Host Selection of Potential West Nile Virus Vectors in Puerto Barrios, Guatemala, 2007

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Abstract. The selection of vertebrate hosts by *Culex* mosquitoes relative to West Nile virus (WNV) transmission in neotropical countries such as Guatemala is not described. This study determined the feeding patterns of *Cx. quinquefasciatus* and *Cx. nigripalpus* and estimated the relative contribution of two common and frequently infected wild bird species, *Turdus grayi* and *Quiscalus mexicanus*, to WNV transmission. Engorged mosquitoes were collected from rural and urban habitats after the dry and wet seasons in the Department of Izabal in 2007. Host selection by *Cx. nigripalpus* varied significantly between urban and rural habitats. Both *Cx. quinquefasciatus* and *Cx. nigripalpus* fed predominantly on chickens and other domestic animals. Blood meals from wild birds were rare, accounting for 1.1% of blood meals identified from *Cx. quinquefasciatus* and 6.5% of blood meals from *Cx. nigripalpus*. Transmission of WNV by these two mosquito species may be dampened by extensive feeding on reservoir-incompetent hosts.

INTRODUCTION

Serologic evidence of West Nile virus (WNV) was first described in Guatemala in 2003 with the confirmation of WNV-seropositive horses in several departments throughout the country.¹ Intensive ecological studies of WNV were initiated within a 80-km² transmission focus that incorporated the city of Puerto Barrios and the rural village of Machacas del Mar in the Department of Izabal. The clay-colored thrush (*Turdus grayi*), great-tailed grackle (*Quiscalus mexicanus*), and domestic chicken (*Gallus gallus*) have been identified as common and frequently infected resident avian hosts within the transmission focus.² In 2007 and 2008, *T. grayi* and *Q. mexicanus* were among the more abundant wild bird species with the highest WNV seroprevalence rates in the transmission focus.² However, the relative importance of these and other tropical vertebrate species to enzootic transmission of WNV is unknown. In Puerto Barrios, WNV infections have been detected in *Culex* (*Culex*) *quinquefasciatus* Say, and *Cx.* (*Cx.*) *mollis*/*Cx.* (*Cx.*) *inflictus*.² WNV has also been detected in *Cx.* (*Cx.*) *interrogator* Dyar and Knab and *Cx.* (*Cx.*) *nigripalpus* Theobald in nearby Chiapas, Mexico.³ WNV isolates were obtained from *Cx. nigripalpus*, *Cx.* (*Cx.*) *bahamensis* Dyar and Knab, *Cx. quinquefasciatus*, and *Cx.* (*Cx.*) *habeator* Dyar and Knab during an outbreak in Puerto Rico in 2007.⁴ However, no study has yet linked the blood-feeding behavior of these potential WNV vectors to virus transmission among vertebrate hosts in Mesoamerica.

The extraordinary biodiversity of the American tropics presents an enormously complex and challenging ecosystem for understanding arbovirus transmission cycles. Currently, there are 18 recognized mosquito species in the subgenera *Culex* (*Culex*) and *Culex* (*Phenacomyia*) in Guatemala⁵ that could potentially serve as vectors of WNV. Vertebrate host selection of these species is poorly understood, and the interactions between these mosquitoes and the myriad of potential WNV-amplifying hosts could vary in response to host availability across habitats and seasons. Comprehensive DNA barcode coverage is available for North American birds, including neotropical migrant species that winter in Guatemala, providing an

excellent DNA reference database with which to match mosquito blood-meal sources.⁶ However, taxonomic coverage of resident wild birds in Guatemala and other tropical vertebrate species likely fed on by mosquitoes is much less complete for both the DNA barcoding gene (mitochondrial cytochrome c oxidase I) as well as mitochondrial cytochrome b.

Understanding how WNV circulates among mosquito vectors and amplifying hosts in tropical ecosystems is a priority for understanding the ecology of WNV and risk of human disease in this part of the world and implementing targeted surveillance and control measures appropriate for this ecological setting. Therefore, the specific aims of this study were to (1) determine the host range of candidate vector mosquitoes in urban and rural habitats and their blood-feeding patterns on different vertebrate hosts during the dry (July) and wet (December) seasons of 2007 and (2) estimate the relative contribution of potential key avian host species to WNV transmission in urban and rural habitats.

MATERIALS AND METHODS

Study site. This study was conducted in a previously identified WNV transmission focus comprising the city of Puerto Barrios and the rural town of Machacas del Mar, located in the Department of Izabal along the Caribbean coast of Guatemala (15°50' N, 88°28' W) (Figure 1). A complete description of the transmission focus is published elsewhere.² Within the WNV transmission focus, 10 1-km² quadrants were selected, each containing a sentinel house at which WNV surveillance activities, including engorged mosquito collections, were conducted. Four of these quadrants were classified as rural, and six sites were urban because of the prevalence of urban facilities (houses, roads, bridges, etc.) and vegetation. Urban quadrants were defined as quadrants having > 30% road and human dwelling infrastructure. In addition to the sentinel houses, six rural microhabitats were also identified for mosquito collections: road, secondary forest, pasture, shaded pasture, river, and hedge.

Mosquito collections. Mosquitoes were aspirated from either the vegetation or 9-in diameter circular plastic flower pots placed horizontally on the ground using a Modified Centers for Disease Control and Prevention (CDC) Backpack Aspirator Model 1412 (John W. Hock Company, Gainesville, FL). For the pot collections, 24 pots were placed in the yard

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FIGURE 1. Mosquito collection sites located in the city of Puerto Barrios and the rural village of Machacas del Mar, Department of Izabal, Guatemala (2007).

surrounding each of four rural and six urban sentinel houses, and 50 pots were placed in each of the six rural microhabitats. Mosquitoes were aspirated from pots during 1 week each month (3–5 collection days per site per month) between July 1–6, 2007 and November 29 to December 7, 2007. Pots were left in place for the entire collection period, and missing pots were replaced as necessary. After aspiration of mosquitoes from pots, approximately 10 minutes were spent at each site aspirating resting mosquitoes from the surrounding vegetation. Collections from pots and vegetation were kept separate. Aspiration cups of mosquitoes were stored on dry ice until sorting. Female mosquitoes were morphologically identified⁷; male mosquitoes were discarded. The identity of a subset of known species (*Cx. quinquefasciatus*, *Cx. (Cx.) lactator*, *Cx. interogator*, *Cx. nigripalpus*, *Cx. (Cx.) chidesteri*, and *Cx. (Cx.) coronator*) and engorged specimens that could only be classified as *Cx. spp.* were confirmed by polymerase chain reaction (PCR).⁸ Mosquitoes were classified as unfed, fully engorged, half-gravid, or gravid, and they were stored individually at ambient temperature in 0.5-mL tubes containing silica gel desiccant and cotton sorted by date and location.⁹ Additional engorged *Cx. quinquefasciatus* collected in 2007 were obtained from CO₂-baited CDC light traps¹⁰ (John W. Hock Company, Gainesville, FL) and gravid traps¹¹ from the same sites within the municipality of Puerto Barrios. *Cx. quinquefasciatus* blood-meal identification data from all collection methods were combined after it was determined that feeding patterns were not significant by trap type.¹²

Vertebrate relative abundance. To generate density and relative abundance data for wild birds and chickens, avian point counts were performed.^{2,13} Point counts were conducted along a single transect in each of the 10 1-km² quadrants. For each quadrant, four 4-minute counts of bird species seen or heard within 50 m were conducted within the first 3 hours of daylight. Mosquito sampling sites were located within 50 m of each transect. Bird surveys were conducted during the same weeks as mosquito collections. Domestic animals, chickens, and humans were counted at each sentinel house while mosquitoes were being collected.

DNA extraction from mosquitoes. Abdomens of engorged mosquitoes in CDC light trap and gravid trap collections were removed over clean microscope slides using clean insect pins and forceps and placed into separate labeled tubes on ice. Engorged abdomens were manually triturated in phosphate buffered saline. DNA was extracted from these homogenates using the QIAamp DNA mini kit (Qiagen, Valencia, CA) according to the manufacturer's instructions. When the QIAamp DNA kit was not available, DNA was extracted with DNAzol reagent (Invitrogen, Carlsbad, CA) according to the manufacturer's instructions. Pellets from DNAzol extractions were incubated overnight at 4°C to maximize pellet rehydration. All DNA samples were stored at –20°C until tested by PCR assays.

Mosquito blood-meal identification. Blood meals from specimens collected by CDC light traps and gravid traps were initially screened in Guatemala for common domestic hosts: cow (*Bos taurus*), human (*Homo sapiens*), pig (*Sus scrofa*), goat (*Capra hircus*), dog (*Canis familiaris*), and chicken (*G. gallus*) were known to be present at the study sites.^{9,14,15} Amplification of chicken DNA was carried out in a separate tube using specific primers UNFOR1029 and Chick1123R under the same reaction conditions.⁹ DNA extracted from whole blood of each vertebrate species tested was used as positive control. For blood meals not identified by multiplexed PCR, vertebrate cytochrome b (cytb) amplicons generated by either avian¹⁶ or mammalian¹⁷ primers and/or vertebrate mitochondrial cytochrome c oxidase 1 (COI) barcoding primers¹⁸ were sent to the CDC in Fort Collins, CO for sequencing. Blood meals from aspirated specimens were first screened using chicken primers as above. Blood meals not containing chicken DNA were identified by PCR amplification and sequencing of a fragment of either the COI or cytb gene.^{16–19} DNA amplifications and sequencing reactions were completed on a DNA Engine PTC-200 thermal cycler (Bio-Rad Laboratories, Inc., Hercules, CA) and analyzed on an ABI 3130 genetic analyzer. Sequences were assembled using Lasergene 9 software (DNA STAR, Inc, Madison, WI). Two databases were used to identify mosquito blood-meal sequences. The

DNA Barcode database was used to identify COI sequences (www.barcodinglife.org) and GenBank was searched to identify cytb sequences. Greater than 98% sequence homology was considered a positive match. To aid in blood-meal identification, novel COI and cytb¹⁶ reference sequences were generated for 40 species of resident birds in Guatemala (GenBank accession nos. EU442290–EU442363).

Blood-meal analysis. The blood-meal host selection of *Cx. quinquefasciatus* and *Cx. nigripalpus* was analyzed by χ^2 to test the null hypothesis that there was no difference in mosquito host selection across habitats or seasons. Preferential host selection favoring one host over another was determined by calculation of the feeding index.²⁰ The feeding index (FI) is expressed as $FI = (Ne/Ne')/(Ef/Ef')$, where Ne is the number of feeds on host I, Ne' is the number of feeds on host II, and Ef/Ef' is the expected proportion of feeds on host I/host II based on their relative abundance. A feeding index of 1.0 indicates equivalent host selection for the two hosts being compared. Values less than and greater than 1.0 indicate lesser and greater host selection, respectively, in feeding on the first host relative the second host. For estimation of the term Ef/Ef', the relative abundance of dogs, humans, cattle, chickens, and wild birds was directly interpreted as the expected proportion of feeds on each species. Raw counts of the domestic animals and chickens and total numbers of wild birds seen or heard along urban or rural point count transects during a given sampling period were, therefore, used in calculation of the feeding indices using the methods in the work by Kay and others.²⁰ All feeding index calculations were set compared with chickens, where chickens were host I.

Relative contribution to WNV transmission. The relative number of WNV-infectious mosquitoes resulting from feeding on *T. grayi* and *Q. mexicanus* was calculated as described previously.^{14,21} For *Cx. quinquefasciatus* and *Cx. nigripalpus* feeding on *T. grayi* and *Q. mexicanus*, the relative number of infectious mosquitoes (F) resulting from feeding on each avian species (i) was estimated by multiplying reservoir competence, C, by the square of the proportion of blood meals (b) from species i each month: $F_i = C_i \times b_i^2$.¹⁴ Avian reservoir competence index values used for *T. grayi* (0.1), *Q. mexicanus* (1.8), and *G. gallus* (0.0) were derived from experimental infection with the Tabasco strain of WNV.²² The relative number of infectious mosquitoes derived from feeding on *Q. mexicanus* compared with *T. grayi* was determined by dividing $F_{Quiscalus}$ by F_{Turdus} .

RESULTS

Host selection of mosquito vectors in urban and rural environments. In total, 686 blood meals from *Culex* mosquitoes were identified during 2007, including blood meals from several potential vectors of WNV in the subgenus *Culex* (*Culex*) (Table 1). Of these blood meals, 486 blood meals were from the aspiration collections in July and November/December of 2007, and 200 blood meals were from CDC light trap and gravid trap collections performed throughout 2007. Regardless of collection method, most blood meals were from domestic animals. Domestic birds fed on in addition to chicken (*G. gallus*) were turkey (*Meleagris gallopavo*), Muscovy duck (*Cairina moschata*), and one white-winged dove (*Zenaida asiatica*). Mammal blood meals included human (*H. sapiens*), cattle (*Bos* spp.), horse (*Equus caballus*), dog (*C. familiaris*), pig (*S. scrofa*),

cat (*Felis catus*), black rat (*Rattus rattus*), Norway rat (*R. norvegicus*), and gray four-eyed opossum (*Philander opossum*).

Only 17 of 686 (2.5%) blood meals identified from *Culex* mosquitoes were obtained from wild birds. Blood meals from *Cx. nigripalpus* included one *Strix* spp. owl, one *T. grayi*, one *Q. mexicanus*, one bare-throated tiger heron (*Tigrisoma mexicanum*), one Northern oriole (*Icterus galbula*), one white-eyed vireo (*Vireo griseus*), one yellow-crowned night heron (*Nyctanassa violacea*), and one unidentified raptor in the family Accipitridae. One *T. grayi* and three *Q. mexicanus* blood meals were identified from *Cx. quinquefasciatus*. One spot-breasted oriole (*I. pectoralis*), one unidentified passerine in the family Thamnophilidae (antshrike spp.), and one *T. grayi* were identified from *Cx. spp.* along with one red-winged blackbird (*Agelaius phoeniceus*) from *Cx. lactator* Dyar and Knab and one *T. grayi* from *Cx. interrogator*. Of these species, *I. galbula* and *V. griseus* are neotropical migrant species, whereas the remainder of species are resident in Mesoamerica all year. Eleven blood meals were obtained from reptile and amphibian species. These blood meals included green iguana (*Iguana iguana*) and a *Gambelia* spp. lizard from *Cx. nigripalpus*, *I. iguana* from *Cx. taeniopus*, and an unidentified skink, unidentified amphibian, unidentified reptile, and three frogs in the family Ranidae from *Cx. spp.* Most of the blood meals obtained from *Cx. spp.* were from *Cx. mollis* Dyar and Knab, *Cx. declarator* Dyar and Knab, or *Cx. inflicus* Theobald. Adult females of these species are difficult to differentiate morphologically, and these three species are not included in the multiplexed PCR assay for *Culex* identification.⁸

The two mosquito species from which the largest numbers of blood meals were obtained were *Cx. nigripalpus* and *Cx. quinquefasciatus*. *Cx. nigripalpus* was collected from both rural and urban habitats, and it had a relatively opportunistic blood-feeding behavior (Table 1). Overall, 36.5% of *Cx. nigripalpus* blood meals came from mammals, 61.5% came from birds, and 1.9% came from reptiles. The host selection of *Cx. nigripalpus* on livestock, humans, dogs, domestic birds, wild birds, and reptiles differed significantly by habitat ($\chi^2 = 18.7$, degrees of freedom (df) = 5, $P < 0.0025$, $\alpha = 0.004$) (Figure 2). No seasonal shift in *Cx. nigripalpus* blood-feeding among humans, dogs, domestic birds, and wild birds was observed in urban areas ($\chi^2 = 6.8$, df = 3, $P > 0.05$, $\alpha = 0.006$) or on livestock, humans, dogs, domestic birds, wild birds, and reptiles in rural areas ($\chi^2 = 7.9$, df = 5, $P > 0.15$, $\alpha = 0.004$). Overall, 52% of identified *Cx. nigripalpus* blood meals came from chickens (Table 1).

FIGURE 2. Host selection as determined by blood meal identification for *Cx. nigripalpus* ($N = 107$) in urban and rural habitats. Variation is principally caused by the lack of livestock and reptilian blood meals detected in urban habitat.

TABLE 1
Number and percentage of blood meals identified from *Culex* mosquitoes collected in Puerto Barrios, Guatemala during 2007

Mosquito species	No. blood meals								Total
	Cattle	Horse	Human	Other mammal	Chicken	Other domestic fowl	Wild bird	Reptile or amphibian	
<i>Cx. (Cx.) quinquefasciatus</i>	2 (0.5%)	0	1 (0.2%)	32 (8.6%)	318 (85%)	16 (4.3%)	4 (1.1%)	0	373
<i>Cx. (Cx.) interrogator</i>	0	0	0	0	15 (83.3%)	2 (11.1%)	1 (5.6%)	0	18
<i>Cx. (Cx.) coronator</i> s.l.	9 (81.8%)	0	1 (9.1%)	0	1 (9.1%)	0	0	0	11
<i>Cx. (Cx.) stigmatosoma</i>	0	0	0	0	1 (100%)	0	0	0	1
<i>Cx. (Cx.) nigripalpus</i>	29 (27.1%)	5 (4.7%)	2 (1.9%)	3 (2.8%)	56 (52.3%)	3 (2.8%)	7 (6.5%)	2 (1.9%)	107
<i>Cx. (Ph.) lactator</i>	0	0	0	0	1 (50%)	0	1 (50%)	0	2
<i>Cx. spp.</i>	41 (33.9%)	7 (5.8%)	2 (1.7%)	6 (5.0%)	53 (43.8%)	2 (1.7%)	4 (3.3%)	6 (5.0%)	121
<i>Cx. (Mel.) taeniopus</i>	29 (56.9%)	8 (15.7%)	2 (3.9%)	4 (7.8%)	7 (13.8%)	0	0	1 (2.0%)	51
<i>Cx. (Mel.) chrysonotum</i>	0	0	0	0	2 (100%)	0	0	0	2

Data include mosquitoes collected by aspiration, CDC light trap, and gravid trap from both urban and rural habitats. *Cx. spp.* refer mostly to *Cx. mollis*/*Cx. inflicus*. *N* = 686.

In contrast, *Cx. quinquefasciatus* was very common in urban areas but more rarely collected in rural areas. Within urban areas, *Cx. quinquefasciatus* blood meals were predominately from chickens (85%) (Table 1). *Cx. quinquefasciatus* blood-meal composition did fluctuate significantly in some months ($\chi^2 = 124.5$, *df* = 32, $P < 0.001$, $\alpha = 0.001$) (Figure 3). However, because of the low number of observations (e.g., only four observations from January to April), temporal fluctuations should be studied more thoroughly. The most common mammalian hosts fed on by *Cx. quinquefasciatus* were dogs (*N* = 27) (Table 1). Other mammalian blood meals included human (*N* = 1), cattle (*N* = 2), cat (*N* = 4), and rat (*N* = 1).

Feeding indices were calculated from the numbers of blood meals from host I compared with host II and the raw counts of these hosts at the collection locations as determined from avian point counts and domestic animal censuses in July and December of 2007 (Table 2). Feeding indices indicated that *Cx. nigripalpus* selected both cattle and dogs over chickens when these hosts were available. Chickens were used over both humans and wild birds in urban and rural habitats (Table 2). Feeding index analysis also suggested that *Cx. quinquefasciatus* selected chickens over humans, dogs, cattle, and wild birds (Table 2).

Relative contribution of key WNV-amplifying hosts. The most common wild bird species fed on collectively by *Culex* mosquitoes were *T. grayi* and *Q. mexicanus*. Of the 17 total *Culex* blood meals from wild birds, 4 each (23.5%) were from *T. grayi* and *Q. mexicanus* (Table 1). We estimated that, for every 1 WNV-infectious *Cx. quinquefasciatus* derived from feeding on *T. grayi*, 162 WNV-infectious *Cx. quinquefasciatus*

were derived from feeding on *Q. mexicanus*, and for every 1 WNV-infectious *Cx. nigripalpus* derived from feeding on *T. grayi*, we estimated that 18 WNV-infectious *Cx. nigripalpus* were derived from feeding on *Q. mexicanus*. However, only 0.003 (0.3%) and 0.008 (0.8%) of *Cx. quinquefasciatus* blood meals originated from *T. grayi* and *Q. mexicanus*, respectively. The proportion of *Cx. nigripalpus* blood meals from each *T. grayi* and *Q. mexicanus* was 0.009 (0.9%). A high proportion of blood meals from each of these mosquito species came from chickens. Chickens are not known to be competent amplifying hosts for WNV,²² and estimates for the relative number of infectious mosquitoes derived from feeding on them were, therefore, zero.

DISCUSSION

This study characterized the seasonal vertebrate host selection of *Cx. quinquefasciatus* and *Cx. nigripalpus* in urban and rural habitats in Guatemala during 2007, and it provided blood-meal identification data from numerous additional mosquito species. Both *Cx. nigripalpus* and *Cx. quinquefasciatus* are widely reported to have opportunistic host selection throughout the Americas.^{23–26} The host selection of *Cx. nigripalpus* in Florida was described as opportunistic, with the majority of blood meals taken from cattle and rabbits, but also, many were from birds, including wading birds.²³ Along the southeastern Pacific coast of Guatemala, *Cx. nigripalpus* fed mostly on birds (61–80% across several years) and occasionally, mammals and reptiles.²⁴ *Cx. quinquefasciatus* is also known to use a wide variety of avian, mammalian, and to a minor extent, reptilian hosts. Whether the mosquitoes were predominantly ornithophilic^{27–30} or mammalophilic^{26,31,32} varied across habitats, geographic locations, and whether collections were performed indoors and/or outdoors.

As opportunists, the host selection behaviors of *Cx. quinquefasciatus* and *Cx. nigripalpus* are, in part, determined by the availability of domestic and free-ranging hosts at different collection sites.^{23,31} In our study, avian blood meals from free-ranging species were rarely detected for both *Cx. nigripalpus* and *Cx. quinquefasciatus*. Less than 1% of blood meals from these candidate WNV vectors were derived from *T. grayi* and *Q. mexicanus*, providing little support that these two wild bird species are significant sources of WNV-infectious mosquitoes. Still, WNV seroprevalence was approximately 11% in *T. grayi* (*N* = 171) in 2007 and 32% in *Q. mexicanus* (*N* = 67), and WNV infection rates in *Cx. quinquefasciatus* were 5.7 per 1,000 in July of 2007 and 15.7 per 1,000 in August

FIGURE 3. The seasonal blood meal composition of *Cx. quinquefasciatus* (*N* = 373) in urban habitat.

TABLE 2

Feeding index values for *Cx. nigripalpus* in urban and rural habitats and *Cx. quinquefasciatus* in urban habitats, Puerto Barrios, Guatemala during 2007

Ratio	Feeding index					
	<i>Culex nigripalpus</i>				<i>Culex quinquefasciatus</i>	
	July: Urban	November/December: Urban	July: Rural	November/December: Rural	July: Urban	November/December: Urban
Chicken:cattle	—	—	0.3	0.1	2.0	—
Chicken:human	—	2.1	7.2	—	54.6	—
Chicken:dog	—	0.2	—	0.3	3.8	1.3
Chicken:wild bird	23.7	—	9.1	8.7	465.9	—

Culex quinquefasciatus blood meals from all four collection methods were combined for calculations. All feeding indices were calculated in comparison with chickens. A feeding index of 1.0 indicates equal host selection for the two hosts being compared. Values greater than 1.0 indicate greater host selection on chickens as compared with the second host, whereas less than 1.0 indicates the reciprocal. A dash indicates that the feeding index value could not be calculated because no blood meals were obtained.

of 2007, indicating a relatively high amount of virus activity in these vertebrate and vector species.²

In this study, the majority of blood meals from wild birds was clustered in May and June of 2007 (Figure 3). It is possible that this spike in mosquito blood-feeding activity on wild birds is the result of mosquitoes capitalizing on the presence of defenseless nestlings and brooding adult birds during the nesting season. Investigations into the importance of nestling birds to WNV transmission have resulted in disparate conclusions. WNV infection/seroprevalence rates in nestling birds were very low in several studies,^{33–35} which concluded that nestlings were not important early-warning sentinel species or amplification hosts for WNV. Mosquito landing rates were also found to be higher on adult birds than on nestlings, and parental brooding reduced landing rates of mosquitoes on nestlings.³⁶ In contrast, mosquito feeding on specific vertebrate hosts in Alabama peaked during the reproductive periods for those hosts.³⁷ The lack of evidence for WNV infection in nestlings during the nesting season may be the result of sampling early in the season, when mosquito and virus activity is relatively low.³⁸ In studies that have highlighted a role for nestling or juvenile birds in WNV amplification, there seems to be strong spatial and seasonal components in the focal of WNV transmission among juvenile birds.^{14,38} The seasonality of mosquito blood-feeding on wild birds in Guatemala may have contributed to the peak in mid-summer WNV amplification observed in 2007 in the work by Morales-Betoulle and others,² and it warrants additional study.

The majority of blood meals for *Cx. quinquefasciatus* and *Cx. nigripalpus* in both urban and rural habitats originated from domestic animals, particularly chickens. Chickens have been previously reported to be heavily used hosts of both *Cx. quinquefasciatus*^{23,27,29,39} and *Cx. nigripalpus*,²³ and this finding likely reflects host composition where mosquito sampling efforts were concentrated. Chickens were very prevalent in both urban and rural peridomestic study sites. Mosquito collections close to the ground in these habitats naturally would yield a large number of blood meals from poultry and other domestic animals from mosquitoes foraging at ground level. Although adult chickens are not considered competent amplifying hosts and may dampen WNV transmission, we recognize that very young chicks (< 1 week old) may be competent amplifying hosts for WNV.⁴⁰ Future research should include blood-meal identification of mosquitoes in locations where domestic animals are not present to further elucidate which mosquito species are feeding on and infecting wild birds.

Mosquito collections from urban sites with substantial human activity produced surprisingly few human-derived

blood meals from *Cx. quinquefasciatus*. In Mexico, humans were the second most common blood source of *Cx. quinquefasciatus*, comprising 9.4% of the blood meals from outdoor collections in one site.²⁹ In Brazil, *Cx. quinquefasciatus* preferred humans to chickens; however, these mosquitoes were collected indoors as well as outdoors.²⁶ *Cx. quinquefasciatus* in Southern California fed predominantly on doves and passerine birds but took approximately 2% of blood meals from humans, showing the capacity of this species to serve as both an enzootic and bridge vector of WNV.⁴¹ Housing in the sampling sites in Guatemala was relatively open, providing mosquitoes free movement in and out of homes. Therefore, the intensive collections performed around houses, yards, and porches using four different collection methods conceivably should have produced human-fed *Culex*. Alternatively, this finding may reflect a highly ornithophilic *Cx. quinquefasciatus* population, with chickens and other domestic animals diverting host-seeking *Cx. quinquefasciatus* away from humans. Zooprophyllaxis through the presence of chickens and other domestic animals in urban areas is one hypothesis to explain the lack of reports of human WNV cases in Puerto Barrios. Additional investigation, including paired indoor-outdoor mosquito collections, is needed to further evaluate the anthropophily of *Cx. quinquefasciatus* in our study sites.

Interestingly, species of *Culex* (*Culex*) and *Culex* (*Melanoconion*), including *Cx. (Culex) nigripalpus* and *Cx. (Mel.) taeniopus*, fed on various species of frogs, skinks, and lizards. These species as well as *Cx. quinquefasciatus* have been previously shown to feed occasionally on reptiles and amphibians.^{23–25,28} The role of these vertebrates as potential arbovirus reservoirs in the tropics has not yet been determined. The green iguana (*I. iguana*) and the North American bullfrog (*Rana catesbeiana*) have been shown to develop only low-titered WNV viremia after experimental infection (< 4.0 log pfu/mL serum).⁴² However, lake frogs (*R. ridibunda*) in Russia⁴³ and juvenile American alligators (*Alligator mississippiensis*)⁴⁴ are known to develop high-titered viremias infectious to feeding mosquitoes. Both *Cx. quinquefasciatus* and *Cx. nigripalpus* were found to have fed on captive alligators in Louisiana.⁴⁵ A high rate of feeding on reptiles and amphibians was reported for *Cx. quinquefasciatus* in Puerto Rico during a period of elevated WNV transmission.⁴⁶

In North America, seasonal shifts in blood-feeding behavior have been well-documented for several species of *Culex* (*Culex*), including *Cx. nigripalpus*.^{14,23,47–49} Such shifts can be epidemiologically significant if fluctuations in particular vector–host contacts result in increased enzootic or epizootic virus transmission.^{23,49,50} In Florida, this shift was attributed

to seasonal patterns of relative humidity and rainfall that regulate *Cx. nigripalpus* movements between resting refugia and open fields, where they encounter primarily mammalian hosts.²³ Although *Cx. nigripalpus* blood meals were analyzed at the end of dry and wet seasons during this study, no significant seasonal shift in blood-feeding behavior was seen for this species in either urban or rural area. More frequent mosquito collections and correlation of blood meals with individual rainfall events may be necessary to detect such a pattern. Similarly, no observable shift in blood-feeding behavior was observed for *Cx. quinquefasciatus*, although its blood-feeding pattern did differ significantly in some months. This variation could be explained by clusters of blood meals from particular vertebrate species in certain months (e.g., Muscovy ducks clustered around a gravid trap one night in June). Seasonal shifts in host selection have been reported previously for *Cx. quinquefasciatus*.^{31,32,51}

Another specific aim sought to estimate the relative contribution of different potential avian amplifying hosts to WNV transmission in Puerto Barrios during 2007. The relative abundance of wild birds used in feeding index calculations was determined from the point count data. Although point counts are an accepted, standardized methodology for performing avian surveys, this method is subject to a number of limitations. For example, the detectability of different bird species by sight and sound, weather conditions, skill level of the observer, distance from the observation point, and time of day that the survey was conducted all influence the recorded abundance of each bird species. Although we designed and conducted our surveys to control for these limitations and performed engorged mosquito collections in the same locations as the bird surveys were conducted, the relative abundance of the wild birds compared with the other vertebrate species available to mosquitoes at each site, particularly at the times that mosquitoes were actively feeding, cannot be precisely determined. Fortunately, *Q. mexicanus* and *T. grayi*, the two potential WNV-amplifying hosts primarily discussed in this study, were very abundant and easily observable at our study sites.² Both *Cx. nigripalpus* and *Cx. quinquefasciatus*, as well as other species of *Culex* (*Cx. spp.* and *Cx. interrogator*) fed on *T. grayi* and *Q. mexicanus*, although apparently at very low rates. Given the high WNV seroprevalence in *Q. mexicanus* in 2007, future mosquito collection efforts should be focused around communal roosts of *Q. mexicanus*. *Q. mexicanus* forms large aggregations in the evening at several locations throughout Puerto Barrios, and it is at these roosting locations where they may be susceptible to mosquito attack rather than at the residential homes where engorged mosquitoes were collected. Communally roosting passerines have previously been hypothesized to be a rich source of blood meals for host-seeking *Culex* mosquitoes as well as a focus of WNV amplification.¹⁴ More work is needed to determine mosquito feeding behavior at communal bird roosts and the contribution of *Q. mexicanus* communal roosts to enzootic WNV amplification.

In conclusion, this study characterized the blood-feeding patterns of *Cx. quinquefasciatus* and *Cx. nigripalpus* in urban and rural habitats in a WNV transmission focus in Guatemala. *Cx. quinquefasciatus* was one of the most common mosquito species collected in urban areas by aspirations, and one from which several WNV isolates have been made within the transmission focus.² This species fed predominantly on chickens,

with few blood meals also identified from humans and wild birds, including *T. grayi* and *Q. mexicanus*. With a highly ornithophilic *Cx. quinquefasciatus* population taking approximately 85% of blood meals from chickens, the effect that these domestic birds have on WNV transmission and the potential role of *Cx. quinquefasciatus* also serving as a bridge vector responsible for transmission of WNV to humans in Puerto Barrios need to be further evaluated. Because of its prevalence in urban and rural areas, previous virus isolations, and propensity to feed on both WNV-amplifying and dead-end hosts, *Cx. nigripalpus* has the potential to serve as both an enzootic and bridge vector of WNV in Guatemala. This species could be involved in circulating WNV among competent wild bird species, including *T. grayi* and *Q. mexicanus*, as well as bridging WNV to chickens, livestock, and humans in both urban and rural settings in Guatemala. However, the high proportion of mosquito blood meals from reservoir-incompetent hosts could function to dampen WNV transmission in Puerto Barrios. Much more work is needed to elucidate vertebrate host selection for the other numerous *Culex* species and the role that those species play in transmission of WNV in this tropical environment.

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