West Nile Virus: Uganda, 1937, to New York City, 1999

CURTIS G. HAYES
Infectious Diseases Directorate, Naval Medical Research Center,
Silver Spring, Maryland 20910-7500

ABSTRACT: West Nile virus, first isolated in 1937, is among the earliest arthropod-borne viruses discovered by humans. Its broad geographical distribution, not uncommon infection of humans, transmission by mosquitoes, and association with wild birds as enzootic hosts were well documented by the mid-1960s. However, West Nile virus was not considered to be a significant human pathogen because most infections appeared to result in asymptomatic or only mild febrile disease. Several epidemics had been documented prior to 1996, some involving hundreds to thousands of cases in mostly rural populations, but only a few cases of severe neurological disease had been reported. The occurrence between 1996 and 1999 of three major epidemics, in southern Romania, the Volga delta in southern Russia, and the northeastern United States, involving hundreds of cases of severe neurological disease and fatal infections was totally unexpected. These were the first epidemics reported in large urban populations. A significant factor that appeared in common to all three outbreaks was the apparent involvement of the common house mosquito, *Culex pipiens*, as a vector. This species had not previously been implicated as important in the transmission of West Nile virus. In addition the epidemic in the northeastern United States was unusual in the association of West Nile virus infection with fatal disease of birds, suggesting a change in the virulence of the virus toward this host. Understanding the risk factors that contributed to these three urban epidemics is important for minimizing the potential for future occurrences. This review will attempt to compare observations on the biology of West Nile virus made over about 60 years prior to the recent epidemics to observations made in association with these urban epidemics.

KEYWORDS: West Nile virus; review; epidemic; vectors; hosts; disease; epidemiology

West Nile (WN) virus has recently emerged as a major public health concern in Europe and the United States with the occurrence of epidemics involving hundreds of cases of meningoencephalitis and encephalitis in three large urban areas since 1996. More human cases of severe neurological disease and more deaths have been reported in these three recent epidemics than in all known past outbreaks of West Nile disease. These are also the first reported epidemics to occur in large urban areas and the first to have the common house mosquito, *Culex pipiens*, implicated as a major vector.

Address for correspondence: Curtis G. Hayes, Infectious Diseases Directorate, Naval Medical Research Center, Silver Spring, Maryland 20910-7500. Voice: 301-319-7455; fax: 301-319-7460. hayesc@nmrc.navy.mil
THE VIRUS

Previous Observations

West Nile virus was originally isolated in 1937 from the blood of an adult female in Uganda.1 The next isolates of WN virus were not obtained until 13 years later in Egypt from the blood of three apparently healthy children.2 However, after that slow beginning, hundreds of additional isolates of WN virus have been obtained, particularly from humans, birds, and mosquitoes, over a wide geographic area ranging from Africa, through the Middle East and Europe, to Asia.3–5

Shortly after the original isolation of WN virus was made, researchers were able to show that this virus was antigenically related to two other arboviruses that were known to cause encephalitis, St. Louis encephalitis (SLE) virus and Japanese encephalitis (JE) virus.6 Later, cross-neutralization studies expanded the relationship of WN virus with other flaviviruses, placing it in an antigenic group with SLE, JE, Murray Valley encephalitis, Kunjin, Usutu, Kokobera, Stratford, and Alfuy viruses.7

Antigenic variation among selected isolates of WN viruses was shown to exist in 1965 when 21 isolates from 6 different countries were compared in a kinetic hemagglutination-inhibition assay.8,9 The isolates were divided into two major groups, the African–Middle-Eastern and Indian antigenic groups, with the exception of one of three isolates tested from South Africa, which was not placed in either group. Several studies have suggested that virulence differences also existed among isolates of WN virus, but interpretation of these studies is complicated by the fact that the isolates compared had undergone different passage histories, usually by intracerebral inoculation of mice, in the laboratory.

With the availability of the improved nucleic acid sequencing technology, an envelope protein gene fragment was compared from 20 WN virus isolates from nine African countries and one isolate from France made between 1951 and 1990.10 This study classified the WN isolates into two lineages (I and II). West Nile viruses belonging to both lineages were found to circulate widely in Africa and overlapped in some areas. This study also classified the closely related Australian flavivirus, Kunjin, as a WN subtype in lineage I.

Current Observations

Improved nucleic acid sequencing capability also has allowed researchers to rapidly determine the genetic relationship of the WN virus isolates made during the course of the three urban epidemics that occurred between 1996 and 1999. Only two WN isolates, one from a mosquito and one from a human, were obtained during the 1996 epidemic in Romania.11 Both isolates, although slightly different from each other, were shown to belong to lineage I of WN viruses. These two isolates were most closely related to several earlier isolates from countries in sub-Saharan Africa, suggesting that they could have been introduced into Romania from migrating birds from that region.

An analysis of gene fragments from WN virus isolates obtained during the 1999 epidemic/epizootic in New York, Connecticut, and New Jersey also showed that they belonged to lineage I.12 All of these isolates had a very high degree of homology (>99.8%) over the 1278 nucleotide regions sequenced, even though they were ob-
tained from humans, birds, and mosquitoes. Unexpectedly, these isolates shared the same high degree of homology with an isolate obtained from a dead goose in Israel in 1998, suggesting that the origin of the WN virus circulating in the northeastern United States was the Mediterranean region.

Nucleic acid sequences of WN virus gene fragments amplified from the brain tissues of seven fatal cases during the Volgograd epidemic were identical to each other and also were placed in lineage I.13 The 165 nucleotide base sequence of the envelope gene used for this analysis was 100% homologous to the same region from the 1996 mosquito isolate made during the Romania epidemic, and showed >98% homology with two isolates obtained in 1999 from a bird and a human in New York City.

**THE VECTOR**

*Previous Observations*

Because WN virus was known to be antigenically related to other viruses transmitted by mosquitoes,5 experimental vector studies were undertaken shortly after its discovery. These studies demonstrated that several species of mosquitoes could be experimentally infected and successfully transmit WN virus in the laboratory.14,15

The first field studies that implicated mosquitoes as important vectors of WN virus were conducted in Egypt from 1952 to 1954.16,17 Over the three years of this study, 17 isolates of WN virus were made from pools of mosquitoes, 5 from *Culex antennatus*, 9 from *Culex univittatus*, and 3 from mixed pools of *Culex pipiens* and *Culex univittatus*. These isolates were made during the same time of the year that WN virus infections were documented in children in the study area. During this same study a number of pools of other arthropods, including ticks, also were tested for WN virus isolation but were negative. Subsequent field studies in South Africa from 1961 to 1964 and Israel from 1964 to 1966 further supported the role of mosquitoes belonging to the *Culex univittatus* complex as major vectors of WN virus.18,19

Other species of mosquitoes, such as *Culex modestus* during an outbreak of WN in the early 1960s in France20 and *Culex vishnui* complex mosquitoes in India and Pakistan, also have been implicated as important vectors of WN virus in different geographical areas.21,22 WN virus has been isolated from many other species of mosquitoes, but their role as vectors has often remained poorly defined.

West Nile virus also has been isolated occasionally from ticks, but their role in the ecology of the virus remains unknown.3–5 Conceivably they could play a role in the dissemination of virus via migrating birds or in the overwintering of WN virus in temperate areas.

*Current Observations*

In all three major WN epidemics that occurred between 1996 and 2000, *Culex pipiens pipiens* was thought to play a major role as a vector for the first time. During the Romanian epidemic, *Culex pipiens pipiens* was the most abundant mosquito species collected, and the single WN virus isolate obtained from mosquitoes was from this species.11 The minimum WN virus infection rate for *Culex pipiens pipiens* per 1000 adult female mosquitoes tested was only 0.19, but all of these females were col-
lected in October at the end of the epidemic. In the epidemic/epizootic that occurred in the northeastern United States in 1999–2000, multiple isolates of WN virus were made from pools of *Culex pipiens pipiens* females collected during the time that transmission to humans and birds also was occurring. Limited vector competence studies have been conducted with four different species of mosquitoes collected in the New York City metropolitan area in 1999 using an isolate of WN virus recovered from the brain of a dead crow found in New York City during 1999. This study supported the field virus isolation data that *Culex pipiens* is a competent vector of WN virus. No entomological observations have been reported from the 1999 epidemic in the Volgograd region of Russia.

Earlier experimental infection studies had demonstrated that *Culex pipiens pipiens* could become infected and transmit WN virus after ingesting a high-titered blood meal. However, only a few isolates of WN virus had been reported previously from this species, and it was generally not considered an important vector because of its low field virus isolation rate compared to the isolation rates from other sympatric mosquito species. Two other interesting observations were made on mosquito vectors during the 1999–2000 WN epidemic in the northeastern United States. West Nile virus was isolated from overwintering *Culex pipiens pipiens* females collected in New York City, and WN virus was isolated or WN virus nucleotide sequences were detected in 12 different species of mosquitoes. The role of *Culex pipiens pipiens* as an overwintering vector for WN virus had previously been suggested by investigators in Egypt when they obtained a single isolate of WN virus from this species during January. However, they speculated that *Culex pipiens pipiens* continued to transmit WN virus at a low level throughout the winter (instead of hibernating), since some females of this species remained active at the ambient winter temperatures in this part of Egypt, and a few children were documented to develop antibodies against WN virus during the winter months. The number of mosquito species from which WN virus has been isolated in the northeastern United States far exceeds the number reported from any other area of the world in association with epidemic or epizootic activity, although WN virus isolates have been reported from a large number of different mosquito species in some tropical countries where endemic/enzootic activity has been monitored.

**THE HOST**

*Previous Observations*

Humans were the first vertebrate hosts recognized to become infected with WN virus by virus isolations obtained from blood as well as the presence of WN neutralizing antibody in blood. Subsequent studies in Israel, however, suggested that humans naturally infected with WN virus usually do not circulate high enough titers of virus in their blood to efficiently infect mosquito vectors. By contrast, experimental infection studies conducted on advanced cancer patients in New York City showed that titers of WN virus in the blood high enough to theoretically infect mosquitoes could occur. Interestingly, this study also found that the clinical severity of a patient’s WN virus infection correlated with the length of their viremia. Many other
studies have confirmed that humans become infected with WN virus in different areas throughout the range of this virus, but their role in the transmission of WN virus remains undefined.\textsuperscript{3–5}

The detailed village-based study on the ecology of WN virus conducted in the Sindbis sanitary district, Egypt from 1952–1954 showed for the first time that wild birds were important hosts in the transmission cycle of WN virus.\textsuperscript{34} In this highly endemic site for WN virus, neutralizing antibody rates in blood samples collected from six of the area’s most abundant bird species were found to range from 48% to 100%. They also found that the antibody rate in the hooded crow population increased from 40% during the late spring to 87% in the summer and winter and suggested the lower rate in late spring reflected the yearly introduction of susceptible juveniles into the population. This also was the first study that isolated WN virus from wild birds and the first to show that experimentally infected wild birds developed titers of WN virus in their blood high enough to infect mosquitoes.

A number of field studies conducted in different geographical areas corroborated the importance of birds in the transmission cycle of WN virus based on the presence of high rates of antibody.\textsuperscript{35–39} In particular, a study conducted in South Africa immediately following a large outbreak of WN in the human population found very high antibody rates among wild birds, suggesting that an avian epizootic had occurred concurrently with the human infections.\textsuperscript{40} Studies in South Africa on the experimental infection of wild birds also showed that nearly all birds were highly susceptible to WN virus and developed high levels of virus in their blood.\textsuperscript{41}

Antibody surveys have shown that many species of wild and domestic mammals, other than humans, become infected with WN virus, and a few WN virus isolates also have been made from naturally infected mammals other than humans.\textsuperscript{3–5} Experimental infection studies have been conducted with a few domestic mammals as well as wild rodents.\textsuperscript{17,42–46} The WN viremia levels have generally been undetectable or low, but only a very limited number of subjects have been included in these studies. On the basis of these early studies most researchers have concluded that the role of mammals, including humans, in the transmission cycle of WN virus is probably not critical to the long-term survival of the virus.

**Current Observations**

High WN antibody rates (41%) were found in domestic birds immediately following the 1996 epidemic in Romania.\textsuperscript{11} These birds were mostly tested from locations where human cases had occurred. A very limited sampling of wild birds was done, and only one out of twelve birds tested was positive for WN virus antibody. These limited results suggested birds played a role in this epidemic. Other than humans, testing of mammals for WN virus antibody was not reported. On the basis of serosurvey results, thousands of human infections occurred during this epidemic.\textsuperscript{47}

A large epizootic in birds clearly occurred in the northeastern United States during the 1999–2000 epidemic. For the first time, thousands of bird deaths attributable to natural WN virus infection were reported during this outbreak.\textsuperscript{30} The largest number of deaths were reported in the common crow, but dozens of other species of birds also were involved. Interestingly, WN virus was recovered from the brain of a dead red-tailed hawk collected during the middle of the winter in New York, suggesting a prolonged infection or a route of transmission other than mosquitoes.\textsuperscript{48} Birds also
have clearly played a major role in spreading WN virus during this epizootic from the four states originally involved in 1999 to twelve states in 2000. Earlier studies had suggested that wild birds were involved in the geographic dispersal of WN virus during their migratory flights, but definitive data were lacking. A number of humans and horses and a few other mammals have been reported infected with WN virus during this epidemic/epizootic. Until better serosurvey data becomes available to determine more accurate infection rates in different species, comparisons are difficult to make, but the preliminary data strongly suggest that birds have been the important vertebrate hosts for WN virus transmission during the epidemic/epizootic in the northeastern United States.

THE DISEASE

Previous Observations

The earliest isolates of WN virus from humans were associated with a mild febrile disease or asymptomatic infections. Several studies were conducted during the early to mid-1950s that provided more detailed information on the clinical presentation of WN virus infection in humans. In Egypt, village-based studies conducted from 1952–1954 on children presenting to a medical clinic found that most WN virus infections in this age group presented as mild acute febrile episodes. These investigators found no evidence of central nervous system involvement in WN virus infections. From these studies it was clear that many asymptomatic infections or infections too mild for the patient to seek medical care also occurred.

Around the same time period, a series of WN epidemics were described in Israel. The first virologically proven epidemic occurred in 1951 among the inhabitants of an agricultural community. Out of 303 inhabitants, 123 (41%) clinical cases were described. Both children and adults presented with acute self-limited febrile disease, but recovery was noted to be more rapid in children compared to adults. Most adults in this study were under the age of 30 years. No fatal cases occurred, and no cases of frank meningitis or encephalitis were diagnosed, although 10 of the children did present with a positive Brudzinski’s sign. A detailed clinical description of 50 additional hospitalized cases of WN confirmed by serology or virus isolation was made during a 1952 epidemic. Most of the patients were young adult soldiers and presented with a typical acute febrile disease course. Only one case of mild aseptic meningitis was reported among these 50 cases.

In 1957 another large epidemic of WN occurred in Israel, and clinical data were described from three distinct patient groups, soldiers living in army camps, children and adults living in and around the town of Hadera, and elderly persons living in two nursing homes. Most patients in the first two groups presented with typical clinical episodes of WN fever similar to what had been described earlier in Israel, although three cases were complicated by meningoencephalitis. From the third group of 45 elderly patients, 12 had a severe course of meningoencephalitis, but all recovered. The other 33 elderly patients presented with only fever. Interestingly, none of these 45 patients presented with lymphadenopathy or rash that had been commonly associated with WN virus infections in the past in Israel. In addition to these 45 serologically confirmed WN infections, four more elderly patients died from diffuse encephalitis during this epidemic, but serological confirmation of these cases was
lacking. These four fatal cases almost certainly represent the first human deaths ever attributed to naturally acquired WN virus infection. This epidemic also provided the first evidence that WN infection may be more severe in elderly persons.

The 1957 epidemic in Israel was the only epidemic of WN prior to the 1996 Romania epidemic in which severe neurological disease, particularly associated with fatalities, was a prominent clinical presentation. However, other sporadic severe cases of WN virus infection have been reported, including fatal cases of encephalitis in children in India53 and fatal cases of hepatitis in the Central African Republic.54

In addition to disease associated with natural WN virus infections, disease occurring following the experimental infection of advanced cancer patients also has been described.33 Of 78 patients experimentally infected with one of the earliest human isolates of WN virus from Egypt, 89% developed fever as their only clinical evidence of disease. However, 11% did develop signs of diffuse encephalitis, but no fatalities were attributed to the WN virus infections.

Other than humans the only animals that had been reported to develop illness following natural WN virus infection prior to the 1999 New York City epidemic/epizootic were horses and a single bird. An isolate of WN virus was recovered from the brain of a 12-year-old horse that died of encephalitis in Egypt in 1959,42 and another isolate was obtained from the lumbar spinal column of a 6-month-old horse with encephalitis in France in 1965.44 During the 1952–1954 field study in Egypt, WN virus was recovered from the brain, spleen, and blood of a sick pigeon.17

In addition to natural infections, several animal species have been experimentally infected with WN virus; however, these studies should be interpreted with caution, since the dose and route of infection usually do not mimic natural exposure. Both horses and wild birds have been reported to develop disease following inoculation of WN virus. In Egypt, two mules, six donkeys, three horses, two sheep, and a water buffalo were infected with WN virus by subcutaneous and/or intravenous inoculation or were exposed to the bites of WN virus–infected mosquitoes, but only two of the donkeys developed low grade fever.42 Apparently some foals inoculated subcutaneously with WN virus in France developed a severe encephalomyelitis, but adult horses did not develop severe disease.44

In Egypt, 13/13 hooded crows and 10/16 house sparrows died after being fed on by WN virus–infected mosquitoes.34 Most deaths occurred between four and seven days postexposure. No deaths were recorded in kestrels, buff-backed herons, and palm doves, which were also infected during these same experiments. In experiments in South Africa, 13 species of wild birds were inoculated intramuscularly with WN virus.41 Although almost all of the birds became infected and some of the birds died during the viremic period, the authors attributed the mortality to handling, not to the infection. Neither house sparrows nor crows were included in these experiments.

Current Observations

Within a period of less than five years, epidemics of WN occurred in and around three large urban areas, producing hundreds of more cases of severe disease in infected humans than had been identified since the discovery of this virus in 1937,13,23,47 Based on serosurvey data collected after the epidemics in southeastern Romania and the northeastern United States, many asymptomatic or mild infections also occurred for each case presenting with neurological disease (less than 1% of infections presented
The most severe and particularly fatal infections mostly occurred in elderly patients, similar to the situation reported during the 1957 epidemic in Israel. The case fatality ratio in the southeastern Romania epidemic was 4.3%, and for the northeastern U.S. epidemic for 1999 the case fatality rate was approximately 11%. On the basis of the limited data presented, the case fatality rate in the Volgograd epidemic was similar. A clinical presentation of severe muscle weakness resembling Guillain-Barré syndrome was prominent during the northeastern U.S. epidemic, and apparently a few similar cases were reported during the 1996 Romania epidemic. Prior to these recent cases, only one other WN infection had been described with a similar clinical presentation in a young adult from Israel. Unfortunately, reliable population-based data on the clinical/subclinical infection ratio and age-specific case fatality rates are not available from earlier epidemics of WN virus infection to compare with these recent epidemics.

Of the three major urban epidemics of WN that have occurred since 1996, only in the northeastern United States has disease been reported in animals other than humans. The most striking feature of the epidemic/epizootic that occurred in New York and surrounding states during 1999–2000 was the thousands of dead and moribund birds that were found infected with WN virus. This is the first time that significant bird mortality has been attributed to natural infection with this virus. The common crow has been the species most frequently reported to be killed by WN virus, but 75 other species have also been found infected with WN virus. Pathological studies of birds have shown that not only the brain but many extraneural tissues, as well, had lesions caused by WN virus infection. The reason why WN virus has been so deadly for birds during this epizootic is not known, but possible reasons are that birds in the Western Hemisphere have not been evolutionarily selected for resistance in the absence of WN virus circulation in this part of the world or that the particular strain of WN virus causing this epizootic has enhanced virulence for birds. The latter possibility is supported by the fact that a WN virus strain recently associated with bird deaths in Israel has been shown by nucleotide sequence comparison to be almost identical to the strain circulating in the northeastern United States.

Clinical cases of WN infection in horses also were reported during the 1999–2000 epidemic/epizootic in the northeastern United States. During this period over 80 clinical cases were confirmed, exceeding the number of cases reported in any past epizootic. Apparently all except one of the horses developing neurological disease have been adults, and their mean age was several years higher than horses that were infected but that did not develop neurological signs.

Interestingly, for the first time during the northeastern U.S. epidemic/epizootic, fatal WN virus infections were identified in mammals other than humans and horses. Fatal infections have apparently been diagnosed in a cat, skunk, squirrel, chipmunk, rabbit, and bats.

THE EPIDEMIOLOGY

Previous Observations

Epidemiological investigations on West Nile virus were conducted in the Nile delta of Egypt from 1952–1954. Villages located in the southern part of the delta were found to be highly endemic for WN virus infection. Transmission was found to
be most intense during the summer months from June through September, with the peak occurring during July. Serosurveys showed that 50% of the four year olds had neutralizing antibody to WN virus that increased to over 90% in twenty year olds, and seroconversion rates exceeding 20% were documented over a three-month period. By contrast, in villages along the northern rim of the Nile delta close to the Mediterranean coast, WN virus activity was found to be much lower. Several factors were found to differ between these two regions that might account for the differences in transmission intensity. Compared to the northern delta villages, the southern villages had a greater human population density (600 versus 200 per square kilometer), a greater amount of land under irrigated cultivation, and a much higher density of the main mosquito vector, *Culex univittatus*.

Around this same time the first epidemics of WN were described in Israel at two major areas located north and south of Tel Aviv along the Mediterranean coastal plain. The seasonal occurrence of these epidemics followed the same summertime pattern as the endemic transmission cycle observed in Egypt. The reason that these epidemics were geographically circumscribed to these two main sites was not determined. Apparently both sites were located in regions previously covered by swamps. Many of the swamps had been converted to fish ponds that still retained some of the earlier ecological characteristics of the swamp habitat.

The isolation of WN virus from sick humans and a horse in the Rhone delta of France and from sick humans in the Volga delta of Russia in the 1960s considerably extended the known epidemic range of WN virus. It is not clear from these early studies if such outbreaks represented a periodic introduction of WN virus into these regions, possibly via migrating birds, or a flare-up of low-level enzootic activity.

Prior to the 1996 epidemic in southern Romania, the largest known epidemic occurred in the Cape province of South Africa in 1974. This epidemic occurred following unusually heavy rains in this normally arid part of the country. Factors contributing to this epidemic were thought to be the unusually heavy rainfall, an increased density of the main mosquito vector, *Culex univittatus*, and the high summer temperatures that prevail in the area where the epidemic occurred.

Prior to the 1974 epidemic, sporadic WN virus infections of humans had been recognized in South Africa, but usually in the moister high inland plateau region. Ecological studies conducted in this region during the 1960s had already provided insight into how manmade modifications of the environment, weather conditions, and mosquito behavior can all impact on the transmission cycle of WN virus.

Studies conducted in other countries from the late 1950s through the early 1990s revealed the widespread geographical distribution of WN virus throughout Africa and its presence in southern Europe and South Asia. However, only a few cases of human disease attributable to WN virus infection, usually among residents of rural areas, were reported during this period.

### Current Observations

Since 1996 large epidemics caused by WN virus have occurred in southern Romania (1996), in the Volgograd region of Russia (1999), and in the northeastern United States (1999). All of these epidemics involved densely populated urban areas as well as surrounding suburban/rural areas and have been characterized by a large number of cases of severe neurological disease. All three sites were located ad-
jacent to large rivers, presumably providing a favorable wetland habitat for attracting both resident and migratory species of wild birds. As mentioned earlier, wild or domestic birds have been implicated as important hosts in both the southern Romania and northeastern U.S. epidemics.

These are the first epidemics reported from large cities and the first epidemics in which *Culex pipiens* has been implicated as a major vector. Apparently all three urban areas had lower than normal rainfall the summers of the epidemics. Such dry conditions presumably could have increased the number of favorable breeding sites available to *Culex pipiens*, which readily lay eggs in stagnant and polluted water sources.

Detailed risk factor data have only been reported from the southern Romania epidemic. A case-control study comparing asymptomatically infected and uninfected persons identified the presence of mosquitoes in the home, more mosquito bites per day, and, for apartment dwellers, having a flooded basement as risk factors for acquiring infection. Several apartment buildings in Bucharest were noted to have basements flooded with a mixture of drinking water and sewage from leaking pipes. A second case-control study comparing patients with WN meningoencephalitis to WN-infected asymptomatic cases found spending a greater amount of time outdoors as a significant risk factor for developing severe disease. Risk factor data has not been published from the epidemics in the Volgograd region or the northeastern United States; however, an initial investigation carried out by the New York City Department of Health on the first cluster of eight cases reported in that city found that all of the patients were active outdoors.

**CONCLUSION**

Comparing knowledge acquired during the three recent urban epidemics about vectors, enzootic/epizootic hosts, virulence of viral strains, and environmental conditions to knowledge gained over the previous 60 years during studies on the ecology of West Nile virus will, we trust, lead to a greater understanding of the factors that contributed to this sudden eruption in epidemic activity. However, to successfully accomplish this, detailed epidemiological and virological studies must be continued and expanded to address the many questions that have arisen from the recent epidemics, such as (1) the role of different mosquito species in the transmission of West Nile virus to birds, humans, horses, and possibly other mammals; (2) the contribution of humans and horses to the infection of mosquitoes with West Nile virus; (3) the importance of nonmosquito transmission, such as direct bird to bird or predator to prey transmission; (4) the importance of genetic differences seen between lineages I and II West Nile viruses and between different clades of West Nile virus within the same lineage in virulence expression and other aspects of the ecology of these viruses; (5) the role of mosquitoes and birds in the overwintering of West Nile virus in temperate regions; (6) the importance of migrating birds in introducing or reintroducing West Nile virus into areas of Europe; and (7) the influence of different natural and man-made environmental conditions on the intensity of West Nile virus transmission. Clearly much research remains to be done on this important emerging human pathogen.
REFERENCES


