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## **West Nile Virus: A Threat to North American Avian Species**

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### **Introduction**

West Nile virus (WNV) was introduced into the United States (US), specifically in New York City (NYC), in 1999; this translocation represented a major shift out of its normal geographical distribution of Africa, the Middle East, Europe and the western parts of Asia (Center for Disease Control 1999a). The route or method of entry into the US is still unknown. WNV is in the genus *Flavivirus*, the family *Flaviviridae* and is closely related to some other viruses in this family, such as Japanese encephalitis virus in Southeast Asia, Murray Valley encephalitis virus in Australia and St. Louis encephalitis (SLE) virus in North and South America. The principal vertebrate hosts for these viruses are wild birds, but few cases of clinical disease or mortality of wild birds were reported previously from natural infection with these viruses, although significant morbidity and mortality occurred in humans and domestic animals (Monath 1988). Natural maintenance of these arboviruses (arthropod-borne viruses) involves their transmission from infected mosquitoes to susceptible birds. A variety of wild birds may become infected, however some species are incompetent hosts for the viruses and do not regularly infect mosquitoes. On the other hand, infections in reservoir competent wild bird species produce high amounts of the virus in their blood (viremia) for the duration of several days and subsequently infect the mosquitoes that feed upon them, completing the transmission cycle. These competent bird species frequently maintain and amplify the particular virus.

Bird populations within the US are frequently infected with the closely related SLE virus, and birds are the source of the virus when humans are infected through mosquitos that feed on both (McLean and Bowen 1980). WNV infects similar wild bird species within its geographic range (Work et al. 1955, Komar et al. 2001b) and fill the same role as a source to infect mosquitoes that transmit

WNV to humans (Marfin et al. 2001). Domestic birds infected with WNV do not develop viremias sufficient to infect vector mosquitoes and are considered incidental hosts for the virus (Langevin et al. 2001), with the exception of domestic geese (Swayne et al. 2001). Domestic livestock, especially equines, and humans are incidental or dead-end hosts as well, since they do not generally contribute to further WNV transmission.

## **West Nile Virus Introduction and Establishment in United States**

The strain of WNV introduced into the US was nearly identical to a new more virulent strain (Isr98) from the Middle East (Lanciotti et al. 1999, Giladi et al. 2001). This invasive virus caused a human epidemic of 62 cases, 7 deaths and extensive mortality in birds in the NYC region before the transmission season ended in November 1999 (Center for Disease Control 1999b). West Nile virus activity expanded from the original epidemic zone in Queens in NYC and from the central cluster of WNV positive birds in the NYC area to more than a 100-mile-wide (over 161 km) epicenter, in 22 counties in three states surrounding NYC (Eidson et al. 2001a). Sightings of dead crows in the region from August to October matched the outward geographic expansion of the laboratory-confirmed, WNV-positive American crows (*Corvus brachyrhynchos*), suggesting that crows were likely responsible for the expansion of WNV out of NYC and that thousands of crows may have died from WNV infection (Eidson et al. 2001b). Analysis of December bird count data from the area showed a decline in the number of crows in the affected zone after the epizootic in 1999, compared to 1998 data (Eidson et al. 2001a). The American crow emerged as the primary indicator of WNV activity because of its high susceptibility to infection. Local and state public health departments began using WNV positive crows to make decisions about human risk. This unique surveillance system integrated state and federal agencies of wildlife health with public health into a coordinated effort to monitor the detection, intensity and geographic expansion of WNV activity. In the US, a total of 295 free-ranging birds of 20 avian species (89% were American crows) were laboratory-confirmed WNV-positive in New York, New Jersey and Connecticut in 1999 (Figure 1), including some captive native and exotic bird species in zoological collections in the area (Steele et al. 2000, Eidson et al. 2001a). Positive birds were collected from August 2 to November 15, 1999.

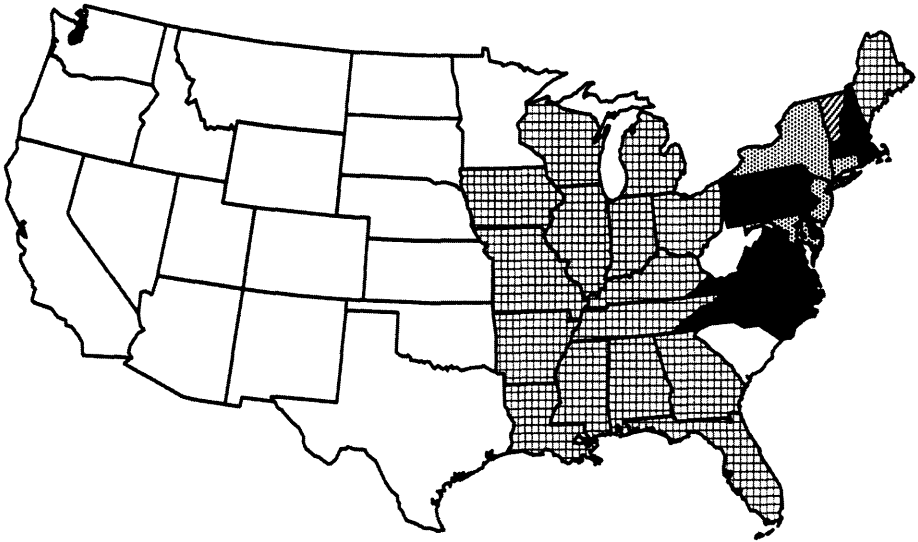


Figure 1. The distribution of WNV activity between 1999 and 2001, in the United States (Eidson et al 2001, Marfin et al. 2001, USGS 2001). Stippling represents WNV presence during 1999 and 2001, diagonal lines represent 2000 only, solid black represents 2000 and 2001, and cross hatching represents 2001 only.

## West Nile Virus Expansion in North America

### *2000 National Surveillance Information*

West Nile virus survived the temperate winter of the northeastern US, where there is no continuous mosquito activity to sustain transmission. But, it re-emerged in 2000 within the same 1999 epicenter in the NYC area, first in American crows in May. Enhanced surveillance of wild birds, sentinel chicken flocks, mosquito vectors and domestic animal and human infections was established initially in 20 states along the Atlantic and Gulf Coast to monitor the geographical dissemination and temporal spread of WNV in the US (Center for Disease Control 2000). Local government officials and the public were enlisted through communication and education campaigns to observe, report and collect dead birds for testing by state and federal laboratories for WNV infection. Data from all of the surveillance components were accumulated, verified and submitted by state health departments to a cooperative WNV national surveillance network, ArboNET, developed and maintained by the Centers for Disease Control and Prevention (Marfin et al. 2001). This

surveillance system provided weekly data summaries and maps of WNV activity throughout the country to monitor its spread, to identify areas of high risk and to assist in the development of prevention and control strategies.

The reporting of WNV activity in 2000 rapidly expanded northward from the 1999 epicenter to the Canadian border during the spring and early summer, then westward to Lake Erie during late summer, and finally southward to North Carolina in the autumn, ultimately including 12 states and the District of Columbia (Figure 1). Additional human cases (21) and two deaths occurred in the NYC metropolitan area (Marfin et al. 2001). Of the total of 104,816 dead birds, reported in 321 counties in 16 states from the state surveillance networks, 12,961 (12%) were submitted for WNV testing, and 4,305 (33%) were virus positive (Table 1). Crows comprised 7,580 (58%) of the birds, and 50 percent of the tested crows were WNV-positive, whereas only 481 (9%) of birds from other species tested (5,381) were positive (Marfin et al. 2001). A significant portion was from New York State (Bernard et al. 2001). The positive percentage of all birds tested in New York was similar to the national positive percentage (Table 1). In upstate New York, north of the epicenter of WNV activity in the NYC area, 23 percent of all birds tested were WNV-positive, versus the 51 percent within the epicenter region. Other bird species and American crows had similar infection rates in the non-epicenter region, whereas 67 percent of dead crows tested from the epicenter were WNV positive (Bernard et al. 2001). Two other states within the epicenter region, Connecticut and New Jersey, reported even higher numbers of WNV positive crows (greater than 1,000) than New York in 2000, but these states concentrated on collecting and testing primarily crows. The percentage of crows testing positive (70%) for WNV infection in the epicenter region of Connecticut (Hadler et al. 2001) was similar to the 67-percent infection rate found in the New York part of the epicenter (Table 2). Five wild mammals (striped skunk, *Mephitis mephitis*; eastern gray squirrel, *Sciurus carolinensis*; eastern chipmunk, *Tamias striatus*; big brown bat, *Eptesicus fuscus*; and little brown bat, *Myotis lucifugus*) were found WNV-positive in 2000 in New York and Connecticut (Marfin et al. 2001).

Dead birds confirmed with WNV infection were reported, the first on February 6, 2000, from a red-tailed hawk (*Buteo jamaicensis*) (Garmendia et al. 2000), and the last on November 17, 2002, from an American crow. However, 85 percent of positive birds were found between July 1 to September 30 (Marfin et al. 2001). This late summer peak of positives represents the amplification of

Table 1. Birds tested for and laboratory-confirmed positive with West Nile virus in 2000 in New York State and for the United States (Bernard et al. 2001, Marfin et al. 2001)

Location/ category	Number positive species	Number tested	Number positive	Percent positive
New York State	61	3,403	1,201	35
Crows only	2	1,732	814	47
Other species	59	1,671	387	23
United States	63	12,961	4,305	33
Crows only	2	7,579	3,823	50
Other species	61	5,382	482	9

WNV transmission in the form of an epizootic in the bird populations. Serologic testing of sentinel bird species for WNV antibody within the epicenter, in 2000 on Staten Island, New York, identified captive pigeons (*Columba livia*) and several wild passerine bird species as possible candidates for use in active WNV surveillance programs (Komar 2001a).

Tens of thousands of birds died in 2000, affecting many new species, from hummingbirds to wild turkeys for a total species list, for the first two years, of 54 native and five non-native, free-ranging species and of six native and five exotic captive species (US Geological Survey 2001a). It is estimated that about 40,000 crows died in New York State alone and of the 12,961 birds tested in the 12 affected states, 4,305 (33%) were WNV-positive (Tables 1 and 3). American crows comprised 3,824 (89%) of all virus positive birds and bluejays (*Cyanocitta cristata*) were 196 (5%). One common raven (*Corvus corvax*) also tested positive in Massachusetts for a total of 93 percent of all

Table 2. *Corvus* spp. tested for and laboratory-confirmed positive with West Nile virus in 2000 in two epizootic states and for the United States (Bernard et al. 2001, Hadler et al. 2001, Marfin et al. 2001)

Location	Number species	Number tested	Number positive	Percent positive
United States	3	7,580	3,824	50
New York State 7	2	1,732	814	47
Non-epicenter				23
Epicenter				67
Connecticut (epicenter)	2	1,574	1,095	70

Table 3. Laboratory-confirmed positive birds with West Nile virus reported in 2000 in the United States (Marfin et al. 2001)

Common name	Scientific name	Number positive	Percent of all infected birds
Crows	<i>Corvus</i> spp	3,824	88.8
Blue Jay	<i>Cyanocitta cristata</i>	196	4.6
Hawks and Falcons	<i>Accipiter</i> , <i>Buteo</i> , <i>Falco</i> spp.	44	1.0
Ruffed Grouse	<i>Bonasa umbellus</i>	27	0.6
Gulls	<i>Larus</i> spp.	26	0.6
House Sparrow	<i>Passer domesticus</i>	20	0.5
American Robin	<i>Turdus migratorius</i>	20	0.5
Mourning Dove	<i>Zenaida macroura</i>	17	0.4
Other Birds	46 other species	131	3.0
Total	63 species	4,305	

positive dead birds as Corvidae. Predatory birds may also be at risk since six hawk and two owl species were positive. Despite the large numbers of birds reported dead and the relatively large number tested for virus, little is known about the effect this virus may have on local or regional populations of birds. The broad expansion of WNV activity in 2000 was probably accomplished by other bird species, most likely some migratory species that do not suffer much mortality (Rappole et al. 2000). The virus was detected as far south as North Carolina by the end of September and even may have reached farther south before the end of the autumn migration of birds.

### ***2001 National Surveillance Information***

Following the 10-fold expansion of the distribution of WNV in the northeastern US in 2000, the virus again survived through the dormant winter season and reappeared in American crows in five separate states in late April and early May 2001. These sites were within the 2000 expanded WNV region in the Northeast. Four of the five locations now represent persistent geographical foci of WNV activity (Connecticut, Maryland, New Jersey and New York) because positive birds were reported there from 1999 to 2001 (Eidson et al. 2001a, Marfin et al. 2001, Center for Disease Control 2001a). A new focus of WNV was detected in northern Florida in June and began to quickly expand in

all directions. This virus focus probably started during autumn of 2000 by migratory birds becoming infected in the northeast and carrying WNV south during their fall migration to and through Florida. The seeding of the virus and the establishment of WNV at this Florida site was certainly influenced by the extended period of mosquito activity that occurs in the warmer Gulf Coast areas of the southeastern states. Transmission of WNV in the bird-mosquito cycle in northern Florida remained below surveillance detection until amplification of transmission was sufficient in June for dead crows to be observed and submitted for WNV testing from this rural area (Center for Disease Control 2001a). Equine clinical cases quickly followed in June and the first human case of the year for the US was reported in an adjacent Florida county with onset of the illness around July 15. Since mosquito transmission within this WNV focus likely occurred weeks before the detection of the virus in June, migrating birds could have become infected while traveling through the area in April and May on their way northward carrying WNV to northern locations, including to some Midwestern states.

Regardless of how the virus was disseminated in the US in 2001, WNV began to be detected in an expanding area from the northeast and southeast to eventually encompass 27 states and Ontario, Canada, by the end of the northern transmission season in November (Figure 1). The original focus in northern Florida gradually expanded throughout that state south to the Florida Keys (Florida Department of Health 2001) and into the neighboring states of Georgia and Alabama. The virus was detected in the Midwestern states of Ohio, Michigan, Wisconsin, Illinois and Indiana, starting in July and August, and it expanded in those states throughout the remainder of the transmission season (Center for Disease Control 2001b). After the initiation of autumn bird migration to the south, states along the Mississippi flyway began detecting WNV-positive dead birds until all of the states on both sides of the Mississippi River, except Minnesota, reported positive birds. Some of the reporting sites were in cities on the river, like Saint Louis, Missouri and Memphis, Tennessee (Center for Disease Control 2001c, US Geological Survey 2001b). Memphis reported 44 positive birds during the months of September and October.

Preliminary surveillance results for 2001 indicate that 58 human cases—with eight deaths—occurred in 10 states, 564 equine cases—in 18 states (US Department of Agriculture 2001, Florida Department of Health 2001)—and 911 pools of mosquitoes (a pool consists of 1-100 mosquitoes, generally of a single

species, collected from one site during one night of trapping) tested positive from 24 mosquito species—in 17 states. From a total of 7,058 birds in 27 states, 5,036 crows (71%) were reported WNV-positive (US Geological Survey 2001b). If the current rate of expansion of WNV continues, doubling the geographical distribution (Figure 1) and the number of dead birds each year (Figure 2), all of the contiguous continental states could be affected and more than 15,000 birds could die from WNV in 2002.

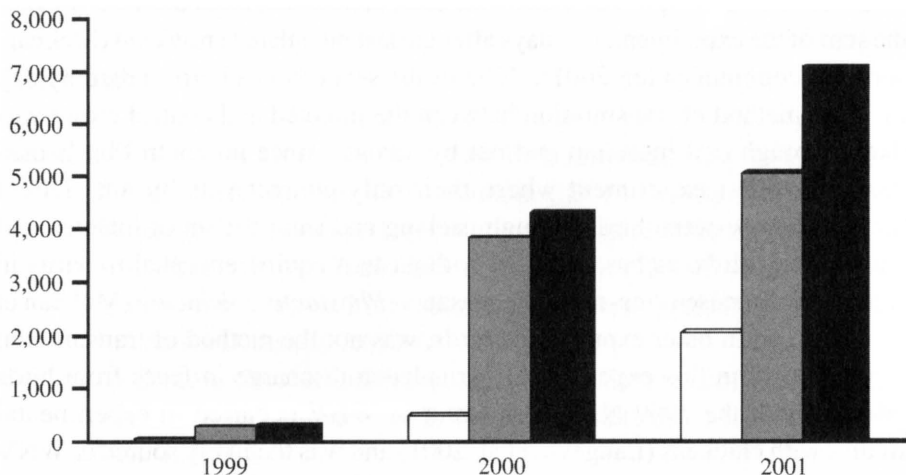


Figure 2. The number of dead birds reported positive with West Nile virus between 1999 and 2001 in the United States (Eidson et al. 2001, Marfin et al. 2001, US Geological Survey 2001). White represents other birds, gray represents American crows and black represents total birds.

## Experimental Studies

Since the information from the dead bird surveillance indicated that crows were particularly susceptible to infection with WNV, experimental studies were initiated in the biosafety level 3 animal facility at the US Geological Service National Wildlife Health Center to determine the extent of their susceptibility. American crows captured in Wisconsin in late winter 2000 were used in two separate experimental infection studies (McLean et al. 2001). Experimental crows were inoculated subcutaneously with a New York 1999 strain of WNV and control birds were inoculated with saline. In the first experiment, the crows were held individually in separate cages and all of the WNV inoculated crows died within four to seven days; the control birds did not become infected.

Viremias in infected crows were sufficient, before they died, to infect vector mosquitoes, indicating that crows are reservoir competent hosts (McLean, personal communication 2001).

In the second experiment, nine WNV inoculated crows, receiving the same dose as in the first experiment, and seven non-inoculated control birds were housed together in the same animal room in a free-flying arrangement that allowed regular contact with each other. Again, all nine inoculated crows died within five to eight days, however control birds began to die 10 days after the start of the experiment, two days after the last inoculated crow died (McLean, personal communication 2001). Five of the seven control crows died by day 21. The method of transmission between the infected and control crows was likely through oral ingestion and not by aerosol, since no control birds died during the first experiment where their only contact was by air. Direct transmission between birds through pecking and cannibalism of infected and clinically ill birds, as has occurred with eastern equine encephalitis virus in commercially raised ring-necked pheasants (*Phasianus colchicus*) (McLean et al. 1985) and in other exotic game birds, was not the method of transmission among crows in this experiment. Virus-laden discharge in feces from birds infected with the 1999 New York strain of WNV occurred in experimental studies with chickens (Langevin et al. 2001) and was the likely source of WNV for control crows in this experiment. The significance of direct transmission of WNV between crows and whether it occurs under natural conditions is unknown at this time. Even though crows die from infection with WNV, they circulate enough virus in their blood for a few days prior to death to infect large numbers of vector mosquitoes and locally perpetuate WNV transmission.

## **Summary and Conclusion**

The introduction and extensive expansion of WNV in the US in the last three years is having a dramatic impact on native wildlife. The disease continues to cause significant mortality in a variety of bird species throughout the eastern US, particularly in American crow and blue jay populations. As the virus expands to new habitats in the southern, midwestern and western states, new bird species will be at risk and different patterns of transmission will develop. In the western states, many additional species of Corvidae (crows, jays, ravens, magpies and nutcrackers) may be affected. Once it becomes well established

in states with warm climates, like Florida where mosquitoes are active year round to sustain almost continuous transmission; these states could serve as annual sources of WNV for migratory birds to re-introduce the virus to northern states in the spring. The rapid increase in geographical distribution of WNV activity that has occurred throughout the eastern US and the rapid increase in the infection and mortality rates in birds during the last three years indicate the emergence of an epizootic disease of major importance to North American birds.

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