

# West Nile Virus

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The evolution of West Nile virus (WNV) has resulted in the emergence of WNV variants that have a significant pathogenicity for humans, horses, and birds. WNV appeared in North America in New York City in 1999 and has since spread throughout the continent into the Caribbean and Mexico and is now believed to be enzootic in much of the United States and southern Canada. Crows, the blue jay, chickadees, hawks, and owls appear to be the most susceptible to WNV disease, although mortality has been reported in nearly 200 species of birds. Disease in companion birds is rare. WNV disease in birds is rapidly fatal and signs, if they occur, are predominately of the central nervous system. Characteristic necropsy findings in birds are intraosseous hemorrhage of the calvaria, an encephalitis, myocarditis, and pancreatitis. Infection can be confirmed by PCR, antigen detection, and virus isolation. Chickens and turkeys are refractory to WNV disease, and chickens have been used to monitor WNV activity. Domestic geese appear to be relatively susceptible to WNV disease. Outbreaks of WNV in Europe, Israel, and the United States have resulted in a significant number of cases of meningitis, encephalitis, and acute flaccid paralysis in people. Encephalitis has also been a common sequella to WNV infection in horses in Europe, North America, and Africa. WNV disease occurs sporadically in many other species of mammals and has been reported to cause significant mortality in a commercial operation of alligators. © 2004 Elsevier Inc. All rights reserved.

**Key words:** West Nile virus; birds; humans; horses

**W**est Nile virus (WNV) is an RNA virus that is a member of the family *Flaviviridae*, genus *Flavivirus*. Within the *Flavivirus* genus, WNV is classified in the Japanese encephalitis virus serocomplex. It is a single-stranded positive-sense RNA virus with approximately 12,000 nucleotides. It has a 30- to 35-nm icosahedral core that is enclosed with a host cell-derived envelope containing two viral membrane glycoproteins (E and M). The total width of the enveloped virus is 45 to 50 nm.

Closely related viruses include the Japanese encephalitis, St. Louis encephalitis, and Murray Valley encephalitis viruses.<sup>1</sup> WNV is not a single virus, but a continuum of closely related viruses, whose pathogenicity to birds and other vertebrates vary significantly and are constantly changing. WNV strains can be divided into two lineages. Lineage 1 can be further divided into three clades (1a, 1b, and 1c). Closely related variants within clade 1 are responsible for outbreaks of disease in humans, horses, and/or birds in North Africa, Israel, Europe, and North America. Clade 1b includes the Kunjin virus that is widespread in Australia and appears to be active in Papua New Guinea and Irian Jaya, and clade 1c has only been isolated in India. Viruses from clades 1b and 1c cause little human or animal disease. WNV lineage 2 is confined predominately to Central and Southern Africa, and while sporadically causing human and animal disease, it is a relatively nonpathogenic virus.<sup>2</sup>

Since 1994 epidemics and epizootics of WNV disease have occurred in Northern Africa, France, Romania, Italy, Russia and Israel.<sup>3</sup> A strain of WNV essentially identical to the strain found in Israel was first identified in New York City, New York in 1999.<sup>2</sup> By the time of this writing, it has spread widely and has been identified in the majority of the States in the United States, several provinces in Canada, multiple islands in the Caribbean, and Mexico.<sup>4-9</sup> It is expected that it will continue to expand its range into Central and South America in the coming years. The recent outbreaks of WNV in Europe, Israel, and the United States are atypical of West Nile virus outbreaks in the past in that the recent outbreaks

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1055-937X/04/1302-0003\$30.00

doi:10.1053/j.sae.2004.01.002

have resulted in an increased incidence of disease in horses and humans, an increase in the severity of the disease seen in humans, and an increased pathogenicity for birds.

### Ecology and Epidemiology of WNV

Birds are the primary vertebrate host for WNV. Mosquitoes are the predominate arthropod vector. For a species of mosquito to be a competent vector for the WNV, the appropriate receptors must be present on endothelial cells lining the mosquito's midgut that allow WNV to invade and replicate in the cell. Additionally, the virus must be able to escape the midgut and then penetrate and replicate in the salivary glands. The most competent mosquito vectors are several species of the ornithophilic members of the genus *Culex*. Examples include *Culex pipiens*, *Cx. nigrapalpus*, *Cx. quinquefasciatus*, and *Cx. restuans*.<sup>10-13</sup>

Bird infection occurs when a bird is bitten by an infected mosquito. After a bird is infected, it remains viremic from 2 to 7 days. The magnitude of the viremia depends on the species infected. Crows and magpies (Family Corvidae), house sparrows (*Passer domesticus*), house finches (*Carduelis mexicanus*), and other passerines appear to develop the highest concentrations of virus in the blood and have the longest duration of viremia. WNV may persist in the skin after the cessation of viremia, allowing mosquito infection for as yet an undetermined period of time.<sup>14,15</sup>

Under the proper environmental conditions, the number of infected birds will reach a point that will allow virus transmission to man and other vertebrates. Transmission occurs by bridge vector mosquitoes, eg, *Aedes vexans* and *Ochlerotatus* spp., that will feed both on birds and mammals. Man, horses, and other mammals are dead-end hosts for WNV, as they do not develop a sufficient viremia to allow mosquito infection. WNV also infects reptiles and amphibians, and it is possible that some of these species may develop adequate blood concentrations of virus to permit mosquito infection.<sup>10,12</sup>

Mosquito-independent transmission between birds may occur. Ingestion of infected mosquitoes, infected mice, and even contaminated water has been shown to result in experimental infection. Therefore, wild crows and other birds consuming birds and mammals that die with WNV infection may themselves become infected.<sup>14</sup>

WNV was first recognized in North America in the late summer of 1999 in New York City, New York, USA. In 4 years, the WNV has spread across

the majority of the United States, southern Canada, and into Mexico and the Caribbean. The rapid spread of the WNV in North America and the Caribbean is believed to have occurred as the result of the movement of infected birds.<sup>16</sup> Outbreaks in Europe and Israel may also result from migration of viremic birds.<sup>3,16,17</sup> Based on the North American experience and studies in Europe, it is clear that once WNV is introduced into an area, it becomes enzootic. Studies in Europe and the USA suggest that seasonal re-emergence of the WNV in Europe and the United States may involve both migrating birds and the overwintering of the virus in mosquitoes.<sup>13</sup> Virus has been isolated from overwintering mosquitoes and can also be transmitted from infected female mosquitoes to a small percentage of their eggs, suggesting that this is the most important means of virus survival through the winter. However, the bird virus cycle of infection may persist year round in the tropics, and recently infected birds migrating north may also re-introduce the virus the following spring. The development of geographic variants of the WNV in the United States, however, argues that reintroduction plays less of a role than infected overwintering mosquitoes.<sup>7</sup>

In the enzootic portions of Europe and North America, infection in wild birds will begin building up in the spring and early summer. Peak mortality in birds is expected from the mid summer to early fall. Human and horse cases are expected to peak from one to a few weeks after bird mortality begins. In the southern United States and in Central and South America, it is assumed that West Nile virus has, or will, establish a year-round presence.

### WNV in Wild and Companion Birds

The impact of WNV on wild birds and companion birds is an evolving story and it most likely will take several years until the actual impact is known. Historically, in its original range, bird deaths were limited. Then, in the 1998 to 2000 Israeli outbreak, significant mortality was observed in domestic geese. Additional deaths occurred in a flock of migrating white storks (*Ciconia ciconia*), a lappet-faced vulture (*Torgos tracheliotus*), and a white-eyed gull (*Larus leucophthalmus*).<sup>3</sup> The first observed WNV disease in birds in North America occurred at the Bronx Zoo (Bronx, NY, USA) and the Queens Wildlife Center (Queens, NY, USA) in August and September of 1999. Twenty-five birds died (Table 1).<sup>18</sup> Since then, nearly 200 native and exotic species have died with WNV in the

**Table 1. Species of Birds That Died in the Initial Outbreak of West Nile Virus at the Bronx Zoo/Wildlife Conservation Park and Queens Wildlife Center, New York, August–September 1999**

| Common Name                | Latin Name                                | Number that Died |
|----------------------------|---|------------------|
| American crow              | <i>Corvus brachyrhynchos</i>              | 5                |
| Fish crow                  | <i>C. Ossifragus</i>                      | 1                |
| Black-billed magpie        | <i>Pica pica</i>                          | 2                |
| Chilean flamingo           | <i>Phoenicopterus chilensis</i>           | 5                |
| Black-crowned night heron  | <i>Nycticorax nycticorax nycticorax</i>   | 1                |
| Guanay cormorant           | <i>Phalacrocorax Bougainvillei</i>        | 3                |
| Laughing gull              | <i>Larus atricilla</i>                    | 2                |
| Bronze-winged duck         | <i>Anas specularis</i>                    | 1                |
| Mallard duck               | <i>A. platyrhynchos</i>                   | 2                |
| Himalayan Impeyan pheasant | <i>Lophophorus impeyanus</i>              | 1                |
| Blyth's tragopan           | <i>Tragopan blythi</i>                    | 1                |
| Northern bald eagle        | <i>Haliaeetus leucocephalus alascanus</i> | 1                |
| Snowy owl                  | <i>Nyctea scandiaca</i>                   | 1                |

Data from Steele et al.<sup>18</sup>

ongoing outbreak. For an updated list of species confirmed to have been infected with WNV, see [http://www.nwhc.usgu.gov/research/west\\_nile/wnvaffected.html](http://www.nwhc.usgu.gov/research/west_nile/wnvaffected.html).

## Passerines

**Corvidae.** The family *Corvidae* contains the crows, ravens, jays, magpies, and nutcrackers. Significant losses of American crows (*Corvus brachyrhynchos*) have been documented in the northeastern United States.<sup>14,19</sup> Experimentally, the American crow has been shown to be highly susceptible to WNV infection and disease. Fish crow (*C. ossifragus*) deaths from WNV infection have also been documented, but this species is less susceptible to disease than the American crow.<sup>14</sup> Although thousands of American crows are known to have died in the first 3 years of the WNV outbreak in the United States, an overall significant impact on numbers of this species has not been documented.<sup>20</sup> Additionally, as WNV spread into the southern and western United States, crow die-offs have not been observed to a similar extent.

Another member of the *Corvidae* that has been highly susceptible to WNV infection and disease is the blue jay (*Cyanocitta cristata*). WNV disease has been observed in the blue jay throughout its range. Again, although it is known that many blue jays have died with WNV disease, overall popula-

tions have not been shown to decline significantly. Experimentally, three of three black-billed magpies (*Pica hudsonia*) died when infected with WNV. The impact WNV will have on these birds in the wild still remains to be determined.<sup>14</sup>

**Other Passerines.** Observations made by bird watchers suggest that WNV has had a significant local impact on the population of black-capped chickadees (*Parus atricapilla*) in the northeastern and midwestern portions of the United States.<sup>21</sup> Experimental infections and field observations suggest that the house finch, house sparrow, and common grackle (*Quiscalus quiscula*) are also susceptible to WNV infection and disease, but mortality in these species will be significantly less than that seen in the American crow and blue jay.<sup>14</sup> Locally, WNV has been observed to be a significant cause of mortality in Eastern bluebirds (*Sialia sialis*), American robins (*Turdus migratorius*) and other thrushes, American cardinals (*Cardinalis cardinalis*), and ruby-throated hummingbirds (*Archilochus colubris*). Estimates of the impact of WNV on small passerine species, especially those wintering in the tropics, will be difficult to determine as nesting bird surveys are not widely done in the United States and Canada.

## Gulls, Flamingos, and Pelicans

The initial outbreak of WNV in New York included two laughing gulls (*Larus atricilla*), and

experimental infections with ring-billed gulls (*Larus delawarensis*) showed them to be susceptible to WNV disease.<sup>14,18</sup> Outbreaks in gulls have not, however, been a significant feature of the WNV outbreak in the United States to date. Five Chilean flamingos (*Phoenicopterus chilensis*) also died in the initial New York outbreak. Subsequent mortality in flamingos has not been documented, but use of the WNV vaccine in collections of these birds may be one of the reasons why.

During the summer of 2003, thousands of deaths occurred in white pelicans (*Pelecanus erythrorhynchos*) in the states of Montana, North and South Dakota, Kansas, and Minnesota. Both adult birds and nestlings were affected, but mortality was highest in prefledglings. WNV was very active in this portion of the United States in other birds, horses, and humans during this period.<sup>22</sup>

### Hawks and Owls

The WNV outbreak that occurred in the midwestern United States during the summer of 2002 is noteworthy in that a large number of hawks and owl species were affected. Red-tailed (*Buteo jamaicensis*) and red-shouldered hawks (*Buteo lineatus*), Cooper's (*Accipiter cooperii*), and Northern goshawks (*Accipiter gentilis*) were highly represented as wild and in falconry birds. Hundreds of great horned owls (*Bubo virginianus*), as well as barred owls (*Strix varia*) and Eastern screech owls (*Otus asio*) were found dead or with WNV disease (B. Dahlhausen, unpublished observations, 2002). A massive die-off of northern owls including snowy (*Nyctea scandiaca*), Northern hawk (*Surnia ulula*), and great gray owls (*Strix nebulosa*) occurred at a breeding and rehabilitation facility in Ontario, Canada (M. Taylor, personal communication, 2003). In contrast to this geographic region of the United States, owl and hawk mortality have not been noted to the same extent in the southern United States (D. Phalen, unpublished observation, 2003).

### Companion Birds

The aviculture community collectively held its breath as WNV swept across the southern United States in the summer of 2001 and 2002. Huge outdoor aviaries of psittacine birds are located in these states. Fortunately, minimal mortality has been observed in parrots in the United States, even in those housed outdoors. Documented cases of WNV have occurred in at least one blue and gold macaw (*Ara ararauna*), cockatiel (*Nymphicus hollandicus*), budgerigar (*Melopsittacus und-*

*uatus*), lorikeet (*Trichoglossus* spp), green-cheeked Amazon parrot (*Amazona viridigenalis*), and two conures (*Aratinga* sp.).<sup>23,24</sup> A study conducted in Louisiana suggested that asymptomatic infection with WNV in psittacine birds was common.<sup>24</sup> Infections trials using the budgerigar and the Quaker parrot (*Myiopsitta monachus*) failed to induce disease in these birds.<sup>14</sup>

Most smaller species of cage bird are housed in doors and would be considered a low risk for being infected with WNV. However, an entire collection of mixed species of Australian finches was lost to WNV disease in Texas during the summer of 2003 (R. deRusha, personal communication, 2003).

### Clinical Findings in Wild and Companion Birds with West Nile Virus Disease

The clinical signs of WNV disease in birds vary from an unexpected death, to nonspecific signs, to those of a progressive neurological disorder. Typically, the duration of the disease is a few hours to a few days. However, birds that are aggressively treated and survive may show signs for several weeks, and some clinical observations suggest that some parrots may have persistent neurological signs after recovery (S. Clubb, personal communication, 2001).

In the first described outbreak in the United States, neurological signs including, ataxia, tremors, abnormal head posture, circling, and convulsions were predominate presenting signs. Evidence of ocular disease including anisocoria and impaired vision were also reported.<sup>15</sup>

Raptors with WNV disease in the midwestern United States outbreak of 2002 presented with a brief (<2 days) history of decreased appetite, decrease in body weight, and a mild, dull mentation. Mutes appeared normal. Affected birds exhibited a progressive clinical course of disease ending in death within 72 to 120 hours after the initial onset of clinical signs. Regurgitation of feedstuffs occurred followed by complete anorexia. Birds exhibited enteritis and an increasing polyuria and biliverdinuria. Altered mentation progressed until the birds became recumbent and nonresponsive to external stimuli. Seizures occurred late in the course of the disease and ended with death of the individual (B. Dahlhausen, unpublished observation, 2002).

During this same outbreak, great horned owls that survived infection for several days developed a continuous "bobbling" of the head that would

cease temporarily when the birds ate. Many of these birds subsequently went blind as the result of a WNV-induced retinitis (P. Redig, personal communication, 2003).

A ne-ne goose (*Branta sandvicensis*) from the Houston Zoo experienced a shifting leg lameness and another became recumbent. Both showed an unusual manifestation of the disease, in that they kept their mouths open and had persistent "tongue flickering."<sup>25</sup>

Experimentally infected house sparrows, house finches, bluejays, laughing gulls, and American and fish crows showed nonspecific signs of lethargy and ruffled feathers, followed by neurological signs including unusual posture, inability to hold their head upright, and ataxia. Birds died within 24 hours of the onset of signs. External hemorrhage from the mouth or cloaca occurred in the American crows.<sup>14</sup> Depression, torticollis, opisthotonus, and death occurred in four 2-week-old geese experimentally infected with WNV.<sup>26</sup>

Mild to moderate neurological signs were observed in several parrots that were positive by the hemagglutination assay for WNV antibodies (S. Clubb, personal communication, 2001). Experimentally infected budgerigars and Quaker parrots did not develop clinical disease.<sup>14</sup>

### Diagnosis of West Nile Virus Infection in the Live Bird

WNV infection should be considered in birds, particularly wild birds and birds housed out of doors, presenting with neurological signs during the warm months of the year. Complete blood counts may reveal a moderate leucocytosis and heterophilia with reactive heterophils and lymphocytes as was observed in raptors in the summer of 2002 (B. Dahlhausen, unpublished observation, 2002). Biochemical findings have not been reported, but myocardial disease is common in some species of birds and elevation in the creatinine phosphokinase is expected with this lesion. Alterations in the electrocardiogram are predicted, but have not been reported. Pancreatitis is also a commonly reported lesion and increased serum amylase concentrations may also occur.

Birds that are sick with WNV disease are likely to be viremic and at least in crows and in some raptors, virus can be detected in the saliva and cloaca.<sup>27,28</sup> These birds then should also be positive with PCR of oral and cloaca swabs and blood (B. Dahlhausen, unpublished observation, 2002).

Serology can also be used to support the hypothesis of a recent WNV infection. A hemaggluti-

nation inhibition assay (HAI) for WNV antibodies is widely available. A plaque reduction assay is also being used, but it not widely available and the turn around time for results has been long. The HAI must be interpreted with caution, as antibodies to other flaviviruses can also cause this assay to be positive.<sup>29</sup> In a survey of birds in an aviary in Louisiana, many of the birds that were positive with the HAI were negative using the plaque reduction assay.<sup>24</sup> This data suggests that the birds that were HAI positive, but negative on the plaque reduction assay, had circulating antibodies to other flaviviruses. Birds may maintain circulating antibody to WNV for some time after infection; therefore, the most conclusive serologic proof that a bird was infected with WNV would be to demonstrate a rising antibody titer in paired serum samples using the plaque reduction assay. Even the plaque reduction assay is influenced by antibodies to other flaviviruses. As a result, scientific researches using this tool need to show that positive antibody titers are higher when using the WNV as compared with other flaviviruses to which the birds might have been exposed.<sup>30,31</sup> Only one of five raptors presenting with confirmed WNV disease were seropositive within 5 days of presentation. All five of these birds, however, were positive for WNV by PCR of blood (B. Dahlhausen, unpublished observation, 2002).

### Treating Birds with West Nile Virus Disease

There have been no controlled studies on the treatment of birds with WNV and the information available from practitioners who have seen this disease is limited. Most birds with WNV disease die quickly before there is time for treatment, and treatment efforts in many birds have been unsuccessful (R. Dahlhausen, unpublished observations). In cases where birds have survived, it is clear that a key to treatment of WNV disease is aggressive supportive care. Antiinflammatory medications such as flumixine meglumide, dexamethasone, and DMSO have been suggested as having a benefit in reducing signs in horses. Mannitol may benefit a bird if it has cerebral edema and vitamin E may assist healing by providing an antioxidant effect.<sup>8,32</sup> Experimental trials in mice and cell culture systems have been done to test the possible benefits of using ribavirin and interferon  $\alpha$ -2b. These experiments suggest that ribavirin may prevent infection, but conflicting results make it unclear whether ribavirin will benefit an animal once the infection is established. In a cell



**Figure 1.** Myocardial necrosis from West Nile virus infection in a Northern Goshawk (*Accipiter gentiles*)

culture system, interferon  $\alpha$ -2b was found to be both protective and therapeutic.<sup>33</sup>

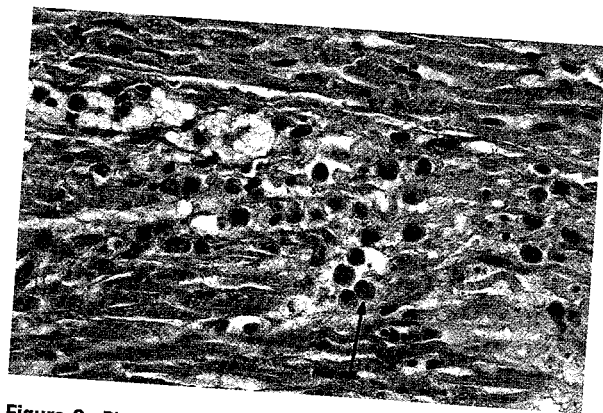
### Postmortem Diagnosis of West Nile Virus Infection in Birds

If one suspects that a bird may have died as the result of WNV infection, it is best to have this animal necropsied by a laboratory with the appropriate biosecurity facilities. Veterinarians and others involved in the necropsy or tissue processing of birds dying during the summer and fall, especially those that had a history of neurological signs should take the following precautions. A mask and goggles should be worn to prevent inhalation or conjunctival exposure to aerosolized body fluids. Double gloving will reduce the risk of direct contact between bird tissues and cuts or sores on hands. Extreme care should be taken when handling scalpel blades, knives, and needles to prevent skin penetration. If unprotected skin is directly exposed to animal tissues or body fluids, it should be immediately and thoroughly washed with soap and water. More detailed biosecurity procedures can be found at [Http://www.nwhc.usgs.gov/research/west\\_nile/wnv\\_guideline.html](http://www.nwhc.usgs.gov/research/west_nile/wnv_guideline.html).

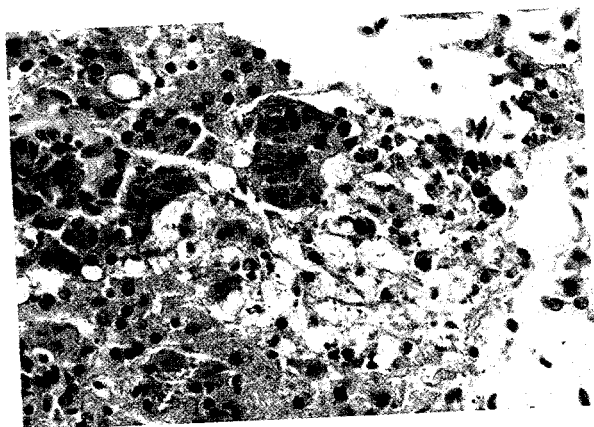
Necropsy findings suggestive of WNV disease include intraosseous hemorrhage of the calvaria, diffuse or localized hemorrhage of the meninges, congestion of the brain, and hemorrhage within the brain. Hemorrhage of the mucosa and serosa of the gastrointestinal tract is also reported. Pancreatic hemorrhage or discoloration may also occur. Splenomegaly is minimal to marked. Focal, linear, or diffuse myocardial pallor (myocardial necrosis) may also be present (Fig 1).<sup>18</sup>

Microscopic lesions are common in the brain, heart, pancreas, and adrenal gland. Multifocal hemorrhage, particularly in the cerebellar folia, but also in other parts of the brain and in the spinal cord is a common lesion.<sup>18</sup> Mild to severe lymphoplasmacytic meningitis is also common. A nonsuppurative encephalitis is most common in the cerebellum and brain stem, but can be found in multiple areas of the brain when the lesions are severe. Necrosis of the Purkinje cells is another common lesion.<sup>18</sup>

Heart lesions include a mild to severe, diffuse lymphoplasmacytic and histocytic epicarditis, myocarditis, and endocarditis (Fig 2). Myocardial degeneration and mineralization is sometimes seen without associated inflammation.<sup>18</sup> Two nestling parrots, with severe diffuse myocardial mineralization were found to be positive for WNV infection by PCR (W. Wigle, personal communication). A lymphoplasmacytic pancreatitis (Fig 3) of varying severity occurs in many birds, as does inflammation of the adrenal gland. Lymphoid depletion or splenic necrosis are additional common histological lesions. Pulmonary hemorrhage and congestion, multifocal hepatic necrosis, severe congestion and dilation of serosal and mucosal vessels of the intestines, and a heterophilic



**Figure 2.** Photomicrograph of myocardial necrosis with plasma cell infiltrate in northern Goshawk (*Accipiter gentiles*) with West Nile virus infection.



**Figure 3.** Photomicrograph of pancreatic necrosis in a Northern Goshawk (*Accipiter gentiles*) with West Nile virus infection.

and lymphoplasmacytic enteritis are less consistent lesions.<sup>18</sup>

Infection in the dead bird can be confirmed by detecting viral antigen in tissues, detecting viral RNA in tissues by PCR or by isolation of the virus in cell culture.<sup>27,28,34,35</sup> A rapid antigen-capture wicking assay (VecTest, Medical Analysis Systems, Camarillo, CA, USA) has been shown to have an approximately 84% sensitivity and 95% specificity when used with oropharyngeal swabs of corvids dying with WNV disease.<sup>27</sup> PCR also has proved to be very useful, and many diagnostic laboratories offer this assay. Oral swabs, brain, heart, kidney, liver, lung, and spleen are all tissues that are expected to be positive in birds with WNV disease.<sup>28,35,36</sup> Similar tissues can be used for virus isolation in Vero cells.<sup>34</sup> However, brain tissues appears to be the best tissue to use for virus isolation. Five of five raptors dying in the midwestern United States outbreak of 2002 were positive on PCR of postmortem tissue swabs of the kidney and adrenal gland (B. Dahlhausen, unpublished observation, 2002). Anti-WNV antibodies and anti-St. Louis encephalitis antibodies can be used for immunohistochemistry in some diagnostic laboratories to detect virus infected cells. Virus particles have also been detected using electron microscopy.

### WNV in Poultry

Chickens and turkeys are relatively resistant to disease caused by WNV and are believed to be dead-end hosts.<sup>36-38</sup> Experimentally, infection with the NY 1999 strain of WNV did not result in clinical signs in chickens, but all chickens seroconverted. Low concentrations of virus in the

blood, insufficient to allow mosquito infection, were only detected for 2 to 3 days.<sup>36</sup> In another infection trial, chicks less than 11 days old were more susceptible to infection and developed higher concentrations of virus in the blood and remained viremic for longer periods of time.<sup>37</sup> Experimental infections of turkeys with the NY 1999 strain resulted in a short-lived, low virus titer, viremia. Clinical signs, virus shedding, and horizontal transmission did not occur.<sup>39</sup> Because of their susceptibility to infection, but resistance to disease, chickens are being used as sentinels to monitor WNV activity. A combination of a capture IgM ELISA and an indirect IgG ELISA was found to be highly effective in detecting chickens infected with WNV.<sup>29,37,39</sup> A blocking ELISA has been developed that has been found useful in multiple species of birds.<sup>40</sup>

Domestic geese, in contrast to chickens and turkeys, have shown a high susceptibility to WNV disease. WNV caused high morbidity and mortality in flocks of domestic geese in Israel from 1997 to 2000. Affected geese had an acute onset of paresis.<sup>41</sup> Experimentally infected geese rapidly develop a viremia that is sufficient to result in mosquito infection. Viremia persists for seven days or less. Virus could also be detected in the oropharynx three to four days after infection. Infected geese showed depression and weight loss. Two birds died. Necropsy findings included a poor body condition, atrophy of the bursa and thymus, a moderate nonsuppurative meningoencephalitis, a severe diffuse heterophilic to lymphocytic myocarditis, and pancreatic acinar cell necrosis. Some of the birds with the meningoencephalitis also had undescribed "ocular lesions."<sup>26</sup>

### Prevention of West Nile Virus Infection in Birds

WNV has created panic in the general bird-owning population in the United States and has been of great concern to wildlife and zoo veterinarians. Immunization studies in hamsters<sup>30</sup> and horses suggest that a vaccine that can induce an antibody response and will protect many animals against infection and may help protect the animals that become infected from developing disease. At the time of this writing, there is only one commercially available vaccine, the West Nile virus vaccine (Fort Dodge Laboratories, Fort Dodge, IA), and it is only approved for use in horses. The ability of this vaccine to protect birds against WNV infection and disease is unclear. The equine dose is 1 mL. Experimental immunization using a 0.5-mL



dose given twice, 2 weeks apart, in cockatiels,<sup>42</sup> 0.2 mL in red-tailed hawks and flamingos,<sup>43</sup> given twice 3 weeks apart, and 1 to 2 mL given to sand hill cranes 3 times at 2-week intervals, failed to illicit a detectable humoral immune response to the vaccine. Six vaccinated and five unvaccinated cranes were challenged with WNV. None of the birds showed signs of disease with the exception of a mild weight loss, but all birds became infected and viremic. The level and duration of the viremia was less in the vaccinated cranes, suggesting that the previous immunizations may have provided some degree of protection. Mild histological lesions were found in all challenged birds.<sup>44</sup>

Many zoo veterinarians have elected to use the commercial equine West Nile virus vaccine to immunize birds. Three species of birds at the Houston Zoo were monitored for the development of an antibody titer following immunization and the susceptibility to natural infection with WNV. Twenty-five Attwater's prairie chickens (*Tyrannus cupido attwateri*) were administered 0.5 mL of the vaccine three times at 3-week intervals. Twenty-four Chilean flamingos and three red-crowned cranes (*Grus japonensis*) were also immunized three times, but with the full 1.0-mL dose. Other birds were immunized, but antibody titers were not determined or not available. The only birds to seroconvert were 15 of the 24 flamingos. During the summer of 2002, five birds died at the Houston Zoo that were found to be infected with WNV. Only one of these birds, a Mauritius pink pigeon (*Columba kubaryi*) had been immunized and it had concurrent chlamydophilosis. Adverse reactions to the vaccine have only been reported in one instance.<sup>25</sup> Four of 18 green-naped lorries died with suspected hemolytic anemia when they were immunized 1 year after a first round of three 1-mL doses. The birds died 2 to 10 days after their booster immunization.<sup>45</sup> The author is not aware of other adverse reactions to the West Nile Virus vaccine in birds.

Based on the above observations, the author's current recommendations regarding measures for preventing WNV infection are to focus first on preventing exposure to mosquitoes. When possible, birds should be kept inside or in a screened enclosure. Eliminating mosquito breeding grounds, using mosquito traps, and judicious spraying will also reduce the risk of exposure. The choice to use the vaccine will be an individual decision based on the knowledge that there appears to be only a small risk associated with its use,

there is also only minimal data to suggest that it will benefit a bird, and the use of the vaccine is a bird is extra label. It appears that most zoos have chosen to immunize high-risk species, such as crows, flamingos, geese, grouse, shorebirds and birds of prey. If an immunization program is to be used, initially, three 1-mL doses of the vaccine should be administered at 3-week intervals. The vaccine can be divided and given in multiple sites in smaller birds. Subsequently, birds immunization should be done every year before the onset of warm weather.

Disease in psittacine birds is rare. Therefore, immunization of psittacine birds is not recommended at this time. Concerned pet owners should keep their birds indoors in the warmer months of the year, particularly during peak periods of mosquito activity.

Research continues on vaccines that may protect birds. Recently, a plasmid vaccine containing the genes for the virus envelop glycoproteins M and E was tested in fish crows. The vaccine was effective at preventing illness and death in the fish crows if administered intramuscularly. It did not, however, prevent infection. The vaccine did not provide protection to crows that were given it orally.<sup>46</sup>

### West Nile Virus in Horses in North America

Several of the new strains of WNV that emerged in the 1990s have proved pathogenic for horses. Before WNV introduction into North America, however, outbreaks were localized and, for the most part, occurred one year and not the next. Outbreaks in Morocco in 1996, Italy in 1998, and France in 2000 only caused disease in horses, and associated bird and human disease were not observed. The strains isolated from the 1998 to 2000 Israel outbreak and the ongoing outbreak in North America, however, are unique in that they are pathogenic for horses, birds, and humans and have recurred year after year.<sup>3,8,32,41,47,48</sup>

WNV is highly infectious to horses but has a low virulence. Only a small percentage of horses infected with WNV ultimately develop signs. The incubation period is not known but may be approximately 8 days. Horses of all ages and breeds appear to be equally susceptible. Clinical signs are those expected of myeloencephalitis. They include ataxia, weakness, muscle fasciculations, fever, hyperesthesia, depression, and recumbency. In one study, depression, seizures, narcoleptic-like episodes, muscle fasciculations, and vestibulo-



lar ataxia, were the signs that were most suggestive of WNV disease. Other signs of WNV disease in horses include cranial nerve deficits including a droopy lip, fasciculations of muscles of the face, and, uncommonly, blindness. Forty-three percent of horses hospitalized for WNV disease, in one study, were euthanized or died. The surviving horses recovered with no or few permanent neurological deficits. WNV infection has been confirmed in 3991 horses in 41 states in the United States from January to the end of October 2003.<sup>8</sup>

The use of antiinflammatory medications including flunixin meglumine, dexamethasone, and DMSO have been suggested to reduce CNS inflammation. Mannitol may be beneficial if CNS edema is present, and vitamin E may provide a positive antioxidative effect. Fluid therapy and nutritional support is required in some animals. Medical care of up to 14 days may be necessary.<sup>8,32</sup>

Definitive diagnosis in the live horse is difficult. Clinical signs are often suggestive of WNV disease, but a number of other diseases may result in similar signs. Abnormalities in the cerebrospinal fluid including a mononuclear pleocytosis and/or an increase in protein are relatively common findings. Hemagglutination inhibition has been the most commonly used serologic assay to verify WNV infection.<sup>8,32</sup>

A killed West Nile Virus vaccine (Fort Dodge Animal Health, Fort Dodge, KS) is available and appears to provide protection against WNV disease in horses. A recombinant DNA vaccine is also being developed and shows promise.

## West Nile Virus Infection in Other Animals

**Mammals.** Two species of North American squirrels and at least one North American chipmunk may be predisposed to WNV disease.<sup>4,49,50</sup> Many mammals including a sheep, llamas, alpacas, dogs, and cats have been infected with WNV and developed disease.<sup>4,41</sup> Immunization of llamas and alpacas has been done in areas where WNV infection in other species has been high. The need and the usefulness of this vaccine in these animals is still to be determined.

Nonhuman primates are susceptible to WNV infection, but generally appear to be resistant to disease when naturally infected. In an extensive study done in a primate breeding facility in Louisiana, a 36% infection rate was detected in 192 primates sampled as judged by serology. All spe-

cies, including rhesus monkeys (*Macaca mulatto*), baboon (*Papio* spp.) and pigtail macaques (*M. nemestrina*) were found to be infected. Significantly, disease was not observed in these animals.<sup>51</sup> Serologic studies have detected antibody-positive lemurs and chimpanzees.<sup>41</sup> The potential impact of WNV on New World primates is not known.

## Reptiles and Amphibians

There is a report of farmed alligators developing WNV disease. The outbreak occurred 2 years in a row. Infected horsemeat was thought to be the source of the infection. Other management factors may also have played a role in this outbreak.<sup>52</sup> Crocodiles in Israel developed antibodies to WNV, but did not become ill.<sup>53</sup> A monitor lizard from Washington, D.C. developed neurologic signs and WNV was isolated from it. A species of frog (*Rana ridibunda*) has been experimentally infected with WNV, and although it became viremic, it did not show signs of disease.<sup>42</sup>

## West Nile Virus in Humans

WNV was first isolated from a woman in Uganda in 1937. It was subsequently isolated from the blood of three children in a village north of Cairo in 1950. Studies during the 1950s showed that WNV infection was enzootic in the Nile river basin and other places in Africa. Infected people either had a transient febrile illness or showed no symptoms. Rarely, WNV infection resulted in central nervous system disease. The first epidemic of WNV occurred in Israel in 1951 and 1952. Subsequent epidemics also occurred in Israel in 1957, 1962, and 1980. Epidemics have also been documented in several African Nations. The largest occurred in 1974 in South Africa when an estimated 18,000 people were believed to be infected with WNV. These epidemics characteristically affected children and resulted in a mild febrile illness accompanied by headache, myalgia, anorexia, abdominal pain, and vomiting. None of these people died from WNV infection. Severe neurological disease was seen for the first time in 1957.<sup>3</sup>

The 1990s brought about a dramatic change in the pathogenicity of WNV for humans. Disease was now more likely to result in neurological manifestation, to affect the elderly, and to result in death. Significant outbreaks in people have been documented in Algeria in 1994, Tunisia in 1997, Romania in 1996, Russia in 1999, and Israel in

1999 and 2000. The Romanian, Russian, and Israeli outbreaks were notable as hundreds of people were hospitalized with neurologic signs in each outbreak and there was a significant mortality rate in these patients.<sup>3</sup>

The greatest human impact of WNV has occurred since its introduction to North America in 1999. Studies on naturally infected people in the North American outbreak suggest that the incubation phase for naturally acquired WNV infection is 2 to 14 days. Most WNV infections in humans are asymptomatic. Twenty percent or less of infected people develop clinical symptoms. The mild form of the disease, West Nile Fever, lasts 3 to 6 days. Signs include malaise, anorexia, nausea, vomiting, eye pain, headache, myalgia and in some cases, a rash that develops over the chest, back, and arms. The rash is an increasingly inconsistent sign.<sup>54-57</sup> As of the first week of November 2003, the Centers for Disease Control in the United States had documented 8219 human cases of WNV for the year 2003. It is assumed that this number represents only a small percentage of the actual number of infections.<sup>4</sup>

Less than 1% of WNV infections result in an acute neurological illness, and this form of the disease is seen predominately in elderly patients and, to a lesser extent, children. Three distinct manifestations of WNV infection of the central nervous system are reported: encephalitis, meningitis, and acute flaccid paralysis. Symptoms of meningitis include nuchal rigidity, Kernig (The leg cannot be fully extended in a sitting position.) or Brudzinski signs (Flexion of the neck usually results in flexion of the hip and knee.), photophobia, phonophobia, as well as, fever or hypothermia. Cerebral spinal fluid analysis typically reveals a pleocytosis and peripheral blood leukocyte counts are usually greater than 10,000 cells per microliter. Neuroimaging findings are consistent with meningeal inflammation. WNV encephalitis causes depression, altered levels of consciousness, lethargy, changes in personality and fever or hypothermia. Results of cerebral spinal fluid and peripheral blood analysis are similar to those found with WNV meningitis. People with acute flaccid paralysis experience a limb weakness with a marked progression over the ensuing 48 hours. Prognosis is guarded for anyone with the neurological manifestations of WNV infection (fatality rates of 4 to 18% are reported) and is grave for people with acute flaccid paralysis. Persistent neurological deficits are common in people who sur-

vive the neurological manifestations of WNV infection.<sup>54-57</sup>

The majority of human infections result from the bite of an infected mosquito. Human infection may also occur under other circumstances. Two microbiologists collecting samples from dead birds infected themselves through skin wounds from contaminated dissecting instruments.<sup>58</sup> Blood products from viremic donors can also transmit WNV to their recipients. In trace-back studies, 15% of 583 people that were given WNV-infected blood products developed West Nile fever. Only 1% developed neuroinvasive disease.<sup>59</sup> Transplantation of organs from infected donors have also resulted in infected recipients.<sup>60</sup> The incidence of severe disease was high in these patients because they were immunologically suppressed. Currently, in the United States, blood and organs are screened for WNV before use. Virus transmission from mother to infant through breast milk is suspected in one case, but this is most likely an extremely rare occurrence.<sup>61</sup>

Infection in humans is confirmed with an IgM capture ELISA using CSF or plasma. IgM can persist in the blood for 500 days, so a rising titer is necessary to confirm a recent infection. Complicating diagnosis further is that cross reactive antibodies to WNV can be found in the blood of individuals previously infected with St. Louis encephalitis virus and Dengue virus and those vaccinated for yellow fever.<sup>54-57</sup>

## Conclusion

Although WNV is not a highly virulent virus for humans, horses, and most birds, it has caused a significant amount of disease and death in all these species. The story of WNV is not complete. There is little doubt that this virus will continue to spread into Central and South America and its impact on the native animals of these areas remains to be seen. It is also likely that it will forever be a present in North America. Again, whether it will continue to cause outbreaks of disease in wild birds or whether disease will be sporadic or occur at a constant but lower level will only be determined with time. Based on the movement of this virus and evolution over the past 10 years, it is most likely that we are only at the beginning of the WNV story and that it will continue to impact peoples and animals around the world for many years to come.

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