West Nile Virus Ecology in a Tropical Ecosystem in Guatemala

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Abstract. West Nile virus ecology has yet to be rigorously investigated in the Caribbean Basin. We identified a transmission focus in Puerto Barrios, Guatemala, and established systematic monitoring of avian abundance and infection, seroconversions in domestic poultry, and viral infections in mosquitoes. West Nile virus transmission was detected annually between May and October from 2005 to 2008. High temperature and low rainfall enhanced the probability of chicken seroconversions, which occurred in both urban and rural sites. West Nile virus was isolated from *Culex quinquefasciatus* and to a lesser extent, from *Culex mollis*/*Culex inflicus*, but not from the most abundant *Culex* mosquito, *Culex nigripalpus*. A calculation that combined avian abundance, seroprevalence, and vertebrate reservoir competence suggested that great-tailed grackle (*Quiscalus mexicanus*) is the major amplifying host in this ecosystem. West Nile virus transmission reached moderate levels in sentinel chickens during 2007, but less than that observed during outbreaks of human disease attributed to West Nile virus in the United States.

INTRODUCTION

West Nile virus (WNV) is a mosquito-borne pathogen that began circulating in the Caribbean Basin in 2001.¹ Ecological studies of WNV in Europe, Asia, the Middle East, and the United States have shown that *Culex* (*Culex*) mosquitoes serve as vectors and that particular species of birds are the principal amplifying hosts.^{2,3} *Culex* mosquitoes and passerine birds appeared to be responsible for WNV amplification in Puerto Rico in 2007.^{4,5} By 2007, serologic evidence for WNV circulation in free-ranging birds and/or horses was reported in numerous tropical locations around the rim of the Caribbean Basin, including Mexico,^{6–8} Guatemala,^{9,10} Costa Rica,¹¹ Colombia,¹² Venezuela,¹³ Guadeloupe,¹⁴ Puerto Rico,¹⁵ Dominican Republic,^{16,17} Haiti,¹⁸ Jamaica,¹⁹ and Cuba.¹⁵

Ecological parameters of WNV transmission have yet to be clearly defined in tropical ecosystems typical of the Caribbean Basin countries.¹ Sero-surveys of free-ranging birds in several countries have identified infections in numerous species of birds but with the exception of a recent report in Puerto Rico,⁴ none of these studies were focused in time and place coincident with active transmission.^{7,8,16,17,19} Similarly, several isolates were derived from various species of *Culex* mosquitoes in tropical America, but except for Puerto Rico,⁵ insufficient data were available to incriminate them as WNV vectors.^{20–22} To determine vectors and amplifying hosts, it is necessary to study WNV ecology within a transmission focus. No such studies have been reported from Central America.

Presumably WNV uses similar hosts and vectors in the tropics as in temperate regions. Therefore, we hypothesized that certain passerine birds and *Culex* mosquitoes would

amplify and transmit WNV in Guatemala, where WNV appears to have been circulating since 2003.⁹ Accordingly, we established a sentinel chicken surveillance network to detect active WNV transmission foci for the development of further ecological studies. Periodic sampling of resident poultry in several Guatemalan departments representing different eco-regions was initiated in 2004. Once an active transmission focus was detected, in the humid Atlantic coast eco-region, we established systematic monitoring of seroconversions in domestic poultry, seroprevalence in free-ranging birds, and viral infections in mosquitoes. Our principal objectives were to describe the vectors, amplifying hosts, and seasonality of WNV transmission. We herein report the findings of our longitudinal investigation, including spatio-temporal patterns of transmission, candidate vectors, and avian amplifying hosts.

MATERIALS AND METHODS

Study sites. In 2004–2005, seven different departments of Guatemala corresponding to different eco-regions were selected for initial prospective monitoring of free-ranging domestic chickens for evidence of local WNV transmission (Figure 1). In 2006, we selected the municipality of Puerto Barrios (15°50'N and 88°28'W), Department of Izabal, for follow-up longitudinal ecology studies of WNV. This Department is located on the Caribbean coast of Guatemala within a subtropical wet forest life zone. Climatic conditions are generally hot and humid without a well-defined dry season. Mean annual precipitation is 3,500 mm. Monthly rainfall and average temperature data from Puerto Barrios were obtained from the Guatemalan Instituto Nacional de Sismología, Vulcanología, Meteorología e Hidrología (NSIVUMEH).

Ecological studies were conducted between March 2006 and March 2009 in an 80 km² geographic area within the Puerto Barrios municipality. Ten 1-km² blocks were randomly selected and a sampling site was selected within each of these blocks. The sampling sites were selected according to the following criteria: 1) access to private property, 2) presence of backyard poultry, and 3) secure vehicular access (Figure 2). In the event of a change in access to a sampling site, the site was relocated to the nearest site that fulfilled the selection

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FIGURE 1. Guatemala departments where domestic chickens were serially sampled to detect West Nile virus (WNV) transmission, 2004–2005. Numbers 1–7 indicate the department, each representing a different eco-region (see text).

criteria. At each site, we surveyed for seroconversion of domestic chickens, mosquito densities, and relative abundance of bird populations.

Each of the 10 sampling points was assigned a macrohabitat category (rural versus urban) according to visual estimates of urbanization and vegetation in an area of ~3 hectares where the bird population surveys were conducted. Urban sites were

defined as having $\geq 30\%$ of road and human dwelling habitats. Rural habitats in the study area included predominantly riparian forest, pasture, and cropland.

Wild birds were captured in two different sites located within the 80 km² study area as explained below.

Chicken monitoring. We placed uniquely numbered aluminum leg bands on 5–10 chicks in each of the 10 sampling sites.

FIGURE 2. Sampling sites for assessment of West Nile virus (WNV) transmission in Puerto Barrios, Izabal, Guatemala, 2006–2009. Markers numbered 1–10 indicate locations of selected study sites where chickens and mosquitoes were sampled. A and B represent locations where wild birds were sampled.

Monthly, blood samples (~1 mL) were collected from the brachial or jugular vein of these marked domestic birds using 1-cc syringes with 26 g 1/2-inch sub-Q needles (Becton-Dickinson, Franklin Lakes, NJ), divided among two 0.6-mL Microtainer serum collection tubes (Becton-Dickinson), and centrifuged for serum separation allowing at least 15 minutes for coagulation. Samples were frozen on dry ice for transport to the Universidad Del Valle de Guatemala (UVG) where they were stored at -20°C . One tube per sample was thawed for antibody detection assays described below. The second tube was available for additional testing if necessary. Initial blood samples were tested to confirm seronegativity for WNV. Results were expressed as the number of seroconversions in the numerator, and the number of chicken-weeks of exposure in the denominator. One chicken exposed to mosquito bites for 1 week represents 1 chicken-week of exposure. Chickens that either seroconverted (became positive for WNV-reactive antibodies), disappeared, or died were replaced to maintain 5–10 birds per sentinel flock. These chickens belonged to and were cared for by private property owners. Supplemental food and preventive veterinary care were provided as needed.

Mosquito surveys. Mosquito population densities were estimated from collections of adult mosquitoes in one CO_2 -baited Centers for Disease Control and Prevention (CDC) light trap (John W. Hock Co., Gainesville, FL) and one gravid trap²³ placed for 1 night per month in each of the 10 sampling sites. In the morning, collection nets were placed in a box containing dry ice to kill the insects by CO_2 asphyxiation. Killed insects were poured onto plastic trays for manual separation of mosquitoes into Nalgene cryovials (Sigma-Aldrich Corp., St. Louis, MO) that were then frozen on dry ice for transport to UVG. In the laboratory, collection cryovials were stored at -70°C until thawed for mosquito identification. Adult female mosquitoes were identified by their morphological characteristics viewed through a stereo microscope (on a chill table) using the Clark-Gil and Darsie key.²⁴ Pools of no more than 50 mosquitoes were separated according to species, location, date, and trap type, and then replaced at -70°C until processed for further testing. Beginning in 2007, supplemental mosquito collections were conducted within 6 weeks following chicken seroconversions using 5–10 traps of each type in the sampling site where the seroconversion event was detected.

Bird counts. To estimate bird abundance, point count surveys²⁵ of birds (wild and domestic) were undertaken by one ornithologist and an assistant at each of the 10 sampling sites. At each site, birds were counted from four different stations located at 150-m intervals along a walking route that began as close as possible to the sentinel chicken flock. At each station, the ornithologist reported visual and auditory observations of all individual birds by species within ~50 m during a 4-minute period. In 2006, point counts were conducted every 3 months. Since May 2007, point counts were conducted monthly.

Relative abundance (no. observed individuals of a species divided by the no. of all birds observed) was calculated seasonally for 119 species that were observed during the survey.

Wild bird antibody surveys. Two locations (A and B; Figure 2) were selected within the 80 km^2 study area to study the prevalence of WNV-reactive antibodies in wild resident birds. Both locations were comprised primarily of secondary forest and pastures. Location A was the Naval Base airport about 1 km from the urban zone of Puerto Barrios. Location B was the rural village of Machacas del Mar about 7 km from the

urban zone. Bird captures were carried out during 1- to 2-week periods ~3 months apart from April 2006 to July 2009. Free-ranging birds were captured using 18–20 mist nets of various mesh sizes and lengths, monitored continuously from sunrise to sunset. Resident birds were identified and aged as juveniles (< 1 year of age) or adults (> 1 year of age) when possible and marked with numbered leg bands. Blood (volume ~1% body mass up to maximum 0.65 mL) was collected from the jugular vein of birds that weighed > 10 g, using 1-cc syringes with 26 g 1/2-inch or 27 g 5/8-inch sub-Q needles (Becton-Dickinson). Mass was measured using a handheld Pesola scale (Avinet, Inc., Dryden, NY). Blood was processed identically as for chickens. Smaller birds and migratory species were released without sampling.

Antibody detection assays. Serum samples from wild and domestic birds were tested using an epitope-blocking enzyme-linked immunosorbent assay (B-ELISA) as described previously.²⁶ Briefly, any sample that blocked both the non-specific flavivirus-reactive monoclonal antibody 6B6C-1 and the WNV-specific monoclonal antibody 3.1112g by $\geq 30\%$ was considered positive. The positive samples and a subset of negative samples were confirmed by the plaque reduction neutralization test (PRNT) using Vero cells as previously described.²⁷ Reference virus strains used in the PRNT were the NY99-4132 strain of WNV and the TBH-28 strain of St. Louis encephalitis virus (SLEV). Serum samples were diluted 1:5 in BA1 media (Hanks M-199 salts, 0.05 M Tris, pH 7.6, 1% bovine serum albumin, 0.35 g/L of sodium bicarbonate, 100 U/mL of penicillin, 100 $\mu\text{g}/\text{mL}$ of streptomycin, 1 $\mu\text{g}/\text{mL}$ of Fungizone) before incubation with an equal volume of virus suspension. To compare titers against both WNV and SLEV, serum samples were tested in serial 2-fold dilutions in duplicate starting with 1:10. Samples that reduced the number of plaques formed by 90% or more for at least one of the reference viruses were considered positive by PRNT, and determined to contain anti-flavivirus antibodies. These antibodies were attributed to either WNV- or SLEV-infection if the reciprocal titer for WNV was at least 4-fold greater than that of SLEV or vice versa, respectively.

Arbovirus detection from mosquitoes. All pools of female *Culex* mosquitoes were homogenized and tested in duplicate for viral plaque growth or cytopathic effects on Vero cell monolayers in 6-well plates or 1-dram shell vials.²⁸ Cultured viruses were harvested in 1 mL of BA1 supplemented with 20% fetal bovine serum, and 140 μL of the virus suspension was RNA-extracted and tested by reverse transcription-polymerase chain reaction (RT-PCR) with group-specific flavivirus, alphavirus, and bunyavirus primers according to published protocols.^{29–31} Positive RT-PCR reactions were followed with specific primers and probes for WNV (and other regional arboviruses) in a real-time RT-PCR format when available.³²

To enhance the detection of WNV and other arboviruses, all *Culex quinquefasciatus* pools were also tested specifically for WNV using WNV-specific primers.³² In addition, since 2007, all pools of *Culex* (subgenus *Culex*) mosquitoes were also tested with *Flavivirus*-consensus primers, including *Culex nigripalpus*, *Culex chidesteri*, and *Culex mollis/Culex inflictus*.²⁹

Mosquito inoculation index. The mosquito inoculation index (*M*) is a measure of the relative number of infectious vector mosquitoes derived from feeding on a vertebrate host population.³³ The value for *M* is derived from the product of

vertebrate host population (P), vertebrate infection rate (I), and vertebrate reservoir competence (C):

$$M = (P)(I)(C).$$

Because the natural process of a vertebrate host infecting hematophagous vectors should incorporate host preference twice (once for the process of infecting the vertebrate host, and once for the process of vectors acquiring the infection from the vertebrate host³⁴), we modified the formula to incorporate the host selection of both infectious and uninfected host-seeking mosquitoes by squaring the vertebrate infection rate term.

$$M' = (P)(I)^2(C).$$

This approach has been used in several studies of WNV in Chicago, IL, Colorado, and the mid-Atlantic, and has been shown to be useful in predicting WNV infection prevalence in mosquitoes and the timing of WNV epidemics.^{35–38} It represents a substantial advance over considering simply the abundance and seroprevalence because some abundant and frequently exposed species may be incompetent hosts, whereas others may be abundant but not frequently exposed, or vice versa.

We used relative abundance values, A , from the bird surveys for the population-based measure, P . Antibody seroprevalence, S , was used in place of I because in the absence of pathogen-attributed mortality, seroprevalence is equal to infection rate, and there is no evidence for WNV-attributed mortality in Guatemalan birds as yet. For vertebrate reservoir competence index values, we used data published for bird species infected with WNV from southern Mexico.³⁹ These values are derived experimentally from the duration and infectiousness of viremia and describe a species' innate potential for infecting mosquitoes.⁴⁰ Thus, our equation for mosquito inoculation rate is

$$M' = (A)(S)^2(C).$$

Statistical analyses. Mosquito infection rates were calculated by the maximum-likelihood estimate for WNV-infected mosquito pools using the PooledInfRate version 3.0 program in Excel.⁴¹ Logistic regression, Fisher's exact and χ^2 tests were used to analyze seroprevalence patterns among wild birds.

RESULTS

Identification of active West Nile virus transmission. We monitored WNV transmission in sentinel chickens from 2004 to 2005 in seven departments of Guatemala (Table 1).

Five seroconversions (change in antibody status from seronegative to seropositive between two sampling time points) in chickens provided evidence of WNV transmission in the Department of Izabal. No evidence for active WNV transmission in chickens was detected in six other departments.

Temporal transmission of West Nile virus and relationship to temperature and rainfall. We monitored seasonality of WNV transmission in Puerto Barrios, Izabal by sampling chicken flocks monthly at 10 sampling sites. Seroconversions to WNV were detected annually, and only occurred during the period from May to October (Figure 3). Peak monthly incidence reached 13.3/1,000 chicken-weeks in 2006, 60.7/1,000 chicken-weeks in 2007, and 6.9/1,000 chicken-weeks in 2008. Monthly rainfall in Puerto Barrios during the period of the study ranged from a minimum of 29.1 mm in May 2007 to a maximum of 775.5 mm in June 2006 (Figure 4A). The temperature fluctuated annually from cooler periods (November–February; average monthly temperatures 23–25°C) to warmer periods (March–October; average monthly temperatures 26–29°C) (Figure 4B). The WNV seroconversions in sentinel chickens only occurred following months when monthly average temperatures were high ($> 27.2^\circ\text{C}$) and increased with decreasing rainfall at these warmer temperatures (Logistic regression: Intercept: $-83.5 \pm (\text{SE}) 14.1$; Temperature coef. = 2.82 ± 0.50 , $P < 0.001$; Rainfall coef. = 0.11 ± 0.04 , $P = 0.005$; Temperature $\times$ Rainfall interaction coef. = -0.0041 ± 0.0014 , $P = 0.004$).

Potential vectors. A total of 117,613 female mosquitoes corresponding to at least 37 species were captured (Supplemental Table S1). Focusing on *Culex* mosquitoes as potential WNV vectors, we tested these for virus plaque formation in Vero cell culture. A subset of pools corresponding to *Cx. quinquefasciatus*, *Cx. nigripalpus* and (since 2007) *Cx. mollis*/*Cx. inflicus* were also tested for flavivirus- ($N = 1,551$) and/or WNV- ($N = 1,732$) specific RNA by standard or real-time RT-PCR, respectively. West Nile virus was isolated from supplemental collections of mosquitoes at locations with recent chicken seroconversions to WNV from two pools of *Cx. quinquefasciatus*, two pools of *Cx. mollis*/*Cx. inflicus*, and three pools of undifferentiated *Culex* mosquitoes collected in July and August 2007. Infection rates in the *Culex* populations sampled at these points in space and time ranged from 0 in *Cx. nigripalpus* to 15.7/1,000 mosquitoes in *Cx. quinquefasciatus* (Table 2). A phylogenetic evaluation of these Guatemalan WNV isolates will be described elsewhere (CDC, unpublished data).

Population dynamics of *Culex* mosquitoes were assessed from standardized routine collections among the 10 sampling sites. *Culex quinquefasciatus* density peaked 1–4 months before the annual peaks of WNV infection incidence in chickens (Figure 3). In contrast, *Cx. nigripalpus* and *Cx.*

TABLE 1
WNV seroconversions and incidence in free-ranging chickens from seven Departments of Guatemala, 2004–2005

Department	Eco-region	N	No. seroconversions	Exposure (chicken-weeks)	Incidence (per 10 ³ chicken-weeks)
Petén	Humid Forest	69	0	1,316	0.00
Alta Verapaz	Humid Montane	24	0	432	0.00
Izabal	Humid Atlantic	224	5	4,215	1.19
Zacapa	Dry Forest	37	0	666	0.00
Chiquimula	Agricultural	23	0	417	0.00
Santa Rosa	Dry Pacific	56	0	1,145	0.00
Escuintla	Humid Pacific	52	0	672	0.00

FIGURE 3. Average mosquito abundance of candidate West Nile virus (WNV) mosquito vectors in relation to monthly incidence of chicken seroconversions across study sites in Puerto Barrios, Guatemala, 2006–2009. Mosquito densities were derived from monthly Centers for Disease Control and Prevention (CDC) gravid and light trap collections at 10 sampling points. Arrows indicate months in which WNV-infected mosquitoes were detected in supplemental collections.

mollis/*Cx. inflicus* densities peaked inconsistently either before or after the detection of WNV infection in chickens depending on the year.

Additional arboviruses were detected and isolated including: *Culex flavivirus*, isolated from *Cx. quinquefasciatus*,²⁸ enzootic strains of Venezuelan equine encephalitis virus

isolated from *Culex taeniopus*, and group C bunyaviruses detected in *Cx. taeniopus* (data not shown).

Vertebrate hosts. Bird relative abundance was assessed during different seasons of North American bird migration (spring migration, breeding season, fall migration, non-breeding season). Almost 24,000 birds were recorded during

FIGURE 4. Climatic data recorded by the Guatemalan National Institute of Meteorology in Puerto Barrios, Guatemala, 2006–2008. (A) Rainfall (mm) and (B) monthly average temperature (°C).

TABLE 2

West Nile virus (WNV) infection rates (determined by maximum likelihood estimate) of *Culex* mosquitoes from supplemental collections in premises where WNV activity in chickens had been recently detected, Puerto Barrios, Guatemala, 2007

<i>Culex</i> species	Site number (corresponding macrohabitat type)/collection date											
	# 7 (rural)/5-Jul-07				# 3 (urban)/3-Aug-07				# 5 (urban)/9-Aug-07			
	No. pools	No. pos pools	No. individuals	IR* (95% CI)	No. pools	No. pos pools	No. individuals	IR (95% CI)	No. pools	No. pos pools	No. individuals	IR (95% CI)
<i>quinquefasciatus</i>	6	1	176	5.7 (0.3–30.7)	3	1	62	15.7 (1.1–107.5)	2	0	26	–
<i>mollis/inflatus</i>	6	1	212	4.7 (0.3–24.7)	2	0	88	–	7	1	267	3.5 (0.2–17.0)
<i>nigripalpus</i>	10	0	456	–	5	0	236	–	12	0	590	–
undifferentiated	9	1	342	2.9 (0.2–14.8)	1	1	50	20.0(NA)	3	1	106	9.0 (0.6–57.5)
All other <i>Cx.</i> species	7	0	116	–	7	0	90	–	13	0	355	–

*IR = WNV infection rate expressed per 1,000 mosquitoes; NA = method to calculate 95% confidence interval for infection rate is not applicable.

standardized surveys comprising 119 species belonging to 41 families and 17 orders (including domestic and wild birds) (Supplemental Table S2). About one-third of these species ($N = 37$) were migratory (transients and winter visitors) and two-thirds ($N = 82$) were permanent residents, including domesticated species and captive birds. Great-tailed grackle (*Quiscalus mexicanus*) was the most abundant species followed by domestic chicken (*Gallus gallus*) and clay-colored thrush (*Turdus grayi*) in all seasons, representing 30–40%, 15–20%, and 5–10%, respectively, of all resident birds surveyed in Puerto Barrios (Figure 5).

A total of 3,833 individual wild birds representing at least 136 different species were captured using mist nets (Supplemental Table S3). Blood samples ($N = 1,799$) were obtained from resident birds. A subset of those samples ($N = 291$) corresponded to “recaptures” of individuals sampled in a previous season or year. A total of 120 birds of

19 species tested positive for prior infection with WNV, as determined by the detection of WNV-specific antibodies (Supplemental Table S4). Seroconversions were detected among recaptures for the following species: clay-colored thrush ($N = 4$), great-tailed grackle ($N = 1$), ferruginous pygmy-owl (*Glaucidium brasilianum*, $N = 1$) and great kiskadee (*Pitangus sulphuratus*, $N = 1$). Seroprevalence differed significantly among habitats (urban versus rural), species, year and age (juvenile versus adult) (Table 3). Free-ranging birds were more likely to be seropositive for WNV if they were adult, captured near urban habitat, sampled in 2007 or 2008, or one of the following species: melodious blackbird (*Dives dives*), ferruginous pygmy-owl (*G. brasilianum*), gray-headed dove (*Leptotila plumbeiceps*), great-tailed grackle (*Q. mexicanus*), or clay-colored thrush (*T. grayi*). Among these, only the latter two species were abundant throughout the study area.

FIGURE 5. Seasonal variation in percent of all avian detections during point counts for selected bird species in Puerto Barrios, Guatemala (2006–2008). “Spring” represents Spring migration, “Summer” breeding season, “Fall” fall migration and “Winter” non-breeding season.

TABLE 3

Logistic regression analyses of West Nile virus (WNV)-antibody status of 1,150 birds from 17 species that had at least one seropositive and one seronegative individual, as required for logistic regression*

Predictor	Category	Coef. (SE)	Z or χ^2	P value	Odds ratio (95% CI)
Intercept		-5.39 (0.64)	-8.36	< 0.001	
Age	Juvenile	(DF = 2)	15.92	< 0.001	1.0
	Adult	0.89 (0.28)	3.18	0.001	2.45 (1.44–4.37)
	Unknown	1.52 (0.44)	3.45	0.001	4.85 (1.89–10.80)
Habitat	Rural (site B)	(DF = 1)	2.80	0.005	1.0
	Urban (Site A)	0.71 (0.26)			2.04 (1.25–3.41)
Year	2006	(DF = 3)	15.27	0.002	1.0
	2007	0.82 (0.29)	2.83	0.005	2.27 (1.31–4.08)
	2008	1.01 (0.32)	3.15	0.002	2.74 (1.48–5.22)
	2009	0.13 (0.40)	0.32	0.751	1.14 (0.50–2.47)
Species†	<i>Columbina talpacoti</i>	(DF = 16)	54.6	< 0.001	1.0
	<i>Dives dives</i>	2.90 (0.70)	4.13	< 0.001	18.23 (4.61–76.20)
	<i>Glaucidium brasilianum</i>	2.38 (0.76)	3.14	0.002	10.81 (2.32–48.60)
	<i>Leptotila plumbeiceps</i>	2.88 (0.88)	3.27	0.001	17.79 (2.89–99.94)
	<i>Quiscalus mexicanus</i>	2.11 (0.50)	4.20	< 0.001	8.22 (3.34–24.84)
	<i>Turdus grayi</i>	1.59 (0.50)	3.18	0.001	4.88 (2.00–14.66)

*The table shows the coefficients (Coef.), standard errors of coefficients (SE), and odds ratios for categorical predictors relative to the reference level, which is given on the row of the predictor name and underlined. The statistics on the predictor row use χ^2 when there are more than two categories for a predictor. DF = degrees of freedom.

†Only species with significantly different seroprevalence ($P < 0.0029$, $\alpha = 0.05$) with respect to *Columbina talpacoti* (ruddy ground-dove) are shown. Eleven species for which no significant difference in seroprevalence was detected include *Crotophaga sulcirostris* (groove-billed ani), *Euphonia hirundinacea* (yellow-throated euphonia), *Icterus pectoralis* (spot-breasted oriole), *Manacus candei* (white-collared manakin), *Momotus momota* (blue-crowned motmot), *Myiozetetes similis* (social flycatcher), *Pitangus sulphuratus* (great kiskadee), *Salpator atriceps* (black-headed saltator), *Salpator coerulescens* (grayish saltator), *Thraupis abbas* (yellow-winged tanager), and *Thraupis episcopus* (blue-gray tanager).

In addition, the seroprevalence of the two most well sampled species, great-tailed grackle and clay-colored thrush, increased significantly coincident with the period of peak transmission observed in chickens (May–Nov 2007), and remained high thereafter (Figure 6; Table 4).

The force of transmission between vertebrate amplifying host and mosquito vectors was quantified by calculating the modified mosquito inoculation index (M') for great-tailed grackle, clay-colored thrush, and domestic chicken. These calculations predicted that great-tailed grackles infected ~6,000 vector mosquitoes for every one mosquito infected by clay-colored thrushes, and that adult chickens infected no

mosquitoes during the period of peak WNV transmission from May to November of 2007 (Table 5).

DISCUSSION

We sought to characterize ecological parameters of WNV transmission in a tropical ecosystem located in the municipality of Puerto Barrios, on the Atlantic coast of Guatemala.

The mosquito species found infected with WNV, *Cx. quinquefasciatus* and *Cx. mollis/Cx. infictus*, are both members of the subgenus *Culex*, which is known to include important vectors of WNV throughout the world.^{36,42} *Culex quinquefasciatus*, known commonly as the southern house mosquito, is frequently found in close association with human activity in urban and rural areas, and has been implicated as a vector for WNV in the United States.^{43,44} This is the first report of WNV infection in *Cx. mollis/Cx. infictus*. Little is known about the bionomics of *Cx. mollis* or *Cx. infictus*. Larval forms of *Cx. mollis* may be distinguished from *Cx. infictus*, but adults are morphologically similar.²⁴ Our collections probably refer to *Cx. mollis*, as *Cx. infictus* is associated with salt water habitats, where it breeds in crab holes.²⁴ However, such habitats are located just a few kilometers from our study sites; therefore, we could not eliminate this species from consideration. Another recognized WNV vector, *Cx. nigripalpus*, outnumbered other *Culex* (*Culex*) species mosquitoes in the transmission focus, yet was not found to be infected. WNV-infected *Cx. nigripalpus* have been reported elsewhere in the Caribbean Basin (Puerto Rico)⁴⁵ and in Chiapas,

FIGURE 6. Seroprevalence of adult and juvenile clay-colored thrush (*Turdus grayi*), and great-tailed grackle (*Quiscalus mexicanus*) before, during, and after the peak transmission period (May–Nov 2007).

TABLE 4
Logistic regression analyses of West Nile virus (WNV)-antibody status of 624 *Turdus grayi* and *Quiscalus mexicanus**

Predictor	Category	Coef. (SE)	Z or χ^2	P value	Odds-Ratio
(Intercept)		-2.52 (0.44)	-5.77	0.000	
Species	<u><i>Q. mexicanus</i></u>	(DF = 1)			1.0
	<u><i>T. grayi</i></u>	-0.52 (0.28)	-1.84	0.066	0.60 (0.34–1.04)
Habitat	Rural (Site B)	(DF = 1)			1.0
	Urban (Site A)	0.67 (0.29)	2.32	0.020	1.95 (1.12–3.49)
Age	Juvenile	(DF = 2)	12.97	0.002	1.0
	Adult	0.95 (0.34)	2.83	0.005	2.60 (1.39–5.25)
	Unknown	1.66 (0.54)	3.06	0.002	5.26 (1.75–15.11)
Season	Pre-Peak	(DF = 1)			1.0
	Peak/Post-Peak	0.70 (0.31)	2.24	0.025	2.01 (1.11–3.81)

*The table shows the coefficients (Coef.), standard errors of coefficients (SE), and odds ratios for categorical predictors relative to the reference level, which is given on the row of the predictor name and underlined. The statistics on the predictor row use χ^2 when there are more than two categories for a predictor. The Peak and post-Peak samples were not significantly different ($P = 0.77$) and were combined into a single season in this analysis.

the Mexican state that borders Guatemala to the west.⁴⁶ The population of *Cx. nigripalpus* from Honduras, which borders Guatemala to the east, has been shown to be competent for WNV transmission in laboratory studies.⁴⁷ To corroborate that this species was not involved with WNV transmission in Puerto Barrios, we found no association of *Cx. nigripalpus* population fluctuations in relation to WNV transmission. We did notice an increase of urban chicken seroconversions immediately following a strong peak of *Cx. quinquefasciatus* population density in May and June of 2007, but not following smaller early summer peaks in 2006 and 2008. Instead, in these years, low-level transmission in rural sites appeared to follow late season (September) population spikes of *Cx. mollis/Cx. inflicus*. It is likely that this pattern occurred in 2007 as well, although it was somewhat obscured by the large amount of virus and mosquito activity in the urban sections of the municipality.

Determining the importance of mosquito species as vectors of WNV ("vectorial capacity") requires characterization of vector competence, and field estimates of virus infection, relative abundance, and host selection.⁴⁸ Most studied populations of *Cx. quinquefasciatus* have been found to be moderately vector-competent for WNV in laboratory experiments.^{49,50} Although vector competence studies for Guatemalan mosquitoes have not been reported, a laboratory colony of *Cx. quinquefasciatus* from Tegucigalpa, Honduras, was shown to be moderately competent with a Guatemalan strain of WNV.⁵¹ A study of the host selection of selected *Culex* mosquito species in urban and rural areas of the municipality of Puerto Barrios in 2008 found that *Cx. quinquefasciatus* fed primarily on birds, and to a lesser degree on humans and other mammals.⁵² Therefore, these mosquitoes appear to be

serving as WNV vectors particularly in urban areas and represent a risk for WNV transmission to humans and horses. *Cx. mollis* and/or *Cx. inflicus* may play a small role as a WNV vector in rural areas.

Avian hosts that are highly infectious and frequently fed upon by competent mosquitoes will be important in WNV amplification.³⁶ We found that the great-tailed grackle (*Q. mexicanus*), domestic chicken (*G. gallus*), and clay-colored thrush (*T. grayi*) were the three most abundant birds in the transmission focus. Although abundant, most chickens and clay-colored thrushes are incompetent as amplifying hosts for WNV.³⁹ On the other hand, the grackle was determined to be highly competent in a study of Mexican birds.³⁹ Using these data to calculate mosquito inoculation index values suggested that the great-tailed grackle in our study may have infected 6,000-fold more vector mosquitoes than the clay-colored thrush. An alternative calculation derived from mosquito host selection studies also implicated the grackle as the primary amplifying host among free-ranging birds.⁵² The great-tailed grackle appears to be a key avian amplifying host of WNV in Puerto Barrios, Guatemala. A different grackle species, the Greater Antillean grackle (*Quiscalus niger*), was implicated as a putative amplifying host for WNV in Puerto Rico, also during a burst of WNV activity in 2007.⁴

In 2007, increased WNV activity was detected in diverse locations of the Caribbean Basin. In addition to Guatemala and Puerto Rico, transmission was detected in northern Colombia.¹² The widespread activity suggests a possible climatologic basis for the virus activity. The assessment of rainfall and temperature factors in Puerto Barrios supported previous findings from temperate locations that WNV transmission is associated with high temperature and low rainfall. Although rainfall creates new mosquito breeding sites for some mosquito species, it can also flush existing mosquito larvae from their current, productive breeding sites. Low rainfall may also favor population growth of *Cx. quinquefasciatus* by increasing the organic content of the water available for mosquito breeding, and elevated temperature accelerates the viral infection kinetics in the vectors.^{53,54}

The impact of WNV on public health in Guatemala and throughout tropical America remains unresolved.^{1,36} Our study provides an opportunity to compare the enzootic transmission intensity of WNV in a tropical setting with that in temperate North America where relatively large outbreaks of human neurological disease have been observed. The numbers of chicken seroconversions per 1,000 chicken-weeks we observed (30–60 between June and August 2007) is comparable to

TABLE 5

Mosquito inoculation index (M') values for three candidate amplifying hosts during a high West Nile virus (WNV) transmission period in Puerto Barrios, Guatemala, 2007

Host	A*	S†	C	$M' (\times 10^{-5})\ddagger$
<i>G. gallus</i>	0.15	0.23	0.00	0
<i>Q. mexicanus</i>	0.28	0.33	1.80	5,500
<i>T. grayi</i>	0.07	0.12	0.01	1

*Abundance (A) data used in this analysis was selected from surveys conducted in the summer of 2007.

†Seroprevalence (S) used for *G. gallus* was for the period May–August 2007; for *Q. mexicanus* and *T. grayi* combined seroprevalence of adult and juvenile birds captured in June–Nov 2007 was used.

‡ $M' = (A)(S)^2(C)$ with A = relative abundance; S = seroprevalence; C = vertebrate reservoir competence derived from studies conducted with a southern Mexico strain of WNV, and calculated for the vector mosquito *Culex quinquefasciatus*. The units for M' is the relative number of infectious *Cx. quinquefasciatus* mosquitoes derived from the specified vertebrate host population.

those seen in Coachella Valley, California in 2004 (17–67) where few human cases were observed,⁴³ but substantially lower than that seen in Kern County, California (100–300) where the human incidence was 6–17/100,000.⁵⁵ In Puerto Barrios, the density of infected mosquitoes (the product of mosquito abundance and WNV infection rates), summed across mosquito species during July and August, 2007, was 0.19 and 0.20 WNV-infected mosquitoes per trap night, respectively. This is comparable to the density of infected mosquitoes in July and August in Maryland in 2004 (0.16 and 0.12, respectively) when there were 16 cases in a population of 6 million or a yearly incidence of 0.266/100,000.⁵⁶ Given that the population of Puerto Barrios is about 60,000, then, using the observed levels of enzootic transmission in Puerto Barrios, the expected number of human cases in 2007 was fewer than 1. This analysis does not take into account biological differences in the vector communities of temperate and tropical ecosystems. Nonetheless, the intensity of enzootic transmission that we measured in Puerto Barrios suggests that human illness from WNV may be rare enough to be overlooked by health practitioners, especially given the similarity of the symptoms for febrile cases to other more common illnesses such as dengue or malaria.

Periods of intense transmission of WNV are likely to recur in Puerto Barrios and other WNV transmission foci in Guatemala and throughout tropical regions of the Americas. Our results suggest that sentinel chicken surveillance is a sensitive system to detect WNV transmission in a tropical ecosystem and could thus be used as an early warning system to potentially mitigate risk of large outbreaks in humans or horses. Urban populations of *Cx. quinquefasciatus* were the best culicine indicators of WNV transmission activity among the mosquitoes sampled in Puerto Barrios. Although the effort required for monitoring WNV transmission in free-ranging birds is less cost effective for routine arbovirus surveillance, changes in seroprevalence among juvenile grackles and thrushes could be used to detect a recent increase in local WNV transmission, especially where bird mist netting operations are already in place. The level of WNV activity that we detected in Puerto Barrios using these surveillance methods was sufficient to cause an outbreak of human disease in a more populated region. Ecological surveillance of WNV in tropical and temperate ecosystems should be implemented where resources are available to better determine the burden of this invasive flavivirus on human, veterinary, and wildlife health throughout different geographical locations where this virus is likely to be circulating.

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REFERENCES

1. Komar N, Clark GG, 2006. West Nile virus activity in Latin America and the Caribbean. *Rev Panam Salud Publica* 19: 112–117.
2. Hayes CG, 1989. West Nile fever. TP Monath, ed. *The Arboviruses: Epidemiology and Ecology*. Volume V. Boca Raton, FL: CRC Press, 5–88.
3. Hayes EB, Komar N, Nasci RS, Montgomery SP, O'Leary DR, Campbell GL, 2005. Epidemiology and transmission dynamics of West Nile virus disease. *Emerg Infect Dis* 11: 1167–1173.
4. Komar N, Bessoff K, Diaz A, Amador MA, Young G, Perez T, Seda R, Hunsperger E, 2012. Avian hosts of West Nile virus in Puerto Rico. *Vector Borne Zoonotic Dis* 12: 47–54.
5. Barrera R, MacKay A, Amador M, Vasquez J, Smith J, Díaz A, Acevedo V, Cabán B, Hunsperger EA, Muñoz-Jordán JL, 2010. Mosquito vectors of West Nile virus during an epizootic outbreak in Puerto Rico. *J Med Entomol* 47: 1185–1195.
6. Estrada-Franco JG, Navarro-Lopez R, Beasley DW, Coffey L, Carrara AS, Travassos da Rosa A, Clements T, Wang E, Ludwig GV, Cortes AC, Ramírez PP, Tesh RB, Barrett AD, Weaver SC, 2003. West Nile virus in Mexico: evidence of widespread circulation since July 2002. *Emerg Infect Dis* 9: 1604–1607.
7. Fernández-Salas I, Contreras-Cordero JF, Blitvich BJ, González-Rojas JI, Cavazos-Alvarez A, Marlenee NL, Elizondo-Quiroga A, Loroño-Pino MA, Gubler DJ, Cropp BC, Calisher CH, Beaty BJ, 2003. Serologic evidence of West Nile Virus infection in birds, Tamaulipas State, México. *Vector Borne Zoonotic Dis* 3: 209–213.
8. Farfán-Ale JA, Blitvich BJ, Loroño-Pino MA, Marlenee NL, Rosado-Paredes EP, García-Rejón JE, Flores-Flores LF, Chulim-Perera L, López-Urbe M, Pérez-Mendoza G, Sánchez-Herrera I, Santamaría W, Moo-Huchim J, Gubler DJ, Cropp BC, Calisher CH, Beaty BJ, 2004. Longitudinal studies of West Nile virus infection in avians, Yucatán State, México. *Vector Borne Zoonotic Dis* 4: 3–14.
9. Morales-Betoulle ME, Morales H, Blitvich BJ, Powers AM, Davis EA, Klein R, Cordon-Rosales C, 2006. West Nile virus in horses, Guatemala. *Emerg Infect Dis* 12: 1038–1039.

10. Morales-Betoulle ME, Komar N, Martinez LE, Betoulle J-L, Alvarez D, Panella N, Contreras CL, Cordon-Rosales C, 2008. The presence of migratory birds may reduce West Nile virus transmission in Guatemala [Spanish]. *Boletín de la Universidad del Valle de Guatemala* 16: 99–110.
11. Hobson-Peters J, Arévalo C, Cheah WY, Blitvich BJ, Tan CS, Sandis A, Araya LN, Hernández JL, Toye P, Hall RA, 2011. Detection of antibodies to West Nile virus in horses, Costa Rica, 2004. *Vector Borne Zoonotic Dis* 11: 1081–1084.
12. Mattar S, Komar N, Young G, Alvarez J, Gonzalez M, 2011. Seroconversion to West Nile and St. Louis encephalitis viruses among sentinel horses, Colombia. *Mem Inst Oswaldo Cruz* 106: 976–979.
13. Bosch I, Herrera F, Navarro JC, Lentino M, Dupuis A, Maffei J, Jones M, Fernández E, Pérez N, Pérez-Emán J, Guimarães AE, Barrera R, Valero N, Ruiz J, Velásquez G, Martínez J, Comach G, Komar N, Spielman A, Kramer L, 2007. West Nile virus, Venezuela. *Emerg Infect Dis* 13: 651–653.
14. Quirin R, Salas M, Zientara S, Zeller H, Labie J, Murri S, Lefrançois T, Petitclerc M, Martínez D, 2004. West Nile virus, Guadeloupe. *Emerg Infect Dis* 10: 706–708.
15. Dupuis AP 2nd, Marra PP, Reitsma R, Jones MJ, Louie KL, Kramer LD, 2005. Serologic evidence for West Nile virus transmission in Puerto Rico and Cuba. *Am J Trop Med Hyg* 73: 474–476.
16. Komar O, Robbins MB, Klenk K, Blitvich BJ, Marlenee NL, Burkhalter KL, Gubler DJ, Gonzalez G, Pena CJ, Peterson AT, Komar N, 2003. West Nile virus transmission in resident birds, Dominican Republic. *Emerg Infect Dis* 9: 1299–1302.
17. Komar O, Robbins MB, Contreras GG, Benz BW, Klenk K, Blitvich BJ, Marlenee NL, Burkhalter KL, Beckett S, Gonzalez G, Pena CJ, Peterson AT, Komar N, 2005. West Nile virus survey of birds and mosquitoes in the Dominican Republic. *Vector Borne Zoonotic Dis* 5: 120–126.
18. Beatty ME, Hunsperger E, Long E, Schürch J, Jain S, Colindres R, Lerebours G, Bernard YM, Dobbins JG, Brown M, Clark GG, 2007. Mosquito-borne infections after Hurricane Jeanne, Haiti, 2004. *Emerg Infect Dis* 13: 308–310.
19. Dupuis AP 2nd, Marra PP, Kramer LD, 2003. Serologic evidence of West Nile virus transmission, Jamaica, West Indies. *Emerg Infect Dis* 9: 860–863.
20. Elizondo-Quiroga D, Davis CT, Fernandez-Salas I, Escobar-Lopez R, Velasco Olmos D, Soto Gastalum LC, Aviles Acosta M, Elizondo-Quiroga A, Gonzalez-Rojas JI, Contreras Cordero JF, Guzman H, Travassos da Rosa A, Blitvich BJ, Barrett AD, Beaty BJ, Tesh RB, 2005. West Nile virus isolation in human and mosquitoes, Mexico. *Emerg Infect Dis* 11: 1449–1452.
21. Ulloa A, Ferguson HH, Méndez-Sánchez JD, Danis-Lozano R, Casas-Martínez M, Bond JG, García-Zebadúa JC, Orozco-Bonilla A, Juárez-Ordaz JA, Farfan-Ale JA, García-Rejón JE, Rosado-Paredes EP, Edwards E, Komar N, Hassan HK, Unnasch TR, Rodríguez-Pérez MA, 2009. West Nile virus activity in mosquitoes and domestic animals in Chiapas, México. *Vector Borne Zoonotic Dis* 9: 555–560.
22. Velásquez G, Herrera F, Komar N, Montañés H, Francisco A, Rivero J, 2008. Evaluation of West Nile virus vectors in Venezuela, using VecTest for rapid detection of infected mosquitoes [Spanish]. *Entomotropica* 23: 167–172.
23. Reiter P, 1983. A revised version of the CDC gravid mosquito trap. *J Am Mosq Control Assoc* 3: 325–327.
24. Clark-Gil S, Darsie RF Jr, 1983. The mosquitoes of Guatemala, their identification, distribution and bionomics, with keys to adult females and larvae, in English and Spanish. *Mosq Systematics* 15: 151–284.
25. Ralph CJ, Sauer JR, Droege S, 1995. Monitoring bird populations by point counts. Gen. Tech. Rep. PSW-GTR-149. Albany, CA: Pacific Southwest Research Station, Forest Service, U.S. Department of Agriculture.
26. Blitvich BJ, Marlenee NL, Hall RA, Calisher CH, Bowen RA, Roehrig JT, Komar N, Langevin SA, Beaty BJ, 2003. Epitope-blocking enzyme-linked immunosorbent assays for the detection of serum antibodies to West Nile virus in multiple avian species. *J Clin Microbiol* 41: 1041–1047.
27. Beaty B, Calisher CH, Shope RE, 1995. Arboviruses. EH Lennette, DA Lennette, ET Lennette, eds. *Viral, Rickettsial, and Chlamydial Infections*. Seventh edition. Washington, DC: American Public Health Association, 189–212.
28. Morales-Betoulle ME, Monzón Pineda ML, Sosa SM, Panella N, López MR, Cordon-Rosales C, Komar N, Powers A, Johnson BW, 2008. *Culex flavivirus* isolates from mosquitoes in Guatemala. *J Med Entomol* 6: 1187–1190.
29. Kuno G, Chang GJ, Tsuchiya KR, Karabatsos N, Cropp CB, 1998. Phylogeny of the genus *Flavivirus*. *J Virol* 72: 73–83.
30. Powers AM, Ledermann JP, 2010. Venezuelan equine encephalitis virus. D Liu, ed. *Molecular Detection of Human Viral Pathogens*. Boca Raton, FL: CRC Press, 373–382.
31. Lambert AJ, Lanciotti RS, 2009. Consensus amplification and novel multiplex sequencing method for S segment species identification of 47 viruses of the *Orthobunyavirus*, *Phlebovirus*, and *Nairovirus* genera of the family *Bunyaviridae*. *J Clin Microbiol* 47: 2398–2404.
32. González-Reiche AS, Monzón-Pineda Mde L, Johnson BW, Morales-Betoulle ME, 2010. Detection of West Nile viral RNA from field-collected mosquitoes in tropical regions by conventional and real-time RT-PCR. N King, ed. *RT-PCR Protocols*. Second edition. New York: Springer-Walker, 109–124.
33. Komar N, Panella NA, Langevin SA, Brault AC, Amador M, Edwards E, Owen JC, 2005. Avian hosts for West Nile virus in St. Tammany Parish, Louisiana 2002. *Am J Trop Med Hyg* 73: 1031–1037.
34. Garrett-Jones C, Shidrawi GR, 1969. Malaria vectorial capacity of a population of *Anopheles gambiae*: an exercise in epidemiological entomology. *Bull World Health Organ* 40: 531–545.
35. Hamer GL, Kitron UD, Goldberg TL, Brawn JD, Loss SR, Ruiz MO, Hayes DB, Walker ED, 2009. Host selection by *Culex pipiens* mosquitoes and West Nile virus amplification. *Am J Trop Med Hyg* 80: 268–278.
36. Kilpatrick AM, 2011. Globalization, land use, and the invasion of West Nile Virus. *Science* 334: 323–327.
37. Hamer GL, Chaves LF, Anderson TK, Kitron UD, Brawn JD, Ruiz MO, Loss SR, Walker ED, Goldberg TL, 2011. Fine-scale variation in vector host use and force of infection drive localized patterns of West Nile virus transmission. *PLoS ONE* 6: e23767.
38. Kilpatrick AM, Daszak P, Jones MJ, Marra PP, Kramer LD, 2006. Host heterogeneity dominates West Nile virus transmission. *Proc Royal Soc B-Biol Sci* 273: 2327–2333.
39. Guerrero-Sánchez S, Cuevas-Romero S, Nemeth NM, Trujillo-Olivera MT, Worwa G, Dupuis A, Brault AC, Kramer LD, Komar N, Estrada-Franco JG, 2011. West Nile virus infection of birds, Mexico. *Emerg Infect Dis* 17: 2245–2252.
40. Komar N, Dohm DJ, Turell MJ, Spielman A, 1999. Eastern equine encephalitis in birds: relative competence of European starlings (*Sturnus vulgaris*). *Am J Trop Med Hyg* 60: 387–391.
41. Biggerstaff BJ, 2006. *PooledInfRate, version 3.0: a Microsoft Excel Add-In to Compute Prevalence Estimates from Pooled Samples*. Fort Collins, CO: Centers for Disease Control and Prevention.
42. Kramer LD, Styer LM, Ebel GD, 2008. A global perspective on the epidemiology of West Nile virus. *Annu Rev Entomol* 53: 61–81.
43. Reisen W, Lothrop H, Chiles R, Madon M, Cossen C, Woods L, Husted S, Kramer V, Edman J, 2004. West Nile virus in California. *Emerg Infect Dis* 10: 1369–1378.
44. Tesh RB, Parsons R, Siirin M, Randle Y, Sargent C, Guzman H, Wuithiranyagool T, Higgs S, Vanlandingham DL, Bala AA, Haas K, Zerinque B, 2004. Year-round West Nile virus activity, Gulf Coast region, Texas and Louisiana. *Emerg Infect Dis* 10: 1649–1652.
45. Barrera R, Hunsperger E, Muñoz-Jordán JL, Amador M, Diaz A, Smith J, Bessoff K, Beltran M, Vergne E, Verduin M, Lambert A, Sun W, 2008. First isolation of West Nile virus in the Caribbean. *Am J Trop Med Hyg* 78: 666–668.
46. Ulloa A, García-Rodríguez HH, Méndez-Sánchez JD, Danis-Lozano R, Casas-Martínez M, Bond-Compean JG, García-Zebadúa J, Orozco-Bonilla A, Juárez-Ordaz JA, Farfan-Ale JA, García-Rejón J, Rosado-Paredes EP, Edwards E, Komar N, Hassan HK, Unnasch TR, Rodríguez-Pérez MA, 2009. West Nile virus activity in mosquitoes and domestic animals in Chiapas, México. *Vector Borne Zoonotic Dis* 9: 555–560.

47. Mores CN, Turell MJ, Dohm DJ, Blow JA, Carranza MT, Quintana M, 2007. Experimental transmission of West Nile virus by *Culex nigripalpus* from Honduras. *Vector Borne Zoonotic Dis* 7: 279–284.
48. Kilpatrick AM, Kramer LD, Campbell S, Alleyne EO, Dobson AP, Daszak P, 2005. West Nile virus risk assessment and the bridge vector paradigm. *Emerg Infect Dis* 11: 425–429.
49. Goddard LB, Roth AE, Reisen WK, Scott TW, 2002. Vector competence of California mosquitoes for West Nile virus. *Emerg Infect Dis* 8: 1385–1391.
50. 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. *J Med Entomol* 42: 57–62.
51. Kent RJ, Crabtree MB, Miller BR, 2010. Transmission of West Nile virus by *Culex quinquefasciatus* Say infected with *Culex* flavivirus Izabal. *PLoS Negl Trop Dis* 4: e671.
52. Kading RC, Reiche AS, Morales-Betoulle ME, Komar N, 2013. Host Selection of Potential West Nile Virus Vectors in Puerto Barrios, Guatemala, 2007. *Am J Trop Med Hyg* 88: 108–115.
53. Wang G, Minnis RB, Belant JL, Wax CL, 2010. Dry weather induces outbreaks of human West Nile virus infections. *BMC Infect Dis* 10: 38. Available at: <http://www.biomedcentral.com/1471-2334/10/38>. Accessed November 20, 2011.
54. Kilpatrick AM, Meola MA, Moudy RM, Kramer LD, 2008. Temperature, viral genetics, and the transmission of West Nile virus by *Culex pipiens* mosquitoes. *PLoS Pathog* 4: e1000092.
55. Reisen WK, Carroll BD, Takahashi R, Fang Y, Garcia S, Martinez VM, Quiring R, 2009. Repeated West Nile virus epidemic transmission in Kern County, California, 2004–2007. *J Med Entomol* 46: 139–157.
56. Kilpatrick AM, Kramer LD, Jones MJ, Marra PP, Daszak P, 2006. West Nile virus epidemics in North America are driven by shifts in mosquito feeding behavior. *PLoS Biol* 4: 606–610.