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PCR-Based Bloodmeal Analysis of *Aedes aegypti* and *Culex quinquefasciatus* (Diptera: Culicidae) in St. George Parish, Grenada

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Abstract

Blood-feeding patterns of mosquitoes affect the transmission and maintenance of arboviral diseases. In the Caribbean, *Aedes aegypti* (L.) and *Culex quinquefasciatus* Say mosquitoes are the dominant mosquito species in developed areas. However, no information is available on the bloodmeal hosts of these invasive vectors in Grenada, where arboviral pathogens such as dengue, chikungunya, and Zika viruses cause significant human suffering. To this end, *Ae. aegypti* and *Cx. quinquefasciatus* mosquitoes were investigated from five semirural locations near houses in St. George's Parish, from 2017 to 2018. Polymerase chain reaction was conducted on DNA extracted from individual blood-fed mosquitoes using vertebrate-specific cytochrome b primers. The 32 *Ae. aegypti* bloodmeals included humans (70%), mongooses (18%), domestic dogs (6%), a domestic cat (3%), and an unidentified bird (3%). Thirty-seven *Cx. quinquefasciatus* mosquitoes took bloodmeals from seven species of birds (51%), humans (27%), domestic cats (8%), iguanas (5%), a domestic dog (3%), a rat (3%), and a common opossum (3%). The high percentage of human bloodmeal hosts in our study, especially by the normally anthropophilic *Ae. aegypti*, is expected. The bloodmeal sources and the percentage of nonhuman bloodmeals (30%) taken by Ae. *aegypti* are comparable to other studies. The large range of hosts may be explained in part by the semirural nature of most local housing. Accordingly, this may contribute to an exchange of pathogens between domestic, peridomestic, and sylvatic transmission cycles.

Key words: *Aedes aegypti*, *Culex*, Grenada, bloodmeal analysis

From the perspective of medical and veterinary medicine, *Aedes aegypti* (L.) and *Culex quinquefasciatus* Say mosquitoes are two of the most important vector mosquitoes in the New World. Both were accidentally introduced to the Western hemisphere (Fonseca 2006, Powell and Tabachnick 2013), have become dominant species in urban environments throughout their range (Calderón-Arguedas et al. 2008, Medeiros-Sousa et al. 2017), and can ably adapt to rural and semirural environments (Chadee et al. 1998, Kweka et al. 2015).

Aedes aegypti is the primary vector of several important human pathogens that have become endemic to one or more Caribbean islands: all four serotypes of dengue virus, Zika virus, chikungunya virus, and yellow fever virus (Auguste et al. 2010, Gibson et al. 2016). They preferentially feed on humans throughout their introduced range (Powell and Tabachnick 2013), but bloodmeal analysis research demonstrates that they will feed on birds (e.g., chickens, mockingbirds) and other mammals (e.g., cats, dogs, horses, pigs, rabbits, raccoons, and rodents) in low abundance compared with humans (Ponlawat and Harrington 2005, Janssen et al. 2015, Garcia-Rejon et al. 2010, Sivan et al. 2015, Stenn et al. 2018). Furthermore, *Ae. aegypti* bloodmeal sources are more diverse in periurban/semirural environments than strictly urban settings (Sivan et al. 2015). While *Ae. aegypti* is typically considered a container-dwelling species in urban areas, Chadee et al. (1998) suggest that urban insecticide-based *Ae. aegypti*-targeted control programs and the semirural nature of many Caribbean islands may have contributed to selection for *Ae. aegypti* populations that are suited to both natural and man-made breeding sites. This impacts the transmission of pathogens whose sylvatic cycles are comparatively well understood (e.g., yellow fever or dengue in nonhuman primates) as well as pathogens that are considered emerging or re-emerging. For instance, over 40 Old World animal species, including mammals, reptiles, and birds, have tested positive for Zika via serology or virus isolation (Bueno et al. 2016, Vorou 2016), but to date, little is known about which vertebrate hosts play a role in Zika transmission cycles in the Americas.

Host preference studies in the New World have shown that *Cx. quinquefasciatus* mosquitoes are ornithophilic but will feed on a large range of hosts, including mammals and reptiles (Edman 1974, Molaei et al. 2007, Garcia-Rejon et al. 2010, Janssen et al. 2015, Stenn et al. 2018). While pathogens that are potentially transmitted by *Cx. quinquefasciatus* are either not endemic (e.g., St. Louis encephalitis virus [SLEV], Rift Valley fever virus, *Wuchereria bancrofti*) or are rarely reported (e.g., Oropouche virus, West Nile virus [WNV], and other equine encephalitis viruses) in most Caribbean islands, they infect people in the mainland Americas, and their introduction or re-emergence to Caribbean islands remain a concern (Chancey et al. 2015, Gibson et al. 2016). Furthermore, *Culex* mosquitoes are competent vectors of pathogens that affect peridomestic and wild animals as well. Over 100 native bird species have tested positive for WNV by polymerase chain reaction (PCR) or serology (Dusek et al. 2009, Center of Disease Control and Prevention 2019). Also, *Cx. quinquefasciatus* transmits *Plasmodium relictum* and *Dirofialria immitis*—the causative agents of avian malaria and canine/feline heartworm, respectively—both of which are prevalent in the Caribbean (Gibson et al. 2016, Soares et al. 2017).

Grenada represents an excellent location to examine the ecology and behavior of vector mosquitoes in a tropical island setting. *Aedes aegypti* and *Cx. quinquefasciatus* are very abundant in and around houses (Panagos et al. 2005). Human dengue cases occur annually in Grenada, and Zika and chikungunya epidemics have plagued the island nation recently (Panagos et al. 2005, Schiøler and Macpherson 2009, Macpherson et al. 2016, Brenciaglia et al. 2018). Much of Grenada consists of densely populated areas that are adjacent to forested regions with wild animals, and many locals own pets and livestock. Because wild and peridomestic animals may be reservoirs for human disease, this situation may facilitate the exchange of pathogens between domestic, peridomestic, and sylvatic transmission cycles. Mosquito host use and habitat choice vary considerably depending on geography, host availability, and seasonality, among many factors (Edman and Taylor 1968, Chadee et al. 1998, Kilpatrick et al. 2006, Thiemann et al. 2011, Saifur et al. 2012). Without knowing the feeding preferences of the populations of the mosquito species found in Grenada, we cannot be completely certain that these mosquitoes follow the patterns of the same species in other areas or what animals and humans may be at risk for pathogens they transmit. To this end, this study uses previously validated PCR techniques to identify the bloodmeals of *Ae. aegypti* and *Cx. quinquefasciatus* in Grenada.

Materials and Methods

Twice each week from October 2016 to January 2018, mosquitoes were captured at two to five houses in St. George Parish, Grenada (Figs. 1 and 2). For each site, one Biogents Sentinel (BG-S, Regensburg, Germany) trap baited with octenol and yeast-based carbon dioxide attractants (Aldridge et al. 2016) and three to five Biogents Gravid Aedes Traps (GAT) with 500-ml alfalfa infusion (Ritchie et al. 2014) were placed within 3 m of houses to attract mosquitoes. After 24 h, traps were collected, and mosquitoes were dispatched at −80°C. Subsequently, mosquitoes were identified to species by morphological analysis; because morphological keys that include recently introduced invasive taxa are not available for Grenada or Caribbean islands, Darsie and Ward (2005) and Lane (1953) were used to discriminate between species known to occur in Grenada according to the Walter Reed Biosystematics Unit (2019). Noticeably, engorged *Ae. aegypti* and *Cx. quinquefasciatus* females caught in BG-S traps and all *Ae. aegypti* caught in GAT traps were processed individually.

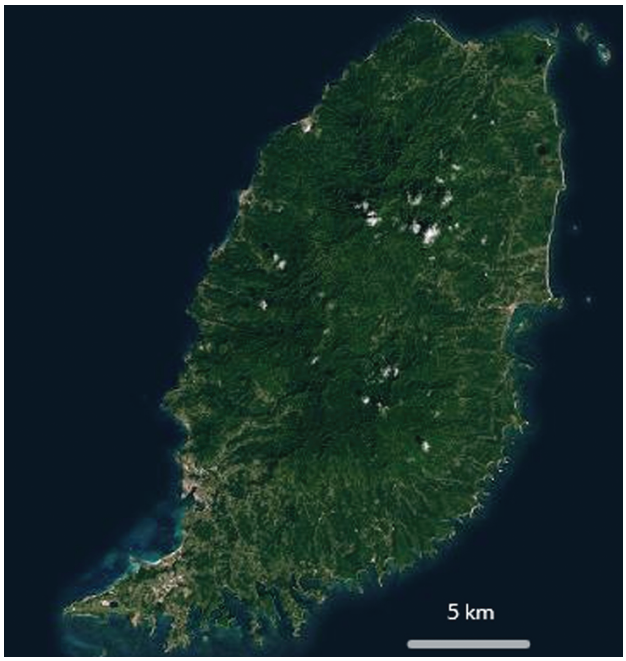

Fig. 1. Map of Grenada, West Indies. Map made with software on www.scribblemaps.com.

Of the 15 sites sampled in St. George parish, mosquitoes captured from nine sites (Fig. 2) were used in this study. Because several hundred bloodfed *Cx. quinquefasciatus* were captured, subsets of mosquitoes from the dry season (March 2017), wet season (October 2017), and transitional periods between these seasons (January 2017 and January 2018) were used in subsequent analysis. Heads were removed using a sterile scalpel blade to prevent PCR inhibition (Beckmann et al. 2014). DNA was extracted using the QIAGEN DNEasy Blood and Tissue kit (Hilden, Germany). A 20-µl PCR with Platinum PCR SuperMix High Fidelity master mix (Thermofisher, Waltham, MA), 1.5-mM $MgCl_2$, and ~50 ng extracted DNA was performed using 0.5 µM of primers from Boakye et al. (1999) that amplify an ~380 bp region of any vertebrate's cytochrome b (*cytb*) mitochondrial gene using the following thermocycler conditions: 50 cycles of denaturation at 94°C for 30 s, annealing at 50°C for 45 s, and primer extension at 72°C for 30 s, with a final extension at 72°C for 10 min. Amplicons of expected size were extracted from gels using the QIAquick Gel Extraction Kit (Hilden, Germany) following the manufacturer's protocol. Amplicons were sent to the Plant-Microbe Genomics Facility at The Ohio State University for direct Sanger sequencing. Raw sequence data were manually edited using Chromas 2.6.4 software and then compared with the sequence database using the NIH's Basic Local Alignment Search Tool (BLAST) to determine percent identity to known *cytb* gene sequences.

Results

Of the 70 putative bloodmeals from *Ae. aegypti* mosquitoes that we processed and analyzed by PCR, 32 total bloodmeals—7 of 43 mosquitoes (16%) from the GAT traps and 25 of 27 mosquitoes (93%) from the BG-S traps—produced unambiguous sequence data for which the best matches were vertebrate *cytb* sequences (Table 1). The other samples did not produce bands in PCR analysis, failed to produce single readable sequences in the Sanger sequencing process, or were identified as off-target amplification of the mosquito host. *Aedes aegypti* bloodmeals that were identified came from five species:

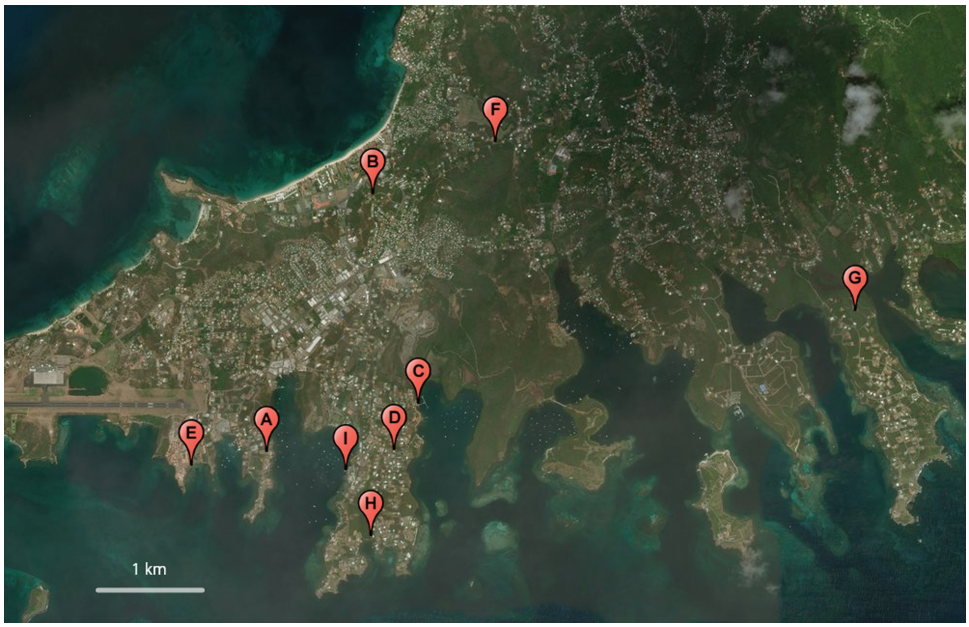

Fig. 2. Map of collection sites in St. George Parish in the southwestern region of Grenada. Map made with software on www.scribblemaps.com.

Table 1. Bloodmeals from *Ae. aegypti* in Grenada

Order of bloodmeal host	Closest match in GenBank		Accession number of closest match	Nucleotide identity		Capture site	Date
Mammalia	*Canis familiaris lupis*	Domestic dog	DQ309764.1	274/274	100%	A	20 Sep 2017
			JX849650.1	170/174	98%	B	25 Aug 2017
	Felis catus	Domestic cat	AB194813.1	285/285	100%	C	19 Jan 2017
	Herpestes auropunctatus	Small Indian mongoose	FJ848672.1	278/279	99%	B	5 Oct 2017
			FJ848672.1	276/277	99%	B	13 Oct 2017
			FJ848672.1	272/273	99%	B	13 Oct 2017
			FJ848672.1	276/277	99%	B	19 Oct 2017
			FJ848672.1	277/277	100%	B	20 Oct 2017
			FJ848672.1	280/281	99%	D	13 Oct 2017
	Homo sapiens	Human	MG561401.1	278/279	99%	A	24 Jan 2018
			MG831412.1	282/282	100%	B	24 Jan 2017
			MG970575.2	295/295	100%	B	25 Aug 2017
			MG970575.2	291/291	100%	B	1 Sep 2017
			MG182042.1	283/284	99%	B	15 Sep 2017
			MG182042.1	279/279	100%	B	15 Sep 2017
			MG571220.1	280/280	100%	B	28 Sep 2017
			MG649328.1	278/279	99%	B	28 Sep 2017
			MG831412.1	279/279	100%	B	31 Oct 2017
			MG970575.2	285/285	100%	C	18 Aug 2017
			MG182042.1	278/279	99%	C	22 Sep 2017
			MG182042.1	279/279	100%	C	14 Sep 2017
			MG831412.1	279/279	100%	C	29 Nov 2017
			MF696072.1	278/279	99%	E	14 Dec 2017
			KP900938.1	278/278	100%	E	27 Dec 2017
			MG831412.1	279/280	99%	E	12 Jan 2018
			KX440321.1	279/279	100%	E	24 Jan 2018
			MH194581.1	274/274	100%	F	2 Feb 2017
			MG182027.1	278/279	99%	G	14 Sep 2017
			MG182042.1	279/279	100%	G	22 Sep 2017
			MG182042.1	278/279	99%	G	22 Sep 2017
			MG182042.1	269/276	97%	G	31 Oct 2017
Aves	*Melopsittacus undulatus*[a]	Budgerigar parakeet	AY724765.1	254/278	91%	C	30 Nov 2017

[a]*Melopsittacus undulatus*, a parakeet, has not been reported in Grenada. No wild psittacid birds reported to live in Grenada (Lepage and Warnier 2014), but pet parrots are common, and migratory flocks of parrots are anecdotally reported.

Table 2. Bloodmeals from *Cx. quinquefasciatus* in Grenada

Order of bloodmeal host	Closest match in GenBank		Accession number of closest match	Nucleotide identity		Capture site	Date
Mammalia	*Canis lupis familiaris*	Domestic dog	DQ309764.1	200/200	100%	C	30 Mar 2017
	Didelphus marsupialis	Common opossum	KT437726.1	275/277	99%	C	27 Oct 2017
	Felis catus	Domestic cat	AB194813.1	275/275	100%	C	30 Mar 2017
			KX348260.1	254/258	98%	E	24 Jan 2017
			KX348260.1	272/275	99%	E	24 Jan 2017
	Homo sapiens	Human	KY824954.1	263/264	99%	C	2 Mar 2017
			MH029820.1	242/242	100%	E	23 Mar 2017
			MG970575.2	277/277	100%	C	20 Oct 2017
			MG272941.1	278/278	100%	D	18 Jan 2018
			MH029820.1	278/279	99%	B	25 Jan 2018
			MH029820.1	280/280	100%	B	25 Jan 2018
			MH029820.1	280/281	99%	B	25 Jan 2018
			MH029820.1	283/283	100%	B	25 Jan 2018
			MH029820.1	279/279	100%	B	25 Jan 2018
			MH029820.1	281/281	100%	B	25 Jan 2018
	Rattus rattus	Black rat	KT232247.1	281/282	99%	C	27 Oct 2017
Aves	*Coereba flaveola*	Bananaquit	EF567840.1	274/275	99%	C	15 Mar 2017
			EF567840.1	272/273	99%	C	16 Mar 2017
			EF567853.1	260/260	100%	C	16 Mar 2017
			EF567853.1	273/275	99%	C	16 Mar 2017
			EF567853.1	276/277	99%	A	27 Oct 2017
			EF567853.1	271/271	100%	B	11 Jan 2018
	Loxigilla noctis	Lesser Antillean bullfinch	AF310041.1	276/276	100%	A	27 Oct 2017
	Setophaga striata[a]	Blackpoll warbler	EU815688.1	264/282	94%	D	20 Oct 2017
	Tiaris bicolor	Black-faced grassquit	AF489899.1	278/279	99%	C	10 Mar 2017
			AF489899.1	278/281	99%	C	30 Mar 2017
			AF489899.1	282/282	100%	A	27 Oct 2017
			KJ945367.1	267/278	96%	A	19 Jan 2018
	Toxostoma curvirostre[b]	Curve-billed thrasher	AF287548.1	268/279	96%	A	20 Oct 2017
			AF287548.1	272/283	96%	A	27 Oct 2017
			AF287548.1	272/283	96%	A	27 Oct 2017
			AF287548.1	272/282	96%	D	27 Oct 2017
	Toxostoma rufum[b]	Brown thrasher	AF130237.2	265/279	95%	I	9 Mar 2017
	Zenaida auriculata	Eared dove	HM640211.1	102/102	100%	A	16 Oct 2017
			HM640211.1	280/280	100%	C	25 Jan 2018
Reptilia	*Iguana iguana*	Green iguana	KX610610.1	212/226	94%	D	17 Jan 2018
			KX610610.1	272/279	97%	E	18 Jan 2018

[a]The 94% identity matched to *Setophaga striata* suggests a close but incorrect identification. There are several *Setophaga* species and other warblers in Grenada without *cytb* sequence entries in GenBank that are probably the source.

[b]Five bloodmeals ostensibly came from two *Toxostoma* species (Family: Mimidae). However, only four mimid species occur in Grenada: *Allenia fusca* (scaly breasted thrasher), *Cinclocerthia ruficauda* (brown trembler), *Margarops fuscatus* (pearly eyed thrasher), and *Mimus gilvus* (tropical mockingbird) (Lepage and Warnier 2014). Of these, only *C. ruficauda* and *M. fuscatus* have *cytb* sequence entries in GenBank, and these entries have 94% identity or less to the five bloodmeal PCR sequences identified as *Toxostoma*, suggesting that other birds served as bloodmeal sources.

humans (70% of the identified bloodmeals), small Indian mongoose (*Herpestes auropunctatus*) (18%), domestic dogs (6%), a domestic cat (3%), and an unknown bird (3%). According to BLAST, the unknown bird bloodmeal sequence was 91% identical to several parrot species (Family *Psittaculidae*) not native to Grenada.

Overall, of the 44 visibly engorged *Cx. quinquefasciatus* mosquitoes that were caught with BG-S traps and analyzed by PCR, 37 (84%) of the bloodmeals were identified (Table 2). The other seven samples either failed to amplify with the *cytb* primers or produced off-target amplification of the *Cx. quinquefasciatus cytb* gene. Thirteen host species were identified overall. Sixteen bloodmeals came from mammals: humans (27%), domestic cats (8%), a domestic dog (3%), a rat (*Rattus rattus*) (3%), and a common opossum (*Didelphus marsupialis*) (3%). Two reptilian bloodmeals were from green iguanas (*Iguana iguana*) (5%). Nineteen *Cx. quinquefasciatus* bloodmeals came from avian hosts, with best matches in GenBank were as follows: bananaquits (*Coereba flaveola*; 16%), curve-billed thrashers (*Toxostoma curvirostre*; 11%), black-faced grassquits (*Tiaris bicolor*; 11%), eared doves (*Zenaida auriculata*; 5%), a brown thrasher (*Toxostoma rufum*; 3%), a Lesser Antillean bullfinch (*Loxigilla noctis*; 3%), and a warbler (*Setophaga striata*; 3%).

Discussion

Generally, our results show that the host preference patterns of *Ae. aegypti* and *Cx. quinquefasciatus* in Grenada are similar to other areas of the world. Humans were the most common bloodmeal source for both mosquito species, but both were willing to feed on other synanthropic and wild animals, which may allow for zoonotic pathogen spillover opportunities. Interestingly, we noted a

considerable difference between the percentage of bloodmeals that could be identified from *Ae. aegypti* mosquitoes caught with BG-S traps (93%) and those caught in GAT traps (19%). We attribute this to the nature of the mosquitoes caught; only noticeably engorged *Ae. aegypti* from the BG-S traps were assayed, whereas all *Ae. aegypti* mosquitoes caught in the GAT traps, regardless of state of engorgement, were analyzed by PCR.

All but one of best matches for the host species identified from *Ae. aegypti* bloodmeals are found in Grenada, supporting the molecular identifications (Table 1). *Aedes aegypti* are considered highly anthropophagic, often living in close proximity to humans. Consistent with this, nonhuman bloodmeals comprised a minority of positive bloodmeal identifications in this study (30%). This is considerably higher than in some studies (Ponlawat and Harrington 2005 [1%], Stenn et al. 2018 [13%]), comparable to several other studies (Janssen et al. 2009 [25%], Sivan et al. 2015 [22% in areas with medium vegetation], Khaklang and Kittayapong 2014 [30%]), and lower than one study (Tempelis et al. 1970 [46%]). We identified *Ae. aegypti* (as well as *Cx. quinquefasciatus*) bloodmeals derived from domestic cats and dogs, which implicate them these mosquitoes as *Dirofilaria immitis* vectors (Brito et al. 1999, Tiawsirisup and Nithiuthai 2006). Interestingly, the percentage of *Ae. aegypti* bloodmeals from mongooses (*Herpestes auropunctatus*) was much higher than expected. Introduced more than 100-yr ago, mongooses are now highly abundant on the island due to high reproductive rates and lack of predators, so the results are not surprising (Nellis and Everard 1983). However, all mongoose bloodmeals were taken in same month (October 2017), and five of six were collected from a single site (Table 1); a clear explanation of this phenomenon is not possible at this time. Little is known about the status of mongooses as a reservoir of dengue, chikungunya, or Zika viruses. Granted that our study had a small sample size, the high percentage of mongoose-derived bloodmeals warrants further investigation into their competence as arboviral reservoir hosts.

The best host matches for 32 of 37 *Cx. quinquefasciatus* bloodmeals have been reported in Grenada (Table 2). *Culex quinquefasciatus* have a wide host preference range in Grenada, which is consistent with other studies (Garcia-Rejon et al. 2010, Janssen et al. 2015, Stenn et al. 2018). Normally considered an ornithophagic mosquito, *Cx. quinquefasciatus* took more bloodmeals from humans than any other species in this study. Janssen et al. (2015) also observed high rates of human-derived bloodmeals when trapping *Cx. quinquefasciatus* in and around houses in two Mexican cities. This study's results, like theirs, imply that the proximity to houses likely contributed to abnormally high rates of human-derived bloodmeals. Regardless, the diversity of bloodmeals—51% from wild avian hosts, 43% from mammalian hosts, and 5% from reptilian hosts—suggests that, like in much of the world, *Cx. quinquefasciatus* can act as an important potential bridge vector between domestic, peridomestic, and sylvatic arboviral transmission cycles when pathogens are present. Viremic cases of WNV are very rarely reported in humans or animals in Caribbean island nations (Gibson et al. 2016). However, several avian bloodmeal sources in this study have previously tested positive for WNV exposure by viral isolation or by serology (Dupuis et al. 2005, Bosch et al. 2007, Dusek et al. 2009, Center of Disease Control and Prevention 2019).

Aedes aegypti is the sole *Aedes* species mosquito in Grenada and is traditionally considered the primary local vector of dengue, chikungunya, and Zika viruses. Our finding that *Ae. aegypti* took a majority (70%) of total blood meals from humans supports incrimination of this species in transmission of these arboviruses in Grenada. Our finding that *Cx. quinquefasciatus* in Grenada feeds upon both avian and mammalian hosts in roughly equivalent percentages has important implications for transmission of pathogens that utilize birds as reservoir hosts (e.g., equine encephalitis viruses). While the competence of *Cx. quinquefasciatus* as a vector of Zika virus is still being debated (Guo et al. 2016, Epelboin et al. 2017, van den Hurk et al. 2017, Smartt et al. 2018), no other human pathogens definitively transmitted by *Culex* spp. mosquitoes have been reported in Grenada. However, our data imply that Grenada has efficacious bridge vectors in abundance if those pathogens are introduced. These data are part of a larger study on arboviral disease ecology and surveillance in Grenada. PCR-based detection of arboviral pathogens in human-associated and wild animals as well as in the mosquito species investigated in this study is underway. By elucidating the host preferences of these and other species of mosquitoes, our study contributes to a growing body of work on the transmission cycles of the arboviruses of human and veterinary concern in the Caribbean.

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