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Vertebrate Hosts of *Aedes aegypti* and *Aedes mediovittatus* (Diptera: Culicidae) in Rural Puerto Rico

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Abstract

The distribution of *Aedes (Stegomyia) aegypti* (L.), the main vector of dengue viruses (DENV) worldwide, overlaps with *Aedes (Gymnometopa) mediovittatus* (Coquillett), the Caribbean treehole mosquito, in urban, suburban, and rural areas. *Ae. mediovittatus* is a competent vector of DENV with high rates of vertical DENV transmission in the laboratory. This study determined whether *Ae. mediovittatus* feeds on humans and compared its feeding patterns with co-occurring *Ae. aegypti* in two rural communities of Puerto Rico. Adult mosquitoes were captured for three consecutive days every week from July 2009 to May 2010 using BG-Sentinel traps with skin lures that were placed in the front yard of houses in both communities. Three methods were used to identify the 756 bloodmeals obtained in this study: a multiplex polymerase chain reaction (PCR) for humans and dogs targeting cytochrome b; a PCR targeting the 16S rRNA; and a nested PCR targeting cytochrome b. *Ae. mediovittatus* fed mostly on humans (45–52%) and dogs (28–32%) but also on cats, cows, horses, rats, pigs, goats, sheep, and chickens. *Ae. aegypti* fed mostly on humans (76–79%) and dogs (18–21%) but also on cats, horses, and chickens. Our results indicate that *Ae. mediovittatus* may have a relatively high rate of vector–human contact, which might facilitate virus transmission or harborage in rural areas of Puerto Rico.

Keywords

Aedes aegypti; *Aedes mediovittatus*; dengue; vector; vertebrate host

Aedes (Stegomyia) aegypti (L.), the main vector of dengue viruses (DENV), co-occurs with other mosquito species that exploit natural and artificial containers with water to complete their development. *Aedes (Stegomyia) albopictus* (Skuse) is among those species; however, it seems to play a lesser role as a DENV vector, partially because it has lower vector competence and because its females prefer to feed on a variety of vertebrate hosts other than humans that are not DENV reservoirs in urban areas (Lambrechts et al. 2010). *Ae. aegypti* also shares its habitat with *Aedes (Gymnometopa) mediovittatus* (Coquillett) (Cox et al.

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2007, Smith et al. 2009), the Caribbean treehole mosquito, which is native to the Greater Antilles (Belkin et al. 1970). Moreover, *Ae. aegypti*, *Ae. albopictus*, and *Ae. mediiovittatus* have been reported to co-occur in the same localities of Cuba (Aguilera et al. 2000, Marquetti et al. 2000) and the Dominican Republic (Pena et al. 2003). *Ae. mediiovittatus* is a competent vector of DENV and exhibits high vertical transmission for all four DENV (Freier and Rosen 1988, Gubler et al. 1985). Gubler et al. (1985) suggested that *Ae. mediiovittatus* may contribute to the maintenance of DENV in Puerto Rico during interepidemic periods. If *Ae. mediiovittatus* acts as a secondary vector or reservoir of DENV, this species must frequently bite humans in nature (Fay and Keirans 1971, Gubler et al. 1985). The objectives of the current study were to determine whether *Ae. mediiovittatus* feeds on humans or other vertebrates and to compare its feeding patterns with the co-occurring *Ae. aegypti* in two rural communities of Puerto Rico where these species overlap extensively by using high specificity molecular techniques to differentiate the source of bloodmeals (Kent 2009).

Materials and Methods

Study Sites

The study was conducted in two communities of the municipality of Patillas, southeastern Puerto Rico: Providencia (17° 59'16"N; 66°0'0" W) and Recio (17° 58'36" N; 65° 57'19" W). The former community is located at a low elevation (33.4 m above sea level [a/s/l]) with mean annual minimum and maximum temperatures of 20.9 and 31.0°C, respectively; mean total annual precipitation of 1,476 mm; and contains 359 houses (747 inhabitants). Recio is also located at low elevation (34.4 [a/s/l]) with mean annual minimum and maximum temperatures of 20.6 and 30.5°C, respectively; mean total annual precipitation of 1,622 mm; and contains 489 houses (1,029 inhabitants). Most houses in both the locations are one-story buildings with backyards or gardens.

Mosquito Samples

Adult specimens of *Ae. aegypti* and *Ae. mediiovittatus* were captured for three consecutive days every week by using 28 BG-Sentinel traps with BG-lure (Biogents AG, Regensburg, Germany) in Providencia (3,780–415 failures = 3,365 samples) and 47 BG traps (5,391–500 trap failures = 4,891 samples) in Recio, from July 2009 to May 2010. BG-lures were replaced with new ones every 3 mo. Trap failures were mainly because of the presence of ants or lizards, low battery voltage, disconnection from the battery, or broken collection bags. The BG traps used in each community were dispersed uniformly over the entire urbanized area by keeping sufficient distances between the traps to minimize trap interactions and spatial autocorrelations. Inter-trap distances were 88 m in Providencia and 90 m in Recio. The traps were placed outdoors on the porch or at the front of the houses in partial shade.

Bloodmeal Analyses

Individual mosquitoes were homogenized in 225- μ l phosphate buffered saline (PBS, pH 7.4). DNA was prepared using a DNeasy Blood and Tissue Kit (Qiagen, Valencia, CA) by following the manufacturer's protocols. The process was automated using the Qiacube

system (Qiagen). Isolated DNA was stored at -80°C until further testing. The extracted DNA served as a template for three polymerase chain reaction (PCR)-based assays. The initial assay was a species-specific duplex PCR with primers that were designed to amplify mitochondrial cytochrome b as described previously (Kent and Norris 2005). Previous universal primer studies have indicated that humans and dogs are the most common hosts for *Ae. aegypti* (Jansen et al. 2009); therefore, primer sets that amplified cytochrome b sequences of humans (*Homo sapiens*) and dogs (*Canis lupus*) were used to initially classify the samples. Kent and Norris (2005) showed that these primer sets have high specificity and could be duplexed. Samples that did not amplify with the two species specific primers were then tested by a PCR that amplified 16S rRNA sequences with a universal primer set targeting all vertebrates as described previously (Kitano et al. 2007, Burkett–Cadena et al. 2008). The remaining samples that did not amplify in the species specific and universal 16 s rRNA assays were tested in a nested PCR designed to amplify cytochrome b sequences with a universal primer set targeting all vertebrates as described previously (Hassan et al. 2003). Nested PCR has been shown to be useful in samples with small amounts of DNA (Hassan et al. 2003). Positive samples were treated with Exosap-IT (USB, Cleveland, OH) for purification and were then either sequenced using a pyrosequencer (PyroMark Q96 ID; Qiagen) or sent to the GENEWIZ sequencing facility (South Plainfield, NJ) for analysis. Sequences were entered into the National Center for Biotechnology Information Basic Local Alignment Search Tool (<http://blast.ncbi.nlm.nih.gov/Blast.cgi>) database for identification, and only those sequences with a match percentage $\geq 95\%$ were accepted as belonging to the identified bloodmeal source as described previously (Hassan et al. 2003).

Statistical Analyses

To determine whether there was a difference in the feeding patterns of *Ae. aegypti* or *Ae. mediovittatus* between locations, we used a two (sites) \times 2 (humans, other vertebrates) contingency table analysis ($\alpha = 0.05$). The null hypothesis that *Ae. aegypti* and *Ae. mediovittatus* fed with the same frequency on humans and other vertebrates was tested using a two (mosquito species) \times 2 (humans, other vertebrates) contingency table ($\alpha = 0.05$). Statistical tests were done using SPSS (ver. 19; IBM, Armonk, NY).

Results

We captured more female *Ae. aegypti* (11,789 and 24,012 in Providencia and Recio, respectively) than *Ae. mediovittatus* (1,242 and 2,475; Table 1). All the female specimens of *Ae. mediovittatus* that had blood ($n = 358$) were processed for bloodmeal identification, of which 110 were collected in Providencia (8.9% of all the females captured) and 248 in Recio (10% of all the females captured). Because we collected almost 10 times more *Ae. aegypti* than *Ae. mediovittatus*, we took a subsample of *Ae. aegypti* specimens with blood to match the numbers of *Ae. mediovittatus* with blood that were collected in each month. One *Ae. aegypti* specimen with blood was taken at a time from each vial containing the specimens captured by each trap (in no particular order) within a given month until the total number in the subsample approximately matched the number of *Ae. mediovittatus* collected in the same month. Thus, we processed a total of 398 *Ae. aegypti* specimens with blood, of

which 132 were collected in Providencia and 266 were collected in Recio. The total number of processed mosquito specimens was 242 from Providencia and 514 from Recio (Table 1).

Primer sets specific for dog and human bloodmeals (Kent and Norris 2005) were initially used to classify the samples, as preliminary studies had suggested that these two species were the most commonly fed upon in the mosquitoes collected (data not shown). In total, 194 (48.7%) of the *Ae. aegypti* samples produced amplification products from the species specific PCR assay, allowing them to be classified as having been derived from either dog or human hosts (Table 2). None of the *Ae. mediovittatus* samples produced amplification products with the species specific PCR assay. Those samples that did not amplify were then used as a template in a universal assay targeting the 16S rRNA gene. The amplicons from this assay were identified by direct sequencing and BLAST analyses as described in Materials and Methods. Of the 562 samples tested in this assay, 347 (61.7%) produced a positive result (Table 2). The remaining 215 samples that were negative in the species specific and universal 16S rRNA assays were then used as a template in a nested PCR assay targeting the cytochrome b gene. Of the 215 samples tested in the nested PCR, 68 (31.6%) produced an amplicon (Table 2). These amplicons were identified to the species level by direct DNA sequencing. In total, 147 samples (19.4% of all those analyzed) did not produce a product in any of the three assays.

The hosts of mosquitoes were identified from the bloodmeals of 199 mosquito specimens from Providencia (82.2%) and 410 (79.8%) specimens from Recio (Table 1). Blood was identified in most *Ae. aegypti* specimens (98.2%) and in a smaller percentage of *Ae. mediovittatus* specimens (60.9%). Most bloodmeals of *Ae. aegypti* were from humans (76.2% in Providencia and 78.9% in Recio) and dogs (20.8% in Providencia and 18.4% in Recio), with one mixed meal of dog and human blood (Table 3). Most *Ae. mediovittatus* also fed on humans (52.2% in Providencia and 45.6% in Recio) and dogs (27.5% in Providencia and 32.2% in Recio) and on a greater variety of domestic vertebrates than those detected for *Ae. aegypti* (Table 3). The percentage of bloodmeals from birds was very low for both species, and no bloodmeals from amphibians or reptiles were detected. The feeding patterns of *Ae. aegypti* ($\chi^2 = 0.48$; df = 1; $P > 0.025$) and *Ae. mediovittatus* ($\chi^2 = 0.81$; df = 1; $P > 0.025$) on humans and other vertebrates did not differ between the study sites. The null hypothesis that *Ae. aegypti* and *Ae. mediovittatus* fed with the same frequency on humans and other vertebrates was rejected ($\chi^2 = 59$; df = 1; $P < 0.001$). In fact, *Ae. aegypti* proportionally fed more often on humans, and *Ae. mediovittatus* fed more often on other vertebrates. The detection of blood of several vertebrates (e.g., goat, sheep, cow, pig, rat, and so forth) in *Ae. mediovittatus* specimens indicates the presence and use of these hosts by *Ae. mediovittatus* in the study areas. Thus, the less diverse diet of *Ae. aegypti* (Table 3) was not because of the absence of alternate vertebrates. Our results also indicate that both the mosquito species are mainly mammalophagic.

Discussion

Our observations in two rural communities of Puerto Rico indicate that *Ae. mediovittatus* fed on people to a considerable extent (45–52% of all blood-meals); therefore, this species should be considered anthropophagic as suggested earlier (Fay and Keirans 1971, Gubler et

al. 1985). *Ae. aegypti* was more anthropophilic (76–79% of all bloodmeals) than *Ae. mediovittatus*. Thus, our results suggest that *Ae. mediovittatus*—a competent vector of DENV in the laboratory—has a relatively high rate of vector–human contact that might facilitate virus transmission or harborage in this rural area of Puerto Rico.

Comparisons of host utilization by mosquitoes may be influenced by collection method and habitat (indoor, outdoor). In this study, the BG traps baited with a BG-lure were used outdoors (Krockel et al. 2006). These are efficient devices for capturing host-seeking females of *Ae. aegypti* but can also capture blood-fed specimens (9–10% of all females). A previous study that used BG traps without a lure in urban areas of Australia showed similar results of host utilization by *Ae. aegypti*: 75.3% human, 13.2% dog, and 4% mixed human-dog bloodmeals (Jansen et al. 2009). Another study using BG traps in urban areas of Barcelona, Spain showed that *Ae. albopictus* fed only on humans (Muñoz et al. 2011). Most studies on host utilization by *Ae. aegypti* have used mechanical aspirators to capture mosquitoes in or around houses and on vegetation. For example, Scott et al. (2000) found that most *Ae. aegypti* specimens that were captured indoors had fed on humans (97%), while those captured around houses had lower percentages of human blood in urban Puerto Rico (79%), which was similar to the results obtained outdoors in the current study (Table 3). It is generally observed that *Ae. aegypti* mostly feeds on humans in or around houses in Thailand (Chow et al. 1993; Scott et al. 1993, 2000; Ponlawat and Harrington 2005; Siriyasatien et al. 2010). In Africa, specimens of *Ae. aegypti* from plantations and native vegetation not around houses had lower percentage of human blood (52–57%; MacClelland and Weitz 1963); however, specimens from in and around houses in the Hawaiian Islands had similar values (53–56%; Tempelis et al. 1970).

Previously, identification of arthropod bloodmeals used immunoassays that limited differentiation to order or family (Tempelis 1975, Washino and Tempelis 1983). We used highly conserved mitochondrial and ribosomal genes with low evolutionary rates, which are the most commonly used markers for this type of analysis and allows for identification of over 200,000 possible species from GenBank. However, this method of identifying bloodmeals does have limitations. Many samples (19.4%) did not have enough vertebrate DNA in the abdomen of the mosquito to properly identify the bloodmeal (Table 1.) These samples may have been highly digested by the mosquito, leaving little DNA for analysis. For this reason, several PCR methods were used to try to identify the bloodmeals. The initial method of identification involved group specific PCR primers to differentiate among two common hosts; this method is useful when encountering few potential blood hosts (Kent and Norris 2005, Kent 2009). Nested PCR has been shown to be useful in samples with small amounts of DNA (Hassan et al. 2003), and was therefore used in the final PCR. The use of DNA sequencing to identify the PCR amplicons is the most specific method currently available.

Although we did not determine the abundance and diversity of vertebrate hosts in the study areas, the more diverse bloodmeals found in *Ae. mediovittatus* show that this species feeds on a wider range of domestic vertebrates (dogs, cats, cows, horses, rats, pigs, goats, sheep, and chicken) than *Ae. aegypti* (dogs, cats, horses, and chickens). With the exception of an incidental observation made in Puerto Rico, which reported one specimen of *Ae.*

mediovittatus containing horse blood (Barrera et al. 2011), there are no previous studies documenting the sources of blood of *Ae. mediovittatus*. The feeding behavior of *Ae. mediovittatus* resembles that of *Ae. albopictus*, which is known for its diverse feeding patterns (Niebylski et al. 1994, Savage et al. 1993, Richards et al. 2006). The composition, abundance, and availability (e.g., screening, air conditioning, house construction) of vertebrate hosts influence the observed proportion of host bloodmeals taken by mosquitoes. For example, *Ae. albopictus* fed on a greater percentages of humans (76–96%) in urban areas of Rome than in rural areas (23–55%) where it fed more commonly and frequently on cattle and horses (Valerio et al. 2010). Because *Ae. mediovittatus* is more frequently collected from suburban and rural areas (Cox et al. 2007, Smith et al. 2007), it is likely that this species encounters a variety of domestic hosts. The use of alternate hosts to humans is important because it implies a lower vector–human contact rate. For example, a DENV-infected *Ae. mediovittatus* mosquito might feed subsequently on a domestic vertebrate that would not amplify DENV, whereas an infected *Ae. aegypti* mosquito was most likely to feed next on another human. Determining whether *Ae. mediovittatus* acts as a DENV vector or reservoir in Puerto Rico will require isolation of DENV from field-collected mosquitoes. Recent studies have produced spatial models that predict the co-occurrence of *Ae. mediovittatus* and *Ae. aegypti* (a proxy for human–virus presence) based on environmental and remote sensing data that will be used to orient the collection of specimens for virus isolation (Little et al. 2011).

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Table 2

Number of bloodmeals identified by each PCR assay

Mosquito species	No. bloodmeals identified			No. unidentified bloodmeals
	Species-specific PCR (dog/human)	16S rRNA PCR	Cytochrome b nested PCR	
<i>Aedes aegypti</i>	194	174	23	7
<i>Aedes mediovittatus</i>	0	173	45	140
Total	194	347	68	147

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Table 3

Percentages of mosquitoes that fed on various vertebrate hosts in two communities in rural Puerto Rico from July 2009 to May 2010

Mosquito species/locality/host	<i>Aedes aegypti</i>		<i>Aedes mediiovittatus</i>	
	Providencia	Recio	Providencia	Recio
Human	76.2	78.9	52.2	45.6
Dog	20.8	18.4	27.5	32.2
Cat	2.3	1.1	4.3	2.7
Cow	–	–	7.2	6.7
Horse	0.8	–	2.9	2.7
Rat	–	–	2.9	–
Pig	–	–	1.4	0.7
Goat	–	–	–	8.1
Sheep	–	–	1.4	–
Chicken	–	1.1	–	1.3
Dog/human	–	0.4	–	–