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Distribution and Abundance of Host-seeking Culex Species at Three Proximate Locations with Different Levels of West Nile Virus Activity

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Abstract. Culex species were monitored at three proximate sites with historically different West Nile virus (WNV) activities. The site with human WNV transmission (epidemic) had the lowest abundance of the putative bridge vectors, Culex pipiens and Cx. salinarius. The site with horse cases but not human cases (epizootic) had the highest percent composition of Cx. salinarius, whereas the site with WNV-positive birds only (enzootic) had the highest Cx. pipiens abundance and percent composition. A total of 29 WNV-positive Culex pools were collected at the enzootic site, 17 at the epidemic site, and 14 at the epizootic site. Published models of human risk using Cx. pipiens and Cx. salinarius as the primary bridge vectors did not explain WNV activity at our sites. Other variables, such as additional vector species, environmental components, and socioeconomic factors, need to be examined to explain the observed patterns of WNV epidemic activity.

INTRODUCTION

Mosquitoes of the genus Culex have been implicated as major vectors of West Nile virus (WNV) worldwide.1 In the northeastern United States, the virus enzootic transmission cycle is maintained primarily by ornithophilic Culex pipiens and Cx. restuans mosquitoes.2–4 Most wild and domestic mammals, as well as humans, appear to be dead end or incidental hosts.1,3 Several species of mosquito have been implicated as potential bridge vectors, including Cx. salinarius, Cx. pipiens, and various other Aedes and Culex species, but questions remain as to the importance of each species overall and at individual sites.3,5 Culex salinarius has been proposed to be the main bridge vector because of its abundance during the peak transmission season, indiscriminate feeding habits, vector competence, and a considerable number of WNV isolates.2,4,6–8 An alternative hypothesis implicates Cx. pipiens as the major epidemic vector on the basis of a risk assessment model, vector competence, high number of WNV isolates, and a shift in feeding behavior from avian to mammalian hosts in the late summer.9,10 The two hypotheses for these Culex species enable additional species, such as Aedes vexans and Ae. japonicus, to be minor epidemic vectors.2,9,10,11 A third hypothesis is that Cx. pipiens and Cx. salinarius are responsible for equine and human WNV transmission, and several non-Culex species serve as occasional, or locally important, bridge vectors.13

Little is known about the relationships between mosquito populations and the incidence of WNV within and outside recognized WNV foci,2 and studies that address population dynamics and abundance of mosquito vectors, specifically Culex species, in areas of WNV transmission are relatively scarce, although such knowledge is invaluable for disease management and vector control.14 Limited knowledge on geographic dimensions of WNV exists,15 especially on the subcounty scale, thus obscuring risk patterns on a finer spatial scale relevant to vector ecology.16 Recent studies have suggested highly focal WNV transmission patterns with fine-scale spatial and temporal dynamics.16–21 To address this issue, we examined the three bridge vector hypotheses in terms of Culex species abundance, composition, and population dynamics within three proximate areas located inside the original WNV epicenter in Suffolk County, New York2 and characterized by different levels of historical WNV epidemic and epizootic activities. We selected sites and sizes of sample areas by analyzing historical data using geographic clustering software. We then compared actual data on mosquito distribution and WNV infection to predictions from current models of WNV transmission.

MATERIALS AND METHODS

Selection and characterization of study sites. Data on locally acquired WNV human and equine cases, positive mosquito pools, and positive birds were provided by Suffolk County and New York State agencies (Table 1). All spatial data were processed using ArcGIS 9.1 software (Environmental Systems Research Institute Inc., Redlands, CA). Spatial cluster detection SaTScanTM software22 was used to identify, rank, and determine the spatial extent of the geographic clusters of WNV human and equine cases that occurred from 1999 through 2004. SaTScan™ uses a circular window (set to the maximum radius of 10 km) to detect potential clusters using a pre-determined coordinate file (tracts of census centroids in this study). The larger spatial extent of the most probable human cluster (5.5 km, approximately 3 miles) was used to define the three study areas. These areas were ranked based on Centers for Disease Control and Prevention (CDC) (Atlanta, GA) WNV guidelines23 as either epidemic (high-to-outbreak human transmission risk, categories 4–5 with human and equine cases), epizootic (moderate-to-high human transmission risk, categories 3–4 with equine cases only), or enzootic (low-to-moderate human transmission risk, categories 2–3 with positive birds only). Area 1 (the most probable human cluster, Figure 1) was thus classified as epidemic, area 3 as epizootic (equine cluster only, no human cases), and area 2 as enzootic (no evidence of mammalian transmission). The human population density was calculated for each area using the 2000 U.S. Census Bureau block data.24

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To select unbiased study sites (Sites 1–3), one-mile radius circles were delineated around the centroids of polygons formed by either interconnecting human and equine WNV cases within each of areas 1 and 3, or those outside area 2 (Figure 1). The resulting sites were characterized by land use/cover (LUC) using on-screen digitizing of 2001 aerial ortho-photography of Suffolk County, and validated by ground-truthing (Table 2). Three LUC types within each study site were monitored: residential, commercial, and natural (forested, open water, and wetlands). These areas were considered the most suitable for *Culex* spp. because of larval habitat (catch and retention basins, open water, wetlands), resting places (vegetation), and hosts (humans and wildlife). Residential areas were assumed the most likely locations where WNV human and equine infection could be acquired.

**Collection and identification of mosquitoes.** Trapping was carried out weekly (n = 36 weeks total) from June 1 through September 30 in 2005 and 2006. One CDC miniature light trap baited with dry ice and one CDC gravid trap baited with rabbit chow infusion were placed approximately 20 meters apart in residential, commercial, and natural areas at each of three sites specifically selected for enhanced surveillance (Figure 1). Mosquitoes were anesthetized and identified to species level except for *Cx. pipiens/restuans* and *Cx. salinarius*. Routine identification of female mosquitoes of these three species can be difficult or not possible because of irregular morphology and damage during the collection process. Thus, a rapid molecular identification method using a polymerase chain reaction (PCR) was used. Briefly, legs from individual *Culex* mosquitoes treated with proteinase K were subjected to a multiplex PCR with species specific primers, and the products visualized after electrophoresis on a gel.

**Mosquito processing for WNV testing.** *Culex* and other species females were pooled in groups of 5 to 50 mosquitoes by date, site, LUC, and trap type (combined if < 5). The pools

<table>
<thead>
<tr>
<th>Area</th>
<th>Human cases 1999–2004</th>
<th>Equine cases 1999–2004</th>
<th>WNV activity</th>
<th>Human population‡</th>
<th>Density (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>5</td>
<td>1†</td>
<td>3</td>
<td>4</td>
<td>17</td>
</tr>
<tr>
<td>2</td>
<td>None</td>
<td>None</td>
<td>None</td>
<td>None</td>
<td>32</td>
</tr>
<tr>
<td>3</td>
<td>None</td>
<td>None</td>
<td>8</td>
<td>20</td>
<td>72</td>
</tr>
</tbody>
</table>

* No of human and equine cases, mosquito pools, and positive birds are shown.
† No. of persons per square mile (density) and percentage of the total county population (%) are indicated.
‡ Pre-study, 1999–2004 (mosquito trap locations used for routine surveillance were different from those used for the study).
§ Study, 2005–2006 (enhanced surveillance with nine new trap locations used in this study).
¶ One additional human case occurred approximately 0.5 miles mile the south of area 1.
were submitted to New York State Department of Health Arboviruses Laboratories for WNV testing by reverse transcription–PCR (RT-PCR) as a part of routine WNV surveillance to ensure timely results prior to our molecular species identification carried out in the off-season. Historically, combined Cx. pipiens/Cx. restuans pools represented most (approximately 90%) of the total Culex spp. pools tested by New York State Department of Health, with some pools likely containing Cx. salinarius. Weekly WNV minimum infection rate (MIR) was calculated using a Microsoft Excel add-in program supplied by CDC.

Statistical analysis of Culex species abundance and composition. Data from 2005 and 2006 were combined to increase the power of the analysis. Abundance (mean weekly catch per light trap per night) and species percent composition (the proportion of each species in light traps) were calculated for Cx. pipiens, Cx. restuans, and Cx. salinarius for each site and LUC type using SPSS software (SPSS Inc, Chicago, IL). The mean weekly catch was log (x + 1) transformed and analyzed LUC type using SPSS software (SPSS Inc, Chicago, IL). The routine mosquito trapping at locations different respectively. Routine mosquito trapping at locations different and 3 had 3 (5%) and 8 (12.5%) of 64 WNV equine cases, recorded in Suffolk County from 1999 through 2004 (pre-standardized residuals considered significant if > 2.0 or < −2.0. Significant results at contingency tables were analyzed using Pearson’s chi-square test.

Characterization of study sites. Of 19 human WNV cases recorded in Suffolk County from 1999 through 2004 (pre-study), 5 (26%) occurred within area 1 (Table 1). Areas 1 and 3 had 3 (5%) and 8 (12.5%) of 64 WNV equine cases, respectively. Routine mosquito trapping at locations different from those established for this study resulted in 4 (1.5%) and 24 (9%) of 272 WNV-positive mosquito pools collected in areas 1 and 3, respectively. In contrast, area 2 had no indication of WNV activity except positive birds, whose distribution, as well as mosquito surveillance efforts and human population density were comparable among the three areas.

The three sites selected for this study (Figure 1) represented typical suburban environments with residential LUC occupying from approximately half (sites 2 and 3) to two-thirds (site 1) of the total area (Table 2). Site 3 had the highest natural LUC (approximately 32%), followed by site 2 (approximately 22%) and site 1 (approximately 12%). Commercial LUC occupied approximately 10% of each site.

Abundance, composition, and population dynamics of Culex species. More than two-thirds of 15,302 Culex spp. females were caught in the gravid traps (Table 3). In 2005, approximately 50% of these specimens, a representative sample from the weekly gravid traps, were identified by PCR and 98% of those were Cx. pipiens. In 2006, a randomly selected sample produced similar results. Accordingly, the gravid trap collection was considered primarily Cx. pipiens. More than 97% of all female Culex caught in CDC light traps were identified by PCR. Overall, Cx. pipiens was the predominant species with 55% in 2005 and 69% in 2006. Culex restuans percent composition decreased from 35% in 2005 to 22% in 2006, and that of Cx. salinarius remained at approximately 10% each year.

In a multivariate model with Culex species abundance as the dependent variable, the main effects of year, site, and LUC, as well as the interaction effect of site × LUC were significant (Table 4). Significantly fewer Cx. restuans were caught in 2006 than in 2005. The abundance of Cx. pipiens and Cx. salinarius differed among the sites, with significantly more Cx. pipiens collected at site 2 and significantly fewer Cx. salinarius caught at site 1 (Tables 4 and 5). Culex pipiens and Cx. restuans had significantly higher abundance in residential LUC, followed by commercial and natural areas (Tables 4 and 5).

In terms of Culex species composition, Cx. pipiens was significantly overrepresented at site 2, and underrepresented at sites 1 and 3, whereas Cx. restuans was significantly under-represented at site 2 and overrepresented at site 1 (Table 5). Culex salinarius was significantly overrepresented at site 3, and underrepresented at sites 1 and 2. Culex restuans and Cx. salinarius were more prevalent in natural areas compared with Cx. pipiens, which had significantly higher percent composition in commercial areas. In addition, Cx. restuans was significantly overrepresented in residential areas.

Populations of Cx. pipiens were characterized by several spikes of approximately 15–20 of Cx. pipiens females per trap night. Culex restuans populations reached approximately 12–15 mosquitoes per trap night during the early summer, decreasing

### Table 2
Comparative analysis of percentage land use/cover at three study sites (one-mile radius), Suffolk County, New York

<table>
<thead>
<tr>
<th>Site</th>
<th>Bar</th>
<th>Comm</th>
<th>For</th>
<th>Resd</th>
<th>Rec</th>
<th>Wat</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>4.0</td>
<td>13.0</td>
<td>9.2</td>
<td>71.2</td>
<td>0.0</td>
<td>2.4</td>
</tr>
<tr>
<td>2</td>
<td>11.2</td>
<td>8.7</td>
<td>21.5</td>
<td>48.9</td>
<td>8.9</td>
<td>0.5</td>
</tr>
<tr>
<td>3</td>
<td>6.0</td>
<td>9.2</td>
<td>25.8</td>
<td>47.0</td>
<td>6.1</td>
<td>3.0</td>
</tr>
</tbody>
</table>

* Bar = barren (open grassy or sandy areas); Comm = commercial (large stores and strip malls); For = forested (sylvan habitat > 80% tree cover); Resd = residential (mostly single-family homes and small apartment complexes); Rec = recreational (golf courses); Wat = wetland (natural [New York State Department of Environmental Conservation] and human-made areas). Wat = open water (natural [lakes and ponds] and human-made [recharge basins]).

### Table 3
Combined Culex spp. collected in gravid (G) and Centers for Disease Control and Prevention light (L) traps, Suffolk County, New York, during the 2005–2006 study period

<table>
<thead>
<tr>
<th>Year</th>
<th>Trap</th>
<th>No. collected</th>
<th>No. tested (%)</th>
<th>No. identified (%)</th>
<th>PIP no. (%)</th>
<th>RES no. (%)</th>
<th>SAL no. (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>2005</td>
<td>G</td>
<td>5,887</td>
<td>2,898 (49.2)</td>
<td>2,782 (96.4)</td>
<td>2,729 (98.1)</td>
<td>44 (1.6)</td>
<td>9 (0.3)</td>
</tr>
<tr>
<td>2006</td>
<td>G</td>
<td>5,215</td>
<td>83 (1.6)</td>
<td>82 (98.8)</td>
<td>81 (98.8)</td>
<td>1 (1.2)</td>
<td>0</td>
</tr>
<tr>
<td>2005</td>
<td>L</td>
<td>2,124</td>
<td>2,095 (98.6)</td>
<td>2,023 (96.5)</td>
<td>1,117 (55.2)</td>
<td>696 (34.4)</td>
<td>210 (10.4)</td>
</tr>
<tr>
<td>2006</td>
<td>L</td>
<td>2,076</td>
<td>2,006 (96.6)</td>
<td>1,953 (97.4)</td>
<td>1,341 (68.7)</td>
<td>428 (21.9)</td>
<td>184 (9.4)</td>
</tr>
</tbody>
</table>

* PIP = Cx. pipiens; RES = Cx. restuans; SAL = Cx. salinarius.
Table 4

Multivariate analysis of variance test for variability in *Culex pipiens* (PIP), *C. restuans* (RES), and *C. salinarius* (SAL) abundance (dependent variables) as a function of years, site, and land use/cover (LUC) type (independent variables), Suffolk County, New York*.

<table>
<thead>
<tr>
<th>Source</th>
<th>Wilks λ</th>
<th>Hypothetical df</th>
<th>Error df</th>
<th>F</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Intercept</td>
<td>0.23</td>
<td>3</td>
<td>304</td>
<td>343.23</td>
<td>0.001</td>
</tr>
<tr>
<td>Year</td>
<td>0.96</td>
<td>3</td>
<td>304</td>
<td>3.68</td>
<td>0.012</td>
</tr>
<tr>
<td>Site</td>
<td>0.80</td>
<td>6</td>
<td>608</td>
<td>12.21</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td>LUC</td>
<td>0.70</td>
<td>6</td>
<td>608</td>
<td>19.71</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td>Year × site</td>
<td>0.96</td>
<td>6</td>
<td>608</td>
<td>2.30</td>
<td>0.033</td>
</tr>
<tr>
<td>Year × LUC</td>
<td>0.98</td>
<td>6</td>
<td>608</td>
<td>0.82</td>
<td>0.551</td>
</tr>
<tr>
<td>Site × LUC</td>
<td>0.82</td>
<td>12</td>
<td>805</td>
<td>5.18</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td>Year × site × LUC</td>
<td>0.95</td>
<td>12</td>
<td>805</td>
<td>1.19</td>
<td>0.284</td>
</tr>
</tbody>
</table>

*Statistically significant results are indicated in bold letters and numbers. df = degrees of freedom; NA = not applicable.

In the northeastern United States, *C. pipiens* and *C. restuans* are commonly recognized as primary enzootic vectors of WNV, whereas *C. pipiens* and *C. salinarius* have been proposed as the main epidemic vectors of the virus. The risk of human infection from a bridge vector is postulated to increase with its population density and human blood index, i.e., the proportion of blood meals taken from humans. Similarly, the vectorial capacity of a bridge vector is postulated to increase with its population density and human blood index, i.e., the proportion of blood meals taken from humans. Because the most important risk factor for acquiring WNV is exposure to infected mosquitoes, the human risk should increase proportionately to the species' vectorial capacity and thus abundance, as demonstrated for some arboviruses (including WNV) in the field. For example, a Colorado study found that census tracts with increased *C. tarsalis* abundance also had higher WNV disease incidence. In this study, the level of WNV human risk at a particular location was defined by the CDC guidelines as high (epidemic transmission with recurring human and equine cases, site 1), moderate (epizootic transmission, equine cases only, site 3), or low (enzootic transmission, positive birds only, site 2). The spatial extent of these areas was determined by spatial cluster detection software and represented a sub-county scale, which enables optimal characterization of the spatial variability of WNV risk. Accordingly, certain epidemiologic predictions could be examined in light of the two main hypotheses proposing a single *Culex* species, either *C. pipiens* or *C. salinarius* as the main WNV bridge vector and thus WNV risk factor in our region.

The probability that a species of mosquito will infect a human with WNV, i.e., human risk = A × Fm × P × Cv, where A is the species proportion or percent composition (termed relative abundance by the authors), Fm is a fraction of mammalian blood meals, P is WNV prevalence, and Cv is vector competence. Given the study sites geographic proximity, Fm and Cv were assumed similar, and P did not differ significantly among the sites, and was, in fact, slightly higher at the enzootic site. Thus, the location with elevated human risk (site 1) is expected to have a higher proportion of the mosquito species serving as a bridge vector according to this model. This epidemiologic assumption was found to be incorrect for both *C. pipiens* and *C. salinarius* in this study. Because *C. pipiens* was the predominant mosquito at all three sites, a cursory analysis might implicate this species as the primary vector. However, its distribution was not correlated with WNV activity, and epidemic activity was greatest where this species was least abundant. Site 2, where *C. pipiens* was most abundant, also had the highest number of WNV isolations, mostly from *C. pipiens* in gravid traps. These findings are indicative of increased vectorial capacity of *C. pipiens* and together with WNV competence of this species’ local populations and increased abundance in the residential areas would be expected to lead to a higher human risk of exposure to WNV. Additionally, if the same vector contributes to the mammalian and avian cycles, pronounced disease activity is likely where this species

to fewer than 5 by the late summer (Figure 2). *Culex salinarius* populations gradually increased to approximately 5 per trap night by mid-August and then decreased. Analysis of the combined data by week showed some variation among the three sites (Figure 3). Site 2 was dominated by *C. pipiens*, whereas *Culex restuans* was more common at sites 1 and 3 especially in June and July. *Culex salinarius* was more abundant with higher percent composition at site 3, where it reached up to 40% of the total *Culex* species by late summer.

**Activity of West Nile virus.** In 2005, a total of 37 *Culex* WNV positive pools were collected from the study sites, with the first pool obtained on July 12. A single non-*Culex* positive pool containing *Uranotaenia sapphirina* was collected at site 3 (natural LUC). The *Culex* WNV-positive pool ratio (site 1/site 2/site 3) was 4/8/2 for light traps and 8/10/5 for gravid traps, respectively. The MIR curve for *Culex* species displayed two peaks, a smaller one in the late August and a higher one in mid-September (Figure 2). In 2006, a total of 23 *Culex* WNV-positive pools were collected, with the first pool obtained on July 11, with no non–*Culex* positive pools. The *Culex* WNV-positive pool ratio (site 1/site 2/site 3) was 2/5/3 for light traps and 3/6/4 for gravid traps. The MIR curve for *Culex* species displayed three peaks, a smaller peak in the late July, a higher peak in the late August, and the highest peak in mid-September (Figure 2). The total number of mosquito pools collected for the study and during routine surveillance and number of positive birds obtained in 2005–2006 are shown in Table 1. No equine cases occurred during the study period, most likely as a result of equine WNV vaccine, which was introduced in 2001. This intervention was followed by a steep decrease in equine WNV cases in Suffolk County to 4 in 2002, 3 in 2003, 1 in 2004, and 0 in 2005–2006. One human case occurred within area 1 in 2005 (Figure 1); an additional human case was reported in close proximity south of the area’s boundary (approximately 0.5 miles). The closest human case to area 2 was that of area 1, whereas the closest human case to area 3 occurred approximately 2.5 miles south of the area’s boundary.

**DISCUSSION**
Culex pipiens, Cx. restuans, and Cx. salinarius abundance and composition by site and by land use/cover type (LUC), Suffolk County, New York*

<table>
<thead>
<tr>
<th>Species</th>
<th>Site</th>
<th>Comm</th>
<th>Nat</th>
<th>Resd</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cx. pipiens</td>
<td>1</td>
<td>2</td>
<td>3</td>
<td></td>
</tr>
<tr>
<td>No. collected</td>
<td>517</td>
<td>1,264</td>
<td>677</td>
<td>860†</td>
</tr>
<tr>
<td>% within site/LUC</td>
<td>55</td>
<td>70</td>
<td>55</td>
<td>73</td>
</tr>
<tr>
<td>Standardized residuals</td>
<td>–2.7</td>
<td>4.5</td>
<td>–3.1</td>
<td>5.1</td>
</tr>
<tr>
<td>Cx. restuans</td>
<td>371</td>
<td>386</td>
<td>367</td>
<td>184</td>
</tr>
<tr>
<td>No. collected</td>
<td>39</td>
<td>21</td>
<td>30</td>
<td>16</td>
</tr>
<tr>
<td>% within site/LUC</td>
<td>6.4</td>
<td>–5.4</td>
<td>1.0</td>
<td>–8.1</td>
</tr>
<tr>
<td>Standardized residuals</td>
<td>–4.0</td>
<td>6.0</td>
<td>1.0</td>
<td>5.0</td>
</tr>
<tr>
<td>Cx. salinarius</td>
<td>55†</td>
<td>150</td>
<td>189</td>
<td>127</td>
</tr>
<tr>
<td>No. collected</td>
<td>39</td>
<td>21</td>
<td>30</td>
<td>16</td>
</tr>
<tr>
<td>% within site/LUC</td>
<td>5.1</td>
<td>–5.1</td>
<td>1.0</td>
<td>–5.1</td>
</tr>
<tr>
<td>Standardized residuals</td>
<td>–4.0</td>
<td>6.0</td>
<td>1.0</td>
<td>5.0</td>
</tr>
<tr>
<td>Total</td>
<td>943</td>
<td>1,800</td>
<td>1,233</td>
<td>1,171</td>
</tr>
</tbody>
</table>

*No. collected = total numbers of collected specimens identified by polymerase chain reaction. To obtain mean trap night catch, each number should be divided by 36 (no. of trap weeks). Percent species composition within each site or LUC category and standardized residuals are shown. Results with statistically significant difference (standardized residuals > 2.0) for species percent composition within each site or LUC categories are indicated by bold standardized residuals. Comm = commercial areas; Nat = natural areas; Resd = residential areas.
†Statistically significant differences ($P < 0.05$) in the abundance of each species among site or LUC categories by Dunnett’s T3 post hoc test.

is abundant. However, of the three sites, site 2 had the lowest WNV activity among mammals. One possible explanation for this result is that host preferences of Cx. pipiens have been found to be highly ornithophilic in this region, supporting Cx. pipiens as the main enzootic vector of WNV in Suffolk County.

Another potential bridge vector, Cx. salinarius, has exhibited WNV competence in the laboratory, often harbors the virus in Connecticut and New York, and is described as an indiscriminate feeder on a wide range of mammalian and avian hosts in this region. However, this species was also least abundant at the site with greatest WNV epidemic activity (site 1), and higher abundance and percent composition of Cx. salinarius were not associated with elevated risk of human infection at either sites 2 or 3. White-tailed deer, a large mammal, has been identified as the source of approximately two-thirds of Cx. salinarius mammalian blood meals.

Feeding preponderance of this species for large domestic animals in the vicinity of natural wetlands was also noted in some studies. Culex salinarius was overrepresented at the study area (site 3) with numerous horse properties adjoining extensive and deer-free parkland (i.e., natural areas), which may account, in part, for the elevated number of WNV equine cases seen in that area before the vaccine was introduced. Conversely, this vector species may play a greater role in WNV epidemic transmission in coastal areas of the Suffolk County’s south shore, where a second most probable cluster of WNV human cases was located. These areas also support extensive tidal salt marshes, the main Cx. salinarius larval habitat in Suffolk County, and therefore experience much higher adult abundance of this species.

Unlike Cx. pipiens and Cx. salinarius, the highest percent composition of Cx. restuans was observed at the epidemic site (site 1) and the lowest at the enzootic site (site 3) corresponding...
to the levels of human risk predicted by the model. Although much higher in June and July, \textit{Cx. restuans} abundance was comparable with that of \textit{Cx. salinarius} during the peak WNV transmission in August and September (Figure 3), especially at sites 1 and 2 with smaller natural areas and located further inland. Like \textit{Cx. pipiens}, \textit{Cx. restuans} was significantly more abundant in residential areas, where larvae of both species were frequently found in groundwater retention basins (Rochlin I, Campbell SR, unpublished data). The local populations of \textit{Cx. restuans} were vector competent for WNV,\textsuperscript{42} and the virus has frequently been detected in field collected specimens from New York\textsuperscript{32} and Connecticut.\textsuperscript{2,6} However, this species is not generally considered a potential epidemic vector\textsuperscript{36} because of its primarily early season activity and blood meal preferences.\textsuperscript{26,36,43} The host preference results that suggest ornithophily are not unequivocal; mammalian feeding was observed in New York,\textsuperscript{31,45} and mammalian blood was found in approximately 15–30\% of the \textit{Cx. restuans} specimens (a higher proportion than in \textit{Cx. pipiens}) in the same studies.\textsuperscript{20,36,43} The limited sample size of 10–40 blooded specimens in these studies raises the possibility of selection and technical (i.e., preferential amplification) biases. Additionally, \textit{Cx. restuans} adults are often morphologically indistinguishable from those of \textit{Cx. pipiens},\textsuperscript{20,31} which might have led to confusion between the two species in the past.\textsuperscript{30,31} Abundance data from this study, both spatial (high versus low human risk sites) and temporal (\textit{Cx. restuans} was less numerous during 2006 when lower WNV activity was also recorded) clearly indicates a continuing need for more research on the role of this species in WNV transmission.

Our analysis focused on vector abundance and viral prevalence as the key components of vectorial capacity and human risk, and other factors, such as feeding preferences, local ecology, and human behavior,\textsuperscript{1} were assumed to have little variation among the three study sites caused by geographic proximity, similar LUC composition, and comparable human population density. The apparent lack of association between the risk of WNV epidemic transmission and the abundance of \textit{Cx. pipiens} and \textit{Cx. salinarius} at these three sites raises important questions about these species’ role as the only primary epidemic vectors and suggests additional variables that might determine human risk.

One possibility is the presence of additional mosquito vectors, such as \textit{Ae. vexans}, \textit{Ae. japonicus}, \textit{Ae. sollicitans}, \textit{Ae. triseriatus}, and \textit{Ae. trivittatus},\textsuperscript{9,48} (found in low numbers at our study sites), which therefore might transmit WNV from birds to humans under favorable environmental and demographic conditions.\textsuperscript{13} Environmental heterogeneity over
spatial micro-scales might have been another factor. Droughts were found to induce WNV amplification in Florida by bringing the hosts and the vectors together.\(^9\) A similar process might have occurred in our study, where the epidemic site’s small and isolated but well preserved natural areas with wetlands served as focal points for birds and mosquitoes. These wetland areas were also surrounded by heavily residential areas, potentially leading to greater human exposure, and more extensive and contagious parkland at the epizootic site or dry forested habitat at the enzootic site did not provide the same level of human exposure. Subtle socioeconomic, demographic, and behavioral differences not captured by human population density might also have contributed to the different transmission patterns.\(^9\)\(^,\)\(^50\)

A combination of unique environmental and demographic factors was more likely as an underlying cause of conditions appropriate for human transmission than was the presence of a particular bridge vector species. West Nile virus human infection occurs sporadically in Suffolk County despite high levels of enzootic viral activity, suggesting that conditions conducive to viral epidemic transmission are also intermittent, and that they may change through time. Epidemiologic and ecologic research on a subcounty level has been proposed as a priority for nationwide development to elucidate the spatial patterns of virus-borne disease risk.\(^9\) Consequently, this study should contribute to our understanding of WNV risk factors and to develop strategies for disruption and prevention of WNV epidemic transmission.

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