

Review

The West Nile virus: its recent emergence in North America

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ABSTRACT – West Nile fever emerged in New York in the summer of 1999 when seven people, several horses and thousands of wild birds died. It was soon established that the human disease and the mortality of birds were related. Continued surveillance detected West Nile virus in mosquitoes, birds, horses, small mammals, bats and humans, and has shown its spread to several northeastern states. These events confirm the establishment of West Nile virus endemically in the United States.
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West Nile virus / North America

1. Brief review on West Nile fever

West Nile virus (WNV) was first isolated from the blood of a woman with a febrile disease in the West Nile district of Uganda in 1937 [1]. It was subsequently recovered from people, birds and mosquitoes in Egypt in the 1950s [2]. Soon it was recognized to be one of the most widespread of the flaviviruses, with a vast geographic distribution, which now includes North America. WNV is small single-stranded RNA viruses of 40-nm size with a lipid envelope (*figure 1*), a member of the Japanese encephalitis antigenic group within the family Flaviviridae. It is closely related to the Kunjin virus, which exists in Australia and Southeast Asia [3]. All of these viruses are transmitted among various animal species by mosquitoes. Man and some species of domestic animals represent accidental hosts, becoming infected when reservoir hosts and the appropriate vectors occur in large numbers. In both man and animals, infection with the WNV induces various responses, from subclinical disease, to short duration flu-like illness, to frank encephalitis and death. It has been known for some time that this virus is maintained in nature by a sylvatic cycle, i.e. bird-to-bird transmission via a mosquito vector, similar to such cycles in other arthropod-borne encephalitides.

Recently, a major outbreak of West Nile fever occurred in Romania (1996 and 1997) during which there were more than 500 human cases, with approximately 10% mortality [4, 5]. The WNV was recovered from *Culex pipiens* mosquitoes, and antibodies to the virus were found in 41% of domestic fowl [4]. In geographic areas

such as Africa, the Middle East and Southwestern Asia, where the disease is endemic, the virus is maintained in nature in enzootic bird–mosquito–bird cycles, which are nearly impossible to eliminate. In these areas, WNV has been isolated from many species of mosquitoes, predominantly of the genus *Culex*, but also from *Aedes* and *Coquillettidia* [2]. Sporadic cases of West Nile fever of humans and equines occurred in France and Russia (1962–1964), Belarus and Ukraine (1970s and 1980s), the Czech Republic (1997) and Italy (1998). Many cases have been documented from Israel and South Africa. Fifty cases with over 10% mortality were recorded in Algiers in 1994. Other outbreaks have been observed in Azerbaijan, Central African Republic, Democratic Republic of Congo, Egypt, Ethiopia, India, Madagascar, Nigeria, Pakistan, Senegal, Sudan and several European countries [2]. Serologic evidence of exposure to WNV has been detected in humans in Armenia, Borneo, China, Georgia, Iraq, Kenya, Lebanon, Malaysia, the Philippines, Sri Lanka, Sudan, Syria, Thailand, Tunisia and Turkey [6–8]. Most recently an outbreak of meningoencephalitis caused by the WNV was reported in the Astrakhan, Krasnodar and Volgograd regions in southern Russia, where approximately 1 000 cases and 40 deaths were reported [9].

2. Emergence of West Nile fever in North America in 1999

West Nile fever emerged in the northeastern United States in the late summer and early autumn of 1999 [10–13]. At its peak, the WNV claimed the lives of seven people in Queens, New York and caused the death of hundreds of wild birds, principally American crows (*Cor-*

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Figure 1. WNV isolated from a crow brain in Vero cells. This crow was one of several hundreds of birds that were examined at our laboratory for pathologic and virologic evidence of West Nile fever during the 1999 outbreak. The virions (arrows) are 40–45 nm in diameter, spherical and enveloped ($\times 37\,500$). Electron microscopy was performed by Drs A.B. West and A. Kumar, Department of Pathology, New York University Medical Center.

us brachyrhynchus), in New York, New Jersey and Connecticut and of horses on Long Island, New York. A lethal infection of a cat was also reported in New Jersey [14]. This outbreak constituted the first to appear on the American continent. Although initially thought to be Saint Louis encephalitis (SLE), a closely related flavivirus, WNV was subsequently identified as the etiologic agent of encephalitis observed both in humans and birds [4, 8, 11, 13]. The distribution of West Nile fever in the years 1999 and 2000 is shown in *figure 2*.

3. Human deaths from West Nile encephalitis

The death of two people with encephalitis late in August 1999 marked the beginning of a limited outbreak of West Nile fever that would eventually claim the lives of five others. In New York a total of 61 individuals were known to have been infected [15]. However, by extrapolation from a serosurvey conducted by the Centers for Disease Control and Prevention (CDC) in October 1999, it is estimated that approximately 2.6% of residents of Queens, New York, the epicenter of the outbreak, may have been

infected. The majority of affected individuals were from the borough of Queens; fewer cases occurred in the Bronx, Manhattan, Brooklyn, and Westchester and Nassau counties [16]. The clinically ill had fever (90%), muscle weakness (57%), headache (50%), alterations in mental status (47%), rash (24%) and stiff neck (21%). Profound muscle weakness requiring respiratory support was a prominent condition observed in four individuals [11, 17]. One patient with concurrent HIV had a 1-week history of headache, fever, neck stiffness, photophobia and vomiting [18]. In this instance, a lumbar puncture revealed increased cerebrospinal fluid pressure, pleocytosis and increased protein.

Early on, the cluster of cases was thought to be SLE [11], a viral encephalitis indigenous to the southwestern United States. Concurrent with the human disease, a regional die-off of crows was recognized, and there were fatalities among exotic birds at the Bronx Zoo. Viruses were recovered from tissues of birds from the latter site, and these were identified as agents closely related to the WNV. The virus that caused the deaths in New York was initially identified by genomic analysis as Kunjin/West Nile-like flavivirus [12]. Sequencing soon established that it was in reality the WNV. Immunohistochemistry and PCR later demonstrated this virus in brain tissues of four individuals who had died [19]. Focal Glial nodules, with lymphocytes and histiocytes, and sparse perivascular cuffs that sometimes extended to the meninges characterized the encephalitis. Cranial nerve roots were affected as well in two patients. One person had concomitant pancreatitis.

Studies completed after the fact have shown that sera and cerebrospinal fluid specimens that were reactive to SLE by IgM ELISA had a much higher reactivity to WNV [11]. PCR detected viral sequences in the cerebrospinal fluid of four individuals who died with West Nile fever, but only in one of four individuals who survived [20]. The retesting of serum samples from patients with encephalitis yielded stronger positive responses to WNV than to SLE.

4. Bird deaths from WNV infection

During the peak of the 1999 outbreak a significant mortality of native wild birds and exotic birds in captivity was reported in Queens, New York and around the Bronx Zoo. In Connecticut, the WNV was first recovered from crows (*C. brachyrhynchus*), and later in a Cooper's hawk (*Accipiter cooperii*) and *C. pipiens* and *Aedes vexans* mosquitoes [10]. WNV was isolated from a crow in Baltimore, the only place outside the three states, New York, Connecticut and New Jersey, most affected. The virus isolates were found to be closely related to two Romanian and one Israeli isolate of WNV. The isolation of WNV from relevant invertebrate vectors [2], along with environmental conditions and potential animal reservoir hosts in the affected regions strongly suggested that WNV would become endemic in the United States. Reservoirs within vertebrate and invertebrate species may have already been established in the affected areas. In fact, in the year 2000 WNV re-emerged in New York, New Jersey, Massachusetts, Rhode Island and Connecticut.

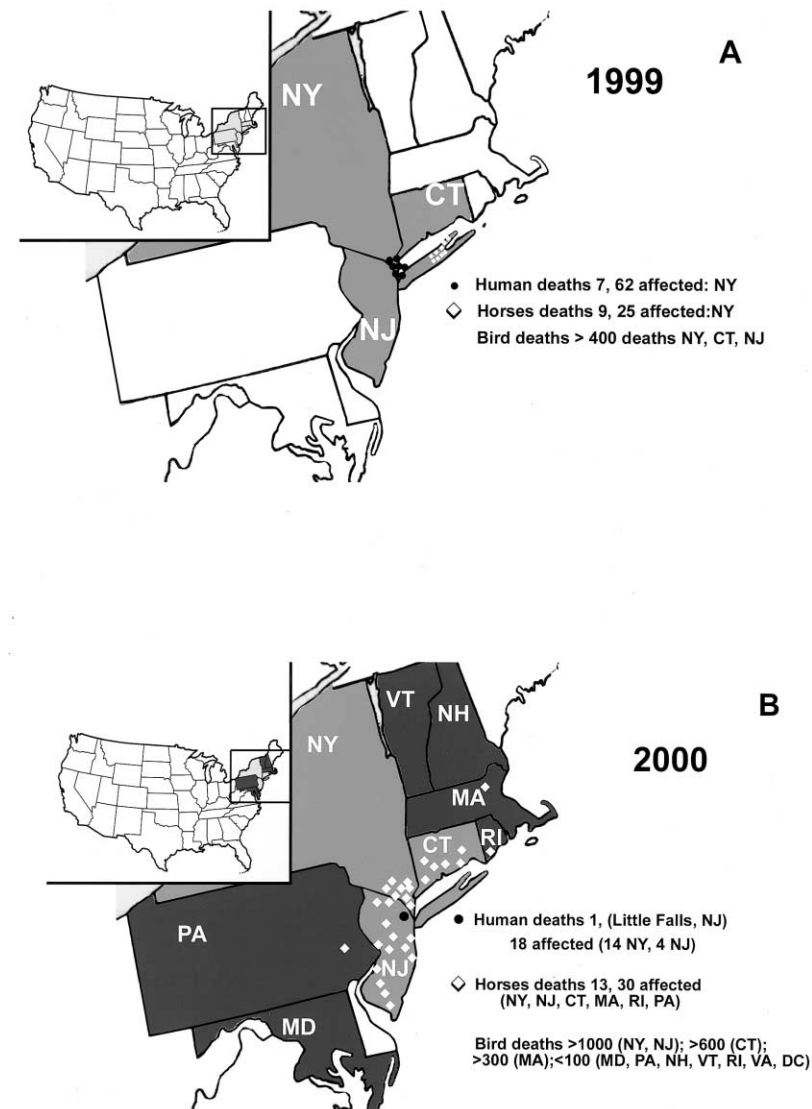


Figure 2. Distribution of West Nile fever cases in the United States. A: Human fatalities (●); states where the highest bird mortality was recorded (shaded areas), area where horse fatalities occurred (◇) in the year 1999. B: Human fatality (●); states where bird fatalities occurred (darker shades indicate new states where the virus has been detected); and horse cases and fatalities (◇) in the year 2000.

The West Nile outbreak in birds in the United States presented unique epizootic characteristics, which differed in some aspects from those described in Africa, the Middle East, western Asia and more recently in eastern Europe [2]. For one, the North American WNV caused large-scale mortality of wild native and exotic birds that did not occur in Romania [4] or the Czech Republic [21] during outbreaks there. Interestingly, during a recent WN outbreak in Israel, geese were reported to be susceptible and to have died from it [22, 23], while deaths in geese were not reported in the United States. However, sub-clinical infections in mallards, pigeons and Canada geese were discovered [14]. Hundreds of birds died in New York and Connecticut, many of which were examined for pathologic and virologic evidence of WNV. The majority were crows. A relatively small proportion of the birds examined had encephalitis, most appearing to have died from multisystem disorders. The few that had encephalitis had perivascular cuffing and some death of neurons, with satellitosis

and neuronophagia. Glial nodules did not occur in the wild species we tested.

Veterinary pathologists at the Bronx Zoo and a wildlife pathologist in upstate New York suggested, independently, that the deaths of birds in southern New York might be related to the encephalitis of humans, both of which occurred during August and September. At the Bronx Zoo, necropsy of two Chilean flamingos, a cormorant and an Asian pheasant revealed encephalitis and myocarditis. It was tissue specimens from these birds that yielded virus. Cross-reactivity between WNV with SLE accounted for an initial consideration of SLE. Comparative analysis of a virus isolate (WN-New York 1999) from a Chilean flamingo (*Phoenicopterus chilensis*) and from tissues from humans showed that the same virus was responsible for both the avian and the human disease [13]. Phylogenetic analysis of the E-glycoprotein separated WNV, Kunjin virus and JE virus into two lineages, with WN-New York 1999 being most closely related to the WN-Israel 1998

strain [24]. The sequence similarities of WN-New York 1999 with several other WNV isolates from birds and humans from the United States are indicative of the presence of a single WNV strain, which is circulating [13].

A study done on the tissues of birds that died during the WN fever outbreak at the Bronx Zoo and the Queens Wildlife Center has been published [25]. This report provides data from 27 birds, representing eight orders (Passeriformes, Ciconiiformes, Pelecaniformes, Charadriiformes, Anseriformes, Galliformes, Falconiformes and Strigiformes) and 14 species (*C. brachyrhynchos*, *C. ossifragus*, *Pica pica*, *P. chilensis*, *Nycticorax nycticorax*, *Phalacrocorax bougainvillii*, *Larus atricilla*, *Anas specularis*, *Anas platyrhynchos*, *Lophophorus impeyanus*, *Tragopan blythii*, *Haliaeetus leucocephalus alascanus*, *Nyctaea scandiaca*). In these specimens, it was shown that brains (88%), hearts (96%), spleens (83%), livers (70%), kidneys (100%), lungs (42%) and ovaries (50%) tested positive for WNV by virus isolation, immunohistochemistry, in situ hybridization, reverse-transcription (RT)-PCR or electron microscopy.

The widespread distribution of the virus in different systems of organs suggests that the virus is pantropic. It appeared to have targeted glial cells, myocardial fibers, macrophages and blood monocytes, renal tubular epithelium, adrenal cortical cells, pancreatic acinar and islet cells, intestinal crypt epithelium, oocytes and fibroblasts. Purkinje cells were especially immunoreactive, except in crows and magpies. Calvarial and meningeal hemorrhage and splenomegaly were prominent gross lesions. Meningoencephalitis and myocarditis were the most prominent microscopic changes. Gross and histologic lesions were common in the cerebellum of many birds. These included hemorrhage in the molecular and granular layers, Purkinje cell necrosis, gliosis, and inflammatory infiltrates. The lesions found in other portions of the brain were generally less severe and included lymphocytic perivascular cuffing, gliosis or glial nodules. Lymphocytic myocarditis, focal necrosis of the liver and spleen, pancreatitis, pulmonary hemorrhage and inflammation of the adrenal gland were lesions found among the birds tested. The virus was more consistently isolated or detected by RT-PCR from the heart and kidneys than from any other organ, including the brain. The death of numerous birds of many different species is a surprising and unique feature of the West Nile outbreak in the United States, one that may reflect little or no co-evolutionary adaptation to WNV [25].

To date WNV has been demonstrated in at least 18 native bird species in the northeastern United States, including the American crow (*C. brachyrhynchos*), ring-billed gull (*Larus delawarensis*), yellow-billed cuckoo (*Coccyzus americanus*), rock dove (*Columba livia*), sandhill crane (*Grus canadensis*), blue jay (*Cyanocitta cristata*), bald eagle (*H. leucocephalus*), laughing gull (*Larus atricilla*), black-crowned night heron (*Nycticorax nycticorax*), mallard (*A. platyrhynchos*), American robin (*Turdus migratorius*), fish crow (*C. ossifragus*), red-tailed hawk (*Buteo jamaicensis*), broad-winged hawk (*Buteo platypterus*), Cooper's hawk (*A. cooperii*), belted kingfisher (*Ceryle alcyon*), American kestrel (*Falco sparverius*) and herring gull (*Larus argentatus*) [26].

5. Horse deaths in Aquebogue, Long Island, New York

From August to November 1999, 31 horses from Aquabogue, New York became ill and had neurologic signs. These included ataxia, trembling, staggering, incoordination, lip dropping, muscle fasciculations, refusal to turn to one side, anxiety, recumbence and paresis (M. Andresen, RN, MPH, personal communication). At the time of the initial examinations, equine protozoal myelitis, rabies, and cerebral herpesvirus infection (rhinopneumonitis) were considered. Late in September a brain from one horse was found to have multiple encephalitic lesions. Brain tissue was then sent to the CDC and the National Veterinary Service Laboratories, in Ames Iowa, which reported the isolation of WNV. A US Department of Agriculture (USDA) Early Response Team investigated clinically ill horses. Of these, 20 (68%) had West Nile antibodies, nine tested negative and two were not tested. PCR tests conducted at the CDC with brain tissue from some of the horses with neurologic signs were positive. Nine of the positive horses had died or were euthanized while some of the serologically positive horses, which had clinical signs, have recovered completely. Experimental inoculation of four ponies with a WNV which had been isolated from crows resulted in a viremia of short duration and seroconversion in all four ponies; however, only one of the animals showed mild neurologic signs but recovered completely (Drs J. Lubroth and T. McKenna, personal communication). This suggested that other yet undefined factors, including age, breed, level and mechanism of exposure, etc., may influence the outcome of infection in horses.

Finding the WNV in horses indicated that the disease had spilled over, beyond wild birds to domestic species. Recognition that horses in the United States became infected with the WNV resulted in embargoes against horses from affected counties, especially for export to the European Union.

6. West Nile in Connecticut

Much like in New York, an excessive number of deaths among wild birds, principally American crows, was noticed in Fairfield and New Haven Counties in Connecticut in August and September 1999. On September 13 two dead crows collected in Westport were received at our Department. One of the crows had encephalitis; the other was decomposed. Brain tissue of the former yielded a virus isolate in Vero cell culture. This was achieved at the Connecticut Agricultural Experiment Station (CAES) in New Haven, Connecticut, marking the first reported recovery of live WNV in North America [10]. Thereafter, virus isolations were made from 27 of 30 crows collected in 18 towns in the two affected counties and from a Cooper's hawk (*A. cooperii*) [10]. Within days of the initial recovery of WNV at New Haven our laboratory isolated the virus and confirmed its identification. We observed calvarial or meningeal hemorrhage and less frequently coelomic hemorrhages, petechiae or echymoses [10]. Many of the

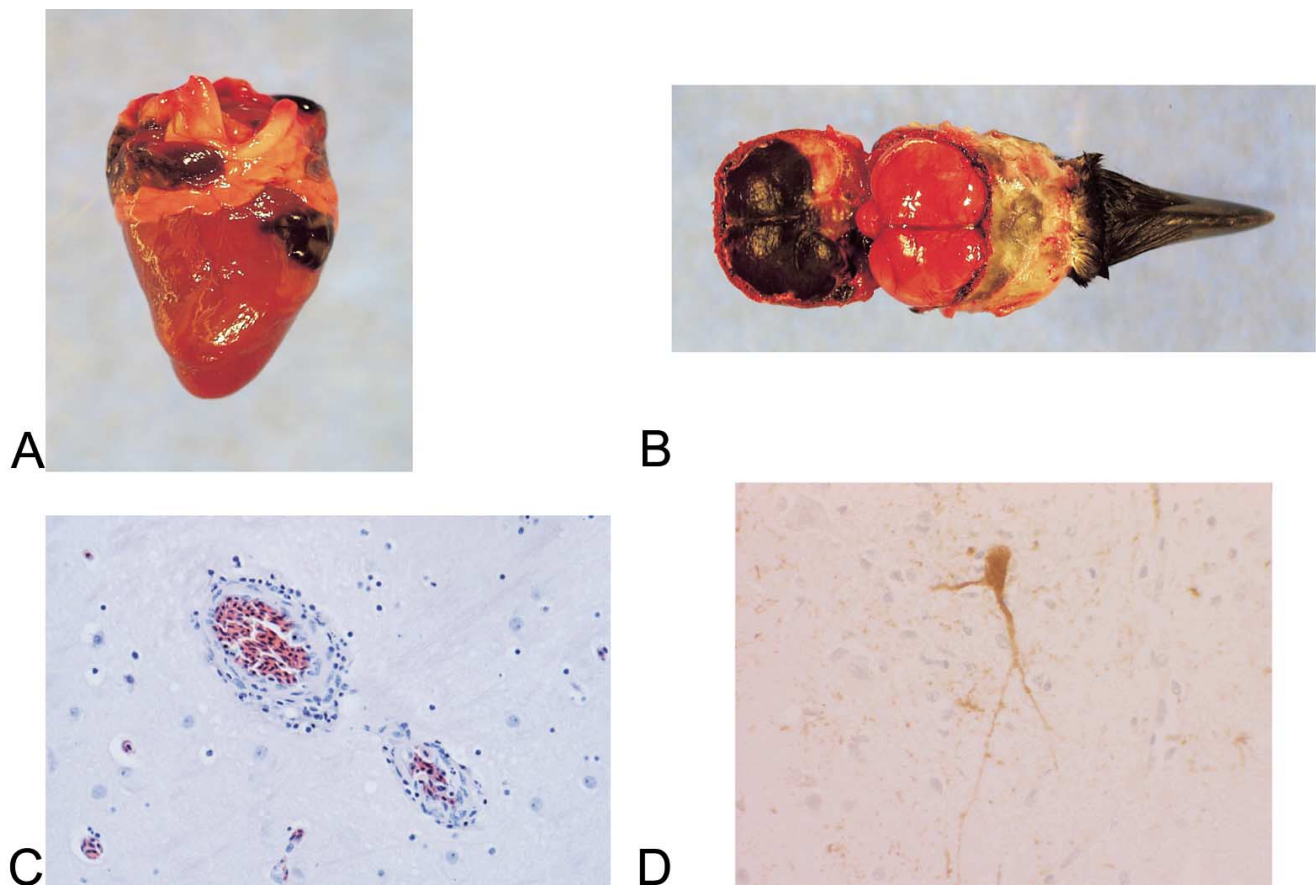


Figure 3. A and B: Hemorrhages in the heart and calvarium and meninges, respectively, of a crow that died from West Nile fever. C: Perivascular lymphocytic cuff in the brain. D: WNV antigens in a neuron of a hawk that tested positive, shown by immunohistochemistry.

affected crows were emaciated. Microscopically, encephalitis was rare. Lesions and viral antigens found in tissues of birds testing positive to WNV are shown in *figure 3*.

C. pipiens and *A. vexans* mosquitoes that were collected in mid-September at a country club in Greenwich, contained the WNV and were thought to be the vectors in the bird cases. Sequence analysis of a portion of the genome of viral isolates from these mosquitoes, the crow from Westport and the Cooper's hawk isolate revealed a close genetic relation with Romanian and Israeli WNV isolates [10]. The sequence of WNV-New York 1999, a virus isolated from the brain of a Chilean flamingo (*P. chilensis*), was most closely related to a WNV isolated from a goose in Israel in 1998 [13]. The partial genomic sequences derived to date from human brain and from zoo birds, crows, horses and mosquito pools are identical [10, 13, 24].

7. First isolation of WNV in the year 2000 from birds

After the end of the 1999 mosquito season our group continued to have an interest in hawks and owls, concerned about their exposure to the virus. In February 2000

a red-tailed hawk (*B. jamaicensis*) that had died (Feb 6th) in Westchester County, New York was tested in our laboratory. The hawk had encephalitis, characterized by glial nodules, with lymphocytes and histiocytes, similar to those that occur in man, and it had foci of necrosis in the spleen, liver and kidney. Virus was isolated from the hawk's brain [27]. The identity of the virus was determined by ELISA, fluorescent antibody test and RT-PCR, then confirmed by independent isolation at the CAES, New Haven, CT. Electron micrographs of infected Vero cells showed typical virions, 40 nm in diameter, in cytoplasmic vesicles. Finding an apparent acute case of West Nile encephalitis in mid-winter suggested that the virus survived beyond the mosquito season and that transmission via the oral route may have occurred, by the consumption of a reservoir host [27, 28]. Transmission via the oral route or via consumption of infected offspring has been documented in laboratory mice [29].

8. WNV surveillance in the year 2000

During the 1999 West Nile outbreak, federal and state agencies initiated emergency measures for surveillance and control. A plan is now in place called the National West Nile Virus Surveillance System, 2000. The Depart-

ment of Human Health and Services, the United States Geological Service, the CDC, the USDA, State Public Health Departments and Natural Resource Agencies [30] coordinate surveillance. The objectives of this plan include: 1) monitoring the spread of WNV over the eastern and southern United States; 2) development of national public health strategies for WN surveillance, prevention and control; 3) providing information to public health officials, elected government officials and the public, and 4) determining the resource needs. CDC released funds for 19 State Health Departments for surveillance. The programs include mosquito testing and the testing of sentinel chickens, wild birds, domestic animals and humans with neurologic disease for WNV.

Through the surveillance system the first WNV-positive crows, detected by virus isolation, occurred in June 2000 in Rockland County, New York. Subsequently, positive birds were found in Bergen County, New Jersey and Westchester and Suffolk Counties in New York. Then a red-tailed hawk in Westchester County, New York and crows in Jamaica Plains, Massachusetts, Stamford and Columbia in Connecticut and Rhode Island tested positive for WNV. New Hampshire, Vermont, Maryland, Pennsylvania, Washington, DC, Virginia and North Carolina also had a few positive birds. An elderly man in Little Falls, New Jersey died of West Nile fever and at least 18 individuals had clinical illness in New York and New Jersey. As of October 2000 more than 20 horses with West Nile fever had died or had been euthanized in New York, New Jersey, Connecticut, Pennsylvania, Massachusetts and Rhode Island. More than 350 mosquito pools have tested positive. The virus was also detected in bats, cats, raccoons, domestic rabbits, squirrels and a chipmunk. Mosquito control measures have been selectively applied wherever the virus is found and where there is risk of infection of humans. The reappearance of WNV in the spring and summer of 2000 confirmed earlier suspicions that the virus had survived the winter. The mechanism of overwintering however, remains to be clarified. Crows have been recognized as a more sensitive indicator of the presence of WNV than mosquitoes. While crows would be categorized as dead-end hosts by convention, they may contribute significantly to its distribution. The pattern of spread to upstate New York, and through Connecticut, Rhode Island, Massachusetts, etc., makes the latter assumption feasible. While mosquitoes are tremendously important in transmission to man, it may be the crows that deserve credit, or blame, for the distribution.

9. Summary and conclusions

In the fall of 1999 a new disease occurred in the greater New York area that ultimately caused the death of seven people. While it would later be shown that 2.6% of the population (of Queens) seroconverted, only 61 were ill; most affected individuals had non-encephalitic symptoms and signs. Those with encephalitis were thought initially to have had SLE. Concurrently, large-scale deaths of crows, other wild birds and exotic birds at local zoos prompted discussion that the bird die-offs and the human cases

might be one-and-the-same. PCR on human brain tissue placed the virus among the flaviviruses.

A connection between the human disease and bird mortality made by veterinarians at the Bronx Zoo and a wildlife pathologist in upstate New York, followed by the isolation and sequencing of a virus from a Chilean flamingo by USDA and CDC scientists, and the isolation of virus from crows, a Cooper's hawk and mosquitoes by Connecticut scientists provided critical information on the identification of WNV in North America.

It became apparent that crows died in greater numbers than other species, and hundreds were necropsied both in New York and Connecticut. Exotic birds that had died at several zoos had encephalitis and focal glial nodules like those that occurred in the human disease. Virus was recovered repeatedly from the brains of crows that had died; however, less than 10% had evidence of encephalitis, and focal glial nodules did not occur.

Concurrent with the deaths of humans and wild birds, an epicenter of equine neurologic disease of a 2.5-mile radius occurred in Aquabogue, New York, half-way out on Long Island and remote from the New York City events. The differential diagnosis of these cases included rabies, equine protozoal myelitis and a few other neurological diseases of horses; however, examination of brain tissue from several animals yielded positive isolations of WNV, and others tested positive by PCR or serology. Nine horses died or were euthanized; a number of others recovered.

In Connecticut WNV was recovered from *C. pipiens* and *A. vexans* and from the brain tissue of a Cooper's hawk and numerous crows. Infected mosquitoes were found on only one occasion and in only one town, the southernmost, whereas infected crows were found in 18 towns along a 62-mile stretch of coastal Connecticut. After the 5th of November no additional cases were recognized until February, when necropsy of a red-tailed hawk from Westchester County, New York turned out positive for WNV. It had focal glial nodules indicative of encephalitis, and WNV was identified by multiple methods. This bird represented the last known case of the 1999 epidemic and suggested that the virus had persisted beyond the mosquito season and that prey-to-predator transmission had occurred.

Surveillance has been in place since April 1, 2000 including the testing of mosquitoes, wild birds that have died, sentinel chickens, domestic animals, and humans with neurologic signs. In March, mosquitoes trapped in New York City were found to contain WNV RNA; and in May crows that died in Rockland County, New York and Bergen County, New Jersey were shown to have died of WNV infection. Since then, hundreds of birds have tested positive in New York, New Jersey and Connecticut, and positive birds were found for the first time in Massachusetts, Rhode Island, Pennsylvania, New Hampshire, Vermont, Washington, DC and Virginia. An elderly man who died from West Nile fever in New Jersey is the first and only known fatal case in the year 2000 thus far. Several horses had also died from West Nile fever in the tri-state area and part of New England. The full endemic and epidemic proportions of West Nile fever in North America are yet to be defined. The American crow appears to be a

very important species in the epidemiology and spread of WNV in North America.

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