

## Host Feeding Pattern of *Culex quinquefasciatus* (Diptera: Culicidae) and Its Role in Transmission of West Nile Virus in Harris County, Texas

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**Abstract.** The vertebrate hosts of 672 blood-engorged *Culex quinquefasciatus* Say, collected in Harris County, Texas, during 2005, were identified by nucleotide sequencing PCR products of the *cytochrome b* gene. Analysis revealed that 39.1% had acquired blood from birds, 52.5% from mammals, and 8.3% were mixed avian and mammalian blood meals. Most frequent vertebrate hosts were dog (41.0%), mourning dove (18.3%), domestic cat (8.8%), white-winged dove (4.3%), house sparrow (3.2%), house finch (3.0%), gray catbird (3.0%), and American robin (2.5%). Results are interpreted in conjunction with concurrent avian and mosquito West Nile virus (WNV) surveillance activities in Harris County. We conclude that *Cx. quinquefasciatus* is an opportunistic feeder and principal mosquito vector of WNV in this metropolitan area; however, transmission by other mosquito species or by other modes of infection, such as ingestion, must account for the high WNV infection rates among local blue jays and American crows.

### INTRODUCTION

After detection of West Nile virus (WNV) in the New York City metropolitan area in 1999,<sup>1,2</sup> the virus rapidly spread west across the continental United States and southern Canada. WNV was first detected in Texas in June of 2002 in a dead bird collected in Houston.<sup>3</sup> Continuous WNV surveillance since 2002 indicates that the virus has now become endemic in the Houston metropolitan area (Harris County) with high levels of activity during the summer months (June to September) and lower levels of activity during the rest of the year.<sup>4</sup>

WNV is thought to be maintained in an enzootic cycle, involving various species of *Culex* mosquitoes as the principal vectors and wild birds as the major vertebrate reservoirs. Because of its local abundance and seasonally high WNV field infection rates, *Cx. quinquefasciatus* Say is presumed to be the principal mosquito vector in Harris County.<sup>3</sup> *Cx. quinquefasciatus* breeds locally in storm sewer catch basins, clean and polluted ground pools, ditches, animal waste lagoons, effluent from sewage treatment plants, and other sites with organic wastes.

Previous reports of the host preferences of *Cx. quinquefasciatus* indicate that this species acquires blood from a diverse range of birds and mammals,<sup>5–19</sup> depending upon the relative abundance and availability of vertebrate hosts within a specific geographic area.

Knowledge of the blood feeding behavior of resident mosquito populations is an essential element in assessing their vectorial capacity within a given locale. To better assess the role of *Cx. quinquefasciatus* in WNV transmission in Harris County, we undertook a study to determine its specific avian and mammalian hosts, and to evaluate its role in enzootic maintenance of the virus in the region. Blood-fed *Cx. quinquefasciatus* mosquitoes were collected between March 1 and November 9, 2005, in traps placed at 268 locations throughout Harris County; the vertebrate sources of these blood meals

were identified by sequencing PCR products of the *cytochrome b* gene of mitochondrial DNA. The results of these studies are presented and interpreted in conjunction with concurrent avian and mosquito WNV surveillance activities in the Houston metropolitan area.

### MATERIALS AND METHODS

**Study area.** Harris County, Texas, is located in the northern part of the Gulf of Mexico coastal plain, a 50-mile swath along the Texas Gulf Coast. The County has a human population of > 3.5 million and includes the city of Houston; it covers a geographic area of > 1,788 square miles, 27% of which is devoted to farming and ranching. The natural vegetation of the county varies from mainly forested in northern and eastern regions to predominant prairie grassland in the southern and western regions. Twenty-two drainages supply surface water to a number of lakes, rivers, and streams dominated by an extensive network of bayous and human-made canals that are part of the flood-management system. Elevation ranges from 0 to 310 feet above sea level. Because of its abundant rainfall, soil composition, and relatively low elevation, Houston is subject to periodic flooding. Houston's flood-control/drainage infrastructure consists of two parts: a series of six major bayous and an elaborate but aging system of storm sewers and underground tunnels that capture the flood waters. The storm sewers carry rain and other surface and drainage water but exclude wastewater and polluted industrial wastes. This elaborate drainage system creates favorable conditions for mosquito larval development, particularly during relatively dry periods when stagnant water remains in the storm sewer system.<sup>3</sup>

**Collection of mosquitoes.** Mosquitoes were collected throughout the year during 2005 from 268 locations in Harris County (Figure 1). A history and description of the Harris County Mosquito Control program have been given before.<sup>3,20</sup>

Mosquito collections for the current study were made using two different methods and trap types. Storm sewer light traps, a modified version of the CDC-designed light trap, were used for collecting *Cx. quinquefasciatus* females that use the storm drains for daytime resting as well as for oviposition and larval

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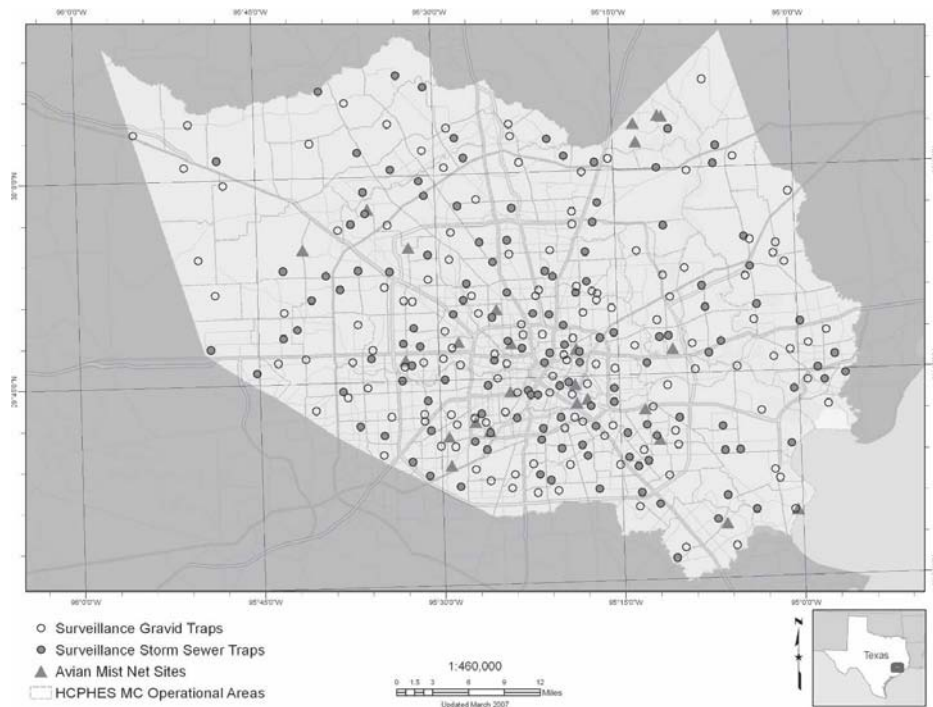


FIGURE 1. Locations of mosquito control operational sites in Harris County, Texas, that yielded mosquito specimens with blood-meals identifiable to host species. Locations of the gravid traps are shown by open circles; locations of the storm sewer light traps are depicted by closed circles; locations of the avian mist net sites are illustrated by closed triangles.

development. In addition, gravid traps baited with hay infusion water were set in areas considered at high risk for WNV activity; these included areas with landscaped vegetation at residential or commercial properties, empty lots and fields, wild brush, wooded areas, and large paved parking lots. Mosquito collections were performed weekly, biweekly, or monthly depending on the historical level of St. Louis encephalitis (SLE) virus found in each area.<sup>20</sup> Traps were set between 1:30 and 5:00 PM and picked up the next morning between 7:30 and 10:30 AM.

**Specimen processing and morphologic identification of mosquitoes.** Field-collected mosquitoes were transported live to the Mosquito Control Division (MCD) of Harris County Public Health and Environmental Services in Houston, inactivated with cold (4–5°C), and transferred to disposable, labeled cardboard boxes. Mosquitoes within boxes were emptied onto chill tables (BioQuip Products, Gardena, CA), identified using appropriate taxonomic keys<sup>21</sup> and sorted into pools of 50 or fewer females for subsequent virus detection. Specimens with visible blood meals were removed from the collections and were transferred to cryotubes labeled with a unique number and held at –70°C in a mechanical freezer. These latter samples were subsequently shipped on dry ice to The Connecticut Agricultural Experiment Station (CAES) for blood-meal identification and detection of WNV. The pools of non-blooded mosquitoes were assayed for WNV at the MCD laboratory in Houston and at the University of Texas Medical Branch (UTMB) in Galveston.

**DNA isolation from blood-fed mosquitoes.** DNA was extracted from the abdominal contents of the blood-fed mosquitoes individually by using DNA-zol BD (Molecular Research Center, Cincinnati, OH) according to the manufacturer's recommendation with some modifications as described

elsewhere.<sup>22–24</sup> Briefly, individual mosquito abdomens were homogenized with the aid of heat-sealed pipette tips or microtube pestles (USA Scientific, Enfield, CT) in 1.5-mL tubes containing DNA-zol BD solution. The homogenates were incubated at room temperature for 5–10 minutes, mixed, and centrifuged at 10,000–13,000g for 10 minutes. After 3–4  $\mu$ L of Poly Acryl Carrier (Molecular Research Center) was added to the supernatant, DNA was then precipitated by using isopropyl alcohol or absolute ethanol. The DNA pellet was washed twice with 75% ethanol, air-dried briefly, reconstituted in 100  $\mu$ L of TE buffer (10 mM Tris-HCl [pH 8.0], 1 mM EDTA), and stored at –20°C for further analysis.

**Blood-meal identification.** Isolated DNA from the mosquito blood meals served as DNA templates in subsequent PCR reactions. PCR primers were based on either multiple alignments of *cytochrome b* sequences of avian and mammalian species obtained from GenBank or published primer sequences.<sup>22</sup> All DNA templates were initially screened with avian- and mammalian-specific primer pairs, using previously described protocols,<sup>22–24</sup> and the sequences were analyzed. Avian-specific PCR primers were 5'-GAC TGT GAC AAA ATC CCN TTC CA-3' (forward) and 5'-GGT CTT CAT CTY HGG YTT ACA AGA C-3' (reverse) with amplified product size of 508 bp. PCR cycling conditions included an initial reaction activation step at 95°C for 5 minutes, followed by 36 cycles of denaturation at 94°C for 30 seconds, annealing at 60°C for 50 seconds, and extension at 72°C for 40 seconds. The final cycle was completed with 7 minutes of extension at 72°C. Mammalian-specific PCR primers were 5'-CGA AGC TTG ATA TGA AAA ACC ATC GTT G-3' (forward) and 5'-TGT AGT TRT CWG GGT CHC CTA-3' (reverse) with amplified product size of 772 bp. Initial PCR reaction activation step was performed at 95°C for 10 minutes, followed by

36 cycles of denaturation at 94°C for 30 seconds, annealing at 55°C for 45 seconds, and extension at 72°C for 1.5 minutes. The final cycle was completed with 7 minutes of extension at 72°C. In a few cases, other primer pairs were additionally used to resolve ambiguous sequences.<sup>22–24</sup> A *Taq* PCR Core Kit (Qiagen, Valencia, CA) was used for all PCR reactions according to the manufacturer's recommendations. A 50- $\mu$ L reaction volume was prepared with 3  $\mu$ L of template DNA, 4  $\mu$ L of each primer (0.1–0.5  $\mu$ M), 5  $\mu$ L of 10 $\times$  QIAGEN PCR Buffer (containing 15 mM MgCl<sub>2</sub>), 1  $\mu$ L of dNTP mix (10 mM each), 0.25  $\mu$ L of *Taq* DNA polymerase (1.25 U/reaction), and 32.75  $\mu$ L of water. PCR reactions were performed with the GeneAmp PCR System 9700 (Applied Biosystems, Foster City, CA), using the above-described thermal cycling conditions. For DNA sequencing, PCR-amplified products of *cytochrome b* gene were purified by using QIAquick PCR purification kit (Qiagen) and sequenced directly in cycle sequencing reactions by using the sequencer, 3730xl DNA Analyzer (Applied Biosystems) at the Keck Sequencing Facility, Yale University (New Haven, CT). Sequences were analyzed and annotated by using ChromasPro, version 1.22 (Technelysium Pty Ltd., Tewantin, Australia) and identified by comparison to the GenBank DNA sequence database (NCBI available online). The performance of the molecular-based assay was previously validated by isolating DNA from the blood of a number of known vertebrate species, subjecting them to PCR amplification, and sequencing as previously described.<sup>22</sup>

**Statistical analysis.** Seasonal changes in the host feeding patterns of *Cx. quinquefasciatus* on selected host species and from avian to mammalian species were analyzed by  $\chi^2$  analysis for trend using GraphPad InStat version 3.0 for Windows (GraphPad Software, San Diego, CA).

**Detection of WNV in blood-fed mosquitoes.** Blood-fed mosquito specimens were also tested at CAES for the presence of WNV by virus isolation in cell culture and using a real-time RT-PCR assay described elsewhere.<sup>25</sup> Briefly, the head and thorax of individual blood-fed mosquitoes were homogenized in 1 mL of phosphate-buffered saline containing 30% heat-inactivated rabbit serum, 0.5% gelatin, and antibiotic/antimycotic by using a Mixer Mill apparatus (model MM300, Retsch Inc., Haan, Germany) as previously described.<sup>26</sup> Mosquito homogenates were centrifuged at 4°C for 10 min at 520g, and then 100  $\mu$ L of the supernatant was inoculated into a 25-cm<sup>2</sup> flask containing Vero cells growing in minimal essential media, 5% fetal bovine serum, and antibiotics/antimycotics. Cells were maintained at 37°C in 5% CO<sub>2</sub> and examined daily for cytopathic effect (CPE) 3–7 days post-inoculation. RNA was extracted from CPE-positive cell cultures by using the viral RNA Kit (Qiagen) and screened for WNV by real-time RT-PCR.<sup>25</sup>

**Detection of WNV in non-blooded mosquitoes.** The pools of non-blooded mosquitoes were divided and assayed for WNV at the MCD laboratory or at the UTMB. Methods used for virus assay at the two institutions differed and are described in an earlier publication.<sup>3</sup> However, for the purposes of this publication, the virus detection results of the two institutions were combined.

**Bird population estimates.** Frequency estimates of local avian species (Figure 2) were based on the bird population analysis, a project developed by the Cornell Laboratory of Ornithology and the National Audubon Society to track the bird abundance in North America. These frequency estimates

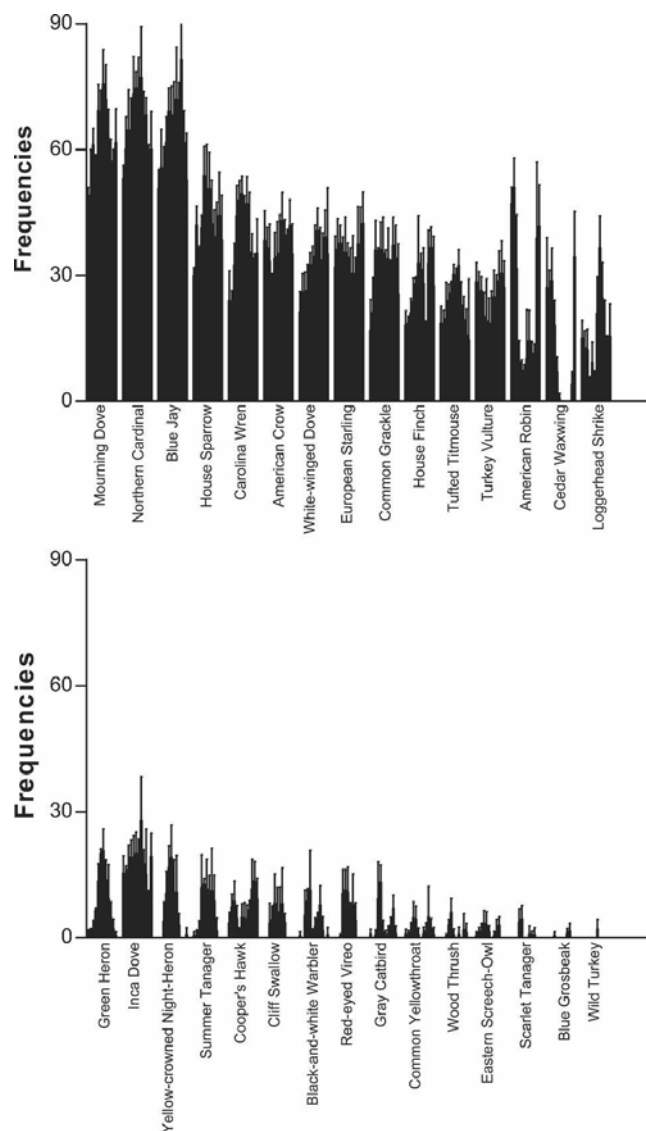


FIGURE 2. Monthly frequency estimates of local avian species determined on the basis of bird population analysis, January 2002–October 2006.

are available through World Wide Web (<http://www.ebird.org>). “Frequency” represents the percentage of checklists reporting the species within a specified date range and region. The frequency data consist of information collected on a weekly basis from January 2002 through October 2006.

**Surveillance for WNV activity in avian population.** WNV activity in the local wild bird population was monitored by two methods: antibody surveillance and testing dead birds for WNV infection. The methods were described in detail in an earlier publication.<sup>3</sup> For antibody surveillance, live birds were captured in mist nets at randomly selected sites in Harris County. Cracked corn and millet seeds were sprinkled around the nets to attract birds. After capture, birds were bled from the jugular vein (0.1–0.3 mL depending on size), identified, and released. Their sera were subsequently tested for the presence of antibodies to WNV and to SLE virus by hemagglutination-inhibition (HI) test at UTMB. Netting of wild birds was done weekly in 2005, from April until November.

After the introduction of WNV into Houston in 2002, the

MCD set up a "dead bird hotline," which has continued to the present time. Residents of the county can telephone the MCD laboratory to report a dead bird; a technician is then dispatched to pick it up. The dead birds are frozen at  $-70^{\circ}\text{C}$  and transferred weekly to the UTMB, where they are tested (culture of brain tissue) for the presence of WNV. Methods were described before.<sup>3</sup>

## RESULTS

**Mosquito collections.** During 2005, a total of 787,636 mosquitoes were collected from locations throughout Harris County, using the predetermined schedule noted before. Collections included predominantly ( $\approx 95\%$ ) *Cx. quinquefasciatus*, and intermittently *Cx. restuans*, *Cx. salinarius*, *Cx. erraticus*, and *Aedes albopictus*. A few specimens of other mosquito species, such as *Cx. nigripalpus*, *Ae. aegypti*, *Ae. taeniorhynchus*, *Ae. triseriatus*, and *Anopheles quadrimaculatus*, were also occasionally captured in the traps. The blood-fed mosquitoes examined in this study were from collections made between March 1 and November 9, 2005.

**DNA analysis.** Blood-meal sources were successfully identified by DNA sequencing from 672 out of 723 *Cx. quinquefasciatus*, of which 263 (39.1%) contained solely avian blood, 353 (52.5%) contained solely mammalian blood, and 56 (8.3%) contained both avian and mammalian blood.

An analysis of 319 avian blood-meal sources is shown in Table 1. Thirty avian species were identified as hosts for *Cx.*

*quinquefasciatus*. These birds were members of six orders, but the majority were species of Columbiformes (pigeons and doves,  $N = 166$ ) and Passeriformes (perching birds,  $N = 140$ ), which together comprised 95.9% of all avian blood meals. The remaining avian blood meals were from Strigiformes (owls) and Ciconiiformes (storks, herons, and relatives; each 1.3%,  $N = 4$ ), Falconiformes (diurnal birds of prey; 0.9%,  $N = 3$ ), and Galliformes (megapodes, curassows, pheasants, quails, and relatives; 0.6%,  $N = 2$ ). The most common avian species that served as blood sources for *Cx. quinquefasciatus* were the mourning dove, *Zenaida macroura* ( $N = 133$ , 41.7% of avian and 18.3% of total); white-winged dove, *Zenaida asiatica* ( $N = 31$ , 9.7% and 4.3%, respectively); house sparrow, *Passer domesticus* ( $N = 23$ , 7.2% and 3.2%); house finch, *Carpodacus mexicanus* ( $N = 22$ , 6.9% and 3.0%); gray catbird, *Dumetella carolinensis* ( $N = 22$ , 6.9% and 3.0%); and American robin, *Turdus migratorius* ( $N = 18$ , 5.6% and 2.5%). The remaining avian-derived blood meals ( $N = 70$ , 22% and 9.6%) were mostly from other Passeriformes birds ( $N = 55$ , 17.2% and 7.6%).

An analysis of the 409 mammalian-derived blood meals identified from *Cx. quinquefasciatus* is shown in Table 2. Thirteen different mammalian species were identified; the most common were domestic dog, *Canis familiaris* ( $N = 298$ , 73% of all mammalian blood meals and 41.0% of total), domestic cat, *Felis catus* ( $N = 64$ , 15.6% and 8.8% respectively), domestic cow, *Bos taurus* ( $N = 15$ , 3.7% and 2.1%), Virginia opossum, *Didelphis virginiana* ( $N = 15$ , 3.7% and 2.1%), and domestic horse, *Equus caballus* ( $N = 4$ , 1.0% and 0.5%).

TABLE 1

Number and percentage of avian blood meals identified from *C. quinquefasciatus* collected at sites in Harris County, Texas, in 2005

Avian host	Family	No.*	% of avian	% of total
Mourning dove, <i>Zenaida macroura</i> †,‡	Columbidae	133	41.7	18.3
White-winged dove, <i>Zenaida asiatica</i> †,‡	Columbidae	31	9.7	4.3
House sparrow, <i>Passer domesticus</i> †,‡	Passeridae	23	7.2	3.2
House finch, <i>Carpodacus mexicanus</i> †,‡	Fringilidae	22	6.9	3.0
Gray catbird, <i>Dumetella carolinensis</i> †	Mimidae	22	6.9	3.0
American robin, <i>Turdus migratorius</i> †,‡	Turdidae	18	5.6	2.5
Cedar waxwing, <i>Bombycilla cedrorum</i> †	Bombycillidae	8	2.5	1.1
Northern cardinal, <i>Cardinalis cardinalis</i> ‡	Cardinalidae	7	2.2	1.0
Summer tanager, <i>Piranga rubra</i> †	Thraupidae	7	2.2	1.0
Common grackle, <i>Quiscalus quiscula</i> ‡	Icteridae	6	1.9	0.8
European starling, <i>Sturnus vulgaris</i> ‡	Sturnidae	6	1.9	0.8
Red-eyed vireo, <i>Vireo olivaceus</i> †	Vireonidae	6	1.9	0.8
Eastern screech-owl, <i>Otus asio</i>	Strigidae	4	1.3	0.5
Green heron, <i>Butorides virescens</i>	Ardeidae	3	0.9	0.4
Loggerhead shrike, <i>Lanius ludovicianus</i> ‡	Laniidae	3	0.9	0.4
Tufted titmouse, <i>Baeolophus bicolor</i> ‡	Paridae	2	0.6	0.3
Turkey vulture, <i>Cathartes aura</i>	Cathartidae	2	0.6	0.3
Wild turkey, <i>Meleagris gallopavo</i>	Phasianidae	2	0.6	0.3
Scarlet tanager, <i>Piranga olivacea</i> †	Thraupidae	2	0.6	0.3
Carolina wren, <i>Thryothorus ludovicianus</i> ‡	Troglodytidae	2	0.6	0.3
Cooper's hawk, <i>Accipiter cooperii</i>	Accipitridae	1	0.3	0.1
Rock dove, <i>Columba livia</i> ‡	Columbidae	1	0.3	0.1
Inca dove, <i>Columbina inca</i> ‡	Columbidae	1	0.3	0.1
Blue jay, <i>Cyanocitta cristata</i> ‡	Corvidae	1	0.3	0.1
Common yellowthroat, <i>Geothlypis trichas</i>	Parulidae	1	0.3	0.1
Wood thrush, <i>Hylocichla mustelina</i> †	Turdidae	1	0.3	0.1
Black-and-white warbler, <i>Mniotilta varia</i>	Parulidae	1	0.3	0.1
Yellow-crowned Night-Heron, <i>Nyctanassa violacea</i> ‡	Ardeidae	1	0.3	0.1
Blue grosbeak, <i>Passerina caerulea</i>	Cardinalidae	1	0.3	0.1
Cliff swallow, <i>Petrochelidon pyrrhonota</i>	Hirundinidae	1	0.3	0.1

\* Includes 56 specimens from which double blood meals were identified.

† Species found with mixed blood meals.

‡ Species from which WNV has been isolated in Harris County, Texas.

TABLE 2

Number and percentage of mammalian blood meals identified from *C. quinquefasciatus* collected at sites in Harris County, Texas, in 2005

Mammalian host	Family	No.*	% of mammalian	% of total
Domestic dog, <i>Canis familiaris</i> †	Canidae	298	72.9	41.0
Domestic cat, <i>Felis catus</i> †	Felidae	64	15.6	8.8
Domestic cow, <i>Bos taurus</i> †	Bovidae	15	3.7	2.1
Virginia opossums, <i>Didelphis virginiana</i> †	Didelphidae	15	3.7	2.1
Domestic horse, <i>Equus caballus</i>	Equidae	4	1.0	0.5
Human, <i>Homo sapiens</i> †	Hominidae	3	0.7	0.4
Black rat, <i>Rattus rattus</i>	Muridae	3	0.7	0.4
White-tailed deer, <i>Odocoileus virginianus</i> †	Cervidae	2	0.5	0.3
Goat, <i>Capra hircus</i>	Bovidae	1	0.2	0.1
Northern raccoon, <i>Procyon lotor</i>	Procyonidae	1	0.2	0.1
Eastern fox squirrel, <i>Sciurus niger</i>	Sciuridae	1	0.2	0.1
Pig, <i>Sus scrofa</i>	Suidae	1	0.2	0.1
Swamp rabbit, <i>Sylvilagus aquaticus</i>	Leporidae	1	0.2	0.1

\* Includes 56 specimens from which double blood meals were identified.

† Species found with mixed blood meals.

Three human-derived blood meals (0.7% of all mammalian blood meals and 0.4% of total) were also identified.

With one exception (in May), the proportions of avian- and mammalian-derived blood meals identified from *Cx. quinquefasciatus* collected during the spring and summer months (March–August) were nearly equal (Figure 3). However, a pronounced seasonal shift from avian to mammalian hosts was detected during the late summer and fall (September to November). The  $\chi^2$  test for linear trend showed that the proportion of avian-derived blood meals decreased significantly ( $P < 0.0001$ ) from June until November. In September, the ratio changed to 27.2% avian and 72.8% mammalian, and by October and November, only 13.3% and 12.5% of the total respective blood meals were avian-derived whereas 86.7% and 87.5% were of mammalian origin.

**Avian population analysis.** Analysis of the seasonal abundance and frequencies of wild birds in Harris County is shown in Figure 2. Certain birds, such as the mourning dove, the northern cardinal, and the blue jay, are abundant throughout the year. We compared the proportion of *Cx. quinquefasciatus* blood meals from a specific avian species with the frequency (abundance) of that species' in this region. The proportion of mosquitoes that had fed on such birds as mourning dove, white-winged dove, and other members of the family Columbidae was as expected on the basis of the abundance of these birds. However, for certain other birds, particularly the blue jay, it was substantially lower than would be expected, on the basis of their estimated frequencies (Figure 2) and on the number of blue jays netted during serosurveys or found dead by local residents.

**Virus isolations from blooded mosquitoes.** WNV was detected in the head and thorax of three blood-fed mosquitoes, suggesting disseminated infection. The sources of blood meals, dates, and collection sites for these 3 WNV-positive mosquitoes were white-winged dove, collected on 11 August within the City of Houston; house sparrow, collected on 18 August from northwest Harris County; and mourning dove collected on 1 September from northwest Harris County.

**Virus recoveries from non-blooded mosquitoes.** During 2005, a total of 391,533 *Culex* mosquitoes (> 98% *Cx. quinquefasciatus*) were assayed for WNV at the MCD and UTMB. Average pool size was 21.4 mosquitoes. From this total, 698 WNV-positive pools were obtained. Most (99.0%) of the

WNV-positive mosquitoes were collected between June and September. These are the four hottest months of the year in Harris County. These data will be presented in more detail in a forthcoming paper.

**Virus isolations from dead birds.** During 2005, a total of 1,334 dead birds from Harris County were processed for WNV. Of this number, 168 birds (12.6%) yielded WNV upon culture. As observed with the mosquitoes, most (91.6%) of the WNV-infected dead birds were also found between the months of June and September. Table 3 shows the WNV isolation rates for the most commonly collected dead bird species. Two species, blue jay and American crow, accounted for 82% of the WNV-positive dead birds.

**Prevalence of WNV antibodies in wild birds.** During 2005, a total of 2,797 wild birds were netted and bled for antibody testing (Table 3). Overall, 312 birds (11.2%) had HI antibodies to WNV. The prevalence of WNV antibodies in the various avian species had little relation to the percentage of dead birds of the same species yielding WNV. Forty-eight percent of 273 dead blue jays were infected with WNV, but only 19.3% of netted (living) blue jays had WNV antibodies. In contrast, comparable WNV antibody rates were found in net-

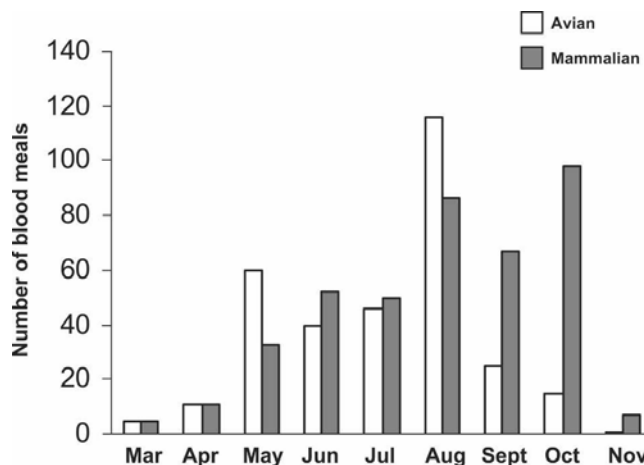


FIGURE 3. Number of identified *Cx. quinquefasciatus* blood meals obtained from avian and mammalian hosts in Harris County, Texas, during 2005.

ted mourning doves, white-winged doves, robins, grackles, starlings, and wrens (17.7%, 15.2%, 22.2%, 11.1%, 12.2%, and 18.7%, respectively), but few if any of these dead birds yielded WNV after culture. These data collectively suggest that many blue jays are infected with the virus but relatively few survive and that WNV infection is less lethal to other species.

## DISCUSSION

It is apparent from our investigation that *Cx. quinquefasciatus* populations from the Houston metropolitan area, located in the south central Gulf region of the United States, use a wide range of vertebrate hosts and indiscriminately feed on both birds and mammals. Overall, 39% of the engorged mosquitoes had acquired blood from birds, 53% from mammals, and 8% from both avian and mammalian hosts. This behavioral characteristic would clearly facilitate transmission of WNV to incidental hosts of concern, including equines and humans, both of which were identified as blood-meal sources, albeit at relatively low prevalence rates. Our findings are consistent with previous studies from other geographic locales that have examined the host feeding preferences of *Cx. quinquefasciatus*: (1) Tucson, Arizona—32% avian and nearly 65% from mammalian hosts, including humans (50%), dogs (12%), and cats (< 3%)<sup>19</sup>; (2) Sao Paulo, Brazil—22% avian and 70% mammalian<sup>17</sup>; (3) Northern Queensland, Australia—29.7% avian and 62.9% mammalian.<sup>27</sup> In contrast, a report from southern Australia found that 70% of the blood meals for this mosquito species were derived from avian hosts and 24% were from mammals,<sup>28</sup> while in another study in southwestern Queensland, Australia, 79% of the identified

blood meals were of avian and only 21% were of mammalian origin.<sup>9</sup> A large study of blood-fed *Cx. quinquefasciatus* ( $N = 10,769$ ) on Oahu island, Hawaii, similarly found that 69% had acquired blood from birds and 31% had fed on mammals.<sup>5</sup> These widely divergent results with regard to the ratio of mammal and bird feedings indicate that populations of *Cx. quinquefasciatus* exhibit considerable variation in blood feeding behavior and are much more opportunistic than *Cx. pipiens*, which in North America is predominantly a bird feeder.<sup>22,29,30</sup> Host availability plays an important role in feeding behavior of mosquitoes in general; however, further experiments are needed to determine the exact role of all contributing factors to the seasonal variation in host feeding patterns of *Cx. quinquefasciatus* noticed in this study.

Our current study revealed that *Cx. quinquefasciatus* in Harris County had acquired blood meals from 30 different avian species, representing 6 orders, primarily Columbiformes and Passeriformes. Virtually all avian host species identified as blood-meal sources in this study have been reported from Harris County, based on direct observation, mist netting records, or bird frequency data.

Columbiformes comprised > 52% of all avian-derived blood meals in our study, and the mourning dove and white-winged dove represented 41.7% and 9.7% of all avian-derived blood meals, respectively. The predominance of mourning doves and white-winged doves suggests an opportunistic feeding behavior for *Cx. quinquefasciatus*. The potential role that these two bird species may play in enzootic cycling of the WNV is unclear. Reservoir competence value, expressed as the duration and magnitude of infectious-level viremias, for the mourning dove have been reported to be relatively low during an experimental infection of birds with the New York

TABLE 3  
WNV antibody rates among netted live birds and WNV recovery rates from dead birds collected in Harris County, Texas, in 2005

Bird identifications Common name (genus and species)	Netted live birds		Dead birds	
	Number tested	WNV antibody rate (%)	Number tested	WNV recovery rate (%)
Mourning dove ( <i>Zenaida macroura</i> )	226	17.7	210	1.9
White-winged dove ( <i>Zenaida asiatica</i> )	46	15.2	66	0
House sparrow ( <i>Passer domesticus</i> )	1,903	8.0	103	17.5
House finch ( <i>Carpodacus mexicanus</i> )	18	5.5	4	0
Gray catbird ( <i>Dumetella carolinensis</i> )	3	0	3	0
American robin ( <i>Turdus migratorius</i> )	18	22.2	29	0
Cedar waxwing ( <i>Bombycilla cedrorum</i> )	0	0	0	0
Northern cardinal ( <i>Cardinalis cardinalis</i> )	201	27.4	35	5.7
Summer tanager ( <i>Piranga rubra</i> )	2	0	0	0
Common grackle ( <i>Quiscalus quiscula</i> )	72	11.1	54	0
European starling ( <i>Sturnus vulgaris</i> )	41	12.2	44	2.3
Red-eyed vireo ( <i>Vireo olivaceus</i> )	0	0	1	0
Eastern screech-Owl ( <i>Otus asio</i> )	1	0	6	0
Green heron ( <i>Butorides virescens</i> )	0	0	2	50.0
Loggerhead shrike ( <i>Lanius ludovicianus</i> )	2	50.0	4	50.0
Tufted titmouse ( <i>Baeolophus bicolor</i> )	1	0	2	0
Turkey vulture ( <i>Cathartes aura</i> )	1	0	0	0
Wild Turkey ( <i>Meleagris gallopavo</i> )	0	0	0	0
Scarlet tanager ( <i>Piranga olivacea</i> )	2	0	0	0
Carolina wren ( <i>Thryothorus ludovicianus</i> )	16	18.7	11	0
Blue jay ( <i>Cyanocitta cristata</i> )	83	19.3	273	48.0
American crow ( <i>Corvus brachyrhynchos</i> )	0	0	7	85.7
Cooper's hawk ( <i>Accipiter cooperii</i> )	0	0	2	0
Rock dove ( <i>Columba livia</i> )	2	50.0	28	0
Inca dove ( <i>Columbina inca</i> )	31	6.4	21	0
Northern mockingbird ( <i>Mimus polyglottos</i> )	43	23.2	68	2.9

1999 strain of West Nile virus. Field-derived information in conjunction with experimental infection studies are required to fully evaluate the importance of reservoir hosts in a specific region.<sup>31</sup> During WNV surveillance in New York State in 2000, 19% of the dead mourning doves tested ( $N = 77$ ) were reported to be WNV-positive.<sup>32</sup> In our study, only 1.9% of 210 dead mourning doves and none of 66 white-winged doves collected in Harris County were WNV-positive. In contrast, the prevalence of WNV antibodies was 17.7% for the mourning dove and 9.7% for the white-winged dove (Table 3). In view of the limited information on the infectious threshold of host viremia and the actual number of virions needed in a blood meal to infect a susceptible mosquito, it may be imprudent to assume that low doses of virus may not result in vector infection and transmission.<sup>33,34</sup>

Nearly 44% of the avian-derived blood meals from *Cx. quinquefasciatus* were determined to be from Passeriformes, including house sparrow, house finch, gray catbird, and American robin. Passerine birds appear to be important reservoir and amplifying hosts for WNV, as reservoir competence values for the common North American Passeriformes are high.<sup>31</sup> Our combined seroprevalence and mortality data (Table 3) implicate passerines, such as blue jays, American crows, house sparrows, house finches, robins, grackles, and cardinals, as important reservoir hosts in Harris County on the basis of their relatively high WNV infection rates and abundance.

The test results with blue jays are noteworthy (Table 3). Blue jays accounted for 78% of all the WNV-positive birds collected in Harris County in 2005. A similar pattern has been observed each year since 2002 in the county WNV surveillance program. During the 5-year period from 2002 to 2006, blue jays comprised 80% of all dead WNV-positive birds ( $N = 1,094$ ) submitted to MCD for examination (R.B. Tesh, unpublished data). Likewise, 19% of 305 live blue jays netted in Harris County during the same period had antibodies to WNV. Yet the proportion of *Cx. quinquefasciatus* that had fed on blue jays (0.3% of all avian feedings and only 0.1% of total) was much lower than would be expected on the bases of frequency data and abundance of blue jays locally.

A similar situation has been observed with another corvid, the American crow. No crow feedings were observed in our study of *Cx. quinquefasciatus* blood meals. Three recent studies that analyzed blood meals of *Cx. pipiens* in the northeastern United States<sup>22,30,35</sup> have likewise reported negligible feeding on crows. Crows are abundant in Harris County and most other regions of the United States, and they also exhibit high mortality after WNV infection.<sup>31,36–38</sup> Like blue jays, they are frequently infected with WNV; but crows do not seem to be a preferred host of these mosquitoes. Then how do so many crows and blue jays get infected with WNV? One plausible explanation could be that corvids acquire the virus by some mechanism in addition to the bite of an infected mosquito, as suggested previously.<sup>22</sup> Crows and blue jays may acquire WNV infection by eating the carcasses of other infected birds. This is the likely route of infection of some raptors (i.e., hawks and owls). Komar and others<sup>31</sup> demonstrated experimentally that American crows fed WNV-infected mice became infected and that many of the infected birds died. Oral infection with WNV has also been demonstrated in other vertebrates as well.<sup>39,40</sup> Blue jays and American crows are omnivorous birds; crows feed on carrion and animal car-

casses, and both crows and blue jays will aggressively attack and eat nestling birds and small mammals.<sup>41</sup> Both bird species may be naturally infected orally by eating sick or dead WNV-infected animals.

Nearly 60% of all identified *Cx. quinquefasciatus* blood meals (including the mixed feedings) contained mammalian blood. Yet of the total, only 3 (0.7%) contained human blood, despite the fact that humans are the most abundant large mammal in the county. These data are compatible with the reported incidence of clinical WNV infection (West Nile fever and neuroinvasive disease) among humans living in Harris County. During 2005, a total of 42 confirmed cases of clinical WNV infection were reported in Harris County. Even allowing for the large percentage ( $\approx 80\%$ ) of asymptomatic human infections that occur with WNV infection,<sup>42</sup> one would expect more clinical disease in a population of  $> 3.5$  million if many people were being bitten by infected *Cx. quinquefasciatus*.

The Harris County blood-meal data and the relative paucity of human cases could be interpreted as indicating that local *Cx. quinquefasciatus* are not very attracted to humans. However, a more likely explanation is that people in Harris County are less exposed to mosquitoes during summer and the period of peak *Cx. quinquefasciatus* and WNV activity. The 4 months of maximum *Cx. quinquefasciatus* density and of most WNV activity in Harris County (June–September) are also the hottest, so many people stay indoors in air-conditioned facilities after dusk, when these mosquitoes are actively feeding. Studies of host feeding patterns of *Cx. quinquefasciatus* in other regions of the United States and the world indicate that this mosquito species readily feeds on humans when accessible.<sup>12,13,17–19</sup>

The mammalian species most frequently identified as a host of *Cx. quinquefasciatus* in the present study was the domestic dog. Dogs accounted for 72.9% of all mammal feedings and 41% of total vertebrate feedings by *Cx. quinquefasciatus* in Harris County. This is credible because dogs are common in Harris County, and the prevalence of WNV infection in local dogs is high. In 2003, 1 year after WNV appeared in Harris County, serum samples from 154 stray dogs ( $> 1$  year of age) were tested for WNV antibody. In that sample, 56.5% of the dogs had HI antibodies to WNV.<sup>3</sup> In the fall of 2006, another 81 canine sera (mixed breeds and  $> 6$  months of age) obtained from local veterinarians were similarly examined; 87.7% of those dogs had WNV antibody by HI test (J. Dennett and R. Tesh, unpublished data). The high antibody prevalence indicates that many dogs in Harris County are infected by WNV, presumably from the bite of infected mosquitoes. A retrospective serologic survey of dogs in New York City after the 1999 outbreak revealed that 10% of local dogs had been infected with WNV.<sup>43</sup> Serological surveys of dogs in the Middle East and Africa also indicate that dogs are frequently infected with WNV.<sup>44,45</sup> Despite these relatively high infection rates, however, dogs are not thought to be important amplifying hosts of WNV. Several studies of experimental WNV infection of canines indicate that dogs, like humans and horses, develop a rather low level and transient viremia after infection.<sup>45–47</sup>

Cats were another mammalian host frequently used as a blood source by *Cx. quinquefasciatus* ( $N = 64$  or 8.8% of total blood meals) in Harris County. Relatively little is known about the pathogenesis of WNV in cats or their potential role in the ecology of WNV. In one reported study, 8 cats were

experimentally infected with WNV by mosquito bite; four of the animals became viremic with peak titers from  $10^3$  to  $10^4$  plaque forming units/mL.<sup>46</sup> Three of the experimentally infected animals developed neurologic signs of disease. Because of the relatively mild climate and open spaces in Harris County, many pet cats and dogs spend a considerable portion of their life outdoors. Thus they are probably much more accessible to blood-seeking *Culex* mosquitoes than humans. In addition, cats are notorious predators of small birds. A bird weakened by WNV would be easy prey for a stalking cat, so oral infection may be another route for cats to be infected.

The preponderance of WNV-infected *Cx. quinquefasciatus* identified in our surveillance activities until now clearly incriminates this mosquito species as the dominant arthropod vector of the virus in Harris County.<sup>3</sup> Between June and September of 2005, a total of 691 WNV-positive pools were identified from 226,880 *Culex* mosquitoes (> 95% *Cx. quinquefasciatus*) also collected and tested. The WNV minimum field infection rate during this 4-month period was 3.0 per 1,000 females. Vector competence studies with *Cx. quinquefasciatus* have shown that it is a competent vector of WNV.<sup>48–51</sup> The results of our blood-meal identifications indicate that *Cx. quinquefasciatus* is an opportunistic mosquito that readily feeds on a variety of birds and mammals, including humans. On the bases of its abundance, feeding habits, and relatively high WNV infection rate, we conclude that *Cx. quinquefasciatus* is the principal vector of WNV in this region, although further research is needed to determine the role of other mosquito species in transmission of the virus. Nevertheless, the mosquito surveillance and control program of the MCD is largely focused on *Cx. quinquefasciatus*.

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