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. taeniorhynchus* 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).
We defined the infection and dissemination rates as the per-
quito was considered to have a disseminated infection (16).
recovered from both the body and leg suspensions, the mos-

nondisseminated infection limited to its midgut. If virus was
body but not its legs, the mosquito was considered to have a
mosquito tissue suspension. If virus was recovered from its
bodies were triturated separately in 1 mL of diluent.

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 $10^{4.2}$ PFU/mL were pre-
pared 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.

Virus and Virus Assay
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dead crow found in the Bronx, New York, during an epizootic
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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 $10^5$ PFU of WNV 1 to 2 days earlier. Immedi-
ately after the mosquitoes fed, blood was drawn from the jug-
ular vein of each chicken (0.1 mL of blood into 0.9 mL of
heparinized diluent), and the blood suspensions were frozen
at $-70^\circ$ 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
cage overlaid with neutral red stain, was added 2 days after the first
overlay.

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 mos-
quito was considered to have a disseminated infection (16).
We defined the infection and dissemination rates as the per-
centages of mosquitoes tested that contained virus in their
body or legs, respectively. Chickens used in the transmission
attempts were bled from the jugular vein 2 days after mos-
quito 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 $10^{4.2}$ PFU of
WNV/mL (10-0.7 PFU/mosquito), held 7 to 14 days, and
allowed to feed on 1- to 2-day-old chickens. Mosquitoes and
blood specimens from these chicks were processed as
described for the orally exposed mosquitoes.

To estimate transmission rates by species, we deter-
minated the percentage of mosquitoes with disseminated infec-
tion (after either oral exposure or by intrathoracic inoculation)
that transmitted virus by bite. We then multi-
plied 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.

Table 1. Mosquito species tested for susceptibility to infection with
West Nile virus

<table>
<thead>
<tr>
<th>Species</th>
<th>Strain</th>
<th>Source (year collected)</th>
<th>Generation</th>
</tr>
</thead>
<tbody>
<tr>
<td>Culex nigripalpus</td>
<td>Indian</td>
<td>Indian River, FL (2000)</td>
<td>F$_{0}$-1</td>
</tr>
<tr>
<td>Cx. quinquefasciatus</td>
<td>Sebring</td>
<td>Sebring County, FL (1988)</td>
<td>&gt;F$_{30}$</td>
</tr>
<tr>
<td>Cx. quinquefasciatus</td>
<td>Vero Beach</td>
<td>Vero Beach, FL (1999)</td>
<td>F$_{10-12}$</td>
</tr>
<tr>
<td>Cx. restuans</td>
<td>Maryland</td>
<td>Frederick &amp; Prince George's Counties, MD (2000,2001)</td>
<td>F$_{0}$</td>
</tr>
<tr>
<td>Cx. salinarius</td>
<td>Chambers</td>
<td>Chambers Co., TX (1992)</td>
<td>&gt;F$_{30}$</td>
</tr>
<tr>
<td>Cq. perturbans</td>
<td>Laurel</td>
<td>Laurel, MD (2000)</td>
<td>F$_{0}$</td>
</tr>
</tbody>
</table>

**Research**

Viral transmission was determined by recovery of virus from the
mosquito tissue suspensions. 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 mos-
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To estimate transmission rates by species, we deter-
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tion (after either oral exposure or by intrathoracic inoculation)
that transmitted virus by bite. We then multi-
plied 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 dissemina-
tion rates were calculated by SAS 8.0 (18). We used Fisher
exact test to compare transmission rates among dissemi-
nated mosquitoes in each species. Significance was tested at
a level of alpha = 0.05.

**Results**

All mosquito species examined in this study were sus-
ceptible to infection with WNV and developed disseminated
infections (Table 2). Infection rates were 84% in all the
Culex species when the viral titer in the donor chicken was
$10^{6.3}$ 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 dissemi-
nated infection that transmitted virus from Table 3 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 effi-
cient vectors (estimated transmission rates 10% to 55%). In
contrast, Cq. perturbans was an inefficient vector (estimated
transmission rate < 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, $10^{5.5-7.5}$ PFU/mL of
blood, are consistent with levels considered to be low to mod-
erate viremias for hooded crows and house sparrows in
Egypt (19) and experimentally infected North American
house sparrows and other passerine birds (N. Komar, pers.
The Culex species tested in this study were moderately efficient vectors, with estimated transmission rates from 10% to 55% after exposure to viremias >10^6 PFU/mL of blood. For comparison, the estimated transmission rate for Cx. pipsiens, which held under conditions similar to those of our study is 20% (9). Although the Culex species tested were readily susceptible to oral infection, most infections were limited to the midgut and did not disseminate to the hemocoel. This finding is similar to results reported for Cx. pipsiens, in which 81% became infected but only 23% developed disseminated infection (9). Compared with the moderately efficient Culex mosquito vectors of WNV, selected container-breeding Aedes and Ochlerotatus species are highly efficient vectors, and selected floodwater Aedes and Ochlerotatus mosquitoes are inefficient laboratory vectors (7-9). The Cq. perturbans in our study fell into the inefficient vector category.

Cx. nigripalpus has not been found naturally infected with WNV. However, the distribution of WNV in the United States is just beginning to reach the southern half of North Carolina, the northernmost limit of these mosquitoes' geographic distribution. Cx. nigripalpus is likely to become involved in WNV transmission because it is a primary vector of SLE in Florida (10,11) and is a competent laboratory vector of WNV. Furthermore, Cx. nigripalpus is an opportunistic feeder (20,21) and shifts host selection based on the season; feeding on avian hosts in the winter and spring and on mammalian hosts in the summer and fall (22). These factors, coupled with the vector competence data, suggest that Cx. nigripalpus could serve as an epizootic as well as an enzootic vector for WNV.

Our study showed that Cx. quinquefasciatus can transmit WNV by bite. WNV has not been isolated from wild-caught Cx. quinquefasciatus. However, the current distribution of WNV is just beginning to overlap the geographic range of this species (generally the southern United States).

Cx. quinquefasciatus has been implicated (through virus isolation and abundance during outbreaks) in the rural transmission of SLE virus in the western United States (23) and in urban transmission of SLE virus in the southern United States (24). In contrast to Cx. pipsiens, which primarily feeds on birds, Cx. quinquefasciatus shows a preference for avian blood but will feed readily on mammals, including humans (25). The data from this study, the biornomics of Cx. quinquefasciatus, and the mosquitoes’ association with an arbovirus with similar epidemiology to WNV, suggest that Cx. quinquefasciatus may play a role in WNV transmission if—or more likely when—the distribution of the mosquito and the virus overlap to a sufficient degree.

Cx. restuans, which has been found naturally infected with WNV (4), transmitted WNV by bite in our study. Similarly, this species has been implicated as a vector of SLE.

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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 a</th>
<th>No. tested</th>
<th>Infection rate b</th>
<th>Dissemination rate c</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></td>
<td>Indian River</td>
<td>5.7±0.5</td>
<td>132</td>
<td>78 ([70-85], 103)</td>
<td>8 ([4-14], 11)</td>
<td>7</td>
</tr>
<tr>
<td></td>
<td>Indian River</td>
<td>6.8±0.4</td>
<td>127</td>
<td>84 ([77-90], 107)</td>
<td>12 ([7-19], 15)</td>
<td>10</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], 0)</td>
<td>6</td>
</tr>
<tr>
<td></td>
<td>Sebring</td>
<td>7.0±0.5</td>
<td>78</td>
<td>91 ([82-96], 71)</td>
<td>22 ([13-33], 17)</td>
<td>20</td>
</tr>
<tr>
<td></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></td>
<td>Vero Beach</td>
<td>6.3</td>
<td>17</td>
<td>94 ([71-100], 16)</td>
<td>12 ([1-36], 2)</td>
<td>&lt;13</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>

aLog10 PFU/mL of blood.
bPercentage of mosquitoes containing virus in their bodies (95% confidence interval (CI), number infected).
cPercentage of mosquitoes containing virus in their legs (95% CI, number disseminated).

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 virus by bite (Table 3).

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### 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 a</th>
</tr>
</thead>
<tbody>
<tr>
<td>Culex nigripalpus</td>
<td>15</td>
<td>87 ([60-98], 13)</td>
</tr>
<tr>
<td>Cx. quinquefasciatus</td>
<td>18</td>
<td>94 ([73-100], 17)</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)</td>
</tr>
<tr>
<td>Cx. salinarius</td>
<td>16</td>
<td>56 ([30-80], 9)</td>
</tr>
<tr>
<td>Coquillettidia perturbans (Laurel)</td>
<td>17</td>
<td>24 ([7-50], 4)</td>
</tr>
</tbody>
</table>

aPercentage of mosquitoes with disseminated infection that transmitted virus by bite (95% confidence interval, number transmitting). Percent transmissions followed by the same letter are not significantly different at alpha = 0.05 by Fisher exact test.
virus by virus isolations from field-collected specimens (26,27), and its role is supported by laboratory transmission studies (13). Cx. restuans breeds in ground pools or container habitats, is widespread in its distribution in the United States, and adults are active early (by mid-May) in the eastern United States (28). This early season abundance, along with coinciding isolations of SLE virus from this species in early summer, implies that it may be involved in the overwintering or amplification of SLE virus (26). The isolation of WNV from Cx. restuans in July in Connecticut (29), relatively early in the WNV transmission season, raises concern that the role of Cx. restuans in WNV transmission may be similar to the one suggested for SLE virus. Cx. restuans feeds primarily on avian hosts (30), but whether it feeds on humans remains unclear (31). Given the lack of firm data on host preference, the role of this species as an enzootic or epizootic vector of WNV is still uncertain.

Our study indicated that Cx. salinarius transmits WNV efficiently by bite. During 2000, evidence of WNV infection was reported in 35 pools of this species, second in number only to the number of positive pools (126) of Cx. pipiens (4). To date, no summary of the data (e.g., minimum infection rates) from the 2000 season has been published, so the relative importance of these isolates cannot be compared. In general, Cx. salinarius appears to be mammalophagic in studies of blood meals, but its host feeding pattern is thought to be opportunistic, depending on host availability, innate host preference, or combination of these factors (20,25,32,33). Given the number of WNV-positive pools, its vector competence for WNV, and its feeding behavior, Cx. salinarius may be an ideal bridge vector between the enzootic avian cycle of WN 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

We thank T. McNamara for providing the Crow 397-99 strain of WNV, D. Shroyer for providing Cx. nigripalpus, J. Nayar for providing the Vero Beach strain of Cx. quinquefasciatus, N. Achee for providing Cq. perturbans, and J. Olsen for providing the Sebring strain of Cx. quinquefasciatus and Cx. salinarius. We also thank J. Blow, R. Leon, and K. Kenyon for critically reading the manuscript.

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 bionomics of mosquitoes in the Amazon Basin region of Peru.

References


