

Short Report

Serologic Evidence of West Nile Virus Infection in Birds, Tamaulipas State, México

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ABSTRACT

Following the introduction of West Nile virus (WNV) into North America in 1999, surveillance for WNV in migratory and resident birds was established in Tamaulipas State, northern México in December 2001. Overall, 796 birds representing 70 species and 10 orders were captured and assayed for antibodies to WNV. Nine birds had flavivirus-specific antibodies by epitope-blocking enzyme-linked immunosorbent assay; four were confirmed to have antibody to WNV by plaque reduction neutralization test. The WNV-infected birds were a house wren, mourning dove, verdin and Bewick's wren. The house wren is a migratory species; the other WNV-infected birds are presumably residents. The WNV-infected birds were all captured in March 2003. These data provide the first indirect evidence of WNV transmission among birds in northern México. Key Words: West Nile virus—*Flavivirus*—Mexico—Bird—Surveillance. Vector-Borne Zoonotic Dis. 3, 209–213.

West Nile virus (WNV; family *Flaviviridae*, genus *Flavivirus*) is usually maintained in cycles between birds and *Culex* species mosquitoes (Burke and Monath 2001). Humans, horses, and other mammals are typically dead-end hosts. WNV has a wide geographic distribution, including Africa, Eurasia, Australia, and more recently North America (Campbell et al. 2002). The initial outbreak of WNV in North America took place in New York City in 1999. Since then, the virus has rapidly dispersed across the Western Hemisphere. WNV activity has now been reported throughout most of the

United States (Centers for Disease Control and Prevention 2002) and southern Canada (Health Canada 2003), as well as several Mexican states (Blitvich et al. 2003a, Dupuis et al. 2003, Loroño-Pino et al. 2003, Ulloa et al. 2003, Mexican Agricultural Ministry 2003), the Caribbean islands (Dupuis et al. 2003, Komar et al. 2003), and possibly El Salvador (Nart 2003).

In response to the introduction of WNV into New York, we established avian and equine infection surveillance in the northeastern states of México in 2001. Tamaulipas State is bordered on the east by the Gulf of México and on the

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north by Texas. The coast of the Gulf of México is a major migratory route for many species of birds migrating from the northeastern, mid-western, and southern United States (Howell and Webb 1995). Many of these birds migrate during or at the end of the arbovirus transmission season in the United States. We hypothesized that WNV could be introduced into Tamaulipas State, and elsewhere in México, via migrating birds. WNV activity has been reported in >80% of Texas counties (Texas Department of Health 2003). Thus, another possible mode of entry of WNV into Tamaulipas State was by its gradual southward spread by short-distance movement of birds (Rappole and Hubalek 2003). Recently, we presented serologic evidence for WNV infections in horses in the nearby Mexican State of Coahuila (Blitvich et al. 2003a). Here, we present data from our avian infection surveillance studies in Tamaulipas State. The Mexican Agricultural Ministry recently announced the first evidence of WNV infection in a bird in Mexico: the isolation of WNV from a corvid in Tabasco State, southern México in May 2003 (Mexican Agricultural Ministry 2003). In this report, we provide the first evidence of WNV transmission among birds in northern México.

Trapping was conducted at Laguna Madre, Tamaulipas State from December 2001 to March 2003. Laguna Madre (23–25°N, 97°W) is located just inland of the Gulf of Mexico. It is the largest lagoon in México, being approximately 185 km in length and 2,000 km² in surface area. Dune vegetation, such as sea oats (*Uniola paniculata*), occurs at the lagoon margins. The average annual temperature is 22–24°C. The average rainfall is 300–750 mm/year.

Birds were trapped at Laguna Madre for 4 days every 2 weeks. Study sites were established in four locations: El Carrizo, El Mezquital, El Refugio, and Las Carretas (Fig. 1). Trapping was performed using strategically placed mist nests, and at least 25 mist nests were used at each study site. Birds were identified according to species, and the migratory or resident status of each bird was also determined (Sibley 2001). Bird species with both migratory and year-round resident populations in Tamaulipas State were classified as presumed residents. Birds were bled from the jugular or ulnar vein,

and blood samples were immediately diluted fivefold using field diluent (sterile phosphate buffered saline containing 0.75% bovine albumin, 100 units/mL penicillin, 100 µg/mL streptomycin, 250 µg/mL amphotericin B). Samples were placed on wet ice (4°C), transported to the Universidad Autónoma de Nuevo León, centrifuged, and stored at –70°C.

In total, 796 birds belonging to 70 species, 24 families, and 10 orders were sampled. The most highly represented orders were Passeriformes ($n = 573$), Columbiformes ($n = 159$), Piciformes ($n = 25$), and Anseriformes ($n = 12$). Overall, 164 (20.6%) birds were identified as migrants, and 632 (79.4%) birds were identified as residents. Migratory birds were most frequently captured in June ($n = 25$) and October ($n = 26$). The most highly represented migratory species were the brown-crested flycatcher (*Myiarchus tyrannulus*, $n = 30$) and Lincoln's sparrow ($n = 17$). The most frequently trapped resident bird species were the common ground dove (*Columbina passerina*, $n = 125$), northern mockingbird (*Mimus polyglottos*, $n = 112$), white-eyed vireo (*Vireo griseus*, $n = 55$), and northern cardinal (*Cardinalis cardinalis*, $n = 46$).

All sera were screened at 1:10 for antibodies to flaviviruses by epitope-blocking enzyme-linked immunosorbent assay (ELISA; Blitvich et al. 2003b). Blocking ELISAs were performed using the flavivirus group-reactive monoclonal antibody (MAb), 6B6C-1, or the WNV-specific MAb, 3.1112G. Sera positive for antibodies to flaviviruses were then tested for neutralizing antibodies to WNV and Saint Louis encephalitis virus (SLEV) by plaque reduction neutralization assay (PRNT). PRNTs were conducted in the BSL-3 facilities at Colorado State University. Isolates of WNV (strain NY99-35261-11) and SLEV (strain TBH-28) were obtained from the World Health Organization Center for Arbovirus Reference and Research, maintained at the Centers for Disease Control and Prevention, Division of Vector-Borne Infectious Diseases, Fort Collins, Colorado. It was important to test for neutralizing antibody to SLEV because this virus is enzootic in the Americas, and antibodies to WNV and SLEV may cross-react. PRNTs were performed using Vero cells. Sera were initially tested at a dilution of 1:20, and titers were expressed as

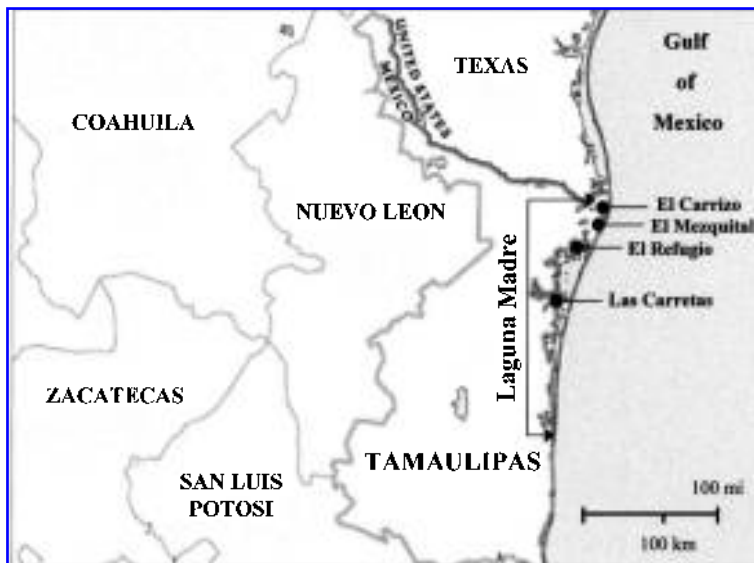


FIG. 1. Geographic location of West Nile virus study sites near Laguna Madre in Tamaulipas State, Mexico.

the reciprocal of serum dilutions, yielding $\geq 90\%$ reduction in the number of plaques (PRNT₉₀).

Nine (1.15%) serum samples had antibodies to flaviviruses as determined by the blocking ELISA test (Table 1). All nine birds had appeared healthy when captured. Neutralizing antibodies to WNV were detected in four of these birds. All four birds were considered seropositive for WNV infection because their WNV PRNT₉₀ titers were at least fourfold greater than their corresponding titers to SLEV. These four seropositive birds were a mourning dove (*Zenaida macroura*, LM-19), verdin (*Auriparus flaviceps*, LM-26), house wren (*Troglodytes aedon*, LM-37), and Bewick's wren (*Thryomanes bewickii*, LM-124). The house wren is a migratory species. The verdin and Bewick's wren are residents of Tamaulipas State, and the mourning dove is a presumed resident. These four birds were captured in March 2003.

Neutralizing antibodies to SLEV were not detected in any birds when using a diagnostic criterion of PRNT₉₀, but were detected in three birds using the less stringent PRNT₇₀ (Table 1). The birds were a verdin (LM-26), northern cardinal (LM-70), and common-ground dove (LM-75). The verdin was positive for WNV infection by PRNT₉₀; the latter two birds were consid-

ered to be infected with an undetermined flavivirus. Three birds were positive in the blocking ELISA that utilized the group-reactive MAb 6B6C-1, but had no detected neutralizing antibody to WNV or SLEV, and presumably had antibodies to a flavivirus of unknown etiology. A Lincoln's sparrow (LM-69) was positive in the WNV-specific blocking ELISA, but had no detectable levels of neutralizing antibodies to WNV; thus, this bird was considered to have antibodies to an undetermined flavivirus.

The overall prevalence of antibodies to WNV was 0.51%. Similarly, we reported a low prevalence (0.06%) of WNV antibodies in 8611 birds trapped in Yucatán State, México, from March 2000 and April 2003 (Farfán-Ale et al. 2003). We reported considerably higher infection rates in horses from Coahuila and Yucatán States (62.5% and 1.19%, respectively; Blitvich et al. 2003a, Loroño-Pino et al. 2003). Furthermore, the first WNV-seropositive horse in northern Mexico was sampled in December 2002, 3 months before we captured the first WNV-seropositive bird in the present serosurvey. Equine surveillance may provide a more timely and efficient means to detect potential WNV activity in regions in Central and South America, possibly due to the mammalophilic feeding habits of *Cx. quinquefasciatus* (Tempelis 1974). However, equine surveillance in Central

TABLE 1. SUMMARY OF BIRDS WITH EPI TOPE-BLOCKING ELISA AND/OR NEUTRALIZING ANTIBODIES TO FLAVIVIRUSES COLLECTED IN LAGUNA MADRE, TAMAULIPAS STATE, MEXICO, 2001–2003

ID number	Common name (species name)	Family (order)	Resident status	Blocking ELISA ^b		PRNT ₉₀ titer		Diagnosis
				3.1112G ^c	6B6C-1 ^d	WNV	SLEV	
LM-19	Mourning Dove (<i>Zenaidura macroura</i>)	Columbidae (Columbiformes)	R ^a	72	91	≥320	<20	WNV
LM-26	Verdin (<i>Auriparus flaviceps</i>)	Remziidae (Passeriformes)	R	81	78	80	<20 ^e	WNV
LM-37	House Wren (<i>Troglodytes aedon</i>)	Troglodytidae (Passeriformes)	M	76	38	≥320	<20	WNV
LM-124	Bewick's Wren (<i>Thryomanes bewickii</i>)	Troglodytidae (Passeriformes)	R	93	70	≥320	<20	WNV
LM-69	Lincoln's Sparrow (<i>Melospiza lincolni</i>)	Emberizidae (Passeriformes)	M	36	22	<20	<20	Flavivirus
LM-70	Northern Cardinal (<i>Cardinalis cardinalis</i>)	Cardinalidae (Passeriformes)	R	20	46	<20	<20 ^e	Flavivirus
LM-73	White-eyed Vireo (<i>Vireo griseus</i>)	Vireonidae (Passeriformes)	R ^a	28	31	<20	<20	Flavivirus
LM-74	White-eyed Vireo (<i>Vireo griseus</i>)	Vireonidae (Passeriformes)	R ^a	25	47	<20	<20	Flavivirus
LM-75	Common-ground Dove (<i>Columbina passerina</i>)	Columbidae (Columbiformes)	R	12	34	<20	<20 ^e	Flavivirus

^aPresumed resident; this species is found year round in Tamaulipas State, although both resident and migratory populations may be present (Sibley 2001).

^bInhibition values ≥30% are considered significant.

^cMAb 3.1112G is WNV-specific.

^dMAb 6B6C-1 is flavivirus-group reactive.

^ePRNT₇₀ titer: 20.

ELISA, enzyme-linked immunosorbent assay; PRNT₉₀, reciprocal 90% plaque reduction neutralization test titer; WNV, West Nile virus; SLEV, Saint Louis encephalitis virus; M, migrant; R, resident.

and South America requires the differential diagnosis of other equine encephalitides found in these regions, such as Venezuelan, eastern and western encephalitis viruses, and rabies virus.

In summary, we have obtained serologic evidence that WNV is active in Tamaulipas State, México. The potential health risk that this introduction poses to humans, as well as equines and avians, in México remains to be determined. Thus, continued surveillance for WNV activity in México is warranted.

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REFERENCES

- Blitvich, BJ, Fernandez-Salas, I, Contreras-Cordero, JF, et al. Serologic evidence of West Nile virus infection in horses, Coahuila State, Mexico. *Emerg Infect Dis* 2003a; 9:853–856.
- Blitvich, BJ, Marlenee, NL, Hall, RA, et al. Epitope-blocking enzyme-linked immunosorbent assays for the detection of serum antibodies to West Nile virus in multiple avian species. *J Clin Microbiol* 2003b; 41:1041–1047.
- Burke, DS, Monath, TP. Flaviviruses. In: Knipe, DM, Howley, PM, eds. *Fields Virology*, 4th ed. Philadelphia: Lippincott Williams and Wilkins; 2001:1043–1126.
- Campbell, GL, Marfin, AA, Lanciotti, RS, et al. West Nile virus. *Lancet Infect Dis* 2002; 2:519–529.
- Centers for Disease Control and Prevention. Provisional surveillance summary of the West Nile virus epidemic—United States, January—November 2002. *Morb Mortal Wkly Rep* 2002; 51:1129–1133.
- Dupuis, AP, Marra, PP, Kramer, LD. Serologic evidence

- of West Nile virus transmission, Jamaica, West Indies. *Emerg Infect Dis* 2003; 9:860–863.
- Farfán-Ale, JA, Blitvich, BJ, Loroño-Pino, MA, et al. Longitudinal studies of West Nile virus infection in avians, Yucatan State, Mexico. *Vector-Borne Zoonotic Infect* (accepted for publication).
- Health Canada. Population and Public Health Branch WNV surveillance updates [On-line]. Available www.hc-sc.gc.ca/pphb-dgspsp/wnv-vwn/mon_e.html#sitrep, 2003.
- Howell, SNG, Webb, S. *A Guide to the Birds of Mexico and Northern Central America*. Oxford: Oxford University Press, 1995.
- Komar, O, Robbins, MB, Klenk, K, et al. West Nile virus transmission in resident birds, Dominican Republic. *Emerg Infect Dis* 9:1299–1302.
- Loroño-Pino, MA, Blitvich, BJ, Farfán-Ale, JA, et al. Serologic evidence for West Nile virus infection in horses, Yucatán State, México. *Emerg Infect Dis* 2003; 9:857–859.
- Mexican Agricultural Ministry—Diario Oficial de la Federación, June 2003, 2003 [On-line]. Available: <http://dof.terra.com.mx>, 2003.
- Nart, P. El Salvador—health authorities confirm the presence of West Nile virus. *PromED*. April 29, 2003 [On-line]. Available: www.promedmail.org [archive number: 20030504.1116], 2003.
- Rappole, JH, Hubalek, Z. Migratory birds and West Nile virus. *J Appl Microbiol* 2003; 94:47–58.
- Sibley, DA. *The Sibley Guide to Birds*. New York: Chanticleer Press, 2001.
- Tempelis, DH. Host-feeding patterns of mosquitoes, with a review of advances in analysis of blood meals by serology. *J Med Entomol* 1974; 11:635–653.
- Texas Department of Health. West Nile virus in Texas [On-line]. Available: www.tdh.state.tx.us/zoonosis/diseases/Arboviral/westNile/default.asp, 2003.
- Ulloa, A, Langevin, SA, Mendez-Sanchez, JD, et al. Serologic survey of domestic animals for zoonotic arbovirus infections in the Lacandon Forest region of Chiapas, Mexico. *Vector-Borne Zoonotic Dis* 2003; 3:3–9.

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