Identification of blood meal sources in Aedes vexans and Culex quinquefasciatus in Bernalillo County, New Mexico

Jacob A. Greenberg¹a, Daniel A. Lujan¹b, Mark A. DiMenna²c, Helen J. Wearing¹d, Bruce V. Hofkin¹e

¹Department of Biology, University of New Mexico, 167 Castetter Hall MSC03 2020, Albuquerque, New Mexico 87131-0001
²Urban Biology Division, City of Albuquerque Environmental Health Department, P.O. Box 1293, Albuquerque, New Mexico 87103

Abstract

Culex quinquefasciatus Say (Diptera: Culicidae) and Aedes vexans Meigen are two of the most abundant mosquitoes in Bernalillo County, New Mexico, USA. In this study, a polymerase chain reaction based methodology was used to identify the sources of blood meals taken by these two species. Ae. vexans was found to take a large proportion of its meals from mammals. Although less specific in terms of its blood meal preferences, Cx. quinquefasciatus was found to feed more commonly on birds. The results for Ae. vexans are similar to those reported for this species in other parts of their geographic range. Cx. quinquefasciatus appears to be more variable in terms of its host feeding under different environmental or seasonal circumstances. The implications of these results for arbovirus transmission are discussed.

Keywords: host feeding, mosquito, West Nile virus, arbovirus
Abbreviations: PCR, polymerase chain reaction; WNV, West Nile Virus
Correspondence: a jgreenbe@unm.edu, b dlujan5@unm.edu, c mdimenna@cabq.gov, d hwearing@unm.edu, e brunoh@unm.edu
*Corresponding author
Received: 7 February 2012 Accepted: 10 October 2012
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ISSN: 1536-2442 | Vol. 13, Number 75

Cite this paper as: Greenberg JA, Lujan DA, DiMenna MA, Wearing HJ, Hofkin BV. 2013. Identification of blood meal sources in Aedes vexans and Culex quinquefasciatus in Bernalillo County, New Mexico. Journal of Insect Science 13:75. Available online:
http://www.insectscience.org/13.75
Introduction

Mosquito blood meal identification has been used to better understand the feeding behaviors of various mosquito species in many areas across the United States and the world (Irby and Apperson 1988; Zinser et al. 2004; Gingrich and Williams 2005; Molaei and Andreadis 2006; Molaei et al. 2006, 2007; Kay et al. 2007; Garcia-Rejon et al. 2010; Sawabe et al. 2010; Barrera et al. 2011). Here, the results of a similar study, using a polymerase chain reaction (PCR) based methodology designed to assess the host feeding patterns of *Aedes vexans* Meigen (Diptera: Culicidae) and *Culex quinquefasciatus* Say in Bernalillo County, New Mexico, USA, are presented.

*Ae. vexans* and *Cx. quinquefasciatus* are two of the most abundant mosquito species in Bernalillo County (DiMenna et al. 2006). *Ae. vexans* mosquitoes are known to feed aggressively on mammals, including humans (Tempelis et al. 1965; Cupp and Stokes 1973; Magnarelli 1977a, b; Ritchie and Rowley 1981; Nasci 1984; Irby and Apperson 1988; Apperson et al. 2002, 2004; Lee et al. 2002; Hassan et al. 2003; Gingrich and Williams 2005; Molaei et al. 2006). *Cx. quinquefasciatus*, alternatively, is less specific in its feeding, but typically takes a majority of its blood meals from avian hosts. (Bohart et al. 1978; Kilpatrick et al. 2006; Savage et al. 2007; Garcia-Rejon et al. 2010). In some cases, *Cx. quinquefasciatus* feeds equally on both birds and mammals, or can show a mammalian bias. (Zinser et al. 2004; Kay et al. 2007; Molaei et al. 2007; Muturi et al. 2008; Swabe et al. 2010).

Both of these mosquito species are known arbovirus vectors of medical and veterinary importance. *Ae. vexans* is known to transmit St. Louis encephalitis and Western Equine Encephalitis, and is a potential bridge vector for West Nile virus (WNV) (Turell et al. 2001; Cupp et al. 2004; Kilpatrick et al. 2005; DiMenna et al. 2006; Molaei and Andreadis 2006; Blitvich 2008). Likewise, *Cx. quinquefasciatus* is known to be a highly competent vector for WNV in North America, and because of its strong tendency to feed on birds, it may play a particularly important role in viral amplification. (Andreadis et al. 2001; Bernard et al. 2001; Kulasekera et al. 2001; Nasci et al. 2001; Sardelis et al. 2001; Turell et al. 2001; White et al. 2001; Goddard et al. 2002; Turell et al. 2002, 2005; Anderson et al. 2004; Andreadis et al. 2004; Solomon 2004; Ebel et al. 2005; Kilpatrick et al. 2005; Hayes and Gubler 2006). Both of these mosquito species, along with a third species, *Culex tarsalis*, have tested positive for WNV infection in Bernalillo County (DiMenna et al. 2006).

Blood meal source, along with other aspects of mosquito biology such as biting frequency, dispersal ability, and local abundance, are important components of vector capacity (Seagerman et al. 2008; Chaves et al. 2010). A greater understanding of host utilization by mosquitoes in Bernalillo County can help clarify the dynamics of local arbovirus transmission, specifically the amplification cycle of vector-borne viruses in their natural hosts, and their transmission to humans and other domestic animals. Such data might consequently lead to more effective and focal vector control.

Materials and Methods

Bernalillo County, in central New Mexico, includes the greater Albuquerque metropolitan area, which accounts for 32.5% of the state’s population (City of Albuquerque 2010). The Rio Grande flows directly through the county.
from north to south, forming the Rio Grande Valley. Much of the valley retains a rural character. The riverbanks are generally heavily wooded and form a riparian forest known as the Rio Grande Bosque. Outside of Albuquerque, the lands adjacent to the Bosque are often devoted to agriculture or grazing. Most mosquito activity, and consequently most arbovirus transmission in Bernalillo County, occurs in the Rio Grande Valley (DiMenna et al. 2007). Over 50 mosquito species have been collected in New Mexico (Wolff and Nielsen 2007). This study focuses on Cx. quinquefasciatus and Ae. vexans, two of the most common species in central New Mexico.

Mosquito collection, identification, and WNV screening
Mosquitoes were collected weekly throughout the mosquito season (May through October) from 2006 through 2010. Each year, between 18 and 22 trapping sites were established along the Rio Grande Bosque in Bernalillo County (Figure 1). Two types of mosquito trap were used at each site. CDC light traps were suspended 1.5 m from the ground and baited with a thermos canister containing approximately 1.5 kg of dry ice, and gravid traps were baited with non-chlorinated water infused with horse manure, grass clippings, and bacterial culture (Pro-pump Liquid Live Bacteria High Count, Ecological Laboratories, www.microbelift.com), which was allowed to ferment for two weeks. Traps were set in the late afternoon, left overnight, and collected the following morning. Collected mosquitoes were immediately placed on dry ice and were subsequently stored at -80°C. Mosquitoes were identified to species using dichotomous keys (Carpenter and Lacasse 1955; Pratt and Barnes 1959; Darsie and Ward 1981). Blood-engorged mosquitoes were set aside for blood meal analysis. Mosquitoes not clearly engorged with a blood meal were tested for WNV as described by Lanciotti et al. (1999). Maximum likelihood estimate of infection and their 95% confidence intervals were determined using the Biggerstaff (2007) Add-in for Microsoft Excel (www.microsoft.com). Relative abundance of each mosquito species was calculated by dividing the number of each species collected by the combined total of collected mosquitoes for each collection season.

Blood meal analysis
Blood-engorged mosquitoes were placed individually on a microscope slide under a dissecting microscope. The midgut and abdomen were removed using a razor blade and sterile forceps. A new slide and blade were used for each mosquito. Genomic DNA was then extracted from each midgut and abdomen with a modified DNAzol BD (Molecular Research Center, www.mrgene.com) procedure as previously described by Molaei et al. (2006).

The source of each blood meal was determined by subjecting each sample of genomic DNA to two separate PCR reactions, one to identify mammalian and one to identify avian DNA. Mammalian blood meals were identi-
fied using mammalian-specific primer pairs for a 772 bp portion of the *cytochrome b* gene (Ngo and Kramer 2003). Likewise, avian blood meals were identified by amplifying a 508 bp fragment of the *cytochrome b* gene with the avian-specific primers (Cisneros and Johnson 2001). Each 50 µl reaction contained 300–400 ng of genomic DNA serving as template, 5 µl of 10x buffer (Roche Applied Science, www.roche-applied-science.com), 8µl dNTPs (200 µM of each; Applied Biosystems, www.invitrogen.com), 8 µL MgCl₂ (4 mM; Roche Applied Science), 5 µl forward primer (0.5 µM), 5 µl reverse primer (0.5 µM), and 0.25 µl TaqGold Polymerase (1.25 U per reaction; Roche Applied Science). Sterile water (Sigma-Aldrich, www.sigmaaldrich.com) was added to bring the total reaction volume to 50 µl. Primer sequences and cycling conditions have been previously published by Greenberg et al. (2012). A negative water control lacking template was included with all PCR reactions. A second negative control consisted of a DNAzol extract lacking mosquito midgut. All reactions also included a positive control containing either mammalian (*Mus musculus*) or avian (*Zenaida macroura*) genomic DNA serving as a template. If a sample did not amplify, a second PCR reaction was performed. After two failed amplifications, the sample was archived in a -80º C freezer.

Amplified PCR products were purified with one of several methods, including a size select e gel (Invitrogen, www.invitrogen.com), PCR purification kit (Qiagen, www.qiagen.com), minielute column (Millipore, www.millipore.com), or exo-sap (Affymetrix, www.affymetrix.com). Amplicons were directly sequenced with a Big Dye 3.1 sequencing kit, using the big dye step protocol PCR regime (Platt et al. 2007). Samples were then sequenced on an ABI 3130 DNA Sequencer (Applied Biosystems, Foster City, CA, USA) at the University of New Mexico, Department of Biology Molecular Facility. Sequences were edited using Sequencher version 4.10.1 (Gene Codes, www.genecodes.com) and identified to species through a BLAST search comparison with the GenBank DNA database (www.ncbi.nlm.nih.gov/blast/Blast.cgi). Those comparisons with a blast error value < 1e⁻²⁰ were included in our analysis.

### Statistical analysis

To determine if each of the two mosquito species under investigation were more likely to feed on either mammalian or avian hosts, all successfully identified blood meals for each species were scored as either “mammalian” or “avian” and subjected to a z-test to determine significant deviation from 50/50 mammalian/avian feeding for the mosquitoes collected in this study. Confidence intervals of 95% were found for each species’ tendency to feed on either mammalian or avian hosts, and the species were compared for overlap. Calculations with non-overlapping 95% confidence intervals and found to be two standard deviations from the null hypothesis of no deviation were considered to be significant.

### Results

A total of 75,619 mosquitoes from 24 species were collected from 2006–2010. Of these mosquitoes, 55,871 (73.9% of total) were *Ae. vexans*, while 9,853 (13.0% of total) were *Cx. quinquefasciatus* (Figure 2). These two species accounted for 65,724 (86.9%) of the total number of mosquitoes collected. The maximum likelihood estimate of infection of *Ae. vexans* and *Cx. quinquefasciatus* were found to be .05/1000 (95% confidence interval of 0.00-0.25) and 1.77/1000 (95% confidence interval of 0.97-3.01) respectively.
A total of 309 out of 337 amplified blood meals (91.7%) were successfully identified to species of origin (Tables 1, 2). Of these, 37 (11.2%) were identified as exotic animals housed in the Rio Grande Zoo. *Ae. vexans* consistently fed largely on mammalian hosts, most commonly cows, horses, and cottontail rabbits (Figure 3). Of the 213 successfully identified blood meals from this species, 206 (96.7 ± 2.4%) were identified as mammalian in origin, while 7 (3.3 ± 2.4 %) were avian. The majority of identified *Cx. quinquefasciatus* blood meals were consistently taken from avian hosts, the most common of which were American robins, house sparrows, and mourning doves (Figure 4). Of the 96 successfully identified blood meals, 77 (80.2 ± 7.9%) were avian in origin, while 19 (19.8 ± 7.9%) were mammalian. No mixed mammalian and avian meals were identified for either species.

**Discussion**

In this study, a PCR-based method was used to identify the blood meal sources of two of the most common mosquito species in Bernalillo County, New Mexico. The results largely confirm those of Loftin et. al. (1997), who al-
fied as mammalian in origin, thus confirming Loftin’s lab-reared mosquito results with field-collected mosquitoes.

Furthermore, the results are in general agreement with those of others who have considered the feeding behavior of *Ae. vexans* in other geographic areas. Across its geographic range, this species shows a strong tendency to feed on mammals. *Cx. quinquefasciatus*, however, may be more strongly influenced by local or seasonal conditions. In Harris County, Texas, and in Tucson, Arizona, for instance, it was found that *Cx. quinquefasciatus* fed more frequently on mammals (Zinser et al. 2004; Molaei et al. 2007), while in Yucatan, Mexico, this species tended to feed on birds (Garcia-Rejon et al. 2010).

The factors that influence the feeding behavior of *Cx. quinquefasciatus* await elucidation. One possible contributing factor may be the seasonal shift in hosts that some species, including *Cx. quinquefasciatus*, have been shown to undergo in other regions. For example, in Harris County, Texas, *Cx. quinquefasciatus* feeds primarily on birds early in the mosquito season. As the season progresses, mammals make up an increasingly larger proportion of their blood meals (Molaei et al. 2007). There are not as yet sufficient data to determine whether or not a similar feeding shift occurs in Bernalillo County. If, however, such a shift is a general feature of *Cx. quinquefasciatus* feeding biology, it may at least in part explain the variable feeding behavior that has been described for this species in different parts of its geographic range.

The data also highlight the fact that neither *Cx. quinquefasciatus* nor *Ae. vexans* are strongly host-specific towards a particular species. *Cx. quinquefasciatus*, which was found to more commonly feed on birds, utilizes a wide range of avian species as sources for blood meals. Likewise, *Ae. vexans*, which fed almost exclusively on mammals, takes blood from a variety of mammalian hosts. Even exotic species housed at the Rio Grande Zoo, where several of the mosquito trapping sites were located, served as blood meal sources. Many of these species are rarely if ever normally encountered in their natural habitat by mosquitoes native to New Mexico. Furthermore, without data on the numbers of potential avian and mammalian hosts in a particular collection area, it is premature to suggest that the results indicate feeding preferences on the part of the mosquitoes that were collected. However, given the widely different feeding patterns observed in mosquitoes from the same collection sites, it seems unlikely that the observed patterns merely reflect host availability. Because *Cx. quinquefasciatus* occasionally feeds on reptiles and amphibians (Savage et al. 2007), at least some of the failed amplifications may reflect the fact that blood meals were of neither mammalian nor avian origin. Such blood meals, however, are likely to be infrequent, and this possibility would not have substantially altered the results.

Because the source of its blood meals is an important component of a particular mosquito species’ vector capacity, the results may help to clarify the roles of *Cx. quinquefasciatus* and *Ae. vexans* in arbovirus transmission in central New Mexico. Both of these species have regularly tested positive for WNV in Bernalillo County (DiMenna et al. 2006). Elsewhere in North America, *Culex* spp. mosquitoes, in particular *Cx. quinquefasciatus*, *Cx. restuans*, *Cx. pipiens*, and *Cx. tarsalis*, have been implicated as the most important WNV vectors (Andreadis et al. 2001; Bernard et al. 2001; Kulasekera et al. 2001; Nasci et al.
Turell et al. (2005) have also demonstrated WNV vector competence for *Ae. vexans*. Because *Ae. vexans* shows such a strong tendency to feed on mammals, it may play an especially important role in bridge transmission to mammals, including humans and horses. It is important to note, however, that *Ae. vexans* is only very rarely found to be infected with WNV in local collections, making its role in WNV transmission questionable. Alternatively, *Cx. quinquefasciatus*, with a significant tendency to feed on birds, may be especially important in viral amplification in avian reservoir hosts such as the American robin (*Turdus migratorius*) (Hamer et al. 2009). A third mosquito species, *Cx. tarsalis*, has also tested positive for WNV in Bernalillo County (DiMenna et al. 2006). Because *Cx. tarsalis* is relatively uncommon at the trapping sites in our study, there are correspondingly few identified blood meals from this species. Determination of this species’ host preference awaits further results.

If the findings regarding the feeding behavior of *Cx. quinquefasciatus* and *Ae. vexans*, along with similar data for *Cx. tarsalis*, can be considered with other aspects of vector capacity, it may be possible to use such information to more effectively control arbovirus transmission. These results increase the understanding of the vector-host relationship in the Bernalillo County area, and provide a more definitive picture of the dynamics of WNV transmission in this environment. Existing knowledge of seasonal trends in abundance and spatial distribution of these species, as well as active surveillance, could help determine the most effective allocation of mosquito control resources in order to provide the most effective control measures possible. As additional data becomes available, future research will be directed towards determining how blood meal sources may vary throughout the mosquito season in this study area, providing further opportunities to refine mosquito control strategies and improve the understanding of local mosquito ecology.

**Acknowledgements**

We gratefully thank Gourdarz Molaei of the Connecticut Agricultural Experiment Station, New Haven, CT, for technical assistance and advice. We also thank Coen Adema and Ben Hanelt, of the Department of Biology, University of New Mexico for their contributions. The blood meal analysis was conducted in the laboratories of Robert Miller and Charles Cunningham of the University of New Mexico Biology Department. Mosquito collections were provided by the City of Albuquerque Urban Biology Division, supported in part by the New Mexico Department of Health. This project was supported in part by a grant from the National Institute of General Medical Sciences award number T34GM00851. The content is solely the responsibility of the authors and does not necessarily represent the official views of the National Institute of General Medical Sciences or the National Institute of Health. Additional support was provided by the New Mexico Horse Council and by the University of New Mexico, Research Allocation Committee, Grant # 06-32.

**References**

Andreadis TG, Anderson JF, Vossbrinck CR. 2001. Mosquito Surveillance for West Nile Virus in Connecticut, 2000: Isolation from *Culex pipiens, Cx. restuans, Cx. salinarius,* and *Culiseta melanura.* Emerging Infectious Disease 7(4): 670–674.
Apperson CS, Harrington BA, Unnasch TR, Hassan HK, Irby WS, Savage HM, Aspen SE, Watson DW, Rueda LM, Engber BR, Nasci RS. 2002. Host-Feeding Habits of Culex and Other Mosquitoes (Diptera: Culicidae) in the Borough of Queens in New York City, with Characters and Techniques for Identification of Culex Mosquitoes. *Journal of Medical Entomology* 39(5): 777–785.

Apperson CS, Hassan HK, Harrison BA, Savage HM, Aspen SE, Farajollahi A, Crans W, Daniels TJ, Falco RC, Benedict M, Anderson M, McMillen L, Unnasch TR. 2004. Host Feeding Patterns of Established and Potential Mosquito Vectors of West Nile Virus in the Eastern United States. *Vector Borne and Zoonotic Diseases* 4(1): 71–82.

Barrera R, Amador M, Young G, Komar N. 2011. Mosquito (Diptera: Culicidae) bloodmeal sources during a period of West Nile virus transmission in Puerto Rico. *Journal of Medical Entomology* 48(3): 701–704.

Bernard KA, Maffei JG, Jones SA, Kauffman EB, Ebel GD, Dupuis AP, Ngo KA, Nicholas DC, Young DM, Shi PY, Kulasekera VL, Eidson M, White DJ, Stone WB, Kramer LD. 2001. West Nile virus infection in birds and mosquitoes, New York State, 2000. *Emerging Infectious Disease* 7(4): 679–685.

Biggerstaff BJ. 2007. *PooledInfRate, version 3.0: a Microsoft Excel add-in to compute prevalence estimates*. Center for Disease Control and Prevention.

Blitvich BJ. 2008. Transmission dynamics and changing epidemiology of West Nile virus. *Animal Health Research Reviews* 9(1): 71–86.

Bohart RM, Washino RK. 1978. *Mosquitoes of California, 3rd edition*. University of California-Berkeley, Division of Agricultural Science.

Carpenter HJ, Lacasse WJ. 1955. *Mosquitoes of North America (North of Mexico)*. University of California Press.

Chaves LF, Harrington LC, Keogh CL, Nguyen AM, Kitron U. 2010. Blood feeding patterns of mosquitoes: random or structured? *Frontiers in Zoology* 7(3): 1–11.

Cicero C, Johnson NK. 2001. Higher-level phylogeny of new world vireos (Aves: Vireonidae) based on sequences of multiple mitochondrial DNA genes. *Molecular Phylogenetic Evolution* 20(1): 27–40.

City of Albuquerque. 2010. City of Albuquerque Census. Available online: [http://www.cabq.gov/](http://www.cabq.gov/)

Cupp EW, Tennessen KJ, Oldland WK, Hassan HK, Hill GE, Katholi CR, Unnasch TR. 2004. Mosquito and arbovirus activity during 1997-2002 in a wetland in northeastern Mississippi. *Journal of Medical Entomology* 41(4): 495–501.

Cupp EW, Stokes GM. 1973. Identification of blood meals from mosquitoes collected in light traps and dog baited traps. *Mosquito News* 33(1): 39–41.

Darsie RF, Ward RA. 1981. *Identification and geographical distribution of the mosquitoes of North America, north of Mexico*. University Press of Florida.

DiMenna MA, Bueno R, Parmenter RR, Norris DE, Sheyka JM, Molina JL, LaBeau EM, Hatton E, Glass GE. 2006. Comparison of
mosquito trapping method efficacy for West Nile virus surveillance in New Mexico. *Journal of the American Mosquito Control Association* 22(2): 246–253.

DiMenna MA, Bueno R, Parmenter RR, Norris DE, Sheyka JM, Molina JL, LaBeau EM, Hatton E, Glass GE. 2006. Emergence of West Nile Virus in Mosquito (Diptera: Culicidae) Communities of the New Mexico Rio Grande Valley. *Journal of Medical Entomology* 43(3): 594–599.

DiMenna MA, Bueno R, Parmenter RR, Norris DE, Sheyka JM, Molina JL, LaBeau EM, Hatton E, Glass GE. 2007. Urban habitat evaluation for West Nile virus surveillance in mosquitoes in Albuquerque, New Mexico. *Journal of the American Mosquito Control Association* 23(2): 153–160.

Ebel GD, Rochlin I, Longacker J, Kramer LD. 2005. *Culex restuans* (Diptera: Culicidae) relative abundance and vector competence for West Nile Virus. *Journal of Medical Entomology* 42(5): 838–843.

Garcia-Rejon JE, Blitvich BJ, Farfan-Ale JA, Loroño-Pino MA, Chi Chim WA, Flores-Flores LF, Rosaldo-Parades E, Baak-Baak C, Perez-Mutul J, Suarez-Solis V, Fernandez-Salas I, Beaty BL. 2010. Host-feeding preference of the mosquito, *Culex quinquefasciatus*, in Yucatan State, Mexico. *Journal of Insect Science* 10:32. Available online: [http://www.insectscience.org/10.32](http://www.insectscience.org/10.32)

Gingrich JB, Williams GM. 2005. Host-feeding patterns of suspected West Nile virus mosquito vectors in Delaware, 2001–2002. *Journal of the American Mosquito Control Association* 21(2): 194–200.

Greenberg JA, DiMenna MA, Hanelt B, Hofkin BV. 2012. Analysis of Post-Blood Meal Flight Distances in Mosquitoes Utilizing Zoo Animal Blood Meals. *Journal of Vector Ecology* 37(1): 83–89.

Hassan HK, Cupp EW, Hill GF, Katholi CR, Klingler K, Unnasch TR. 2003. Avian host preference by vectors of eastern equine encephalomyelitis virus. *American Journal of Tropical Medicine and Hygiene* 69(6): 641–647.

Hayes EB, Gubler DJ. 2005. West Nile Virus: epidemiology and clinical features of an emerging epidemic in the United States. *Annual Review of Medicine* 57: 181–194.

Irby WS, Apperson CS. 1988. Hosts of Mosquitoes in the Coastal Plain of North Carolina. *Journal of Medical Entomology* 25(2): 85–93.

Kay BH, Boyd AM, Ryan PA, Hall RA. 2007. Mosquito feeding patterns and natural infection of vertebrates with Ross River and Barmah Forest viruses in Brisbane, Australia. *American Journal of Tropical Medicine and Hygiene* 76(3): 417–423.

Kilpatrick AM, Kramer LD, Campbell SR, Alleyne EO, Dobson AP, Daszak P. 2005. West Nile virus risk assessment and the bridge
vector paradigm. *Emerging Infectious Disease* 11(3): 425–429.

Kilpatrick AM, Daszak P, Jones MJ, Marra PP, Kramer LD. 2006. Host heterogeneity dominates West Nile virus transmission. *Proceedings of the Royal Society of Biology* 273: 2327–2333.

Kulasekera VL, Kramer L, Nasci RS, Mostashari F, Cherry B, Trock SC, Glaser C, Miller JR. 2001. West Nile virus infection in mosquitoes, birds, horses and humans, Staten Island, New York, 2000. *Emerging Infectious Disease* 7(4): 722–725.

Lanciotti R, Roehrig JT, Deubel V, Smith J, Parker M, Steele K, Crise B, Volpe KE, Crabtree MB, Sherret JH, Hall RA, MacKenzie JS, Cropp CB, Panigrathy B, Ostlund E, Schmitt B, Malkinson M, Banet C, Weissman J, Komar N, Savage HM, Stone W, McNamara T, Gubler DJ. 1999. Origin of the West Nile virus responsible for an outbreak of encephalitis in the northeastern United States. *Science* 286(5448): 2333–2337.

Lee JH, Hassan H, Hill G, Cupp EW, Higazi TB, Mitchell CJ, Godsey MS, Unnasch TR. 2002. Identification of mosquito avian-derived blood meals by polymerase chain reaction-heteroduplex analysis. *American Journal of Tropical Medicine and Hygiene* 66(5): 599–604.

Loftin KM, Byford RL, Loftin MJ, Craig ME, Steiner RL. 1997. Host preference of mosquitoes in Bernalillo County, New Mexico. *Journal of the American Mosquito Control Association* 12(1): 71–75.

Magnarelli LA. 1977a. Host feeding patterns of Connecticut mosquitoes (Diptera: Culicidae). *American Journal of Tropical Medicine and Hygiene* 26(3): 547–552.

Magnarelli LA. 1977b. Physiological age of mosquitoes (Diptera: Culicidae) and observations on partial bloodfeeding. *Journal of Medical Entomology* 13(4): 445–450.

Molaei G, Andreadis TG. 2006. Identification of Avian- and Mammalian-Derived Blood-meals in *Aedes vexans* and *Culiseta melanura* (Diptera: Culicidae) and Its Implication for West Nile Virus Transmission in Connecticut, U.S.A. *Journal of Medical Entomology* 43(5): 1088–1093.

Molaei G, Andreadis TG, Armstrong PM, Anderson JF, Vossbrinck C. 2006. Host Feeding Patterns of *Culex* Mosquitoes and West Nile Virus Transmission, Northeastern United States. *Emerging Infectious Disease* 12(3): 468–474.

Molaei G, Andreadis TG, Armstrong PM, Bueno R, Dennett JA, Real SV, Sargent C, Bala A, Randle Y, Guzman H, Travassos da Rosa A, Wuihiranyagool T, Tesh RB. 2007. Host Feeding Pattern of *Culex quinquefasciatus* (Diptera: Culicidae) and Its Role in Transmission of West Nile Virus in Harris County, Texas. *American Journal of Tropical Medicine and Hygiene* 77(1): 73–81.

Muturi EJ, Muriu S, Shiliulu J, Mwangangi JM, Jacob BG, Mbogo C, Githure J, Novak RJ. 2008. Blood-feeding patterns of *Culex quinquefasciatus* and other culicines and implications for disease transmission in Mwea rice scheme, Kenya. *Parasitology Research* 102(6): 1329–1335.

Nasci RS. 1984. Variations in the blood-feeding patterns of *Aedes vexans* and *Aedes*...
**trivittatus** (Diptera: Culicidae). *Journal of Medical Entomology* 21(1): 95–99.

Nasci RS, White DJ, Stirling H, Oliver JA, Daniels TJ, Falco RC, Campbell S, Crans WJ, Savage HM, Lanciotti RS, Moore CG, Godsey MS, Gottfried KL, Mitchell CJ. 2001. West Nile virus isolates from mosquitoes in New York and New Jersey, 1999. *Emerging Infectious Disease* 7(4): 626–630.

Platt AR, Woodhall RW, George AL. 2007. Improved DNA sequencing quality and efficiency using an optimized fast cycle sequencing protocol. *BioTechniques* 43: 58–62.

Pratt HD, Barnes RC. 1959. *Identification keys for common mosquitoes of United States*. CDC Training Guide U.S. Department of Health, Education and Welfare, Public Health Service.

Ritchie S, Rowley WA. 1981. Blood-feeding patterns of Iowa mosquitoes. *Mosquito News* 41(2): 271–275.

Saegerman C, Berkvens D, Mellor PS. 2008. Bluetongue Epidemiology in the European Union. *Emerging Infectious Disease* 14(4): 539–544.

Savage HM, Aggarwal D, Apperson CS, Katholi CR, Gordon E, Hassan HK, Anderson M, Charnetzky D, McMillen L, Unnasch EA, Unnash TR. 2007. Host Choice and West Nile Virus Infection Rates in Blood-Fed Mosquitoes, Including Members of the *Culex pipiens* Complex, from Memphis and Shelby County, Tennessee, 2002–2003. *Vector-Borne and Zoonotic Diseases* 7(3): 365–386.

Sawabe K, Isawa H, Hoshino K, Sasaki T, Roychoudhury S, Higa Y, Kasai S, Tsuda Y, Nishiumi I, Hisai N, Hamao S, Kobayashi M. 2010. Host-Feeding Habits of *Culex pipiens* and *Aedes albopictus* (Diptera: Culicidae) Collected at the Urban and Suburban Residential Areas of Japan. *Journal of Medical Entomology* 47(3): 442–450.

Solomon T. 2004. Flavivirus encephalitis. *New England Journal of Medicine* 351(4): 370–378.

Tempelis CH, Reeves WC, Bellamy RE, Lofy MF. 1965. A three-year study of the feeding habits of *Culex tarsalis* in Kern County, California. *American Journal of Tropical Medicine and Hygiene* 14: 170–177.

Turell MJ, O’Guinn ML, Dohm DJ, Jones JW. 2001. Vector competence of North American mosquitoes (Diptera: Culicidae) for West Nile virus. *Journal of Medical Entomology* 38(2): 130–134.

Turell MJ, Sardelis MR, O’Guinn ML, Dohm DJ. 2002. Potential vectors of West Nile virus in North America. *Current Topics in Microbial Immunology* 267: 241–252.

Turell MJ, Dohm DJ, Sardelis MR, O’Guinn ML, Andreadis TG, Blow JA. 2005. An Update on the Potential of North American Mosquitoes (Diptera: Culicidae) to Transmit West Nile Virus. *Journal of Medical Entomology* 42(1): 57–63.

White DJ, Kramer LD, Backenson PB, Lukacik G, Johnson G, Oliver JA, Howard JJ, Means RG, Eidson M, Gotham I, Kulasekera V, Campbell S. 2001. Mosquito surveillance and polymerase chain reaction detection of West Nile virus, New York state. *Emerging Infectious Disease* 7(4): 643–649.
Table 1. Avian blood meals identified from either *Aedes vexans* or *Culex quinquefasciatus*. Exotic avian species, indicated by (*), were identified from mosquitoes captured at the Rio Grande Zoo.

| Species name | Common name | Avian species | Aedes vexans Avian total (n=179) | Percent | Culex quinquefasciatus Avian total (n=196) | Percent |
|--------------|-------------|---------------|----------------------------------|---------|------------------------------------------|---------|
| *Acipiter cooperi* | Cooper's hawk | 3 | 143 | 0.5 | 1 | 13 |
| *Anas platyrhynchos* | Mallard duck | 1 | 143 | 0.5 | 1 | 13 |
| *Coughto mewiata* | Marsh duck | 1 | 28.6 | 1 | 9 | 1.4 |
| *Chamaeleo salmoni* | Goldfinch | 1 | 1 | 13 | 1 |
| *Cnemadocetes megalops* | House finch | - | - | 7 | 9.1 |
| *Crania jamaicensis* | Pigeon | 1 | 5 | 6.5 |
| *Dromas armeniaca* | Emu* | 1 | 143 | 0.5 | 1 | 13 |
| *Gryllus palustris* | Chicken | 1 | 1 | 13 |
| *Geococcyx californiana* | Road runner | - | - | 1 | 1 |
| *Heterogryllus gouldi* | Wild turkey | - | - | 2 | 2.6 |
| *Pipile dalmatiana* | House sparrow | - | - | 14 | 18.2 |
| *Phaeomus colubrinus* | Common quail | - | - | 2 | 2.6 |
| *Pteropus melanopterus* | Black-headed gnatcatcher | - | - | 2 | 2.6 |
| *Sturnus vulgaris* | Cocktail | 1 | 1 | 13 |
| *Strix aluco* | Ostrich* | - | - | 1 | 13 |
| *Trichoglossus haematodus* | Rainbow lorikeet* | - | - | 1 | 13 |
| *Tachypholus migratorius* | American robin | 2 | 28.6 | 1 | 16 | 20.8 |
| *Tityrea melanochlora* | Tropical kingbird | - | - | 1 | 13 |
| *Vidua grisula* | Andean cock* | 1 | 1 | 13 |
| *Zenaida macroura* | Mourning dove | - | - | 11 | 14.3 |

Table 2. Mammalian blood meals identified from either *Aedes vexans* or *Culex quinquefasciatus*. Exotic mammalian species, indicated by (*), were identified from mosquitoes captured at the Rio Grande Zoo.

| Species name | Common Name | Mammalian species | Aedes vexans Mammal total (n=206) | Percent | Culex quinquefasciatus Mammal total (n=196) | Percent |
|--------------|-------------|--------------------|----------------------------------|---------|------------------------------------------|---------|
| *Astridopus afghanicus* | Red Panda* | 1 | 0.5 | 0.5 | - | - |
| *Blarina brevicauda* | Arctic fox* | 1 | 0.5 | 0.5 | - | - |
| *Bubalus bubalis* | Cows | 77 | 37.4 | 36.2 | 6 | 31.6 |
| *Bubalus bubalis* | Water buffalo* | 1 | 0.5 | 0.5 | - | - |
| *Camelus bactrianus* | Bactrian camel* | 1 | 0.5 | 0.5 | - | - |
| *Camelus dromedarius* | Dromedary Camel* | 1 | 0.5 | 0.5 | - | - |
| *Canis latrans* | Coyote | 1 | 0.5 | 0.5 | 1 | 53 |
| *Canis lupus familiaris* | Dog | 10 | 4.9 | 4.7 | 2 | 10.5 |
| *Capra hircus* | Goat | 1 | 0.5 | 0.5 | - | - |
| *Canis latrans* | White rhino* | 2 | 1 | 0.9 | 2 | 10.5 |
| *Equus asinus* | Donkey | 1 | 0.5 | 0.5 | - | - |
| *Equus caballus* | Horse | 47 | 22.8 | 22.1 | 3 | 15.8 |
| *Hippopotamus amphibius* | Hippopotamus* | 1 | 0.5 | 0.5 | - | - |
| *Hippopotamus amphibius* | Hartebeest | 1 | 0.5 | 0.5 | - | - |
| *Homo sapiens* | Humans | 11 | 53 | 52 | - | - |