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Mosquito (Diptera: Culicidae) Bloodmeal Sources During a Period of West Nile Virus Transmission in Puerto Rico

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Abstract

Host bloodmeals of indigenous Caribbean mosquitoes have not been studied previously. We identified vertebrate DNA in 90 blood-engorged mosquitoes belonging to four genera (*Aedes*, *Culex*, *Deinocerites*, and *Uranotaenia*) and 12 species that were collected in Puerto Rico within a geographic and temporal focus of West Nile virus transmission in 2007. It was found that 62 (68.8%) bloodmeals were from reptiles, 18 (20.0%) from birds, and 10 (11.1%) from mammals. Only one bloodmeal of 18 derived from *Culex* (*Culex*) species was passerine, suggesting a preference for nonpasserine birds and other vertebrates (i.e., reptiles) among the candidate WNV vectors. We interpret the results with respect to vectorial capacity for West Nile virus, an emerging arbovirus throughout the Caribbean Basin.

Keywords

West Nile virus; ecology; mosquito; arbovirus vector; reservoir host

West Nile virus (family *Flaviviridae*; genus *Flavivirus*, WNV) is a mosquito-borne arbovirus known for outbreaks of neurologic disease and death among people, horses, and birds in temperate regions of North America. Transmission in the Caribbean Basin has been documented since 2001, but outbreaks have not been detected (Komar and Clark 2006). Intense WNV transmission was detected in Puerto Rico beginning in May 2007, with high infection rates in sentinel chickens and mosquitoes (Barrera et al. 2008, Hunsperger et al. 2009), although no human disease was reported (Torres Aponte et al. 2008). Among the ≈ 40 mosquito species resident in Puerto Rico, three are likely WNV vectors: *Culex nigripalpus* Theobald, *Culex bahamensis* Dyar & Knab, and *Aedes taeniorhynchus* (Wiedemann) (Barrera et al. 2010). The ecological and epidemiological basis for the low disease incidence remains a mystery. We enhanced our understanding of WNV ecology in Puerto Rico by studying the feeding behavior of several species of mosquitoes collected within a WNV transmission focus. Vertebrate host targets of blood-engorged mosquitoes were identified using nucleic acid amplification and sequencing techniques.

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Resting mosquitoes were collected from the Roosevelt Roads Naval Base and parts of Naguabo and Ceiba municipalities in eastern Puerto Rico (18.20767–18.27408° N, –65.68303–65.59411° W), by using backpack aspirators (Clark et al. 1994) each morning for 15 d in July and August 2007, from 100 black-painted wood-fiber pots (27.9 by 27.9 cm, Western Pulp Products, Corvallis, OR; Komar et al. 1995) placed in 25 clusters of four (facing outward) in wetland, mangrove, deciduous and evergreen forest, rural, and urban habitats. Additional mosquitoes resting on vegetation, crab holes, roots, or artificial structures were collected with backpack aspirators for 15–20 min in each of the six types of habitats (at alternative sites away from the pots) each day. Mosquitoes were killed by freezing on dry ice, and transported with dry ice to the laboratory where they were sorted and identified on chill tables. Mosquito identification was done using several taxonomic keys (Adames 1971, Belkin and Heinemann 1975, Galindo et al. 1954, Sirivanakarn 1982, Tulloch 1937). Blood-engorged mosquitoes were stored individually until they were processed for bloodmeal identification.

Engorged mosquitoes were placed individually in 1.5-ml microcentrifuge tubes and frozen at –20°C until processed for detection of vertebrate DNA. DNA was extracted from whole mosquitoes following a modified salt extraction as described previously (Kent and Norris 2005) with dried pellets resuspended in 100 µl of HPLC water. After extraction, a DNA fragment was amplified and sequenced using primers specific for either cytochrome *c* oxidase 1 (CO1) or cytochrome *b* (cytb) vertebrate mitochondrial genes by using protocols described previously (Kent et al. 2009). Vertebrate blood was identified using the DNA Barcode database (www.barcodinglife.org) for CO1 sequences or GenBank (www.ncbi.nlm.nih.gov) for cytb sequences.

In total, 207 blood-engorged mosquitoes was captured (67 from wood-fiber pots and 140 from natural resting places) in the following numbers and species: one *Aedes mediiovittatus* (Coquillett), 17 *Ae. taeniorhynchus*, eight *Aedes tortilis* (Theobald), eight *Culex* (*Melanoconion*) *taeniopus* Dyar & Knab, one *Cx. (Melanoconion) erraticus* (Dyar & Knab), 85 *Cx. (Mel.)* spp., eight *Cx. (Culex) bahamensis*, one *Cx. (Cux.) habilitator* Dyar & Knab, six *Cx. (Cux.) janitor* Theobald, 16 *Cx. (Cux.) nigripalpus*, 28 *Cx. (Cux.) quinquefasciatus* Say, seven *Cx. (Cux.)* spp., 11 *Deinocerites magnus* (Theobald), six *Uranotaenia cooki* Root, and four *Uranotaenia* spp. Vertebrate host identities could be determined for 90 engorged specimens of 12 mosquito species, including 62 (68.8%) with reptilian blood, 18 (20.0%) with avian blood, and 10 (11.1%) with mammalian blood (Table 1). Among the avian bloodmeals, six were derived from chickens, presumably the same birds used as sentinels to monitor WNV transmission. The other 12 came from a variety of birds including ardeids ($n = 7$, three species of herons and egrets), columbids ($n = 2$, two species of doves), passerines ($n = 2$, both red-legged thrush), and one grebe (Table 1). Insufficient numbers of any one mosquito species were collected to permit an analysis of avian host preferences among candidate vector mosquito species. Among *Culex* species within the subgenus *Culex* (*Cx. quinquefasciatus*, *Cx. nigripalpus*, *Cx. janitor*, and *Cx. bahamensis*), 18 bloodmeals were identified, including 11 (61%) from reptiles and seven (39%) from birds. Only two bloodmeals were identified from passerine birds, both red-legged thrush, from a *Cx. nigripalpus* and a *Uranotaenia* species. Similarly, most *Culex* (*Melanoconion*) sp.

mosquitoes had reptilian blood, representing 44.4% of all mosquito bloodmeal identifications.

Three abundant and locally widespread mosquito species were found with WNV disseminated infections during the 2007 epizootic in Puerto Rico: *Cx. nigripalpus*, *Cx. bahamensis*, and *Ae. taeniorhynchus* (Barrera et al. 2010). In this study, *Cx. nigripalpus* showed a broad host bloodmeal diet that included birds and reptiles, whereas *Ae. taeniorhynchus* fed upon birds, reptiles, and mammals (Table 1). Previous studies have shown that these two species use a broad spectrum of vertebrate hosts as sources of blood, including reptiles (Edman 1974, Christensen et al. 1996). The only bloodmeal analyzed of *Cx. bahamensis* came from a reptile (Table 1). This mosquito species bites birds and mammals in the laboratory (Edman 1974). The ample feeding on reptiles that was observed in unidentified species of the subgenus *Melanoconion* of *Culex* in this study was previously observed for *Cx. (Mel.) pilosus* (Dyar and Knab) and *Cx. (Mel.) peccator* (Dyar and Knab) in Florida (Edman 1979).

A focus of intense WNV transmission in eastern Puerto Rico during summer 2007 provided an opportunity to investigate the ecology of WNV in Puerto Rico. Reptiles were the principal vertebrate host identified from the mosquito bloodmeals. Interestingly, of the 18 avian bloodmeals, only two were passerine, suggesting a potentially zooprophyllactic role for nonpasserine species, such as columbids (pigeons and doves) and ardeids (herons, egrets). Furthermore, only one bloodmeal of 18 derived from *Culex* (*Cx.*) species (*Cx. nigripalpus*) was passerine, suggesting a preference for nonpasserine birds and other vertebrates (i.e., reptiles). This is significant because a study of the relative abundance of the local bird species found that the order Passeriformes accounted for the majority of resident birds, and most passerine birds are competent amplifying hosts of WNV (Komar et al. 2003, unpublished). A larger bloodmeal analysis study is needed to determine mosquito preferences among these passerine birds. In particular, blood-engorged mosquitoes should be collected from the arboreal canopy, if possible, and other substrates where birds roost nocturnally.

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Morphological identities of 113 blood-engorged mosquitoes matched with DNA sequence-derived identities of the vertebrates that provided the respective bloodmeals, including 23 unidentified vertebrates

Table 1

Vertebrate	Mosquito species												
	<i>Aedes mediovittatus</i> (Coquillett)	<i>Ae. taenio-rhynchus</i> (Wiede-mann)	<i>Ae. tortilis</i> (Theobald)	<i>Culex</i> (Mel.) sp.	<i>Cx.</i> (Cx.) sp.	<i>Cx. taeniotopus</i> Dyar & Knab	<i>Cx. bahamensis</i> Dyar & Knab	<i>Cx. janitor</i> Theobald	<i>Cx. nigripalpus</i> Theobald	<i>Cx. quinque-fasciatus</i> Say	<i>Deino-cerites magnus</i> (Theobald)	<i>Urano-taenia cooki</i> Root	<i>Ur. sp.</i>
Mammals													
Human (<i>Homo sapiens</i>)		1	1	1					1				1
Horse (<i>Equus caballus</i>)	1												
Cow (<i>Bos taurus</i>)			1		1								
Black rat (<i>Rattus rattus</i>)		1			3								
Birds													
Red-legged thrush (<i>T. plumbeus</i>)									1				
African collared-dove (<i>Streptopelia roseogrisea</i>)				1									
Zenaida dove (<i>Zenaida aurita</i>)										1			
Cattle egret (<i>Bubulcus ibis</i>)				1									
Yellow-crowned night heron (<i>Nycticorax violaceus</i>)				2					1				
Green heron (<i>Butorides virescens</i>)		2	1										
Pied-billed grebe (<i>Podilymbus podiceps</i>)													1
Chicken (<i>Gallus gallus</i>)		1							2	3			
Reptiles													
Skink spp.				1									1
Puerto Rican crested anole (<i>Anolis cristatellus</i>)				17				4	2		1		4
Sharp-mouthed lizard (<i>Anolis pulchellus</i>)				15									
Cope's leopard lizard													1

Vertebrate	Mosquito species												
	<i>Aedes mediovittatus</i> (Coquillett)	<i>Ae. taenio-rhynchus</i> (Wiede-mann)	<i>Ae. tortilis</i> (Theobald)	<i>Culex</i> (Mel.) sp.	<i>Cx.</i> (Cx.) sp.	<i>Cx. taeniopus</i> Dyar & Knab	<i>Cx. bahamensis</i> Dyar & Knab	<i>Cx. janitor</i> Theobald	<i>Cx. nigripalpus</i> Theobald	<i>Cx. quinque-fasciatus</i> Say	<i>Deino-cerites magnus</i> (Theobald)	<i>Urano-taenia cooki</i> Root	<i>Ur. sp.</i>
(<i>Gambelia copei</i>)													
Iguana (<i>Iguana iguana</i>)	1		1	1	2	1	1	3			7		
Lizard spp.				1									
Unknown	4		2	10		1		1			2	1	2