Vector Competence of Selected North American Culex and Coquillettidia Mosquitoes for West Nile Virus

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To control West Nile virus (WNV), it is necessary to know which mosquitoes are able to transmit this virus. Therefore, we evaluated the WNV vector potential of several North American mosquito species. Culex restuans and Cx. salinarius, two species from which WNV was isolated in New York in 2000, were efficient laboratory vectors. Cx. quinquefasciatus and Cx. nigripalpus from Florida were competent but only moderately efficient vectors. Coquillettidia perturbans was an inefficient laboratory vector. As WNV extends its range, exposure of additional mosquito species may alter its epidemiology.

In 1999, West Nile virus (WNV) was recognized for the first time in the Western Hemisphere, causing human, equine, and avian deaths (1-4). Entomologic investigations of this outbreak resulted in the isolation of WNV from two mosquito species, Aedes vexans and Culex pipiens (2). The distribution of WNV in the United States expanded in 2000 from four northeastern states (Connecticut, Maryland, New Jersey, and New York) to eight additional eastern states (Delaware, Massachusetts, New Hampshire, North Carolina, Pennsylvania, Rhode Island, Vermont, and Virginia) and the District of Columbia (4).

During 2000, evidence of WNV infection was reported in nine additional mosquito species (4). These isolation studies provide preliminary evidence of involvement of several mosquito species in the transmission cycle. However, it is necessary to determine if any of these species are able to transmit WNV by bite before they can be implicated as vectors. In addition, the population density, host preference, feeding behavior, longevity, and seasonal activity of each mosquito species must be considered in determining its relative importance.

In Africa, southern Europe, and western Asia, WNV has been enzootic for many years, with isolations from >40 mosquito species, most in the genus Culex (5,6). Laboratory studies indicate that many Culex and Aedes species in the traditional enzootic range of WNV are competent laboratory vectors (5,6). However, because the introduction of WNV to the United States was recent, little is known about the potential for North American mosquito species to act as vectors of this virus.

Preliminary studies with North American mosquitoes indicate that New York strains of Cx. pipiens and Ae. vexans are competent but only moderately efficient laboratory vectors (7). The vector competence of Ae. aegypti, Ae. albopictus, Ochlerotatus atropalpus, Oc. j. japonicus, Oc. sollicitans, and Oc. taeniorynchus for WNV has since been evaluated (8,9). WNV was isolated from Cx. restuans and Cx. salinarius caught during the 2000 outbreak in New York (4); however, the ability of these species to transmit WNV by bite is unknown. Other viruses circulating in the eastern United States have a similar epidemiology (e.g., St. Louis encephalitis [SLE] and eastern equine encephalomyelitis [EEE] viruses): they are maintained in an enzootic cycle involving birds as amplifying hosts and ornithophilic mosquitoes as enzootic vectors. Based on their association with these other arboviruses, several mosquito species should be considered potential vectors of WNV, although it has not yet spread to areas where these mosquitoes are found.

To assist public health personnel in assessing the risk that a potential mosquito vector represents for transmission of WNV, we conducted laboratory studies to evaluate the vector competence of Cx. nigripalpus, Cx. quinquefasciatus, Cx. restuans, Cx. salinarius, and Coquillettidia perturbans.

Materials and Methods

Mosquitoes

We tested five mosquito species for susceptibility to WNV (Table 1). Cx. nigripalpus was tested because it is the primary vector of SLE virus in Florida (10,11). Cq. perturbans is a potential epizootic vector of EEE virus in the eastern United States (12). Cx. salinarius has been found naturally infected with WNV (4) and has been implicated as a potential epizootic vector of EEE virus (12). Cx. quinquefasciatus has been implicated as a potential enzootic and epizootic vector of SLE virus (13). Cx. restuans has been found naturally infected with WNV (4) and may play a secondary role in the transmission and maintenance of SLE virus (14).
Virus and Virus Assay

The WNV strain (Crow 397-99) used was isolated from a dead crow found in the Bronx, New York, during an epizootic in 1999 (7); it had been passaged once in Vero cell culture. Stocks of virus at a concentration of $10^{4.2} \text{PFU/mL}$ were prepared in a standard diluent (10% heat-inactivated fetal bovine serum in Medium 199 with Earle's salts [GIBCO-BRL, Gaithersburg, MD] NaHCO$_3$, and antibiotics). Viral stocks, triturated mosquito suspensions, and chicken blood samples were tested for infectious virus by plaque assay on Vero cells as described (15), except that the second overlay, containing neutral red stain, was added 2 days after the first overlay.

Vector Competence Studies

Mosquitoes were allowed to feed on 2- to 3-day-old leg-horn chickens (Gallus gallus) that had been inoculated with approximately $10^5 \text{PFU}$ of WNV 1 to 2 days earlier. Immediately after the mosquitoes fed, blood was drawn from the jugular vein of each chicken (0.1 mL of blood into 0.9 mL of heparinized diluent), and the blood suspensions were frozen at $-70^\circ\text{C}$ until assayed for virus to determine viremias at the time of mosquito feeding. After feeding on viremic chickens, engorged mosquitoes were transferred to 3.8-L screen-topped cardboard cages and held at 26°C with a 16:8(L:D)-hour photoperiod. After an incubation period of 12 to 14 days, the mosquitoes were allowed to feed again on 1- to 2-day-old chickens. Immediately after the transmission attempt, the mosquitoes were killed by freezing, their feeding status was determined, and their legs and bodies were triturated separately in 1 mL of diluent.

Infection was determined by recovery of virus from the mosquito tissue suspension. If virus was recovered from its body but not its legs, the mosquito was considered to have a nondisseminated infection limited to its midgut. If virus was recovered from both the body and leg suspensions, the mosquito was considered to have a disseminated infection (16). We defined the infection and dissemination rates as the percentages of mosquitoes tested that contained virus in their bodies or legs, respectively. Chickens used in the transmission attempts were bled from the jugular vein 2 days after mosquito feeding, and the blood was handled as described above. Recovery of virus from this blood indicated transmission (9).

To examine viral transmission more efficiently, some of the unfed mosquitoes were inoculated intrathoracically (17) with 0.3 µL of a viral suspension containing $10^{4.2} \text{PFU/mL}$ of WNV/mosquito. Recovery of virus from this blood indicated transmission (9).

To estimate transmission rates by species, we determined the percentage of mosquitoes with disseminated infection (after either oral exposure or by intrathoracic inoculation) that transmitted virus by bite. We then multiplied that percentage times the percentage of mosquitoes that developed a disseminated infection after feeding on a host with a particular viremia. The result is the estimated transmission rate for those mosquitoes.

Statistical Analysis

Confidence intervals (95%) for infection and dissemination rates were calculated by SAS 8.0 (18). We used Fisher exact test to compare transmission rates among disseminated mosquitoes in each species. Significance was tested at a level of alpha = 0.05.

Results

All mosquito species examined in this study were susceptible to infection with WNV and developed disseminated infections (Table 2). Infection rates were $\geq 84\%$ in all the Culex species when the viral titer in the donor chicken was $\geq 10^{6.3} \text{PFU/mL}$ of blood. In contrast, the infection rate was 18% in Cq. perturbans fed on a chicken with a similar level of viremia. For most mosquito species tested, dissemination rates were approximately one fourth the infection rates.

None of the Culex species tested differed significantly in the percentages of mosquitoes with disseminated infection that transmitted virus (Table 3). However, the percentage of Cq. perturbans with disseminated infection that transmitted WNV was significantly lower than that for Cx. nigripalpus and Cx. quinquefasciatus (Fisher exact test, $p < 0.01$).

We used the percentage of mosquitoes with disseminated infection that transmitted virus from Table 2 and the dissemination rates at 14 days after the infectious blood meal from Table 2 to estimate the transmission rate for each species. Under laboratory conditions and at the highest viral dose tested, the Culex species tested were moderately efficient vectors (estimated transmission rates 10% to 55%). In contrast, Cq. perturbans was an inefficient vector (estimated transmission rate $\leq 2\%$) (Table 2).

Conclusions

Previous laboratory studies indicate that a number of North American mosquito species could serve as vectors of WNV (7-9). Our study indicated that several additional Culex species and Cq. perturbans are potential vectors of WNV. The viremias used in our study, $10^{5-7.5} \text{PFU/mL}$ of blood, are consistent with levels considered to be low to moderate viremias for hooded crows and house sparrows in Egypt (19) and experimentally infected North American house sparrows and other passerine birds (N. Komar, pers.)
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### Table 2. Infection, dissemination and estimated transmission rates for mosquitoes orally exposed to West Nile virus

<table>
<thead>
<tr>
<th>Species</th>
<th>Strain</th>
<th>Viral dose&lt;sup&gt;a&lt;/sup&gt;</th>
<th>No. tested</th>
<th>Infection rate&lt;sup&gt;b&lt;/sup&gt;</th>
<th>Dissemination rate&lt;sup&gt;c&lt;/sup&gt;</th>
<th>Estimated transmission rate</th>
</tr>
</thead>
<tbody>
<tr>
<td>Culex nigripalpus</td>
<td>Indian River</td>
<td>4.6</td>
<td>7</td>
<td>29 ([4-71], 2)</td>
<td>0 ([0-41], 0)</td>
<td>0</td>
</tr>
<tr>
<td>Cx. quinquefasciatus</td>
<td>Sebring</td>
<td>5.5</td>
<td>16</td>
<td>50 ([25-75], 8)</td>
<td>6 ([0-30], 1)</td>
<td>6</td>
</tr>
<tr>
<td>Cx. quinquefasciatus</td>
<td>Vero Beach</td>
<td>5.0</td>
<td>13</td>
<td>46 ([19-75], 6)</td>
<td>0 ([0-25], 0)</td>
<td>0</td>
</tr>
<tr>
<td>Cx. restuans</td>
<td>Maryland</td>
<td>6.6±0.3</td>
<td>11</td>
<td>100 ([72-100], 11)</td>
<td>55 ([23-83], 6)</td>
<td>55</td>
</tr>
<tr>
<td>Cx. salinarius</td>
<td>Chambers</td>
<td>6.6±0.3</td>
<td>20</td>
<td>95 ([75-100], 19)</td>
<td>60 ([36-81], 12)</td>
<td>34</td>
</tr>
<tr>
<td>Coquillettidia perturbans</td>
<td>Laurel</td>
<td>6.6±0.3</td>
<td>11</td>
<td>18 ([2-52], 2)</td>
<td>9 ([0-41], 1)</td>
<td>2</td>
</tr>
</tbody>
</table>

<sup>a</sup>Log<sub>10</sub> PFU/mL of blood.  
<sup>b</sup>Percentage of mosquitoes containing virus in their bodies (95% confidence interval [CI]), number infected.  
<sup>c</sup>Percentage of mosquitoes containing virus in their legs (95% CI), number disseminated.  
<sup>d</sup>The estimated transmission rate = the percentage of mosquitoes that developed disseminated infection 12-14 days after ingesting WNV multiplied by the percentage of mosquitoes with disseminated infection that transmitted infection by bite (Table 3).

### Table 3. Percent of mosquitoes with disseminated infection (after either oral exposure to or intrathoracic inoculation with West Nile virus) that transmitted virus by bite

<table>
<thead>
<tr>
<th>Species (strain)</th>
<th>No. tested</th>
<th>Percent transmission&lt;sup&gt;a&lt;/sup&gt;</th>
</tr>
</thead>
<tbody>
<tr>
<td>Culex nigripalpus</td>
<td>15</td>
<td>87 ([60-98], 13)&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>Cx. quinquefasciatus</td>
<td>18</td>
<td>94 ([73-100], 17)&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>Cx. quinquefasciatus</td>
<td>Not determined</td>
<td></td>
</tr>
<tr>
<td>Cx. restuans (Maryland)</td>
<td>2</td>
<td>100 ([16-100], 2)&lt;sup&gt;a,b&lt;/sup&gt;</td>
</tr>
<tr>
<td>Cx. salinarius (Chambers)</td>
<td>16</td>
<td>56 ([30-80], 9)&lt;sup&gt;a,b&lt;/sup&gt;</td>
</tr>
<tr>
<td>Coquillettidia perturbans (Laurel)</td>
<td>17</td>
<td>24 ([7-50], 4)&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

<sup>a</sup>Percentage of mosquitoes with disseminated infection that transmitted virus by bite (95% confidence interval), number transmitting.  
<sup>b</sup>Percent transmissions followed by the same letter are not significantly different at alpha = 0.05 by Fisher exact test.
Research

As WNV extends its range southward and westward, its vector competence may be an ideal bridge vector between the enzootic avian cycle of WNV and mammalian hosts.

Cq. perturbans was the least efficient WNV vector of those we tested. Contributing heavily to this finding was the presence of a salivary gland barrier. Less than one fourth of Cq. perturbans with disseminated infection transmitted WNV by bite (Table 3). Furthermore, this is the only North American species tested so far that exhibits a substantial salivary gland barrier. Cq. perturbans is generally regarded as mammalophagic (30,34); however, there are reports of its feeding on wading birds and passerines (34-36) and of numerous EEE virus isolates from field-collected specimens (37-40). Despite the low transmission rate, the role of Cq. perturbans as a potential epizootic vector of WNV should not be totally discounted.

Our study extended the list of potential North American mosquito vectors of WNV. None of the North American species tested in this study or others (7-9) was refractory to WNV. However, there is a wide range in vector competence in these species, ranging from nearly incompetent (e.g., Cq. perturbans) to highly efficient (e.g., Oc. j. japonicus). These data are similar to those for Old World mosquito vectors of WNV, in which all Aedes and Culex species tested were competent vectors (5,6). Vector competence studies indicate that North American mosquitoes fall into three general categories depending on genera and, in some instances, breeding habitat: highly efficient, container-breeding Aedes and Ochlerotatus species; moderately efficient, Culex species; and inefficient, floodwater-breeding Aedes and Ochlerotatus and Cq. perturbans.

As WNV extends its range southward and westward, additional mosquito species (e.g., Cx. nigripalpus, Cx. quinquefasciatus, Cx. tarsalis, and Ae. albopictus) will have greater exposure to this virus. Involvement of some of the species, particularly container-breeding Aedes and Ochlerotatus, may alter the epidemiology of WNV and present additional control problems for mosquito abatement personnel. In addition, mosquitoes are more efficient vectors at warmer temperatures (41,42; Dohm, unpub. data), a factor that will further change the epidemiology of WNV as its range extends southward.

Acknowledgments

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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 Pub. No. 86-23, Revised 1996). The facilities are fully accredited by the Association for Assessment and Accreditation of Laboratory Animal Care, International.

Major Sardelis is a graduate student in the Division of Tropical Public Health at the Uniformed Services University of Health Sciences. His research interests focus on the impact of newly invasive mosquito species on arbovirus transmission in the eastern United States and the distribution and biornomics of mosquitoes in the Amazon Basin region of Peru.

References

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