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Research

Vertebrate Hosts of *Aedes aegypti*, *Aedes albopictus*, and *Culex quinquefasciatus* (Diptera: Culicidae) as Potential Vectors of Zika Virus in Florida

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Abstract

Zika virus (ZIKV), once considered an obscure pathogen, spread rapidly from 2014 to 2016 to become an internationally notifiable condition of major public health concern. The relative importance of various *Culex* and *Aedes* species mosquitoes (Diptera: Culicidae) in ZIKV transmission is a topic of debate. Quantifying host use is important in determining the vectorial capacity of a mosquito species for transmitting ZIKV in nature. In the United States, few data are available on host use of *Aedes aegypti* L. (Diptera: Culicidae) and *Aedes albopictus* (Skuse) (Diptera: Culicidae), confirmed and suspected vectors of ZIKV, respectively. Here, we report results of bloodmeal analysis to quantify host use of confirmed (*Ae. aegypti*) and suspected (*Ae. albopictus* and *Culex quinquefasciatus* Say (Diptera: Culicidae)) vectors of ZIKV in two Florida counties. At an auto salvage yard in Indian River County, *Ae. aegypti, Ae. albopictus* and *Cx. quinquefasciatus* fed mainly on humans, taking 90.2, 90.8, and 78.6% of bloodmeals from humans, respectively. At a residential area in Martin County, *Ae. aegypti*, *Ae. albopictus* took 61.5 and 66.7% of bloodmeals from humans, higher than *Cx. quinquefasciatus* (11.1%). Patterns of host use suggest that *Ae. aegypti* and *Ae. albopictus* are the most likely vectors of ZIKV in Florida and that *Cx. quinquefasciatus* would likely play a lesser role in ZIKV transmission in Florida. However, the relative importance of the three species in ZIKV transmission is likely location and population specific. Detailed studies quantifying other parameters of vectorial capacity, including vector competence, are needed in order to determine the actual role for each species in ZIKV transmission.

Key words: mosquito, Zika virus, bloodmeal analysis, vertebrate host

Zika virus (ZIKV, *Flaviviridae*, *Flavivirus*) is a mosquito-borne human pathogen that is associated with the neurological disorder Guillain-Barré syndrome (Oehler et al. 2014) and congenitally infected fetuses, resulting in a variety of permanent congenital neurological and physical sequelae (Rasmussen et al. 2016). Originally considered a relatively obscure virus infecting non-human primates in Africa and later documented in Southeast Asia in the 1960s (Marchette et al. 1969), ZIKV suddenly spread to the South Pacific Islands (Duffy et al. 2009) and the Americas (Duong et al. 2017) over a relatively short period (2007–2016). Autochthonous transmission of ZIKV in Brazil in 2015 (Campos et al. 2015, Zanluca et al. 2015) was intensely investigated in response to perceived links between ZIKV infection and microcephaly in congenitally infected fetuses (Schuler-Faccini et al. 2016). ZIKV spread subsequently throughout the American tropics in 2015 (Ferreira-de-Brito et al. 2016) with Florida documenting the first locally-acquired mosquito-borne cases of ZIKV transmission in the United States in 2016 (Likos et al. 2016). The Florida ZIKV outbreak occurred in urban areas of Miami-Dade County, with 326 human cases documented statewide as of 22 July 2016 and total of 1,467 human cases reported by the end of 2016 (Florida Department of Health 2017). ZIKV is likely to continue to pose a threat to Florida due to large numbers of traveler-imported cases (Florida Department of Health 2017), an abundance of susceptible human hosts, and the presence of confirmed and suspected ZIKV vectors (Likos et al. 2016).

The recent, nearly global emergence of ZIKV raises important questions about which vector species transmit the virus in the newly emerging areas. The anthropophilic mosquito *Aedes aegypti* L. (Diptera: Culicidae) is considered an important vector of ZIKV in emerging regions as field-collected pools of *Ae. aegypti* females

have tested positive for ZIKV in Malaysia (Marchette et al. 1969), Mexico (Guerbois et al. 2016) and Brazil (Ferreira de-Brito et al. 2016, Ayllón et al. 2017). In fact, Weaver et al. (2016) hypothesized that the sudden emergence of the ZIKV is due, in part, to the viral adaptation of more efficient transmission by *Ae. aegypti* mosquitoes. Despite substantial evidence incriminating *Ae. aegypti* in ZIKV transmission, there is still an ongoing discussion with regards to whether other mosquito species transmit ZIKV in nature (Guedes et al. 2017, Ayres et al. 2017, Lourenço and Failloux 2017, Roundy et al. 2017). Two mosquito species that have received substantial attention as putative ZIKV vectors are *Aedes albopictus* (Skuse) (Diptera: Culicidae) and *Culex quinquefasciatus* Say (Diptera: Culicidae). In Brazil *Ae. albopictus* has been suggested as a likely ZIKV vector (Ferreira -de Brito et al. 2016) and ZIKV RNA fragments were detected in *Ae. albopictus* eggs from field sites in Bahia (Smartt et al. 2017). In Gabon, studies implicated *Ae. albopictus* as playing a central role in a ZIKV epidemic, due to the invasion of *Ae. albopictus* in urban areas and the findings of the two *Ae. albopictus* pools testing positive for ZIKV (Grard et al. 2014). The proposition that *Cx. quinquefasciatus* plays a role in ZIKV has been a hotly contested issue (Ayres et al. 2017, Guedes et al. 2017, Lourenço and Failloux 2017, Roundy et al. 2017). While some laboratory infection studies have found *Cx. quinquefasciatus* is capable of transmitting ZIKV by bite after receiving an infectious bloodmeal (Guo et al. 2016, Smartt et al. 2018), other studies have found that *Cx. quinquefasciatus* are refractory to both the African prototype ZIKV strain and currently circulating Asian lineage ZIKV (Kenney et al. 2017, Main et al. 2018). Laboratory competence is just one variable contributing to the overall vectorial capacity of a mosquito species (or population).

While virus detections from field samples and laboratory competence for various virus strains are important criteria in incriminating a putative vector species, natural blood-feeding patterns of the candidate vector species is also critical to understanding the role that a putative vector species may play in pathogen transmission. A highly competent vector that does not feed upon competent host species will not contribute to transmission in natural settings (Hardy et al. 1983). For viruses, such as ZIKV, in which humans are the competent hosts, mosquito species that take a relatively low percentage of bloodmeal from humans are unlikely to serve as principal vector of the virus. By the same rationale, a putative vector that is abundant and takes a large portion of bloodmeals from humans can sustain transmission of the virus, as has been demonstrated for yellow fever virus (Kramer and Ciota 2015). Bloodmeal analysis of field-collected mosquitoes can provide information of patterns of host use by suspected vectors in nature and permits quantification of biting rate, a key variable in the vectorial capacity equation. Despite the perceived importance of these vector mosquitoes in the transmission of a wide diversity of pathogens, only a handful of studies have quantified host use of *Ae. aegypti, Ae. albopictus* and *Cx. quinquefasciatus* in Florida. Tempelis (1975) found that *Ae. aegypti* took 83% of bloodmeals from humans in Florida, far greater than the 53% found in Hawaii by Tempelis et al. (1970). Niebylski et al. (1994) examined host use by *Ae. albopictus* from five U.S. states, including Florida. From 42 bloodmeals analyzed from rural Polk County, Florida, six (14.3 %) were from humans (Niebylski et al. 1994). Edman (1974) found that 9.1–11.9% of *Cx. quinquefasciatus* in Florida were from humans, depending upon site. While these studies provide important baseline data for determining broad patterns of host use for these mosquitoes, the sampling for these collective works spanned unspecified sampling periods, disparate habitats and host communities. These important differences in sampling between these studies (Edman 1974, Tempelis 1975, Niebylski et al. 1994) complicate our ability to draw meaningful conclusions regarding host use of these mosquitoes in relation to transmission of ZIKV.

In the current study, we quantified host use of *Ae. aegypti*, *Ae. albopictus*, and *Cx. quinquefasciatus* from two eastern Florida counties. Blood-engorged females were captured using traps or active aspiration from resting sites then subjected to polymerase chain reaction (PCR)-based assays to identify host species through sequencing and comparison with nucleotide sequences from publicly available database. The goal of this study was to assess the potential roles of these mosquito species in transmission of ZIKV (and other pathogens) in Florida.

Methods

Study Sites

Fieldwork was performed in Martin County (2016) and Indian River County (2017), Florida. In Martin County, a total of 14 households were selected in seven low-density residential neighborhoods. In Indian River County the mosquito collection site was a self-service auto recycling center that was surrounded by low-density residential properties. Both areas had considerable human and non-human animal activity, including domestic, feral, and wild vertebrate animals. No attempt was made to quantify vertebrate animals in either county. Oral consent was obtained from property owners prior accessing property for mosquito collection.

Mosquito Sampling

In Martin County, mosquitoes were sampled weekly from July 1 through 16 November 2016 using modified Biogents (Regensburg, Germany) BG-Sentinel mosquito traps utilizing BG–Lures. One trap was operated at each household (n = 14). Lures were replaced once each month. Each trap was powered by a 12-V, 18-Ah rechargeable sealed lead-acid battery controlled through a programmable digital 12-V DC timer (model CN101A; OKtimer, China; Burkett-Cadena et al. 2016). The intake funnel net of the trap was adapted (Fig. 1) to accept a 50 ml Falcon tube containing 25 ml of preservative media (polypropylene glycol) instead of a mosquito trap catchbag provided. This novel modification allowed trapped mosquitoes to be preserved in the field for 1 wk, preserving the morphological and genetic integrity of specimens for downstream molecular work. Traps were left at the houses and programmed to turn on to collect mosquitoes each day for 2 h in the morning (6:00 a.m. to 8:00 a.m.) and 2 h in the afternoon (6:00 p.m. to 8:00 p.m.). The mosquito samples were retrieved weekly, returned to the laboratory, washed in 95% ethanol, and identified to species using morphological structures of the adult (Darsie and Morris 2003) when possible.

In Indian River County, aspirations were made between 9:30 a.m. and 11:30 a.m., two to four times monthly from September 2016 to June 2017 using a modified Dustbuster (Black and Decker) handheld vacuum. A clear 1.0-m acrylic tube (8.2 cm diameter) served to lengthen the reach of the aspirator to aid in sampling from otherwise inaccessible resting sites. The intake of the aspirator was fitted with a PVC pipe coupler which accepted stainless steel mesh-bottom collection canisters (BioQuip Products, Rancho Dominguez, CA) as described by Bingham et al. (2014). Mosquitoes were sampled from the inside and outside of manmade items (salvage cars, tires) and vegetation (bushes and dense grasses). All blood-engorged females (both counties) were stored individually in 70% ethanol (500 ml) in 1.5 ml microcentrifuge tubes at –20°C until molecular analysis.

Molecular Procedures

Genomic DNA from blood-engorged females was extracted using Qiagen DNEasy Blood and Tissue Kit (Qiagen, Valencia, CA) following the manufacturer's protocol. Host DNA was amplified using PCR with three specific primer pairs targeting *16S rRNA* and *cytochrome b* genes (Blosser et al. 2016) for identification of mammalian, amphibian, reptilian and avian bloodmeal sources (Table 1). The samples were at first screened using H2714 and L2513 mammalian/amphibian primer pair targeting *16S rRNA*. Samples that did not amplify using these primers were screened on 16L1 and H3056_R (reptile) primer pair targeting *16S rRNA* followed by L0 and H1 (avian) primer pair targeting *cytochrome b* gene. PCR reaction mixture and cycling conditions were identical to those of Blosser et al. (2016).

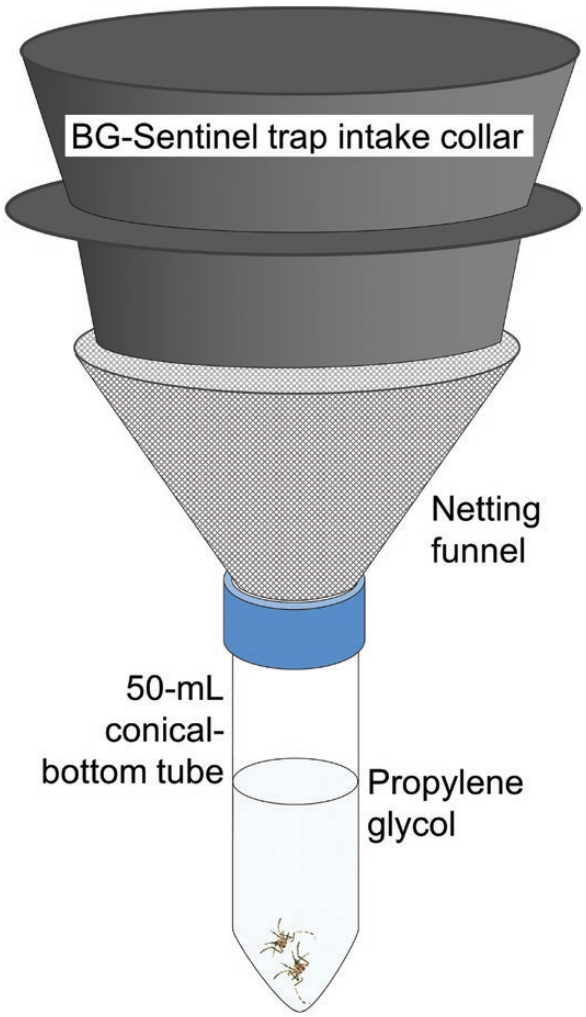

Fig. 1. BG-Sentinel trap intake collar modification for collecting females directly into preservative.

For damaged blood-engorged *Culex* females that could not be identified morphologically, genomic DNA was amplified by PCR using primer pairs targeting mosquito DNA (Table 1). Samples were initially screened using the primers pair LCO1490 and HCO2198 targeting cytochrome c oxidase 1 (Folmer et al. 1994). Samples that failed amplification were then screened using primers pair ACEquin and B1246 to amplify a 274 bp diagnostic fragment of *Cx. quinquefasciatus* as described in Smith and Fonseca (2004). PCR reaction mixes were prepared using the manufacturers protocol (Platinum Green Hot Start PCR 2X MM, Invitrogen 13001013). Cycling conditions for LCO1490 and HCO2198 were 94°C for 2 min, followed by 35 cycles of 94°C for 30 s, 55°C for 30 s, and 72°C for 1 min. Cycling conditions for ACEquin and B1246 were identical to Jones et al. (2012). Positive and negative controls were used in all PCR runs.

All PCR products were loaded onto a 1% agarose gel stained with SYBR Safe at 100V for 35 min and bands visualized with blue light transilluminator. Amplicons were sent to Eurofins Genomic Laboratory (Louisville, KY) to be sequenced in forward directions. Sequences were entered in GenBank (National Center for Biotechnology Information) database for identification and only samples with ≥95% identity matches were accepted for bloodmeal host identification using BLASTn.

To ensure that our methodology produced uncontaminated mosquito and host DNA, a laboratory experiment was performed to simulate the trapping and storage conditions, followed by molecular analysis. In brief, female mosquitoes known to have fed upon chicken blood were placed in polypropylene glycol with two unfed females of the same species, in six replicates. The samples were held outdoors for 1 wk then individual females washed with 95% ethanol, replicating handling methods. Each individual female was then subjected to DNA extraction, bloodmeal PCR, and gel electrophoresis following protocols outline above.

Data Analyses

Chi-square test of independence was used to test for differences between counties in the distribution (Kent et al. 2009) of human and non-human bloodmeals for *Ae. aegypti*, *Ae. albopictus*, and *Cx. quinquefasciatus*. Within each county, chi-square test of independence was used to test for differences between mosquito species in

Table 1. Primers used in host bloodmeal and mosquito species identification

Primer pair	Sequence	Amplicon size (bp)	Target taxa	Citation
H2714 L2513	5′-CTCCATAGGGTCTTCTCGTCTT-3′ 5′-GCCTGTTTACCAAAAACATCAC-3′	300	Mammals, amphibians	Kitano et al. 2007
16L1 H3056_R	5′-CTGACCGTGCAAAGGTAGCGTAATCACT-3′ 5′-CTCCGGTCTGAACTCAGATCACGTAGG-3′	450	Reptiles	Hass et al. 1993
L0 H1	5′-GGACAAATATCATTCTGAGG-3′ 5′-GGGTGGAATGGGATTTTGTC-3	220	Birds	Lee et al. 2008
ACEquin B1246	5′-CCTTCTTGAAT GGCTGTGGCA-3′ 5′-TGGAGCCTCCTC TTCACGG-3′	274	*Culex quinquefasciatus*	Smith and Fonseca 2004
LCO1490 HCO 2198	5′-GGTCAACAAATCATAAAGATATTGG-3′ 5′-TAAACTTCAGGGTGACCAAAAAATCA-3′	710	Mosquitoes	Folmer et al. 1994

the distribution of human and non-human bloodmeals. Alpha for all comparison was set at 0.05.

Results

Ae. aegypti was the most frequently captured species in the both counties, using the two different collection methods (Fig. 2). Blood-engorged females of *Ae. aegypti* constituted 2.1 and 10.7% of total females in Martin County (BG-Sentinel trap) and Indian River County (aspiration), respectively (Fig. 2). *Aedes albopictus* was the second and third most commonly-collected mosquito species in Martin County (BG-Sentinel trap) and Indian River County (aspiration), respectively (Fig. 2). As with *Ae. aegypti*, blood-engorged females of *Ae. albopictus* constituted a larger percentage of total adults and total females in Indian River County (16.5%; aspiration) than in Martin County (2.8%; BG-Sentinel trap). Many of the *Culex* spp. females captured in BG-Sentinel traps using polypropylene glycol as preservative were not identifiable to species based upon morphological characters due to missing scales of the pleuron and were therefore not differentiated. *Culex quinquefasciatus* was the second most commonly-collected mosquito species in Indian River County (aspiration) (Fig. 2). Blood-engorged females constituted 17.1% of total *Cx. quinquefasciatus* females in Indian River County (aspiration). In Indian River County, 34 *Culex nigripalpus* females Theobald ($n = 13$ blood-fed) were collected by aspiration. Very small numbers ($n < 5$ total) of other mosquitoes were sampled, including species of *Aedes*, *Wyeomyia*, *Uranotaenia, Psorophora, Mansonia* and *Anopheles*.

In this study, 284 blood-engorged mosquitoes of three mosquito species (*Ae. aegypti*, *Ae. albopictus* and *Cx. quinquefasciatus*) were collected in two counties, identified to species, and processed for bloodmeal analysis (Table 2). Host identity, sequences with ≥95% identity match to vertebrate hosts in GenBank, was successfully determined for 93.0% of the 284 bloodmeal samples. A slightly higher percentage of bloodmeals were successfully identified from aspirated mosquitoes (93.9% of 245), compared to those from BG-Sentinel traps (88.2% of 39).

Ae. aegypti fed mainly on mammals (99.3% overall) in both Martin (100.0%) and Indian River (99.2%) counties (Fig. 3). Humans comprised 61.5 and 90.2% of bloodmeals in Martin County and Indian River County, respectively. This distribution of human and non-human bloodmeals between the two counties was significantly different based upon chi-square test of independence ($\chi^2 = 8.85$, df = 1, $P = 0.002$). Non-human mammals (12.5% overall) fed upon by *Ae. aegypti* included domestic cat (*Felis domesticus*), domestic dog (*Canis familiaris*), cottontail rabbit (*Sylvilagus palustris*), raccoon (*Procyon lotor*), and native (*Neotoma floridana*) and invasive rodents (*Rattus* spp.). One avian bloodmeal (northern mockingbird, *Mimus polyglottos*) was detected from *Ae. aegypti* in Indian River County. No reptile or amphibian bloodmeals were detected.

Ae. albopictus also fed mainly on mammals in the two counties (Fig. 3) and took most bloodmeals from humans. Human bloodmeals constituted 66.7 (2/3) and 90.8% (59/65) of total *Ae. albopictus* bloodmeals in Martin County and Indian River County, respectively. Non-human mammal bloodmeals were derived from eastern cottontail ($n = 2$), nine-banded armadillo ($n = 1$), raccoon ($n = 1$), and brown rat ($n = 1$). This distribution of human and non-human bloodmeals between the two counties was not significantly different based upon chi-square test of independence ($\chi^2 = 1.80$, df = 1, $P = 0.179$). One reptile bloodmeal was encountered (brown anole lizard). Avian and amphibian bloodmeals were not encountered in any of *Ae. albopictus* samples tested.

Overall, *Cx. quinquefasciatus* took a smaller percentage of total bloodmeals from humans (59.3%) than did *Ae. aegypti* (87.5%) and *Ae. albopictus* (89.7%). The distribution of human and non-human bloodmeals varied significantly ($\chi^2 = 23.59$, df = 1, $P < 0.001$) between Martin County (10.5%) and Indian River County (78.6%). In Martin County, *Cx. quinquefasciatus* fed heavily upon non-mammalian hosts, deriving 50% (9/18) of total bloodmeals from birds and 11.1% (2/18) of total bloodmeals from reptiles. Avian bloodmeals ($n = 2$ or fewer per host species) were observed from domestic

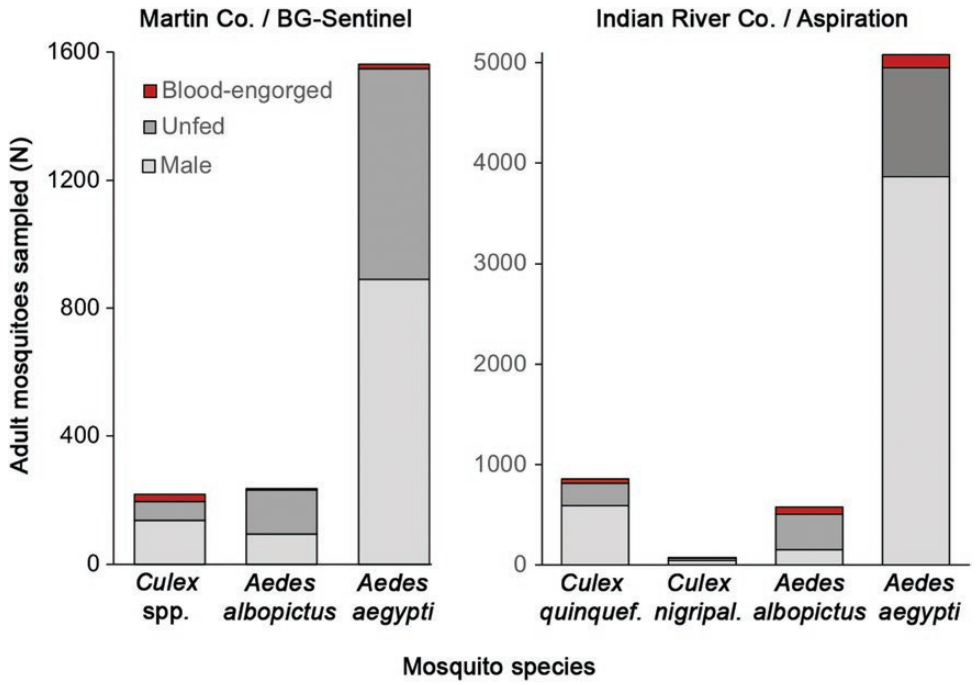

Fig. 2. Total abundance of adult mosquitoes collected from Martin County (BG-Sentinel trap) and Indian River County (aspiration of resting sites). Only species for which >10 individuals were sampled are shown.

Table 2. Bloodmeals from vertebrate hosts of *Aedes aegypti*, *Aedes albopictus*, and *Culex quinquefasciatus* from two Florida counties

County	Species	Total avian	Total mammal	Human	Non-human mammal	Total reptile	Grand total
Indian River	*Aedes aegypti*	1	122	111	11	0	123
	Aedes albopictus	0	65	59	6	0	65
	Culex quinquefasciatus	4	38	33	5	0	42
Martin	*Aedes aegypti*	0	13	8	5	0	13
	Aedes albopictus	0	2	2	0	1	3
	Culex quinquefasciatus	9	7	2	5	2	19

Mosquitoes were collected using a power aspirator at a you-pull-it salvage yard (Indian River County) and BG-Sentinel trap in residential area (Martin County), 2015–2017.

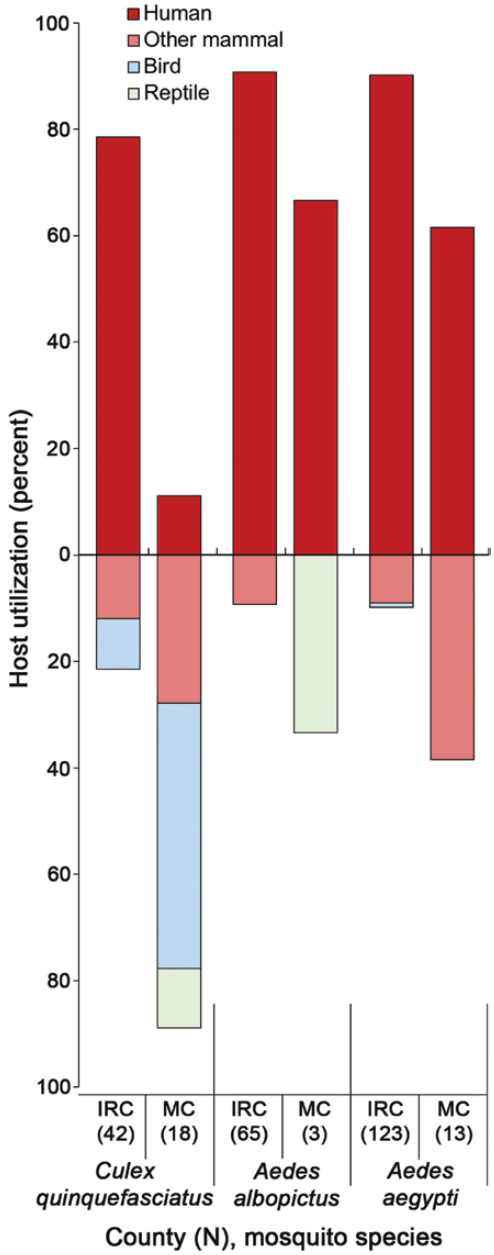

Fig. 3. Results of bloodmeal analysis for suspected and confirmed vectors of Zika virus from residential sites in Martin County (BG-Sentinel trap) and an automobile recycling facility in Indian River County (aspiration of resting sites), Florida, United States, 2016–2017. Bars above the vertical axis represent the percentage of bloodmeals from humans.

chicken (*Gallus gallus*), house sparrow (*Passer domesticus*), mourning dove (*Zenaida macroura*), northern cardinal (*Cardinalis cardinalius*), and northern mockingbird (*Mimus polyglottos*). Non-human mammals constituted 27.8% (5/18) of bloodmeals from Martin County and 11.9% (5/42) from Indian River County. In Martin County, these included northern raccoon (n = 1) and Virginia opossum (n = 4). In Martin County these included domestic dog (n = 1), domestic cat (n = 1), eastern cottontail (n = 1), and northern raccoon (n = 2). Two bloodmeals from exotic lizards (11.1% of county total) were detected in samples from Martin County, namely brown anole (*Anolis sagrei*) and green iguana (*Iguana iguana*).

Significant differences between mosquito species in the proportion of bloodmeals from humans and non-human hosts were observed in Martin County, but not in Indian River County. *Cx. quinquefasciatus* took a significantly smaller proportion of bloodmeals from humans than did *Ae. aegypti* (χ^2 = 8.78, df = 1, P = .002) or *Ae. albopictus* (χ^2 = 5.15, df = 1, P = 0.023) in Martin County. No significant differences were observed between *Ae. aegypti* or *Ae. albopictus* in the proportion of bloodmeals from humans and non-human hosts.

The results of the experiment testing whether our field and molecular methodology produced uncontaminated mosquito and host DNA indicated that our methods were robust to mosquito and host DNA degradation and cross contamination. None of the female mosquitoes (n = 12) held with blood-engorged females in polypropylene glycol in outdoor conditions for 1 wk then processed by PCR for host bloodmeal PCR and gel electrophoresis were found to be positive for host DNA, while all blood-engorged females were positive for host DNA.

Discussion

The high abundance and human host use of *Ae. aegypti* in both Martin and Indian River Counties supports the assertion that this mosquito species could serve as important Zika vector in southern Florida. *Aedes albopictus* was less common, but also demonstrated high anthropophily at both locations, suggesting that this mosquito would likely play a secondary role in ZIKV transmission in southern Florida. *Culex quinquefasciatus* showed the greatest opportunism with respect to host use, feeding upon a greater diversity of hosts than *Ae. aegypti* and *Ae. albopictus*, suggesting that this mosquito would be of lesser importance as a ZIKV vector.

Our finding that *Ae. aegypti* fed mainly on humans in Florida (87.5% combined) were expected and generally agrees with the findings of other studies from abroad. In general, most studies of *Ae. aegypti* host use have reported relatively high (>75%) feeding upon humans, particularly when sampling is performed in suburban and urban areas. In Thailand and India, e.g., multiple studies

demonstrate that *Ae. aegypti* feed mainly on humans (>80%) in domestic and peridomestic areas (Chow et al. 1993; Scott et al. 1993, 2000; Ponlawat and Harington 2005; Siriyasasatien et al. 2010; Sivan et al. 2015; Wilson and Sevarkodiyone 2015). Studies in Australia using BG-Sentinel trap without lure reported *Ae. aegypti* feeding 75.3% on humans (Jansen 2009). In Puerto Rico *Ae. aegypti* collected using BG-Sentinel trap with BG-lure in two communities took 76–79% of bloodmeals from humans (Barrera et al. 2012). A notable departure from the anthropophilic pattern was observed in rural Africa (Kenya and Uganda) where *Ae. aegypti* took less than 25% of total bloodmeals were from humans (McClelland and Weitz 1963). Reptiles and rodents were found to be the dominant hosts in sparsely populated areas. Very few data are available on the host use of *Ae. aegypti* in the United States. Tempelis (1975) reported that *Ae. aegypti* took 83% of bloodmeals from humans in Florida and 53% from humans in Hawaii (Tempelis et al. 1970). Tempelis (1975) did not report the collection location, habitat, sampling method, and number of samples analyzed in Florida. Studies of *Ae. aegypti* host use varied with respect to samples size, collection technique, characteristic of the locations and method of bloodmeal analysis. Even though these variations likely introduce some bias, most studies found that *Ae. aegypti* is primarily anthropophilic, feeding more than 50% on human blood.

The large overall percentage (89.7%) of bloodmeals from humans by *Ae. albopictus* conflicts with the conclusions of a previous study from Florida (Niebylski et al. 1994), which found that just 14.3% of total *Ae. albopictus* bloodmeals were obtained from humans, and a study from Missouri, United States reporting that 8.2% of the bloodmeals derived from humans (Savage et al. 1993). The reason for these discrepancies may be due to differences in sampling environment. Niebylski et al. (1994) sampled from a rural, likely agricultural site, resulting in a large percentage (30.1% overall) of bloodmeals from cow, while Savage et al. (1993) sampled from a tire yard. While most studies comparing host use of *Ae. albopictus* and *Ae. aegypti* have reported a somewhat lower percentage of human-derived bloodmeals in *Ae. albopictus* (Sivan et al. 2015, Wilson and Sevarkodiyone 2015), both species typically feed upon humans at very high rates (Ponlawat and Harrington 2005, Sivan et al. 2015) in urban settings (>90%). Faraji et al. (2014) demonstrated a trend of increasing anthropophily by *Ae. albopictus* across a rural to urban gradient in the northeastern USA. Other studies examining host use of *Ae. albopictus* (without *Ae. aegypti*) have generally found high rates of feeding upon human hosts, including 61.1% in South Korea (Kim et al. 2017), and 100% in Indonesia (Jumali et al. 1979) and Spain (Munoz et al. 2011). Our results from Florida indicate that contact rates between *Ae. albopictus* and humans are sufficient to consider this mosquito species a potential vector of ZIKV.

For *Cx. quinquefasciatus*, a striking contrast in host use was demonstrated between the two sampling locations (Fig. 3). A relatively high level of ornithophily (50%) was observed in *Cx. quinquefasciatus* females from in Martin County, using BG-Sentinel trap in residential areas, compared to anthropophily (78.6% from humans) observed in Indian River County. Unlike *Ae. aegypti* and *Ae. albopictus*, many studies on host use of *Cx. quinquefasciatus* from North America are available. Studies from the southern US have generally reported substantial use of both avian and mammalian hosts, as in Florida (Edman 1974), Texas (Molaei et al. 2007), Tennessee (Savage et al. 2007), New York and New Jersey (Apperson et al. 2004). While our results from residential areas of Martin County (opportunism) agree with these previous studies, the high level of anthropophily observed in the salvage yard in Indian River County are comparable to studies from India (Samuel et al. 2004) and Mexico (Janssen et al. 2015). The variation in host availability at the two sites was likely a driving force in the observed differences in host use between the two locations. However, data on human density and activity estimates from the two areas were not available and no attempts were made to compare the genetics of the two populations. Our findings support previous indications that *Cx. quinquefasciatus* is an opportunistic feeder (Molaei et al. 2007) but under certain circumstances takes a sufficiently large proportion of bloodmeals from humans to play a minor role in ZIKV transmission in Florida. Other parameters of vectorial capacity for this species need to be quantified to validate this assertion.

Due to the absence of ZIKV transmission during the study period, we could not collect field evidence of infection, which represents a limitation in our ability to incriminate these mosquito species as vectors of ZIKV. Furthermore, the use of two different sampling methods in the two counties limits our power to determine with confidence whether the difference in host use observed between the two counties is due to sampling methods or site-specific variation in host availability. The two different environments of mosquito sampling in Martin County and Indian River County likely had a strong effect on the patterns of host use. Many studies have found spatial variation in host use in vector mosquitoes (reviewed in Chaves et al. 2010). A recent examination of the relationship between host use and human economic conditions found that proportion of human bloodmeals from *Ae. albopictus* varied significantly with neighborhood socio-economic status (Goodman et al. 2018) with a greater likelihood of feeding on humans in areas with higher income. Without data on availability of various potential hosts, including humans, it is perhaps not possible to infer whether the differences in host use between the two counties were due to sampling method or to access to humans. The reliance on different sampling methods (attractive traps vs. active aspiration) between the two counties complicates our ability to parse out the effects of environment versus sampling method, as sampling method could affect observed patterns of host use (Kent et al. 2009). The significantly higher proportions of bloodmeals from humans at the auto recycling facility in Indian River County could be due to the number of humans entering the property without proper protections. The low sample size in Martin County (13 *Ae. aegypti*¸ 3 *Ae. albopictus*, and *18 Cx. quinquefasciatus*) limits our ability to draw firm conclusions from that data and should be interpreted with caution. Based upon our results, all three species demonstrated that they could play a role in the transmission of ZIKV under certain circumstances. Other things being equal *Ae. aegypti* would be the most likely vector of ZIKV in southern Florida, given its greater abundance and anthropophily; however, other aspects of vectorial capacity, particularly longevity and extrinsic incubation period, will certainly influence this interpretation.

This study provides the first spatially comparative data on the host use patterns of *Ae. aegypti*, *Ae. albopictus*, and *Cx. quinquefasciatus* in Florida, information that is important in understanding the relative importance of these species with regards to transmission of ZIKV and other pathogens (dengue and chikungunya virus) that share the same vector species and vertebrate hosts. Further, these data help to inform potential mosquito control strategies in Florida based on the knowledge that these three species are biting humans and have varying capabilities to transmit ZIKV. Despite the widely accepted importance of *Ae. aegypti* and *Ae. albopictus* in transmission of arboviruses, very few data on host use by these mosquitoes are currently available for the continental United States.

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