Vector-Host Interactions and Epizootiology of Eastern Equine Encephalitis Virus in Massachusetts

Goudarz Molaei,1 Theodore G. Andreadis,1 Philip M. Armstrong,1 Michael C. Thomas,1 Timothy Deschamps,2 Esteban Cuevas-Incle,3 Walter Montgomery,3 Matthew Osborne,4 Sandra Smole,4 Priscilla Matton,5 Wayne Andrews,5 Curtis Best,2 Frank Cornine III,2 Ellen Bidlack,6 and Tony Texeira6

Abstract

Eastern equine encephalitis (EEE) virus is a highly pathogenic mosquito-borne zoonosis that is responsible for outbreaks of severe disease in humans and equines, resulting in high mortality or severe neurological impairment in most survivors. In the northeastern United States, EEE virus is maintained in an enzootic cycle involving the ornithophilic mosquito, Culiseta melanura (Coquillett) and passerine birds in freshwater swamp habitats. To evaluate the role of Cs. melanura and Culiseta morsitans (Theobald) in recent episodes of EEE virus activity in Massachusetts, we collected blood-fed mosquitoes between June, 2007, and October, 2008, from virus foci in 6 counties, and identified the source of blood meals by PCR amplification of mitochondrial cytochrome b gene and sequencing. Analysis of 529 Cs. melanura and 25 Cs. morsitans revealed that nearly 99% and 96% of mosquitoes, respectively, acquired blood meals solely from avian hosts. American Robin, Turdus migratorius Linnaeus was identified as the most common vertebrate host for Cs. melanura (21.7%, n = 115), followed by Tufted Titmouse, Baeolophus bicolor (L.) (8.7%, n = 46), Black-capped Chickadee, Poecile atricapillus (L.) (8.5%, n = 45), Scarlet Tanager, Piranga olivacea (Gmelin) (6.8%, n = 36), Field Sparrow, Spizella pusilla (Wilson) (6.2%, n = 33), Northern Cardinal, Cardinalis cardinalis (L.) (5.7%, n = 30), and other mostly Passeriformes birds. Mammalian-derived blood meals were identified as white-tailed deer, Odocoileus virginianus Zimmermann, domestic cow, Bos taurus L., and human, Homo sapiens L. There were 4 isolations of EEE virus, West Nile virus, and Highland J virus from Cs. melanura. Our results in conjunction with other lines of evidence, including reservoir competency, prevalence of antibody, and infection in nature, suggest that the American Robin, Tufted Titmouse, Black-capped Chickadee, and a few other passerine birds may play key roles in supporting EEE virus transmission in Massachusetts. Infrequent blood feeding of Cs. melanura on mammalian hosts, including humans, also indicates that this mosquito may occasionally contribute to epidemic/epizootic transmission of EEE virus in this region.

Key Words: Culiseta melanura—Culiseta morsitans—Blood feeding pattern—Mitochondrial cytochrome b gene—Epizootiology—Eastern equine encephalitis virus.

Introduction

Eastern Equine Encephalitis (EEE) virus (family Togaviridae, genus Alphavirus) is a highly pathogenic mosquito-borne agent responsible for periodic outbreaks of severe disease in humans and equines, causing high mortality and severe neurological impairment in most survivors. During the last decade, episodes of EEE virus have reemerged in the northeastern United States, including Massachusetts, where there has been increased virus activity and recurrent human and equine cases (Centers for Disease Control and Prevention 2006). These episodes occur when ecological conditions favor virus amplification followed by overflow into human and equine populations.
EEE virus is amplified in an enzootic cycle involving or-nithophilic mosquitoes, principally *Culicidae melanura* (Co- quillet). and to a lesser extent *Caligus morsitans* (Theobald), and birds, primarily members of Passeriformes (perching birds) (Hayes et al. 1981, Morris and Zimmerman 1981, Morris 1988, Scott and Weaver 1989, Crans et al. 1994, Howard et al. 1994) inhabiting fresh water swamp foci. However, the role that these mosquitoes play in epidemic and epizootic transmission of virus to humans and horses, and the contribution of various bird species as amplification hosts is not well defined. The conventional paradigm posits that *Cs. melanura* and *Cs. morsitans* are involved in enzootic cycling of EEE virus among birds, whereas other mosquito species such as *Culicoides perturbans* (Walker), *Ochlerotatus canadensis* (Theobald), *Aedes vexans* (Meigen), and *Oc. solitans* (Walker) that feed more opportunistically, transmit virus to mammals, including hu-mans and horses (Crans 1977, Crans and Schulze 1986).

However, recent host-feeding pattern studies indicate that populations of *Cs. melanura* in the northeastern United States acquire small proportions of blood meals from mammals in addition to birds as their preferred hosts (Molaei et al. 2006, Molaei et al. 2006a). Furthermore, in a recent study that evaluated the ability of field-collected mosquitoes to acquire, replicate, and potentially transmit EEE virus, *Cs. melanura* had the highest prevalence of infection and virus titers among other potential vectors (Armstrong and Andreadis 2010). These research findings in concert with frequent isolations of EEE virus from field-collected *Cs. melanura* highlight the potential importance of this mosquito species as both primary enzootic and epidemic vector of the virus.

The current research initiative was undertaken to examine the vector–host interactions and blood-feeding patterns of *Cs. melanura* and *Cs. morsitans* and their role in enzootic and epizootic transmission in a region with endemic EEE virus activity. Accordingly, engorged mosquitoes were collected during peak mosquito season from June to October of 2007 and 2008 from 6 Massachusetts counties with focal EEE virus activity. Blood meal sources were identified by sequencing PCR products of the mitochondrial cytchrome *b* gene.

**Materials and Methods**

**Study sites**

Mosquitoes were collected in 30 trapping sites located in Bristol, Essex, Middlesex, Norfolk, Plymouth, and Worcester Counties in Massachusetts (Fig. 1). Most of the area’s estimated human population of 4,758,369 resides in regions consisting of cities and unincorporated communities. In general, the study area is highly urbanized, with a few remnants of agricultural and undisturbed natural landscapes interspersed within highly fragmented residential and commercial developments. Most collection sites were located along the borders of wooded wetland habitats dominated by red maple, *Acer rubrum* L., and Atlantic white cedar, *Chamaecyparis thyoides* (L.) Britton, Sterns & Poggenb. trees. The majority of mosquitoes were collected within established and emergent EEE virus foci in Bristol, Plymouth, and Essex Counties.

**Mosquito sampling**

Engorged mosquitoes were collected weekly between June 4, 2007, and October 15, 2008, from locations within the 6-county study area (Fig. 1) by using primarily resting boxes according to established protocol (Morris 1981). Resting boxes were placed on dry forested uplands within sight of red maple swamp habitats surrounded by shrubs, on manicured garden areas under rhododendron bushes, in the middle of mixed forest in the proximity of pine swamps, and along the edges of Atlantic white cedar, red maple, and maple and high-bush blueberry swamps. Engorged mosquitoes were mostly sampled from locations where positive mosquito pools or human or equine cases of EEE virus had been previously reported. In addition to resting boxes, supplementary samplings also were made by using modified dry ice-baited, CDC-style light traps (John W. Hock Company, Gainesville, FL) (Sudia and Chamberlain 1962). These traps were set along the tree line of wooded Atlantic white cedar, maple, hemlock, and high-bush blueberry swamp areas. Specimens were transported alive in coolers (4–8°C) with ice packs to the various agency labora-tories. Engorged mosquitoes were then speciated using a dissecting microscope and identification key (Andreadis et al. 2005). Specimens with visible blood meals were transferred to microtubes, labeled with a unique number, and transported on dry ice to the Massachusetts Department of Public Health to be held at ~80°C in an ultra-low-temperature freezer. Blood meal analysis and detection of EEE virus from the en-gorged mosquitoes were performed at the Connecticut Agri-cultural Experiment Station (CAES).

**DNA isolation and blood meal identification from engorged mosquitoes**

Mosquito abdomens were removed with the aid of a dissecting microscope and disposable razor blades for blood meal analysis. DNA was isolated from the abdominal content of engorged mosquitoes individually by using DNAzol BD (Molecular Research Center, Cincinnati, OH) or DNaseasy Blood and Tissue Kit (Qiagen, Valencia, CA) according to the manufacturers’ recommendation with some modifications as described elsewhere (Molaei et al. 2006a, 2006b). Isolated DNA from the mosquito blood meals served as DNA templates in subsequent PCR assays with primers based on vertebrate mitochondrial cytchrome *b* sequences using previously described protocols and thermal cycling conditions (Molaei et al. 2006a, 2006b). The source of mosquito blood meals was identified by sequence comparison to the GenBank DNA sequence database (NCBI at http://www.ncbi.nlm.nih.gov/).

**Detection of virus in blood-fed mosquitoes**

Blood-fed mosquitoes were tested for the presence of EEE virus or other arboviruses by virus isolation in cell culture (Armstrong et al. 2011) and with real-time RT-PCR assays (Lanciotti et al. 2000, Lambert et al. 2003). The head and thorax of individual blood-fed mosquitoes were homogenized in 1 mL of phosphate-buffered saline (PBS) containing 30% heat-inactivated rabbit serum, 0.5% gelatin, and antibiotic/anti-myocytic by using a Mixer Mill apparatus (model MM300, Retsch Inc., Haan, Germany), as previously described (Andreadis et al. 2004). Mosquito homogenates were centrifuged at 4°C for 10 min at 520 × g, and then 100 μL of the supernatant was inoculated into a 25-cm² flask containing Vero cells growing in minimal essential media, 5% fetal bovine serum, and antibiotics/antimycotics. Cells were maintained at 37°C.
in 5% CO₂ and examined daily for cytopathic effect (CPE) 3–7 days postinoculation. RNA was extracted from CPE-positive cell cultures by using the viral RNA Kit (Qiagen), and virus was identified by real-time RT-PCR.

Bird population estimates

Frequency estimates of avian species for Bristol, Essex, Middlesex, Norfolk, Plymouth, and Worcester Counties were performed by using data from the online eBird database (http://www.ebird.org/) developed in 2002 by the Cornell Laboratory of Ornithology and National Audubon Society to track bird distribution and abundance in North America. The observation frequency, expressed in decimal format ranging from 0 to 1, represents the percentage of checklists reporting the species within a specified date range and region (Table 1). The estimated frequency data used consist of information obtained from 4918 checklists analyzed on a weekly basis during the mosquito collection season (June through November, 2007–2008). Bird species were ranked in descending order by frequency index, from most to least frequently observed, to assess relative distribution and observation frequency within the region, and for comparison with blood meal results in the present study. It is noteworthy that results of avian surveys (via checklists) could vary depending upon the detectability of songs, habitat, time of day, weather condition, observer’s skills, and other behavioral characteristics. It is likely that some "secretive" birds present at a given site may be underestimated or entirely overlooked. It is also worthy to note that detection of a certain species at a site during the day does not guarantee the species would be accessible to host-seeking mosquitoes at dusk/nighttime. Similarly, differences in bird behavior and other spatial and temporal factors may create conditions that some bird species become more suitable/accessible as hosts for mosquitoes.

Results

Blood meal analysis

Blood meal sources were successfully identified by DNA sequencing from 554 Cs. melanura and Cs. morsitans (Tables 2 and 3). Of the 529 Cs. melanura analyzed, 523 (98.9%) contained avian blood and 6 (1.1%) had mammalian blood. Of the 25 Cs. morsitans analyzed, 24 (96.0%) contained avian blood and 1 (4.0%) had mammalian blood.

We identified 55 different species of avian hosts for Cs. melanura (Table 2). The greatest preponderance of blood meals
Cells highlighted in gray indicate bird species that served as the source of blood meals for *Culiceta melanura*.

(\(n=115, 21.7\%)\) was from American Robin, *Turdus migratorius* Linnaeus. Other frequent hosts included Tufted Titmouse, *Baeolophus bicolor* (L.) (\(n = 46, 8.7\%\)); Black-capped Chickadee, *Poecile atricapillus* (L.) (\(n = 45, 8.5\%\)); Scarlet Tanager, *Piranga olivacea* (Gmelin) (\(n = 36, 6.8\%\)); and Field Sparrow, *Spizella pusilla* (Wilson) (\(n = 33, 6.2\%\)). The 55 species were members of 8 avian orders and 25 families. The order Passeriformes constituted 97.0% (\(n = 507\)) of blood meals acquired by *Cs. melanura*, followed by Columbiformes 1.7% (\(n = 9\)), Cuculiformes 0.4% (\(n = 2\)), and 0.2% (\(n = 1\)) each of the Accipitriformes, Falconiformes, Gruiformes, Pelecaniformes, and Piciformes. Of 25 avian families, Turdidae (thrushes) with 34.6% (\(n = 181\)) of blood meals, Paridae (chickadees) with 17.4% (\(n = 91\)), and Cardinalidae (cardinals) with 14% (\(n = 73\)) were the 3 most frequent hosts.

Mammalian hosts for *Cs. melanura* were identified as whitetailed deer, *Odocoileus virginianus* Zimmermann (\(n = 3, 0.6\%\)), domestic cow, *Bos taurus* L. (\(n = 2, 0.4\%\)), and human, *Homo sapiens* L. (\(n = 1, 0.2\%\) (Table 2)); 3 of these mosquitoes contained mixed blood meals of avian and mammalian origin. Of
Table 2. Number and Percentage of Avian- and Mammalian-derived Blood Meals Identified From Culiseta melanura Sampled in Massachusetts 2007–2008

<table>
<thead>
<tr>
<th>Avian (common name)</th>
<th>Scientific name</th>
<th>Residency Code</th>
<th>Bristol No. (%)</th>
<th>Essex No. (%)</th>
<th>Middlesex No. (%)</th>
<th>Norfolk No. (%)</th>
<th>Plymouth No. (%)</th>
<th>Worcester No. (%)</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>American Robin</td>
<td>Turdus migratorius</td>
<td>P, T</td>
<td>76 (27.2)</td>
<td>8 (8.9)</td>
<td>4 (15.4)</td>
<td>6 (17.6)</td>
<td>17 (20.2)</td>
<td>4 (25.0)</td>
<td>115</td>
</tr>
<tr>
<td>Tufted Titmouse</td>
<td>Baeolophus bicolor</td>
<td>P</td>
<td>32 (11.5)</td>
<td>5 (6.3)</td>
<td>2 (7.7)</td>
<td>2 (5.3)</td>
<td>3 (6.2)</td>
<td>2 (12.5)</td>
<td>46</td>
</tr>
<tr>
<td>Black-capped Chickadee</td>
<td>Poecile atricapillus</td>
<td>P</td>
<td>25 (9.0)</td>
<td>10 (11.1)</td>
<td>1 (3.8)</td>
<td>1 (2.9)</td>
<td>8 (9.5)</td>
<td>45</td>
<td></td>
</tr>
<tr>
<td>Scarlet Tanager</td>
<td>Piranga olivacea</td>
<td>S</td>
<td>7 (2.5)</td>
<td>4 (4.4)</td>
<td>3 (11.5)</td>
<td>1 (2.9)</td>
<td>21 (25.0)</td>
<td>36</td>
<td></td>
</tr>
<tr>
<td>Field Sparrow</td>
<td>Spizella pusilla</td>
<td>S</td>
<td>26 (9.3)</td>
<td>4 (4.4)</td>
<td>1 (3.8)</td>
<td>2 (2.4)</td>
<td>33</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Northern Cardinal</td>
<td>Cardinis cardinals</td>
<td>P</td>
<td>5 (1.8)</td>
<td>17 (18.9)</td>
<td>2 (7.7)</td>
<td>6 (7.1)</td>
<td>30</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Wood Thrush</td>
<td>Hylocichla mustellina</td>
<td>S</td>
<td>8 (2.9)</td>
<td>7 (7.8)</td>
<td>11 (32.3)</td>
<td>1 (1.2)</td>
<td>27</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Red-eyed Vireo</td>
<td>Vireo olivaceus</td>
<td>S</td>
<td>12 (4.3)</td>
<td>1 (1.1)</td>
<td>1 (2.9)</td>
<td>1 (6.25)</td>
<td>15</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Baltimore Oriole</td>
<td>Icterus galbula</td>
<td>S</td>
<td>2 (0.7)</td>
<td>4 (4.4)</td>
<td>6 (17.6)</td>
<td>1 (1.2)</td>
<td>1 (6.25)</td>
<td>14</td>
<td></td>
</tr>
<tr>
<td>Brown-headed Cowbird</td>
<td>Molothrus ater</td>
<td>P, T</td>
<td>10 (3.6)</td>
<td>1 (1.2)</td>
<td>1 (1.2)</td>
<td>10</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Chipping Sparrow</td>
<td>Spizella passerina</td>
<td>S</td>
<td>9 (3.2)</td>
<td>1 (1.2)</td>
<td>1 (1.2)</td>
<td>9</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Common Yellowthroat</td>
<td>Geothlypis trichas</td>
<td>S</td>
<td>4 (1.4)</td>
<td>1 (1.1)</td>
<td>2 (7.7)</td>
<td>1 (1.2)</td>
<td>1 (6.25)</td>
<td>9</td>
<td></td>
</tr>
<tr>
<td>Gray Catbird</td>
<td>Dumetella carolinensis</td>
<td>S</td>
<td>5 (1.8)</td>
<td>1 (2.9)</td>
<td>3 (3.6)</td>
<td>9</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mourning Dove</td>
<td>Zenaida macroura</td>
<td>P</td>
<td>3 (1.1)</td>
<td>4 (4.4)</td>
<td>2 (5.9)</td>
<td>9</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cedar Waxwing</td>
<td>Bombycilla cedrorum</td>
<td>P, T</td>
<td>5 (1.8)</td>
<td>2 (2.2)</td>
<td>1 (1.1)</td>
<td>8</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Grasshopper Sparrow</td>
<td>Ammodramus</td>
<td>S</td>
<td>2 (2.2)</td>
<td>2 (7.7)</td>
<td>2 (5.9)</td>
<td>1 (1.2)</td>
<td>7</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Red-winged Blackbird</td>
<td>Agelaius phoeniceus</td>
<td>P, T</td>
<td>5 (1.8)</td>
<td>1 (1.1)</td>
<td>1 (1.2)</td>
<td>7</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Rose-breasted Grosbeak</td>
<td>Phuecticus ludovicans</td>
<td>S</td>
<td>2 (0.7)</td>
<td>2 (2.2)</td>
<td>1 (3.8)</td>
<td>1 (2.9)</td>
<td>1 (6.25)</td>
<td>7</td>
<td></td>
</tr>
<tr>
<td>House Wren</td>
<td>Troglodotes aerdon</td>
<td>S</td>
<td>3 (1.1)</td>
<td>1 (3.8)</td>
<td>2 (2.4)</td>
<td>6</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Ovenbird</td>
<td>Seiurus aurcapilla</td>
<td>S</td>
<td>4 (1.4)</td>
<td>1 (3.8)</td>
<td>1 (1.2)</td>
<td>6</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Black-and-white Warbler</td>
<td>Mniotilla varia</td>
<td>S</td>
<td>5 (1.8)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>House Finch</td>
<td>Carpodacus mexicanus</td>
<td>P</td>
<td>4 (4.4)</td>
<td></td>
<td></td>
<td>1 (1.2)</td>
<td>5</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Blue Jay</td>
<td>Cyanocitta cristata</td>
<td>P, T</td>
<td>1 (0.4)</td>
<td>2 (2.2)</td>
<td>1 (3.8)</td>
<td>4</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Common Grackle</td>
<td>Quiscalus quiscula</td>
<td>P, T</td>
<td>1 (0.4)</td>
<td>3 (3.6)</td>
<td></td>
<td>4</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Eastern Towhee</td>
<td>Pipila erythropthalmas</td>
<td>S</td>
<td>3 (1.1)</td>
<td></td>
<td>1 (1.2)</td>
<td>4</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Pine Warbler</td>
<td>Dendroica pinus</td>
<td>S</td>
<td>3 (1.1)</td>
<td>1 (1.1)</td>
<td></td>
<td>4</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Savannah Sparrow</td>
<td>Passerculus sandwichensis</td>
<td>S</td>
<td></td>
<td>2 (2.4)</td>
<td>1 (6.25)</td>
<td>3</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Veery</td>
<td>Catharus fuscens</td>
<td>S</td>
<td>3 (1.1)</td>
<td></td>
<td></td>
<td>3</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Yellow Warbler</td>
<td>Dendroica petchias</td>
<td>S</td>
<td>2 (0.7)</td>
<td>1 (1.1)</td>
<td></td>
<td>4</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Yellow-rumped Warbler</td>
<td>Dendroica coronata</td>
<td>P, T</td>
<td>2 (0.7)</td>
<td>1 (1.1)</td>
<td></td>
<td>3</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Other Avian Species</td>
<td></td>
<td></td>
<td>15 (5.4)</td>
<td>7 (7.8)</td>
<td>5 (19.2)</td>
<td>3 (3.6)</td>
<td>5 (31.25)</td>
<td>35</td>
<td></td>
</tr>
<tr>
<td>Mammalian</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>White-tailed Deer</td>
<td>Odocoileus virginianus</td>
<td></td>
<td>1 (0.4)a</td>
<td></td>
<td></td>
<td>2 (2.4)b</td>
<td>3</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cow</td>
<td>Bos taurus</td>
<td></td>
<td>2 (2.2)c</td>
<td></td>
<td></td>
<td>2</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Human</td>
<td>Homo sapiens</td>
<td></td>
<td>1 (1.2)</td>
<td></td>
<td></td>
<td>1</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td></td>
<td></td>
<td>279</td>
<td>90</td>
<td>26 (34)</td>
<td>84</td>
<td>16 (529)</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Residency codes: P, permanent resident (found year round in the state); S, summer resident (present in the state during the nesting season); T, transient (migrates through the state in spring and/or fall).
aBristol County white-tailed deer sample contained mixed blood with a Black-capped Chickadee.
bWhite-tailed deer samples from Plymouth contained mixed blood with a Common Yellowthroat and a Black-capped Chickadee.
cOne cow sample from Essex County contained mixed blood with a House Finch.

These, 1 specimen from Bristol County acquired a blood meal from a Black-capped Chickadee and white-tailed deer; 1 specimen from Plymouth County was identified with mixed blood from Common Yellowthroat, Geothlypis trichas (L.), and white-tailed deer; and 1 specimen from Essex County contained blood from a House Finch, Carpodacus mexicanus (Müller), and domestic cow.

The composition of 25 avian- and mammalian-derived blood meals for Cs. morsitans is shown in Table 3. We identified 14 avian species as hosts for Cs. morsitans. The largest number of blood meals was from Wood Thrush, Hylocichla mustellina (Gmelin) (n=7, 28.0%), followed by Tufted Titmouse (n=3, 12.0%), Black-capped Chickadee, Eastern Towhee, Pipilo erythropthalmas (L.) (n=2, 8.0% each), and 10 other avian species (n=1, 4.0% each). White-tailed deer was the only mammalian host for Cs. morsitans (n=1, 4.0%).

**Virus isolations from blood-fed mosquitoes**

All blood-fed mosquitoes were tested for infection with EEE virus and/or other arboviruses that currently circulate in the region. Four virus isolates were recovered from the head and thorax of individual blood-fed Cs. melanura mosquitoes in Vero cell culture, and later were identified as EEE, West Nile, and Highland J viruses by real-time RT-PCR assays, suggesting disseminated infection. The identity of viruses, trap type, dates of collection, location, and blood meal sources for these mosquitoes are provided in Table 4.
Avian frequency analysis

Residency status of the bird species that frequently served as the source of blood meals for *Cs. melanura* is shown in Table 2. Of these, six species were permanent residents (found year round), 18 were summer residents (present during the nesting season), and seven were permanent residents with part of the population transient in the spring and/or fall.

Estimated frequency patterns of bird occurrence in the 6 counties were similar and thus were combined for analysis (Table 1, Fig. 2). We focused on 43 of more than 330 bird species reported from the region as some of the most commonly encountered birds that reside in swamp localities and share habitats with *Cs. melanura* or reside in adjacent swamp-border areas frequented by host-seeking females. American Robin, Tufted Titmouse, Black-capped Chickadee, Northern Cardinal, *Cardinalis cardinalis* (L.), Gray Catbird, *Dumetella carolinensis* (L.), and Mourning Dove, *Zenaida macroura* (L.) together constituted 48.5\% \((n=254)\) of all avian-derived blood meals by *Cs. melanura*. These birds were also among the most frequently encountered avian species in the region based on the eBird database. Blue Jay, *Cyanocitta cristata* (L.), was identified as the 3rd most common bird species in the region, but constituted only 0.8\% \((n=4)\) of avian-derived blood meals by *Cs. melanura*. Although American Crow, *Corvus brachyrhynchos* Brehm, was the 8th most frequent bird species in the region, we did not identify a single crow–derived blood meal in either *Cs. melanura* or *Cs. morsitans*. Similarly, no *Cs. melanura* specimens were identified with blood meals from American Goldfinch, *Carduelis tristis* (L.) or Downy Woodpecker, *Picoides pubescens* (L.), 2 comparatively abundant bird species in the region. Northern Waterthrush, *Parkesia novaboracensis* (Gmelin); Veery, *Catharus fuscescens* (Stephens); and Field Sparrow were among the bird species that had relatively low frequencies. There were 10 bird species with relatively high frequencies in the study sites (Table 1, Fig. 2); however, these birds were not identified as hosts for *Cs. melanura*.

Discussion

Our study provides insight into the vector–host interactions of *Cs. melanura* and *Cs. morsitans* in Massachusetts, an area in the northeastern United States with multiple EEE virus foci. Our results confirm the strong ornithophilic nature of *Cs.*
FIG. 2. Average estimated frequencies (June through November) of 43 avian species in Massachusetts. Bars highlighted in black indicate frequent avian species that also served as hosts for Culiseta melanura. Bars highlighted in white depict bird species that were not identified as sources of blood meals for Culiseta melanura. Percentages following species’ names show the proportion of blood meals for Cs. melanura from each bird species.
melanura (99%) and Cs. morsitans (96%), in acquiring blood meals from avian hosts; however, we were also able to document, albeit low, that both of these species also acquire mammalian blood meals. Of note, was the identification of 1 human blood meal from Cs. melanura. This finding is particularly relevant to public health surveillance of EEE virus in Massachusetts, especially during the peak season of Cs. melanura abundance and coincident detection of the virus.

During the last several years, EEE virus has been isolated from 16 different mosquito species collected throughout northeastern United States (ArboNET; Centers for Disease Control and Prevention, Atlanta, GA). The overwhelming majority of isolations have been made from Cs. melanura, reaffirming its direct involvement in enzootic amplification of the virus within virus foci. Although EEE virus frequently infects Cs. melanura, this species has generally been considered an unlikely “bridge” vector because it feeds mainly on birds (Scott and Weaver 1989, Komar and Spielman 1994). However, in our study, a small proportion of Cs. melanura and Cs. morsitans acquired blood meals from mammalian hosts, including a human-derived blood meal from Cs. melanura.

Identification of mammalian-derived blood meals from Cs. melanura in the present study is consistent with the results of our earlier investigations on the feeding behavior of this mosquito species collected from 2 sites associated with an EEE virus focus in central New York (Molaei et al. 2006a) and from Connecticut (Molaei and Andreadis 2006). In New York, 0.8% and 5.0% (n = 484) of the blood meals for Cs. melanura were identified from mammals solely or in mixed blood meals, respectively. Similarly, in Connecticut 4.2% (n = 48) of the blood meals originated from mammals including equines. Thus identification of one mammal-derived blood meal from this mosquito species in the present study is credible based on human population density as one of the most abundant mammalian species in the region. The possibility of a potential contamination during laboratory analyses and PCR cannot be entirely ruled out; however, we are reasonably confident that experimentations have been carefully conducted, and that Cs. melanura occasionally feeds on mammals including humans. A recent study from New Jersey also documented 2 human-derived blood meals of 6 specimens with nonavian hosts (Apperson et al. 2004).

Earlier analysis of engorged Cs. melanura, collected from the periphery of 2 red maple-white cedar freshwater swamps in the Taunton River basin in the towns of Easton and Raynham, Massachusetts by using the precipitin technique, reported 0.5% (n = 5) mammalian blood-feeding in mosquitoes collected from the swamp perimeter in 1977 (Nasci and Edman 1981). Similarly, 0.3% (n = 5) and 0.4% (n = 2) of Cs. melanura were identified with mammalian-derived blood meals in mosquitoes collected from the outside swamp and perimeter, respectively, in 1978 (Nasci and Edman 1981). Mammalian species that served as the source of blood meals in the latter study included dog, rabbit, and cat. In the present study, we identified white-tailed deer, cow, and human as hosts for Cs. melanura. Although the rather small percentage of mammalian-derived blood meals does not permit a comprehensive discussion, differences are likely due to variations in collection methods, and abundance of the host species in the 2 studies. In a recent study in Connecticut, Cs. melanura was the predominant source of EEE virus (83 [68%] of 122 virus isolations) and the only species to support consistently high virus titers required for efficient transmission (Armstrong and Andreadis 2010). In retrospect, it is possible that a single EEE virus detection from 554 blood meals may be somewhat an underrepresentation of actual infection of mosquitoes during the study period. During the 2007 and 2008, rates of positive pools detected at these same sites in Massachusetts were 1.4% (25/1726) in 2007 and 1.8% (11/600) in 2008 tested by RT-PCR. These findings further implicate Cs. melanura as a potential “bridge” vector of EEE virus to humans and other mammals throughout the range of distribution, in addition to its prominent role as an enzootic vector.

Enzootic transmission of EEE virus depends on the frequent interaction of Cs. melanura with key virus-competent bird species, some of which may serve as “superspreaders.” In the present study, 97% of all avian-derived blood meals originated from Passeriformes birds, including American Robin, Tufted Titmouse, Black-capped Chickadee, Scarlet Tanager, Field Sparrow, and other avian species possibly serving as reservoirs for EEE virus. We identified American Robin as the most frequent source of blood (n = 115, 21.7%) for Cs. melanura. Similarly, American Robins also served as a frequent source of blood for host-seeking Cs. melanura in neighboring Connecticut (22.9%, n = 11) (Molaei and Andreadis 2006) and New York (9.1%, n = 46) (Molaei et al. 2006a), and for Culex spp. mosquitoes throughout the Northeast and other regions of the United States (Apperson et al. 2002, Apperson et al. 2004, Molaei et al. 2006b, Savage et al. 2007, Hamer et al. 2009), thus implicating these birds as important amplifying hosts in enzootic transmission of arboviruses, including EEE and West Nile viruses.

American Robins are found throughout most of North America. Permanent and transient populations of this species use a wide variety of open and forested habitats in urban/suburban and rural settings, and active in riparian forests, early successional forests (Martin 1973, Hutto 1995, Sallabanks 1995), closed canopy forests, and woodlands (www.epa.gov/region1/getsites/restofriver/reports/final_era/B%20 %20Focus%Species%20Profiles/EcoRiskProfile_american_robin.pdf). American Robins are the most prominent tree-roosting birds in woodland habitats, where large flocks of these birds roost in summer months after nesting ends (Poole 2005). This creates a spatial overlap with activities of Cs. melanura in EEE virus foci throughout the region. Dates of the first clutch of American Robins in early April through late July in southern regions of the Northeast, and early May through early July in northern areas (e.g. northern Maine; http://goo.gl/1GIDB/) overlap temporally with the emergence of the 1st generation of Cs. melanura. American Robins are competent amplifying hosts for EEE virus based on the intensity of infection and duration of viremias after infection by mosquitoes (Komer et al. 1999). EEE virus also has been isolated from American Robins in Massachusetts (Main et al. 1998) and New Jersey (Crans et al. 1994). Moreover, antibody prevalence studies indicate that these birds are frequently exposed to EEE virus throughout the region (Dalrymple et al. 1972, Bast et al. 1973, Morris et al. 1975, Main et al. 1988, Crans et al. 1994, Gettman, personal communication). Our results further suggest that American Robins may play a more important role in early season virus amplification, due to their abundance and greater availability as hosts at this time of the year.

Tufted Titmouse and Black-capped Chickadee were the 2nd and 3rd most frequent hosts and together constituted 17.4% (n = 91) of blood meals for Cs. melanura. The Tufted Titmouse...
is found throughout the eastern United States, and the Black-  
capped Chickadee is common in the east. Our estimated bird  
frequency data analysis also indicated that these two species  
are common in eastern Massachusetts (Table 1, Fig. 2). The  
preferred habitats for Tufted Titmouse and Black-capped  
Chickadee include deciduous and mixed deciduous/conifer-  
ous woodlands, open woods, swamps, and dense canopies  
(http://bna.birds.cornell.edu/). These birds are more com-  
mon near edges of wooded areas, but can be found in the  
middle of large wooded tracts that make them accessible to  
host-seeking Cs. *melanura* and other woodland inhabiting  
mosquitoes. Tufted Titmouse and Black-capped Chickadee  
captured within EEE virus foci in Alabama (Stamm 1968),  
Maryland (Dalrymple et al. 1972), New Jersey (Crans et al.  
1994), New York (Howard et al. 2004), Massachusetts (Main  
and Kale 1971, Hodgson et al. 2001). Frequent  
infection of Wood Thrushes with EEE virus has been reported.  
Of 42 isolations of EEE virus from more than 3000 birds bled  
in south Alabama, there were more from Wood Thrush than  
any other bird species (Stamm 1968). In New Jersey, early-  
season virus isolation from Wood Thrush and a few other bird  
species has been reported as evidence of a cryptic EEE virus  
prevalence (Crans et al. 1994). In Massachusetts, Wood Thrush had  
the highest EEE virus antibody prevalence rates (26.7%) fol-  
lowed by Swamp Sparrow, *Melospiza georgiana* (Latham)  
(24.5%), American Robin (20.9%), and Ovenbird, *Seiurus  
aurocapilla* (L.) (18.2%) (Main et al. 1988).

We identified several other avian species as the sources of  
blood for *Cs. melanura* and *Cs. morsitans* at moderate or low  
frequency. Our estimated bird frequency data analyses also  
demonstrate that these species occur with varying degrees of  
frequency, but due to the microhabitat differences, they may  
not have been accessible to host-seeking mosquitoes.  
Nonetheless, a number of these species, most notably Scarlet  
Tanager; Northern Cardinal; Red-eyed Vireo, *Vireo olivaceus*  
(L.); Baltimore Oriole, *Icterus galbula* (L.); Common Yellow-  
throat, *Geothlypis trichas* (L.); Red-winged Blackbird, *Agelaius  
phoenicus* (L.); House Wren, *Troglodytes aedon* (Vieillot);  
Ovenbird; Blue Jay; Common Grackle, *Quiscalus quiscula*  
(L.); and Veery have been shown to have high antibody  
prevalence for EEE virus, or virus has been isolated from  
these birds in New Jersey, New York, and Massachusetts  
(Emord and Morris 1984, Main et al. 1988, Crans et al. 1994,  
Kumar and Spielman 1994, Howard et al. 2004). These res-  
ervoir-competent birds have been incriminated in the  
maintenance and amplification of EEE virus in the region  
(Komar et al. 1999).

To better understand vector–host interactions, and gain  
insight into the role of key bird species in EEE virus trans-  
mision, we examined estimated bird frequency data in the  
study region. We compared bird frequency data in the 6  
counties from which enzootic-mosquitoes were collected to  
each other and to the entire region, and because there were no  
appreciable differences, we used data for the entire region in  
our analyses. We identified more than 330 bird species as  
permanent resident, summer resident, and/or transient (birds  
that migrate through the region in spring and/or fall), with  
varying frequencies and abundance. Among 43 avian species  
identified as frequent birds in the region, 33 species also  
served as hosts for *Cs. melanura*, indicating that these mos-  
quitoes acquire blood meals from the most common bird  
species in the region. However, there were a number of fre-  
cquently observed species, including American Goldfinch;  
Downy Woodpecker; and White-breasted Nuthatch, *Sitta  
carolinensis* Latham that were not identified as the source of  
blood meals for *Cs. melanura* and *Cs. morsitans* in our analyses.  
The lack of feedings on these birds might indicate that local  
populations of *Cs. melanura* and *Cs. morsitans* are not attracted  
to these species. However, a more likely explanation is that  
there was no temporal and/or spatial overlap between mos-  
quito and bird activities. Certain birds, such as Woodpeckers,  
spend the night deep inside tree holes and thus are isolated  
from host-seeking mosquitoes (Edman et al. 1972). Defensive  
behavior that prevents or interrupts mosquito blood feedings  
could also be considered as a contributing factor to the lack  
of blood feedings on some bird species as has been previously  
reported for European Starling, *Sturnus vulgaris* L. (Edman  

Bridging transmission of arboviruses by mosquitoes would  
require flexibility in the phenotype, such that an earlier blood  
feeding on birds (virus-amplifying hosts) follows a later  
feeding on mammals particularly humans. Because of the  
predominantly ornithophilic nature of *Cs. melanura* in blood  
feeding, there was little expectation of seasonal shifts from  
avian to mammalian hosts. However, a seasonal shift from  
American Robin to other avian species was noted. The chi-  
squared tests for linear trend showed that the proportion of  
American Robin–derived blood meals was significantly  
higher earlier in the season (*p* < 0.0001); 37.3% and 22.6% of all  
avian-derived blood meals in June and July, respectively,  
were from this avian species (Table 5). This was during the  
time when blood-feeding activity of the 1st generation of *Cs.  
melanura* temporally overlapped with the influx of migrating  
American Robins and start of the breeding season throughout  
the region. However, blood feeding on American Robin de-  
creased gradually as the season progressed. In August and  
September, 14.1% and 7.7% of the blood meals were from this  
species, respectively, and by October it further declined to  
4.8%. Toward the end of the season, a variety of other avian  
species such as Tufted Titmouse, Black-capped Chickadees,  
Scarlet Tanager, and Field Sparrow more frequently served as  
hosts for *Cs. melanura* (Table 5). An earlier study on the blood-  
feeding pattern of *Cs. melanura* in Massachusetts also reported  
a trend toward increasing diversity as the season progressed  
(Nasci and Edman 1981). During spring and early summer,  
passerine birds were among the most frequent source of blood  
meal for *Cs. melanura*, whereas later in the season,
nonpasserines and nonavian hosts were used, and feeding on mammalian hosts peaked during the 1st week of September (Nasci and Edman 1981).

In the present study, a relatively small percentage of Cs. melanura was identified with blood meals from certain avian species, despite our efforts for collecting an increased number of engorged mosquitoes by using numerous sites and 2 trap types. This limitation in conjunction with the scarcity of critical and more recent information on the community structure, breeding, and daily activities of various avian species precludes a comprehensive discussion on the potential shift in host selection of Cs. melanura. Nonetheless, availability and abundance of various vertebrate species may influence temporal heterogeneity in the host-feeding pattern of mosquitoes and potential shifts from avian to mammalian hosts or from a certain members of avian community to the others (Molaei et al. 2006b, Kilpatrick et al. 2007, Hamer et al. 2008). Other factors, such as increased mosquito abundance, physiological changes in mosquito host preference, and defensive behavior in birds, also have been postulated as underlying causes for variations in seasonal blood-feedings patterns (Tempelis et al. 1965, Edman et al. 1974, Nelson et al. 1976, Thiemann et al. 2011). Although the focus of the present study was on the role of Cs. melanura and Cs. morsitans, it is also important to discuss the contribution of other potential “bridge” vectors in the genera of Culex, Aedes, Ochlerotatus, Anopheles, and Coquillettidia to epidemic/epizootic transmission of EEE virus in Massachusetts and other regions of the Northeast. EEE virus has been isolated from these mosquitoes in Connecticut (Andreadis et al. 1998), and throughout northeastern United States (Cran and Schulze 1986, Centers for Disease Control and Prevention 2006). Biting risk of potential epidemic/epizootic mosquito vectors in EEE virus foci in Bristol and Plymouth Counties in southeastern Massachusetts, where human and horse cases have been historically reported, was estimated using carbon dioxide-baited light traps for capturing adult mosquitoes (Moncayo and Edman 1999). It has been suggested that Cq. perturbans, Oc. canadensis, and Culex salinarius Coquillet were more likely vectors of EEE virus in Massachusetts than Ae. vexans, Anopheles punctipennis (Say), and Anopheles quadrinaculatus Say. Susceptibility to per os infection and potential salivary transmission for EEE virus in 6 mosquito species also have been investigated in an earlier study in Massachusetts (Vaidyanathan et al. 1997).

On the basis of estimates of laboratory vector competence, frequent EEE virus isolations from field-collected mosquitoes, distance from forest resting habitats during host seeking, blood-feeding patterns coinciding with human disease, and sufficient host diversity to act as “bridge” vectors from birds to mammals, mosquito species were ranked from the most to least probable epidemic vectors: Cs. salinarius, An. quadrinaculatus, Oc. canadensis, Cq. perturbans, Ae. vexans, and An. punctipennis. Of these, Ae. vexans and An. punctipennis were unable to transmit EEE virus under laboratory conditions (Merrill et al. 1934, Ten Broeck and Merrill 1935, Davis 1940, Chamberlain et al. 1954, Wallis and Main 1974). Comparatively larger number of isolations from Cq. perturbans collected in Massachusetts and New York, where the greatest number of human cases has been correspondingly reported, in concert with catholic feeding habits (Edman 1971, Magnarelli 1977, Apperson et al. 2002, 2004, Molaei et al. 2008), abundance, and vector competence, make this mosquito species a likely suspect involved in epidemic/epizootic transmission of EEE virus to humans and equines in Massachusetts and throughout the region.

In conclusion, our study further clarifies the host associations of Cs. melanura and Cs. morsitans in this region of the northeastern United States. We find that these mosquitoes feed primarily on birds and focus their feeding activity on several common Passeriformes species capable of supporting EEE virus transmission. A small proportion of Cs. melanura and Cs. morsitans acquired blood meals from mammalian hosts including humans, suggesting their potential involvement in epidemic/epizootic transmission of EEE virus to humans and equines.

Acknowledgments

We thank John Shepard and Shannon Finan of the Center for Vector Biology & Zoonotic Diseases (CAES) for technical assistance. We are also grateful to numerous individuals at the Department of Public Health and Mosquito Control Projects in Massachusetts for assistance in collecting, identification, and handling of mosquitoes. Funding for this research was provided in part by Laboratory Capacity for Infectious Diseases Cooperative Agreement Number U50/CCH/6806-01-1 from the Centers for Disease Control and Prevention, and the United States Department of Agriculture (USDA) Specific Cooperative

---

**Table 5. Monthly Prevalence of Avian-Derived Blood Meals for Culiseta melanura in Massachusetts 2007–2008**

<table>
<thead>
<tr>
<th>Avian species</th>
<th>June (n = 153)</th>
<th>July (n = 159)</th>
<th>August (n = 99)</th>
<th>September (n = 91)</th>
<th>October (n = 21)</th>
</tr>
</thead>
<tbody>
<tr>
<td>American Robin</td>
<td>37.3%</td>
<td>22.6%</td>
<td>14.1%</td>
<td>7.7%</td>
<td>4.8%</td>
</tr>
<tr>
<td>Tufted Titmouse</td>
<td>4.6%</td>
<td>10.7%</td>
<td>12.1%</td>
<td>9.9%</td>
<td>4.8%</td>
</tr>
<tr>
<td>Black-capped Chickadee</td>
<td>7.8%</td>
<td>7.5%</td>
<td>6.1%</td>
<td>15.4%</td>
<td>4.8%</td>
</tr>
<tr>
<td>Scarlet Tanager</td>
<td>5.2%</td>
<td>3.1%</td>
<td>1%</td>
<td>18.7%</td>
<td>23.8%</td>
</tr>
<tr>
<td>Field Sparrow</td>
<td>1.3%</td>
<td>7.5%</td>
<td>8.1%</td>
<td>9.9%</td>
<td>9.5%</td>
</tr>
<tr>
<td>Northern Cardinal</td>
<td>1.3%</td>
<td>9.4%</td>
<td>7.1%</td>
<td>4.4%</td>
<td>9.5%</td>
</tr>
<tr>
<td>Wood Thrush</td>
<td>3.3%</td>
<td>6.3%</td>
<td>10.1%</td>
<td>2.2%</td>
<td>0%</td>
</tr>
<tr>
<td>Other avian species</td>
<td>39.2%</td>
<td>32.7%</td>
<td>41.4%</td>
<td>31.9%</td>
<td>42.9%</td>
</tr>
</tbody>
</table>

*Indicates total number of blood meals from various avian species in each month.*
Agreement Number 58-6615-1-218, and USDA-administered Hatch funds to the CAES.

Author Disclosure Statement

No competing financial interests exist.

References


Martin, K. Breeding density and reproductive success of Robins in relation to habitat structure on logged areas of Vancouver


Poole, A. (Editor). The birds of North America online: Cornell Laboratory of Ornithology, Ithaca, NY. Available at http://bna.birds.cornell.edu/BNA/2005/.


Address correspondence to: Goudarz Molaei Center for Vector Biology & Zoonotic Diseases The Connecticut Agricultural Experiment Station 123 Huntington Street PO Box 1106 New Haven, CT 06504 E-mail: Goudarz.Molaei@ct.gov