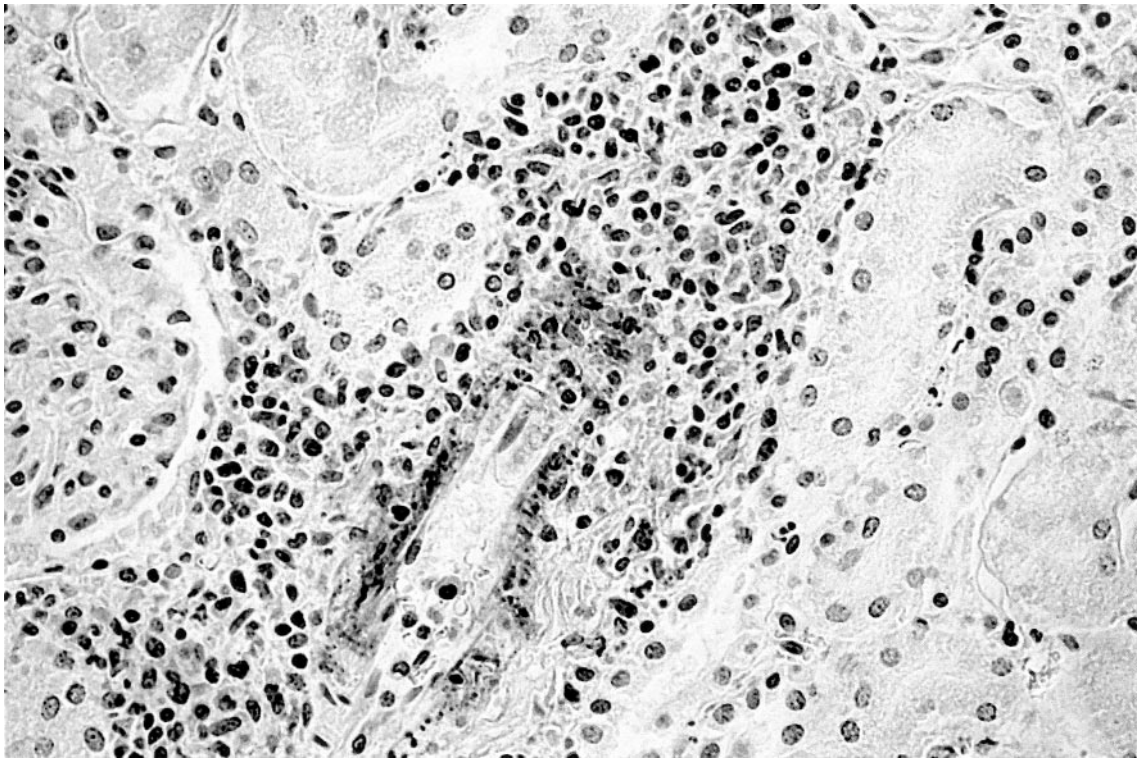

Figure 3. Immunohistochemical section of the kidney of an Arctic wolf pup. Note the WNV-positive smooth muscle cells within the inflamed arteriolar wall. IHC, 400×.

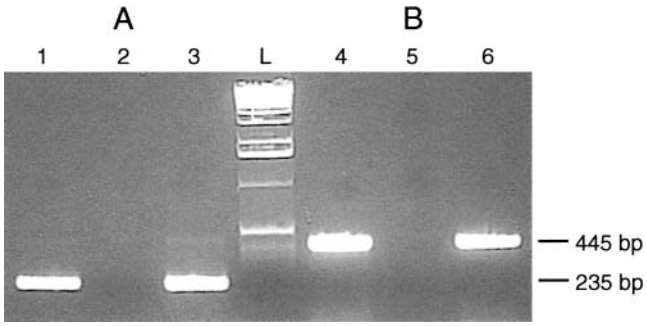

Figure 4. Visualization of RT-PCR product. Reaction A: amplification of a 235-bp fragment from NS3 coding region of WNV. Lane 1: ribonucleic acid extracted from the wolf pup. Lane 2: negative control. Lane 3: positive control (infected avian tissues). Reaction B: amplification of a 445-bp fragment from E coding region of the WNV. Lane 1: ribonucleic acid extracted from the wolf pup. Lane 2: negative control. Lane 3: positive control (infected avian tissues). L: 1-kb DNA ladder.

vovirus. For reverse transcriptase–polymerase chain reaction (RT-PCR) amplification of WNV RNA, 100 mg of fresh brain and kidney were homogenized together with 0.5-mm glass beads at maximum speed for 3 minutes in the Mini-BeadBeater-8.[a] Homogenized tissue (200 μl) was used for RNA extraction following instructions of the QIAamp RNA blood Mini Kit Handbook.[b] Amplification of RNA (5 μl) was performed using the Qiagen one-step RT-PCR kit. The primers targeted the NS3 coding region (M.A. Drebot, personal communication) and the E coding region of the WNV genome.[2] Resulting PCR products were, respectively, 235 and 445 bp amplicons. Reverse transcriptase–polymerase chain reaction products were analyzed by agar gel electrophoresis with ethidium bromide staining and UV visualization.

Many small foci of gliosis with necrotic glial cells and mild neutrophilic infiltrates were found in the cerebrum, mostly in the cortex. Rare and minimal lymphocyte infiltration in Virchow–Robin spaces was seen (Fig. 1). Gram stain did not reveal bacteria in the nervous tissue. There was marked mucopurulent rhinitis with colonization by abundant mixed bacteria. The thymus and Peyer's patches displayed moderate lymphoid depletion. Occasional individual small-intestinal crypts had flattened to cuboidal epithelium and contained necrotic cells. There was diffuse marked hepatic lipidosis, and rare necrotic Kupffer cells were observed. Kidney sections revealed multifocal marked lymphoplasmacytic vasculitis of small arterioles in particular. Fibrinoid degeneration was present in the wall of some small arteries (Fig. 2). Multifocal lymphoplasmacytic cuffing of vessels was observed throughout the renal parenchyma. Silver stain did not reveal argyrophilic bacteria in the kidney or the liver.

The avidin–biotin–peroxidase complex method was used for immunohistochemical demonstration of antigens in paraffin blocks of cerebrum and kidney; analyses were conducted at Prairie Diagnostic Services (CDV and CHV) and at the Ontario Veterinary College (WNV).

Antigens were not identified for CDV and CHV. In the kidney, some glomerular mesangial cells, inflammatory cells infiltrating blood vessels, and smooth muscle cells within degenerate arteriolar walls were positive for WNV (Fig. 3). In the cerebrum, there were few positive glial cells in rare foci of gliosis.

Tissues tested for WNV by RT-PCR were positive for the 2 target regions of the viral genome (Fig. 4). No nested-PCR reaction was used on the wolf pup tissues because the virus replicated in the brain and kidney at a titer high enough for standard RT-PCR detection.

Reports of naturally acquired WNV infection in mammals are limited to horses, squirrels, a rabbit, a cat, a chipmunk, an aged dog, and a juvenile wolf. In WNV-infected horses, neural lesions consist of polioencephalomyelitis of the spinal cord, brain stem, basal nuclei, thalamic gray matter, and midbrain with small foci of gliosis and necrosis.[1] In some horses, mild nonsuppurative myocarditis, scattered hemorrhages of the renal medulla, and splenic lymphoid depletion were also detected.[1] The wolf pup had a different WNV presentation with vascular lesions of the kidneys, and to a lesser extent, of the cortical cerebrum. The hepatic lipidosis was partially associated with anorexia. No specific clinicopathological evaluation was performed on the wolf before death. The renal vascular inflammation and associated vasoconstriction may have resulted in diminished blood flow to the kidney, diminished glomerular filtration rate, and azotemia of renal origin. The origin of the bloody intestinal content was undetermined.

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Sources and manufacturers

a. Biospec Products Inc., Bartlesville, OK.
b. Qiagen, Valencia, CA.

References

1. Cantile CF, Del Piero G, Di Guardo G, Arispici M: 2001, Pathologic and immunohistochemical findings in naturally occurring West Nile Virus infection in horses. Vet Pathol 38:414–421.
2. 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 7:739–741.
3. Komar N: 2000, West Nile viral encephalitis. Rev Sci Tech 19: 166–176.

4. Kramer LD, Bernard KA: 2001, West Nile Virus infection in birds and mammals. Ann N Y Acad Sci 951:84–93.

5. Marfin AA, Gubler DJ: 2001, West Nile encephalitis: an emerging disease in the United States. Clin Infect Dis 33:1713–1719.

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Pathological and immunohistochemical findings in American crows (*Corvus brachyrhynchos*) naturally infected with West Nile virus

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Abstract. Twenty-one American crows were identified as being West Nile virus (WNV) infected by WNV-specific reverse transcriptase–polymerase chain reaction (RT-PCR) performed on fresh brain tissue (cerebrum and cerebellum of 16 crows) or by WNV-specific immunohistochemistry of various organs (21 crows). Consistent gross lesions attributable to WNV infection were not detected. Common histological lesions included necrosis of spleen and bone marrow. West Nile virus antigen was consistently detected in heart and kidney (100%). In addition, bone marrow (92%), duodenum (89%), proventriculus (87%), liver (86%), lung (85%), spleen (80%), pancreas (61%), and brain (45%) contained WNV antigen–positive cells. Infected cells included cardiomyocytes; neurons; endothelial cells and vascular smooth muscle cells; hematopoietic cells of bone marrow; and macrophages of spleen, liver (Kupffer cells), and lungs. Epithelial cells of renal tubules, duodenum, pancreas, and proventriculus were also infected. The diagnostic histopathologist should consider WNV infection in crows in the absence of any inflammatory lesions. Immunohistochemistry of heart and kidney is as reliable in detecting WNV infection in American crows as RT-PCR of fresh brain tissue.

West Nile virus (WNV) is a flavivirus that causes disease and death in numerous avian species and various mammals, such as humans, horses, and less commonly, sheep.[3,10] The virus is primarily transmitted by mosquitoes, but direct contact with infected birds and consumption of carcasses of WNV-infected birds also have been shown to represent routes of transmission in birds, including crows.[6] American crows (*Corvus brachyrhynchos*) are highly susceptible to WNV and frequently die as a result of the infection.[6] The histological lesions and immunohistochemical findings in WNV-infected American crows have only been rarely addressed in the literature.[1,9]

Twenty-one American crows that were submitted to the Department of Veterinary Diagnostic Medicine, University of Minnesota, St. Paul, Minnesota, between July and October 2002, underwent necropsy. Tissue samples, including liver (21 cases), kidney (21 cases), spleen (20 cases), heart (20 cases), brain (20 cases: 20, cerebrum; 17, cerebellum; 13, mesencephalon; 11, medulla oblongata), lung (20 cases), duodenum (19 cases), pancreas (18 cases), eye (17 cases), proventriculus (15 cases), and tibial bone marrow (13 cases), were fixed in 10% buffered formalin. The formalin-fixed tissues were paraffin embedded. Sections were cut at 4 μm and stained with hematoxylin and eosin (HE). Histological lesions such as necrosis and inflammation were subjectively graded as mild or moderate and marked on the basis of the estimated percentage of affected tissue (necrosis) and the estimated number of infiltrating inflammatory cells (inflammation).

A peroxidase-based polymer system[a] was used for immunohistochemical demonstration of WNV antigen. A mouse monoclonal antibody directed against an epitope of the E protein[b] was used[2]. This antibody does not cross-react with Saint Louis encephalitis virus or Japanese encephalitis virus. In brief, 4-μm sections were mounted on poly-L-lysine–coated slides. The sections were rehydrated. For antigen retrieval, the sections were pretreated with proteinase K[a] for 4 minutes. After rinsing with water, the sections were incubated with 3% H_2O_2 diluted in H_2O for 20 minutes to block the endogenous peroxidase activity. The sections were then rinsed in TRIS[a]–buffered saline (TBS, 0.05 M, pH 7.6). For blockage of nonspecific binding sites, the sections were incubated with normal goat serum that was diluted 1:10 in TBS. After draining excess blocking serum off the sections, the anti-WNV antibody was

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