Identification of Avian- and Mammalian-Derived Bloodmeals in Aedes vexans and Culiseta melanura (Diptera: Culicidae) and Its Implication for West Nile Virus Transmission in Connecticut, U.S.A.

GOUDARZ MOLAEI1 AND THEODORE G. ANDREADIS

The Connecticut Agricultural Experiment Station, 123 Huntington Street, P.O. Box 1106, New Haven, CT 06504


ABSTRACT To evaluate the host-feeding patterns of Aedes vexans (Meigen) and Culiseta melanura (Coquillett) as secondary vectors of West Nile virus (family Flaviviridae, genus Flavivirus, WNV) in Northeastern United States, we identified the source of vertebrate bloodmeals by sequencing portions of the cytochrome b gene of mitochondrial DNA. Analysis of polymerase chain reaction products from a total of 119 Ae. vexans revealed that 92.4% of individuals acquired blood solely from mammalian and 2.5% from avian hosts. Mixed bloodmeals from both avian and mammalian hosts were detected in 5% of individuals of this species. Ae. vexans obtained vertebrate bloodmeals most frequently from white-tailed deer (80%) followed by domestic horse, American robin, eastern cottontail, and domestic cat. In contrast, Cs. melanura fed predominantly on avian species (89.6%) but exhibited some inclination for mammalian blood (4.2%). Individual mosquitoes containing mixed bloodmeals were also identified in 6% of Cs. melanura. American robin was the most common source of vertebrate blood for Cs. melanura (23%), followed by wood thrush and gray catbird. American crow represented only 2% of the bloodmeals identified in Cs. melanura, as was similarly found with other recognized Culex vectors of WNV in the northeast. These findings support the view that Ae. vexans is likely to be a relatively important ‘bridge vector’ to large mammals, including deer and horse, whereas Cs. melanura likely plays a secondary role in enzootic transmission of WNV among free-ranging birds in more rural environs.

KEY WORDS mosquito, host-feeding pattern, Aedes vexans, Culiseta melanura, West Nile virus

Since the discovery of West Nile virus (family Flaviviridae, genus Flavivirus, WNV) in North America in 1999 (Anderson et al. 1999, Lanciotti et al. 1999), >60 mosquito species have been found infected with this virus (CDC 2005b). In the Northeastern United States, Culex L. mosquitoes, i.e., Culex pipiens L., Culex restuans Theobald, and Culex salinarius Coquillett, are considered to be the principal vectors (Andreadis et al. 2001, 2004; Bernard et al. 2001; Kulasekera et al. 2001, Nasci et al. 2001, White et al. 2001, Anderson et al. 2004, Ebel et al. 2005, Kilpatrick et al. 2005). Other species, including Aedes vexans (Meigen) and Culiseta melanura (Coquillett), also have been implicated as potential secondary vectors because of their local abundance, demonstrated vector competence in the laboratory (Turell et al. 2005), and frequent infection with WNV in nature (Bernard et al. 2001, White et al. 2001, Andreadis et al. 2004); however, their vectorial capacity and respective role(s) in epidemic transmission to humans and/or enzootic amplification among various wild bird species have not been fully resolved.

Ae. vexans is an aggressive human biter that feeds almost exclusively on mammals and rarely on birds (Tempelis et al. 1967, 1970; Edman 1971; Cupp and Stokes 1973; Magnarelli 1977a,b; Ritchie and Rowley 1981; Nasci 1984; Irby and Apperson 1988; Apperson et al. 2002, 2004; Lee et al. 2002; Hassan et al. 2003; Lee et al. 2004; Gingrich and Williams 2005). Because of its local abundance and biting behavior, it is considered to be a potentially important bridge vector for WNV in the region (Andreadis et al. 2004, Turell et al. 2005). However, its limited preference for feeding on birds, a presumed prerequisite for acquisition of the virus, has been suggested as a major factor that reduces its vectorial capacity (Andreadis et al. 2004). Ae. vexans is also fairly opportunistic in its host-seeking behavior and is known (Nasci 1984) to exhibit local variation depending on host availability. Thus, knowledge of the feeding behavior of resident populations is an essential element in assessing the vectorial capacity of this species within a given locale.

Regional host-feeding studies on local populations of Cs. melanura in the Northeastern United States (Jobbins et al. 1961, Crans 1964, Schober and Collins 1966, Magnarelli 1977a, Nasci and Edman 1981, Ngo and Kramer 2003, Apperson et al. 2004) clearly demonstrate that this species, in contrast to Ae. vexans, has a strong preference for avian hosts, especially Passe-
riformes. Occasional feeding on mammals, including humans, has been noted (Jobbins et al. 1961, Apperson et al. 2004), although this seems to be very rare and a function of local fauna and host accessibility.

With a few exceptions (Apperson et al. 2002, 2004; Lee et al. 2002; Hassan et al. 2003; Ngo and Kramer 2003), the majority of the aforementioned investigations have largely been based on serological identification of bloodmeals by using broadly reactive antisera. This has allowed for identification of mammalian hosts to the species level but for avian hosts, identification has been limited to class and at best, ordinal level only. Ngo and Kramer (2003) implemented a polymerase chain reaction (PCR)-based assay to identify host bloodmeals from mosquitoes by using primers for the cytochrome b gene that allowed them to distinguish between mammalian and avian bloodmeals and differentiate among four avian orders. Recently, in our analysis we have identified vertebrate bloodmeals in mosquitoes by sequencing portions of the cytochrome b gene of mitochondrial DNA (Molaei et al. 2006). This has allowed us to unambiguously identify both avian- and mammalian-derived bloodmeal sources to the species level.

The current research initiative was undertaken to characterize the host-feeding patterns of Ae. vexans and Cs. melanura and to evaluate their contribution as secondary vectors in enzootic maintenance of WNV among wild birds and epidemic transmission to mammalian hosts, including humans.

Materials and Methods

Mosquito Collection. Mosquitoes were collected from 54 sites located within seven counties in Connecticut (Fig. 1) over a 3-yr period (June–October 2002–2004) as part of a statewide surveillance program (Andreadis et al. 2004). The majority of Ae. vexans was obtained from densely populated residential locales where WNV is endemic. These included parks, greenways, golf courses, undeveloped wood lots, sewage treatment plants, dumping stations, and temporary wetlands associated with waterways. Most females of Cs. melanura were collected from more sparsely populated rural locales that included permanent freshwater swamps (red maple/white cedar) and bogs and swamp–forest border environments. Two trap types were used: a CO₂-baited Centers for Disease Control (CDC) light trap (John W. Hock Co., Gainesville, FL) and a CDC gravid mosquito trap (Reiter 1983). Traps were operated overnight and retrieved the next morning. Adult mosquitoes were transported alive to the laboratory where they were identified on chill tables with a stereomicroscope by using descriptive keys (Means 1979, Darsie and Ward 1981, Means 1987, Andreadis et al. 2005). All mosquitoes with fresh or visible blood remnants were transferred into individual 2-ml tubes labeled according to species, date of collection, and locale and stored at −80°C.

DNA Isolation from Blood-Fed Mosquitoes. Mosquito abdomens were removed and reserved for bloodmeal analysis with the aid of a dissecting microscope. Each mosquito was dissected individually on a new microscope slide by using flame-sterilized forceps to avoid cross-contamination. DNA was isolated from the abdominal contents of blood-fed mosquitoes individually using DNA-zol BD (Molecular Research Center, Cincinnati, OH) according to the manufacturer’s recommendation with some modifications (Molaei et al. 2006).

Bloodmeal Analysis. Isolated DNA from the mosquito bloodmeals served as DNA templates in subse-
quently PCR reactions. PCR primers were either based upon a multiple alignment of cytochrome b sequences of avian and mammalian species obtained from GenBank or previously published primer sequences (Table 1). A TaqPCR core kit (QIAGEN, Valencia, CA) was used for all PCR reactions according to the manufacturer’s recommendation. A 50-μl reaction volume was prepared with 3 μl of template DNA, 4 μl of each primer (0.1–0.5 μM), 5 μl of 10× QIAGEN PCR buffer (containing 15 mM MgCl₂), 1 μl of dNTP mix (10 mM each), 0.25 μl of TaqDNA Polymerase (1.25 U/reaction), and 32.75 μl of water. All PCR reactions were performed using the GeneAmp PCR System 9700 (Applied Biosystems, Foster City, CA). PCR-amplified products were purified using QIAquick PCR purification kit (QIAGEN) and sequenced directly in cycle sequencing reactions at the Keck Sequencing Facility (Yale University, New Haven, CT) by using the sequencer 3730xl DNA Analyzer (Applied Biosystems). Sequences were annotated using ChromasPro, version 1.22 (Technelysium Pty Ltd., Tewantin, Australia) and identified by comparison with the GenBank DNA sequence database (National Center for Biotechnology Information, Bethesda, MD: http://www.ncbi.nlm.nih.gov/Genbank/index.html). The performance of the molecular-based assay was previously validated (Molaei et al. 2006).

Results

Bloodmeal sources were successfully identified by DNA sequencing from 119 of 120 Ae. vexans and 48 of 49 Cs. melanura. Of 119 Ae. vexans analyzed, 110 (92.4%) contained mammalian blood only, three (2.5%) avian blood only, and six (5%) both avian and mammalian blood. Of 48 Cs. melanura analyzed, 43 (89.6%) contained avian blood only, two (4.2%) mammalian blood only, and three (6.2%) both avian and mammalian blood.

An analysis of the mammalian and avian bloodmeal sources for Ae. vexans is shown in Table 2. In total, seven mammalian host species were identified for Ae. vexans. White-tailed deer, Odocoileus virginianus (Zimmermann), was the most frequently identified host (79.8% of total), followed by horse, Equus cabalus (L.) (9.2%), eastern cottontail, Sylvilagus floridanus (Allen) (3.4%), and domestic cat, Felis catus (L.) (2.5%). All (including mixed bloodmeals) of the avian-derived bloodmeals found in Ae. vexans were identified as American robin, Turdus migratorius (L.).

The composition of avian- and mammalian-derived bloodmeals for Cs. melanura is shown in Table 3. In total, 18 species of birds were identified. The most frequently identified hosts were American robin (23.0% of total); wood thrush, Hylocichla mustelina (Gmelin) (12.5%); gray catbird, Dumetella carolinensis (L.) (10.4%); tufted titmouse, Baeolophus bicolor (L.) (6.3%); veery, Catharus fuscens (Stephens) (6.3%); song sparrow, Melospiza melodia (Wilson) (6.3%); and common grackle, Quiscalus quiscula (L.) (6.3%). Only one (2.1%) American crow-derived bloodmeal was identified. The most common mammalian species was white-tailed deer (6.3% of total) followed by domestic cat (2.1%) and northern raccoon, Procyon lotor (L.) (2.1%).

Discussion

Our investigations of the host-feeding patterns of local populations of Ae. vexans and Cs. melanura, as determined by bloodmeal analysis, provide meaningful information relative to their respective roles as
potential secondary vectors of WNV in this region of the Northeastern United States.

We find that Ae. vexans feeds mainly on mammalian species, white-tailed deer and horse. This finding is in agreement with previous studies conducted in northeastern and other regions of the United States where the species has similarly been shown to have a decided preference for large mammals (Tempelis et al. 1967; Edman 1971; Suyemoto et al. 1973; Magnarelli 1977a,b; Ritchie and Rowley 1981; Nasci 1984; Irby and Apperson 1988, Ngo and Kramer 2003, Apperson et al. 2004). The identification of moderate numbers (7.6%) of avian-derived bloodmeals from a competent reservoir (American robins) (Komar et al. 2003) as well as mixed bloodmeals (5.0%) from both avian and mammalian hosts are particularly significant as these findings demonstrate a minimally necessary condition for acquisition of the virus from an avian host and subsequent transmission to mammals.

The apparent affinity of Ae. vexans for deer over other mammalian species in the current study is likely the function of their local abundance and availability. Although no formal census of host density was taken, white-tailed deer are the most abundant large mammals in the region after humans and were likewise found to be the predominant mammalian host for Cx. salinarius collected from similar locales within the state (Molaei et al. 2006). Nasci (1984) also has suggested that large mammals such as deer may have a greater tolerance for mosquito biting, resulting in more successful feeding on these hosts. The identification of a moderate number of horse-derived bloodmeals (9.2%) is significant and is consistent with the view that Ae. vexans is likely to be a relatively important “bridge vector” to horses in this region, given its abundance, prevalence of WNV infection (Andreadis et al. 2004), and moderate vector competence (Turell et al. 2001, 2005).

Table 3. Number and percentage of avian and mammalian bloodmeals identified from Cx. melanura collected in Connecticut, 2002–2004

<table>
<thead>
<tr>
<th>Host</th>
<th>No.</th>
<th>% total</th>
</tr>
</thead>
<tbody>
<tr>
<td>Avian</td>
<td></td>
<td></td>
</tr>
<tr>
<td>American robin</td>
<td>11</td>
<td>22.9</td>
</tr>
<tr>
<td>Wood thrush, Hylocichla mustelina (Gmelin)</td>
<td>6</td>
<td>12.5</td>
</tr>
<tr>
<td>Gray catbird</td>
<td>5</td>
<td>10.4</td>
</tr>
<tr>
<td>Tufted titmouse</td>
<td>3</td>
<td>6.3</td>
</tr>
<tr>
<td>Veery, Catus fuscusceus</td>
<td>3</td>
<td>6.3</td>
</tr>
<tr>
<td>Song sparrow</td>
<td>3</td>
<td>6.3</td>
</tr>
<tr>
<td>Common grackle</td>
<td>3</td>
<td>6.3</td>
</tr>
<tr>
<td>Cx. melanura</td>
<td>2</td>
<td>4.2</td>
</tr>
<tr>
<td>Cedar waxwing, Bombyx cedrorum (Veillot)</td>
<td>1</td>
<td>2.1</td>
</tr>
<tr>
<td>Northern cardinal, Cardinalis cardinalis (L)</td>
<td>1</td>
<td>2.1</td>
</tr>
<tr>
<td>American crow, Corvus brachyrhynchos (Brehm)</td>
<td>1</td>
<td>2.1</td>
</tr>
<tr>
<td>Brown-headed cowbird, Molothrus ater (Boddart)</td>
<td>1</td>
<td>2.1</td>
</tr>
<tr>
<td>Northern parula, Parula americana (L)</td>
<td>1</td>
<td>2.1</td>
</tr>
<tr>
<td>Black-capped chickadee, Parus atricapillus (L)</td>
<td>1</td>
<td>2.1</td>
</tr>
<tr>
<td>House sparrow, Passer domesticus (L)</td>
<td>1</td>
<td>2.1</td>
</tr>
<tr>
<td>Scarlet tanager, Piranga olivacea (Gmelin) (Boddaert)</td>
<td>1</td>
<td>2.1</td>
</tr>
<tr>
<td>Ovenbird, Setaria assulciaus (L)</td>
<td>1</td>
<td>2.1</td>
</tr>
<tr>
<td>Mourning dove, Zenaida macrura (L)</td>
<td>1</td>
<td>2.1</td>
</tr>
<tr>
<td>Mammalian</td>
<td></td>
<td></td>
</tr>
<tr>
<td>White-tailed deer</td>
<td>3</td>
<td>6.3</td>
</tr>
<tr>
<td>Domestic cat</td>
<td>1</td>
<td>2.1</td>
</tr>
<tr>
<td>Northern raccoon</td>
<td>1</td>
<td>2.1</td>
</tr>
</tbody>
</table>

* Includes 3 specimens from which double-blood meals were identified.
ies of local populations from several metropolitan areas in the vicinity of New York City (Komar et al. 2001, Nasci et al. 2002) have further detected neutralizing antibodies in American robin, gray catbird, northern cardinal, and house sparrow. American robin has been correspondingly shown to be the most common source of avian blood for local northeastern populations of other ornithophilic mosquitoes, Cx. pipiens and Cx. restuans (Apperson et al. 2004, Molaeti et al. 2006), further implicating the potential role of this bird as an important amplifying host in the WNV transmission cycle in this region. Although the vector competence of Cs. melanura for WNV has not been fully evaluated, the species is susceptible and develops disseminated infections after feeding on viremic birds, a necessary condition for transmission (Turell et al. 2005). Its abundance in sylvan environments and frequent isolation of WNV in nature (Andreadis et al. 2004), in concert with the present findings on its host feeding preferences for reservoir competent birds, are consistent with the view that this species plays a secondary role in enzootic transmission of WNV among free-ranging birds in more rural environments.

Acknowledgments

We thank Louis A. Magnarelli for providing vertebrate blood samples and Philip M. Armstrong for comments on an earlier version of the manuscript. We also acknowledge John Shepard, Michael Thomas, and our mosquito/arbovirus support group for collection and identification of mosquitoes. Funding for this research was provided by Laboratory Capacity for Infectious Disease Cooperative Agreement U50/CCU6506-01-1 from the Centers for Disease Control and Prevention, United States Department of Agriculture (USDA) Specific Cooperative Agreement 55-6615-1-218, and USDA-administered Hatch funds CONH00768.

References Cited


Received 9 January 2006; accepted 5 April 2006.