INTRODUCTION

The recent 1999 outbreak of illness due to infection with West Nile (WN) virus in the New York City Metropolitan Area represents the first documented introduction of WN virus into the New World. West Nile virus is a member of the Japanese encephalitis virus serogroup in the family Flaviviridae. In Africa and southern/central Europe, where this virus is enzootic, it has been associated with numerous species of mosquitoes. However, most virus isolations have been made from various Culex (Culex) spp. Despite knowledge of potential vectors in Africa and southern Europe, little is known about the potential for North American mosquito species to serve as a vector for this virus.

We evaluated mosquitoes collected from the epizootic area in New York during the 1999 outbreak of WN, for their potential to become infected with and to transmit WN virus. Third and fourth instar larvae and pupae were collected from September 30 to October 3, 1999, in Bronxville and Larchmont (Westchester County, New York) and reared to the adult stage in New York at ambient temperature. Bronxville and Larchmont are about 20 km northeast of the borough of Queens, the epicenter of the WN outbreak. Adult mosquitoes were collected in Centers for Disease Control miniature light traps (Hausherr’s Machine Works, Toms River, NJ) baited with dry ice on the nights of October 3 and 4, 1999, at the Marshland Conservancy in the Town of Rye which is also in Westchester County, and approximately 27 km from the borough of Queens. Adult mosquitoes were transported at ambient temperature to a biological safety-level–3 laboratory at the United States Army Medical Research Institute of Infectious Diseases at Fort Detrick, Maryland; provided with apple slices as a carbohydrate source; and held at 26°C for 3 to 6 days until exposure to WN virus. Voucher specimens were deposited at the National Museum of Natural History, Smithsonian Institution, Washington, DC.

In conducting research using animals, the investigators adhered to the Guide for the Care and Use of Laboratory Animals prepared by the Committee on Care and Use of Laboratory Animals of the Institute of Laboratory Animal Resources, National Research Council (NIH Publication No. 86–23, Revised 1996). Laboratory facilities are fully accredited by the Association for Assessment and Accreditation of Laboratory Animal Care, International.

In the laboratory, collected mosquitoes were allowed to feed on 1- to 3-day-old leghorn chickens that had been inoculated subcutaneously with $10^{3.3}$ plaque-forming units (pfu) of WN virus 1 to 3 days earlier. We used an unpassed isolate of WN virus (Crow 397–99 strain) obtained from the brain of a crow that had died in the borough of the Bronx on September 14, 1999. Immediately after the mosquitoes fed, 0.1 ml of blood was obtained from the jugular vein of each viremic chicken and added to 0.9 ml of diluent (10% fetal bovine serum in medium 199 with Earle’s salts and antibiotics). The blood suspensions were frozen at −70°C until tested by plaque assay on Vero-cell monolayers (American Type Culture Collection, Rockville, MD) to determine the viremias at the time of mosquito feeding. Procedures for plaque assay were similar to those of Gargan and others except that the second overlay containing neutral red was added 2 rather than 4 days after the original assay. After exposure to the viremic chickens, engorged mosquitoes were transferred to 3.8-liter, screen-topped cardboard cages held at 26°C under a 16:8 hr (light:dark) photoperiod. After an incubation period of 13 or 14 days, these WN virus-exposed mosquitoes were allowed to feed on 1- to 2-day-old uninfected chickens (i.e., “transmission” chickens) to determine if mosquitoes could transmit WN virus by bite. Immediately after the transmission attempt, the mosquitoes were killed by freezing, were identified as to species, and had their legs removed. Bodies and legs were triturated separately in 1 ml of the above diluent.

If virus was recovered from its body, but not its legs, the mosquito was considered to have a nondisseminated infection limited to its midgut. In contrast, if virus was recovered from both body and leg suspensions, the mosquito was considered to have a disseminated infection. Each of the transmission chickens was bled from the jugular vein 2 days after mosquitoes had fed, and the blood handled and analyzed as described above. Recovery of virus indicated transmission.

To further examine virus transmission, some of the original, unfed mosquitoes were inoculated intrathoracically with 0.3 μl of a virus suspension containing $10^{4.3}$ pfu of WN virus/ml ($10^{3.1}$ pfu/mosquito) and allowed to feed on a susceptible chicken 7 to 14 days later. The inoculated mosquitoes and blood specimens from fed-upon chickens were processed as described above.

Viremias in the inoculated chickens fell into two groups: $10^{2.2/0.3}$ and $10^{2.2/0.4}$, and for each of the mosquito species tested, infection rates were higher in the mosquitoes that had ingested blood containing the higher dose of WN virus (Table 1). The Bronxville and Larchmont Culex pipiens had
similar infection and dissemination rates at each infectious dose (Fisher’s exact test, $P = 0.43$). Thus, data from the two geographic populations were combined for further analysis. Overall, 81% of the Cx. pipiens that had ingested blood from a chicken with a viremia of $10^{3.2\pm0.4}$ plaque-forming units (pfu/ml became infected. Although only a small sample was tested, the susceptibility to infection of New York Aedes sollicitans and Aedes taeniorhynchus was consistent with observations made on specimens of these same two species collected on the eastern shore of Virginia (Turell M, unpublished data) (Table 1). For each of the mosquito species tested in the current study, specimens with a disseminated infection were shown to transmit virus by bite. Similar results were found for Ae. sollicitans and Ae. taeniorhynchus collected on the eastern shore of Virginia (Turell M, unpublished data) (Table 1). For each of the mosquito species tested in the current study, specimens with a disseminated infection were shown to transmit virus by bite. Similar results were found for Ae. sollicitans and Ae. taeniorhynchus collected on the eastern shore of Virginia (Turell M, unpublished data). Thus, there was no evidence of a significant salivary gland barrier in any of the species of mosquitoes tested, and individuals that develop a disseminated infection would be expected to transmit virus by bite during their next blood feeding. Although WN virus was isolated from field-collected Aedes vexans during the recent outbreak in the New York City Metropolitan Area, this species was only a moderately efficient laboratory vector in our study, and was also an inefficient vector of the closely related Flavivirus, St. Louis encephalitis. Culex pipiens appears to have been the principal epizootic vector during the outbreak in the New York City Metropolitan Area in 1999 as evidenced by the following: 1) this species was abundant during the New York outbreak, 2) it is strongly ornithophilic, 3) West Nile virus was isolated from Cx. pipiens in New York, and 4) the current study demonstrated that it is a relatively competent laboratory vector of WN virus. However, the number of mosquitoes tested in this study is small and additional information is needed to determine potential bridge vectors between viremic avian hosts and humans and equines.

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Table 1

<table>
<thead>
<tr>
<th>Species of Mosquito</th>
<th>Viremia (pfu ingested)</th>
<th>Number tested</th>
<th>Infection rate*</th>
<th>Dissemination rate†</th>
<th>Disseminated transmission rate [N]</th>
<th>Estimated transmission rate §</th>
</tr>
</thead>
<tbody>
<tr>
<td>Aedes taeniorhynchus</td>
<td>5.2 ± 0.2</td>
<td>2</td>
<td>0</td>
<td>0</td>
<td>n.t.</td>
<td>0</td>
</tr>
<tr>
<td>Aedes vexans</td>
<td>5.2 ± 0.2</td>
<td>3</td>
<td>0</td>
<td>0</td>
<td>n.t.</td>
<td>0</td>
</tr>
<tr>
<td>Culex pipiens</td>
<td>5.2 ± 0.2</td>
<td>46</td>
<td>17</td>
<td>2</td>
<td>100 (1)</td>
<td>2</td>
</tr>
<tr>
<td>Aedes sollicitans</td>
<td>7.2 ± 0.2</td>
<td>1</td>
<td>100</td>
<td>0</td>
<td>n.t.</td>
<td>?</td>
</tr>
<tr>
<td>Aedes taeniorhynchus</td>
<td>7.2 ± 0.2</td>
<td>2</td>
<td>0</td>
<td>0</td>
<td>n.t.</td>
<td>0</td>
</tr>
<tr>
<td>Aedes vexans</td>
<td>7.2 ± 0.2</td>
<td>13</td>
<td>46</td>
<td>8</td>
<td>100 (1)</td>
<td>8</td>
</tr>
<tr>
<td>Culex pipiens</td>
<td>7.2 ± 0.2</td>
<td>83</td>
<td>81</td>
<td>16</td>
<td>86 (7)</td>
<td>14</td>
</tr>
</tbody>
</table>

* Percentage of mosquitoes containing virus in their bodies.
† Percentage of mosquitoes with a disseminated infection that transmitted virus (number refeeding).
‡ Percentage of mosquitoes with a disseminated infection after ingestion of a viremic blood meal × the percentage of mosquitoes with a disseminated infection that transmitted WN virus to chickens by bite.

n.t. = not tested.

Disclaimer: The views of the authors do not purport to reflect the positions of the Department of the Army or the Department of Defense.

Authors’ addresses: Michael J. Turell and Monica L. O’Quinn, Vector Assessment Branch, Virology Division, United States Army Medical Research Institute of Infectious Diseases, 1425 Porter Street, Fort Detrick, Maryland 21702–5011. JoAnne Oliver, Arthropod-borne Disease Program, New York State Department of Health, Room 133 Illick Hall, State University of New York, College of Environmental Science and Forestry, Syracuse, New York 13210.

REFERENCES