West Nile Virus Isolates from Mosquitoes in New York and New Jersey, 1999

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An outbreak of encephalitis due to West Nile (WN) virus occurred in New York City and the surrounding areas during 1999. Mosquitoes were collected as part of a comprehensive surveillance program implemented to monitor the outbreak. More than 32,000 mosquitoes representing 24 species were tested, and 15 WN virus isolates were obtained. Molecular techniques were used to identify the species represented in the WN virus-positive mosquito pools. Most isolates were from pools containing *Culex pipiens* mosquitoes, but several pools contained two or more *Culex* species.

In late August 1999, an outbreak of human encephalitis was detected in New York City (NYC) (1). The first cases occurred in a small area in northern Queens and were immunoglobulin M seropositive against St. Louis encephalitis (SLE) virus. The etiologic agent was West Nile (WN) virus (2,3), a member of the Japanese encephalitis virus complex (genus *Flavivirus*, family Flaviviridae), which includes other mosquito-transmitted human pathogens such as Japanese encephalitis virus, SLE virus, Murray Valley encephalitis virus, and Kunjin viruses (4). Both SLE virus, which is a native North American arbovirus, and WN viruses are zoonotic agents maintained in a transmission cycle involving bird and mosquito species (4,5).

Outbreak investigations identified human and animal cases, virus-positive dead birds, seropositive live birds, and virus-positive mosquitoes, indicating widespread virus transmission throughout the NYC metropolitan area (6,7). Sixty-two laboratory-confirmed human cases with clinical illness occurred (46 in NYC, 15 in surrounding suburbs in Westchester and Nassau counties, and 1 in a Canadian tourist who visited NYC) (8). The earliest detected onset of human illness occurred during the first week of August and the latest during the third week of September 1999 (2). In this report, we describe the mosquito surveillance program conducted in response to the outbreak and discuss mosquito species associated with WN virus transmission in 1999.

Materials and Methods

Surveillance designed to monitor mosquito populations associated with the outbreak and determine the species and proportion of mosquitoes carrying the virus was initiated in NYC and surrounding counties during the first 2 weeks of September. NYC and most surrounding counties had not maintained systematic mosquito surveillance and control programs before this outbreak. As a result, no information was available about the density or distribution of mosquito species in the area (1). The exceptions were Nassau and Suffolk counties, NY, and all counties in New Jersey (NJ), where comprehensive mosquito control programs, including surveillance for eastern equine encephalitis (EEE) virus activity, had been in effect for many years. As widespread virus transmission became apparent, mosquitoes were collected from a broader geographic area. Existing mosquito control programs participated by expanding mosquito sampling and providing specimens for testing.

Mosquitoes were collected from September 2, 1999, through October 29, 1999. Some *Culex* species mosquitoes collected earlier in the season as part of long-term EEE virus monitoring programs were provided by Suffolk and Nassau counties to assess evidence of infection in mosquitoes before the onset of human cases. Carbon dioxide-baited CDC miniature light traps (9) or traps of similar design were used to collect host-seeking adult female mosquitoes of various species. CDC gravid traps (10) or traps of similar design were used to collect gravid female mosquitoes (i.e., those that had taken a blood meal and were searching for a site to lay eggs) of the genus *Culex.* Although WN virus has been isolated from >40 mosquito species and several species of ticks (11), *Culex* species mosquitoes have been frequently associated with transmission of SLE and WN viruses (4,12,13).

Mosquitoes were placed in labeled tubes, frozen and held at -70°C, and shipped to the Centers for Disease Control and Prevention, Fort Collins, Colorado. The specimens were identified to species if possible, but the condition of certain morphologically similar *Culex* mosquitoes often prevented this. Morphologic characteristics essential for accurate species identification are often damaged during mosquito

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collection and shipping (and as a result of natural aging of mosquitoes). Therefore, many specimens were only identified to the level of genus or to a species group (e.g., *Cx. pipiens/restuans* group, which includes the morphologically similar *Cx. pipiens* and *Cx. restuans* species). All specimens, including those that appeared to contain blood meals or partially digested blood meals, were tested for virus. Therefore, the virus infection rate in the mosquito population reflects the proportion of mosquitoes that had contacted a viremic host. Specimens were grouped into pools of 50 (by species, date, and location of collection) and were tested for virus. Every mosquito pool was tested by a Vero cell plaque assay (14), which is sensitive to all North American mosquito-transmitted pathogenic viruses and many nonpathogenic mosquito-transmitted viruses. After WN virus was determined to be the etiologic agent, a WN virus-specific reverse transcriptase-polymerase chain reaction (RT-PCR) assay (15) was used in conjunction with the Vero cell plaque assay to detect and identify WN virus in mosquito pools. Other viruses isolated in the plaque assay were identified by virus-specific RT-PCR (R. Lanciotti, unpub. data). The identity of the mosquitoes in virus-positive pools was subsequently determined or verified by species-diagnostic PCR (16). This technique, based on interspecific nucleic acid sequence variation, identifies *Cx. pipiens*, *Cx. restuans*, or *Cx. salinarius* (in combination or alone) in a pool of 50 mosquitoes.

Results

During the surveillance program, 32,814 mosquitoes representing 25 species were collected and tested for WN virus in 1,853 pools (Table 1). More than half of mosquitoes tested (18,016) were in the genus *Culex*; most of these could not be identified to species but were likely *Cx. pipiens* or *Cx. restuans*. In the remaining specimens, the predominant species were the floodwater mosquitoes *Aedes vexans* and *Ae. trivittatus*. The collection period, number of *Culex* mosquitoes, and number of other mosquito species tested for each of the 10 NY and 10 NJ counties providing specimens are listed in Table 2. The number collected and tested was not a good representation of the relative population density of *Culex* and other species mosquitoes because sampling was not consistent across participating counties. The total number collected was higher in areas where sampling was more intense. The numbers of *Culex* and other species within a county were representative of the relative abundance of various mosquito larval habitats where mosquito traps were placed (e.g., permanent water sites appropriate for *Cx. pipiens* and *Cx. restuans* development vs. floodwater habitats appropriate for *Ae. vexans* and *Ae. trivittatus*).

Table 1. Mosquito species identification by morphologic characteristics, New York and New Jersey, 1999

Genus	Species	Total
Aedes	*albopictus*	8
	canadensis	26
	cantator	55
	cinereus	426
	japonicus	64
	sollicitans	178
	sticticus	175
	taeniorhynchus	187
	triseriatus	132
	trivittatus	3,274
	vexans	7,956
	unidentified *Aedes* sp.	901
Anopheles	*bradleyi*	1
	punctipennis	23
	quadrimaculatus	77
	walkeri	32
	unidentified *Anopheles* sp.	12
Coquillettidia	*perturbans*	155
Culiseta	*melanura*	587
Culex	*erraticus*	4
	pipiens	511
	pipiens/restuans	4,686
	restuans	215
	salinarius	1,866
	territans	8
	unidentified *Culex* sp.	10,726
Psorophora	*ferox*	245
	unidentified *Psorophora* sp.	6
Uranotaenia	*sapphirina*	31
Unidentified genus	unidentified mosquito sp.	256
Total		32,814

Table 2. Mosquito species tested for West Nile virus, New York and New Jersey, 1999

	Collection dates		No. tested	
	From	Through	*Culex* sp.	Other sp.
New York counties (borough)				
Bronx[a]	9/2/99	10/26/99	166	4,679
Kings (Brooklyn)[a]	9/11/99	10/26/99	122	24
New York (Manhattan)	9/11/99	10/26/99	1,344	93
Queens[a]	9/10/99	10/26/99	6,245	156
Richmond (Staten Island)	10/2/99	10/26/99	18	38
Nassau[a]	8/19/99	10/22/99	1,301	846
Orange	9/13/99	9/13/99	80	16
Rockland	9/13/99	10/5/99	171	1,877
Suffolk[a]	6/8/99	10/20/99	6,849	1,217
Westchester[a]	9/8/99	10/19/99	334	1,206
New Jersey counties				
Bergen	9/22/99	10/20/99	48	328
Burlington[b]	10/4/99	10/26/99	0	234
Camden[b]	10/4/99	10/25/99	0	53
Cape May[b]	9/15/99	10/30/99	0	90
Essex	9/24/99	10/12/99	18	521
Hudson[a]	9/9/99	10/20/99	1,281	3,255
Middlesex	9/24/99	9/30/99	9	25
Ocean[b]	9/29/99	9/29/99	0	3
Salem[b]	9/29/99	10/28/99	0	142
Warren	10/28/99	10/28/99	7	3

[a]Counties in which West Nile virus-infected *Culex* species mosquitoes were collected.
[b]Other species tested are primarily *Culiseta melanura* collected as part of New Jersey's long-term eastern equine encephalitis surveillance program.

Suffolk County, NY, was an exception. Total collections in Suffolk County were very large, and *Culex* species mosquitoes were selectively submitted for testing. Several NJ counties provided mainly *Culiseta melanura* mosquitoes for testing. This species feeds almost exclusively on birds and is the primary enzootic vector of EEE virus. These specimens were solicited to determine if WN virus-infected birds were being fed upon as they migrated south in late summer and early fall. WN virus-infected mosquitoes were collected in six NY counties and one NJ county.

WN virus was isolated from 15 pools of mosquitoes (Table 3). All isolates were from *Culex* species. Identification of the species composition of these pools by molecular techniques indicated that six pools contained exclusively *Cx. pipiens* and

Table 3. West Nile virus-positive mosquito pools, New York and New Jersey, 1999

County	Collection date	Species[a] (morphologic id.)	Species[b] (molecular id.)
Queens, NY	9/12/99	*Culex pipiens*	*Cx. pipiens*
	9/13/99	*Cx. pipiens*	*Cx. pipiens*
	9/13/99	*Cx.* species	*Cx. pipiens*/*restuans*
	9/19/99	*Cx.* species	*Cx. pipiens*
	9/20/99	*Cx.* species	*Cx. pipiens*
	10/10/99	*Cx. pipiens*/*restuans*	insufficient sample
Kings (Brooklyn), NY	9/12/99	Cx. species	*Cx. pipiens*
	9/15/99	*Cx.* species	*Cx. restuans*/*salinarius*
Bronx, NY	9/12/99	*Cx*. species	*Cx. restuans*/*salinarius*
Nassau, NY	9/29/99	*Cx. pipiens*	*Cx. pipiens*
	10/3/99	*Cx.* species	*Cx. pipiens*/*restuans*/*salinarius*
	10/10/99	*Cx. pipiens*/*restuans*	*Cx. pipiens*/*restuans*/*salinarius*
Suffolk, NY	10/4/99	*Cx. species*	*Cx. restuans*/*salinarius*
Westchester, NY	10/1/99	*Cx. restuans*	*Cx. restuans*/*salinarius*
Hudson, NJ	9/28/99	*Cx. pipiens*	insufficient sample

[a]Species identification by morphologic characteristics.
[b]Species identification by species-specific polymerase chain reaction primers.

seven contained two or more *Culex* species (combinations of *Cx. pipiens*, *Cx. restuans*, and *Cx. salinarius*). Two pools contained insufficient material for molecular species identification. The only evidence that another species was involved in WN virus transmission in 1999 was the isolation of WN virus from a pool of *Ae. vexans* mosquitoes collected on September 14, 1999, in southwestern Connecticut (7). The earliest WN virus isolates in NY and NJ came from collections made on September 12, 1999, in Queens, Brooklyn, and the Bronx. The latest WN virus isolate came from collections made on October 10, 1999, in Queens and Nassau County. Most isolates were from Queens, which was the location of most human WN-virus infection cases (6).

Other viruses were isolated from mosquitoes during the surveillance program (Table 4). Flanders virus was isolated from 11 pools of *Culex* species mosquitoes, most of which contained combinations of species. Flanders virus is a widely distributed rhabdovirus frequently found in birds and bird-feeding mosquitoes and apparently nonpathogenic in vertebrates (17). EEE virus was isolated from a pool of *Cs. melanura* collected in Burlington County, NJ. Three isolates of a California serogroup virus were obtained from pools of *Ae. trivitattus* collected in the Bronx and Nassau County, NY. Numerous California serogroup viruses are present in this region of North America (18). Although these California serogroup isolates were not specifically identified for this study, they are likely trivitattus virus, a generally nonpathogenic member of the California serogroup commonly found in *Ae. trivittatus* (19).

The minimum infection rate (MIR) of WN virus in *Culex* mosquitoes, expressed as the number infected per 1,000 specimens tested, was calculated by county for the sampling periods (weeks) during which WN virus was isolated from mosquitoes (Table 5). MIR for a given period and location is an indicator of prevalence of virus in the habitat and of transmission intensity and, in many circumstances, is related to the risk for human disease. All *Culex* mosquitoes collected in a county during a particular week, except *Cx. territans,* which feeds predominantly on amphibians, were combined to determine the denominator for this value because many of the *Culex* specimens could not be identified below genus or species levels. As a result, MIR estimates probably underestimate the infection rate for certain *Culex* species and overestimate the rate for others. MIR for WN virus-infected *Culex* in this outbreak was 0.7/1,000 to 57.1/1,000, although the 95% confidence intervals are very large around MIR estimates calculated from small sample sizes.

Conclusion

Mosquito surveillance, although not implemented until late in the outbreak (well after most transmission to humans that resulted in clinical cases), provided information about transmission dynamics that may prove useful in developing

Table 4. West Nile virus-positive mosquito pools containing viruses other than West Nile virus, collection location, date, species composition, and virus identification

County	Collection date	Species[a] (morphologic id.)	Species[b] (molecular id.)	Virus identification
Bronx, NY	9/9/99	*Aedes trivittatus*	not done	California serogroup
	9/12/99	*Ae. trivittatus*	not done	California serogroup
Nassau, NY	10/15/99	*Culex pipiens*/*restuans*	*Cx. pipiens*/*restuans*/*salinarius*	Flanders
	10/16/99	*Ae. trivittatus*	not done	California serogroup
Suffolk, NY	6/29/99	*Cx. pipiens*/*restuans*	*Cx. pipiens*/*restuans*	Flanders
	6/29/99	*Cx. pipiens*/*restuans*	*Cx. restuans*	Flanders
	7/7/99	*Cx. pipiens*/*restuans*	*Cx. pipiens*/*restuans*	Flanders
	7/27/99	*Cx. pipiens*/*restuans*	*Cx. pipiens*/*restuans*/*salinarius*	Flanders
	8/3/99	*Cx. pipiens*/*restuans*	*Cx. pipiens*/*restuans*/*salinarius*	Flanders
	8/10/99	*Cx. pipiens*/*restuans*	insufficient sample	Flanders
	8/10/99	*Cx. pipiens*/*restuans*	insufficient sample	Flanders
	8/16/99	*Cx. pipiens*/*restuans*	insufficient sample	Flanders
	9/28/99	*Cx. restuans*	*Cx. restuans*/*salinarius*	Flanders
Hudson, NJ	9/22/99	*Cx. pipiens*	*Cx. pipiens*	Flanders
Burlington, NJ	10/11/99	*Culiseta melanura*	not done	Eastern equine encephalitis

[a]Species identification by morphologic characteristics.
[b]Species identification by species-specific polymerase chain reaction primers.

Table 5. West Nile (WN) virus infection rates in *Culex* species mosquitoes, New York and New Jersey, 1999

Sampling period	County	#WN virus-positive pools	Total *Culex* specimens tested[a]	MIR[b]	(95% Confidence Interval)
9/12-9/19	Queens	3	820	3.7	(0.8-10.7)
	Kings (Brooklyn)	2	35	57.1	(7.0-191.6)
	Bronx	1	48	20.8	(0.5-110.7)
9/19-9/25	Queens	2	862	2.3	(0.3-8.3)
9/26-10/2	Nassau	1	198	5.1	(0.1-27.8)
	Hudson, NJ	1	138	7.2	(0.2-39.7)
	Westchester	1	92	10.8	(2.0-54.5)
10/3-10/9	Nassau	1	214	4.7	(0.1-25.8)
	Suffolk	1	810	1.2	(0.03-6.9)
10/10-10/16	Queens	1	1496	0.7	(0.02-3.7)
	Nassau	1	135	7.4	(0.2-40.6)

[a]Excluding *Culex territans*.
[b]Minimum infection rate expressed as number infected per 1,000 specimens tested.

new surveillance systems. *Culex* mosquitoes, particularly *Cx. pipiens*, appear primarily responsible for epizootic transmission. *Cx. pipiens* was quite common in Queens, NY, and other areas where isolates were obtained and transmission activity was documented by avian and human surveillance programs. *Cx. restuans* and *Cx. salinarius* were also implicated in virus transmission. Since these species were found only in combination in WN virus-positive pools, their importance is difficult to assess. *Cx. pipiens* and *Cx. restuans* are ornithophilic, feeding mainly on birds and occasionally on mammals (20). *Cx. salinarius*, which is a pest species common in the region (21), feeds readily on humans and other mammals (20), which suggests that it may be involved in epidemic transmission of WN virus.

Relatively high MIR values in areas where human cases occurred validate use of mosquito-based surveillance to estimate risk for virus transmission to humans. MIRs found in this study are consistent with MIRs calculated for WN virus in mosquitoes reported in other areas. MIR estimates for the primary vector species during WN virus outbreaks range from 0.8/1,000 for *Cx. fatigans* in India (22) to as high as 25.0/1,000 for *Cx. univittatus* in South Africa (23). While it is difficult to associate a quantified risk for human disease to an MIR value, evidence from *Cx. pipiens*-borne SLE outbreaks indicates that widespread transmission to humans is likely when MIR exceeds 3/1,000 but may occur at much lower infection rates (24).

Mosquito-based virus surveillance has its limitations. Adequate estimates of virus distribution and transmission require extensive field and laboratory resources to obtain and process large sample sizes over relatively large geographic areas. In addition, identification of field-collected *Culex* mosquito specimens to species by morphologic characters is difficult, and verification of species composition in pools often requires use of molecular techniques not commonly available to mosquito surveillance programs. The importance of accurate mosquito species identification is underscored by the indication that *Cx. salinarius* may have been involved in WN-virus transmission during 1999. This information was not evident from morphologic identification and was determined only by molecular techniques. Accurate identification of species is essential in estimating risk for transmission to humans and directing mosquito control programs.

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