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Serologic Surveillance for West Nile Virus and Other Flaviviruses in Febrile Patients, Encephalitic Patients, and Asymptomatic Blood Donors in Northern Mexico

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Abstract

A clinical and serological investigation was performed to determine the presence of West Nile virus (WNV) among febrile and encephalitic patients in northern Mexico. In addition, asymptomatic blood donors were serologically assayed for WNV to determine the seroprevalence of WNV in the general population. The study cohort consisted of 1432 individuals (588 febrile patients, 44 encephalitic patients, and 800 asymptomatic blood donors). All subjects were negative for WNV IgM. Sixty subjects were reactive for dengue virus (DENV) IgM (16 blood donors and 44 febrile patients). A subset ($n = 425$) of individuals was also screened by ELISA for flavivirus IgG. The prevalence of flavivirus IgG in febrile patients, encephalitic patients, and blood donors ranged from 40% to 59%. A subset ($n = 147$) of sera reactive for flavivirus IgG was further tested by plaque reduction neutralization test. Six individuals with no history of travel during the preceding 12 months were seropositive for WNV. Another 65 individuals were seropositive for DENV1 and 24 were seropositive for DENV2. The high prevalence of dengue antibodies in northern Mexico appears to limit the incidence of WNV infection in this region.

Article Summary Line: Antibodies to WNV, DENV-1, and DENV-2 were identified in humans in northern Mexico.

Key Words: Blood donor—Dengue virus—Flavivirus—Human—Mexico—Surveillance—West Nile virus.

Introduction

West Nile virus (WNV) (family *Flaviviridae*, genus *Flavivirus*) was first isolated in 1937 from the blood of a febrile woman in the West Nile district of Uganda (Smithburn 1940). The virus was first reported in the Western Hemisphere in 1999 during an outbreak of human, equine, and avian encephalitis in New York City (Lanciotti et al. 1999, Nash et al. 2001). Since then, WNV has dispersed across the Western Hemisphere and is now found throughout the United States, Canada, Mexico, and the Caribbean, and parts of Central and South America (Komar et al. 2006, Blitvich 2008, Kramer et al. 2008). In the United States, WNV has been responsible for more than 27,000 cases of human illness with over 1000

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deaths. In contrast, the introduction of WNV into Mexico has been relatively benign despite data from equine and avian infection surveillance providing evidence of widespread WNV circulation in Mexico since 2003 (Blitvich et al. 2003, Estrada-Franco et al. 2003, Fernandez-Salas et al. 2003, Lorono-Pino et al. 2003). The Mexican Secretary of Public Health has reported seven human cases of WNV in Mexico (Komar et al. 2006, Blitvich 2008). The cases occurred in the States of Chihuahua ($n = 4$), Sonora ($n = 1$), and Nuevo Leon ($n = 1$) in 2003, and Sonora ($n = 1$) in 2004. These states are in northern Mexico, and all border the United States. In addition, WNV RNA has been detected in the Mexican blood supply (Sanchez-Guerrero et al. 2006). The viremic donor was identified as an asymptomatic 41-year-old man from Chihuahua State and was also positive for WNV IgM, but not IgG, suggesting that he had a recently acquired WNV infection.

The reasons for the low incidence of WNV illness in Mexico as compared to the United States are not known. One explanation is that preexisting immunity to another flavivirus is providing partial protection to humans and other vertebrates from subsequent WNV infection. Indeed, laboratory studies have shown that prior immunization of hamsters with heterologous flaviviruses (Japanese encephalitis virus, St. Louis encephalitis virus [SLEV] and Yellow fever virus) reduces the severity of subsequent WNV infection (Tesh et al. 2002). The four serotypes of dengue virus (DENV1–DENV4) and SLEV are endemic in many regions of Mexico and therefore could be reducing the incidence and/or severity of WNV infections in humans in Mexico (Gubler 2002, 2006, Hayes and Gubler 2006, Blitvich 2008, Kramer et al. 2008). Other explanations include under-reporting, the emergence of attenuated WNV variants, and geographic differences in the species composition, relative abundance, and susceptibility of vertebrates or vectors.

The majority of WNV infections are subclinical with no apparent symptoms, but during recent outbreaks in the United States, approximately 20% of infections have resulted in a mild flu-like illness known as West Nile fever (WNF), and 1 in 150 infections has resulted in severe neuroinvasive disease (WNND) (Mostashari et al. 2001, Gubler 2002, Hayes and Gubler 2006). WNF is characterized by a variety of nonspecific symptoms (fever, headache, myalgia, nausea, fatigue, weakness, vomiting, and diarrhea) that cannot be distinguished from other infectious illnesses on clinical examination. The illness typically lasts for 2–5 days, but in more severe cases, fatigue can persist for over a month. WNND is characterized by encephalitis, meningitis, and/or poliomyelitis-like flaccid paralysis. The fatality rate in patients with WNND is approximately 10%, and long-term neurological sequelae occurs in >50% of patients (Hayes et al. 2005, Hayes and Gubler 2006, Sejvar and Marfin 2006).

The laboratory test most commonly used for WNV diagnosis is the IgM antibody-capture enzyme-linked immunosorbent assay (MAC-ELISA) (Martin et al. 2000, 2002). This assay provides a rapid and sensitive method to detect WNV IgM, which is usually detected in patients at clinical presentation but can persist for more than a year (Roehrig et al. 2003). Another technique routinely used for WNV diagnosis is the indirect IgG ELISA, which is often performed in tandem with the MAC-ELISA (Tardei et al. 2000). This technique exhibits a high degree of sensitivity when used to screen sera for the presence of WNV IgG. However, because of the cross-reactivity of antiflaviviral IgG, the assay is not WNV specific. For this reason, samples positive by flavivirus IgG ELISA are often further tested by plaque reduction neutralization test (PRNT), the most accurate serologic test available for flavivirus diagnosis (Beaty 1995). PRNTs performed using WNV and other flaviviruses endemic in the same area provide the most accurate serologic approach to identify WNV infections and distinguish serologic cross-reactions among flaviviruses.

In this study, we performed a clinical and serological investigation to obtain information that could help to explain the low incidence of WNV illness in northern Mexico. Patients who presented clinical signs consistent with WNF or WNND were recruited for the study and serologically assayed for WNV and other flaviviruses by MAC-ELISA, IgG ELISA, and/or PRNT. In addition, the seroprevalence of WNV in the general population of northern Mexico was estimated using asymptomatic blood donors as a convenience population.

Materials and Methods

Study population

Our study cohort consisted of three groups of individuals: (i) patients with suspected WNF, (ii) patients with suspected WNND, and (iii) asymptomatic blood donors. Our criteria for suspected WNF and WNND were consistent with the U.S. national case definition established by the Centers for Disease Control and Prevention (CDC) (CDC 2009). The clinical criterion for WNF was the presence of documented fever, as measured by the patient or clinician, in the absence of WNND. The clinical criterion for WNND was the presence of fever in addition to at least one of the following (as measured by the clinician): (i) an acutely altered mental status such as disorientation, stupor, and coma, (ii) other acute signs of central or peripheral neurologic dysfunction such as paralysis, sensory deficits, abnormal reflexes, generalized convulsions, and abnormal movements, or (iii) illness clinically compatible with meningitis (e.g., headache or stiff neck).

Patients and asymptomatic blood donors were recruited between October 2005 and September 2007 from the following Health Centers localized in Monterrey, Mexico: the Dr. Jose E. Gonzalez University Hospital School of Medicine (Universidad Autonoma de Nuevo Leon, UANL), a Diagnostic Reference Laboratory from Federal Secretariat of Health, and a third-level Hospital Number 33 from government Social Insurance. Written consent to participate in the study was obtained from each subject. For patients <18 years of age, written consent was obtained from parents or legal guardians. A serum sample was collected from each individual who agreed to participate. Demographic information and other pertinent information (symptoms, travel history, and vaccination status) were obtained for each study participant by self-questionnaire. Individuals who had been immunized against yellow fever virus were not included in this study because they had preexisting flavivirus antibody that could potentially cause serologic cross-reactivity in the diagnostic assays. All data were handled confidentially and anonymously. All research protocols were approved by the institutional review boards at the UANL and Iowa State University (ISU).

Demographic characteristics

A total of 1432 individuals were recruited for the study (Table 1). Of these, 588 were patients with unspecified fever,

TABLE 1. DEMOGRAPHIC AND CLINICAL CHARACTERISTICS OF THE STUDY COHORT

	Study group			
Demographic characteristics	*Febrile patients*	*Encephalitic patients*	*Blood donors*	*Total*
No. participants	588	44	800	1432
No. males	182	27	658	867
No. females	406	17	142	565
Male:female ratio	1:2.2	1:0.6	1:0.2	1:0.7
Age range (years)	1–73	1–79	18–60	1–79
Mean age	28	33	32	31
State of residence:				
(1) Nuevo Leon	588	43	767	1398
(2) Coahuila	0	0	8	8
(3) Tamaulipas	0	1	25	26

44 were patients with encephalitic manifestations, and 800 were asymptomatic blood donors. There were 867 (61%) males and 565 (39%) females. All study participants were from northern Mexico. The majority lived in Nuevo Leon State, the remainder lived in Coahuila State and Tamaulipas State. The ages of the study participants ranged from 1 to 79 years. The mean age was 31 years.

WNV MAC-ELISA

All sera were assayed for WNV IgM using the WNV IgM Capture-ELISA from Focus Technologies (Cypress, CA) in the Biosafety Level 2 facilities at the UANL following the manufacturer's instructions. Test samples and positive and negative controls were diluted 1:100 and then added to microwells coated with anti-human IgM antibodies. After 1 h incubation, wells were washed and WNV antigen was added. After 2 h incubation, plates were washed again and horseradish peroxidase (HRP)–conjugated mouse antiflavivirus conjugate was added. Plates were incubated for 30 min and then washed, and a substrate consisting of tetramethylbenzidine (TMB) and hydrogen peroxide was added to each well. After 10 min, stop solution was added and the absorbance was determined spectrophotometrically at 450 nm. Index values for test sera and controls were obtained by dividing the absorbance of the test or control well by the absorbance of the calibrator (cutoff control). Index values of <0.9 were considered negative, values from 0.9 to 1.1 were considered equivocal, and values of >1.1 were considered positive for IgM.

DENV MAC-ELISA

All sera were assayed for DENV IgM using the Dengue IgM Capture ELISA from PanBio Diagnostics (Brisbane, Australia). Test samples and positive and negative controls were diluted 1:100 and added to the 96-well plates precoated with capture antibody (anti-human IgM). After incubation, wells were washed, and an antigen–MAb complex containing a mixture of DENV 1–4 antigens together with an HRP-labeled MAb specific to the DENV antigens was added. After second 1 h incubation and wash step, TMB substrate was added; after 10 min, stop solution was added and the absorbance was determined spectrophotometrically at 450 nm. Index values of <0.9 were considered negative, values from 0.9 to 1.1 were considered equivocal, and values of >1.1 were considered positive for IgM.

Flavivirus indirect IgG ELISA

A subset of sera were assayed for flavivirus IgG using the WNV IgG ELISA from Focus Technologies and DENV IgG Indirect ELISA from PanBio Diagnostics. Test samples and positive and negative controls were diluted 1:100 and added to the wells of 96-well plates precoated with DENV and WNV antigen (Malan et al. 2004). After 1 h incubation, wells were washed and HRP-conjugated sheep anti-human IgG was added. After a 30 min incubation and wash step, TMB substrate was added; after 10 min, stop solution was added and the absorbance of each well is determined spectrophotometrically at 450 nm. Our results were reported as index values relative to the Cut-off calibrator. Index values for test sera and controls were obtained by dividing the absorbance (OD) of the test or control well by the mean of absorbance values of the Cut-off Calibrator. Index values of <1.3 were considered negative, values from 1.3 to 1.5 were considered equivocal, and values of >1.5 were considered positive for IgG (Hogrefe et al. 2004, Tilley et al. 2005).

PRNT

A subset of sera demonstrated to contain antibodies to flaviviruses by MAC-ELISA, and/or IgG ELISA were subsequently tested by PRNT in the Biosafety Level 3 facilities at ISU according to standard methods (Beaty 1995). PRNTs were done using WNV (strain NY99-35261–11), SLEV (strain TBH-28), DENV-1 (strain Hawaii), DENV-2 (strain NGC), DENV-3 (strain H-87), and DENV-4 (strain 241). SLEV and the four serotypes of DENV were included because they occur in Mexico, and are known to react with antibodies to WNV (Calisher et al. 1989, Burke et al. 2001). Viruses were obtained from the World Health Organization Center for Arbovirus Reference and Research maintained at the CDC, Division of Vector-Borne Infectious Diseases (Fort Collins, CO). The PRNTs were performed using African green monkey kidney (Vero) cells, with the exception of DENV-1 PRNTs, which were performed using Rhesus monkey kidney (LLC MK) cells. Sera were tested in the presence of 8% labile serum factor (Chappell et al. 1971). Sera were initially tested at a dilution of 1:20. Those that reduced the number of plaques by ≥70% ($PRNT_{70}$) were titrated. Titers were expressed as the reciprocal of serum dilutions yielding ≥90% reduction in the number of plaques ($PRNT_{90}$). For etiologic diagnosis, the $PRNT_{90}$ antibody titer to the respective virus was required to be at least fourfold greater than that to the other flaviviruses tested.

WNV case definition

In this study, a patient was considered to have an acute clinical WNV infection if all of the following criteria were met: (i) the patient had symptoms consistent with WNF or WNND, (ii) the patient was positive for the presence for WNV IgM by MAC-ELISA, and (iii) the patient's $PRNT_{90}$ antibody titer to WNV was at least fourfold greater than that to the other flaviviruses tested. An asymptomatic blood donor was considered to have a recent subclinical WNV infection if the donor was positive for the presence of WNV IgM by MAC-ELISA and their $PRNT_{90}$ antibody titer to WNV was at least fourfold greater than that to the other flaviviruses tested.

Results

Screening for WNV and DENV IgM

All study participants were screened by MAC-ELISA for the presence of WNV and DENV IgM. Individuals from all three study groups were negative for WNV IgM. Sixteen blood donors and 44 febrile patients were reactive for DENV IgM, which suggests that they had recent DENV infections. None of the encephalitic patients were reactive for DENV IgM (Table 2).

Screening for flavivirus IgG

A subset ($n = 425$) of participants were screened for antibodies to flaviviruses using the group-reactive WNV and/or DENV IgG ELISA. Of these, 51 were tested by WNV IgG ELISA only, 93 tested by DENV IgG ELISA only, and 281 were tested by both assays. Overall, 248 febrile patients, 42 encephalitic patients, and 135 blood donors were tested. Of these, flavivirus IgG was detected in 142 (57%) febrile patients, 17 (40%) encephalitic patients, and 80 (59%) blood donors (Table 2).

Screening for neutralizing antibodies to flaviviruses

A subset ($n = 147$) of sera that had antibodies to flaviviruses by MAC-ELISA and/or IgG ELISA were further analyzed by PRNT using WNV, SLEV, and all four serotypes of DENV. Six individuals were seropositive for WNV (Table 3). Of these, two were asymptomatic blood donors and four were febrile patients. However, the febrile patients did not meet the case definition for WNV because they were negative for WNV IgM. These data suggest that the patients had a prior WNV infection, but this virus was not responsible for the clinical signs observed at the time of presentation. Similarly, the two asymptomatic blood donors seropositive for WNV were negative for WNV IgM, suggesting that they did not have recently acquired WNV infections. All six individuals seropositive for WNV lived in Nuevo Leon State, and none had traveled outside the state during the preceding 12 months. These data indicate that the six individuals had locally acquired WNV infections. The two blood donors seropositive for WNV were male; one was 19 years of age, and the other was 43 years. Of the four febrile patients seropositive for WNV, three were male and one was female. Their ages ranged from 26 to 45 years. Representative serologic data from 14 flavivirus-positive study participants are shown in Table 4.

Sixty-four individuals were seropositive for DENV-1 and another 23 were seropositive for DENV-2 (Table 3). Of the individuals seropositive for DENV-1, 44 were febrile patients, 9 were encephalitic patients, and 11 were asymptomatic blood donors. Of the individuals seropositive for DENV-2, 20 were febrile patients, 1 was an encephalitic patient, and 2 were asymptomatic blood donors. Twenty-four individuals were seropositive for an undetermined DENV serotype (Tables 3 and 4). An individual was considered to be seropositive for an undetermined DENV serotype when the $PRNT_{90}$ antibody titer to one DENV serotype was at least fourfold greater than that to WNV and SLEV, but not all of the other DENV serotypes. Twenty-two individuals were considered to have a secondary flavivirus infection as indicated by the high $PRNT_{90}$ antibody titers to all viruses tested. Eight individuals had no neutralizing antibodies to flaviviruses. No study participants were seropositive for DENV-3, DENV-4, or SLEV.

Discussion

We performed a clinical and serological investigation to determine the incidence of WNV illness in febrile and encephalitic patients in northern Mexico. Our main rationale for undertaking this investigation was to obtain information that could explain why a major outbreak of WNV illness has not been observed in this region. Of the 632 patients who presented with suspected WNF or WNND, all were negative for WNV IgM; thus, none of the patients recruited for this study met the CDC case definition for WNV. Based on these

TABLE 2. PREVALENCE OF WEST NILE VIRUS IgM, DENGUE VIRUS IgM, AND FLAVIVIRUS IgG IN EACH STUDY GROUP

	Study group		
Serologic test	*Blood donors*	*Febrile patients*	*Encephalitic patients*
WNV MAC-ELISA	0/800 (0%)	0/588 (0%)	0/44 (0%)
DENV MAC-ELISA	16/800 (2.0%)	44/588 (7.5%)	0/44 (0%)
Flavivirus IgG ELISA	80/135 (59.3%)	142/248 (57.3%)	17/42 (40.5%)

Data are presented as the number of individuals tested/number of individuals positive.

WNV MAC-ELISA, West Nile virus IgM antibody-capture enzyme-linked immunosorbent assay; DENV MAC-ELISA, dengue virus IgM antibody-capture enzyme-linked immunosorbent assay.

TABLE 3. SEROLOGIC SUMMARY OF THE SUBSET OF FEBRILE PATIENTS, ENCEPHALITIC PATIENTS, AND ASYMPTOMATIC BLOOD DONORS TESTED BY PLAQUE REDUCTION NEUTRALIZATION TEST

	Study group			
PRNT diagnosis	*Febrile patients*	*Encephalitic patients*	*Blood donors*	*Total*
WNV	4	—[a]	2	6 (4%)[b]
SLEV	—	—	—	—
DENV-1	44	9	11	64 (43.5%)
DENV-2	20	1	2	23 (15.6%)
DENV-3	—	—	—	—
DENV-4	—	—	—	—
DENV (undetermined serotype)	16	3	5	24 (16.3%)
Secondary flavivirus infection	22	—	—	22 (14.9%)
Negative	6	1	1	8 (5.4%)
Total	112	14	21	147 (100%)

[a] 0.

[b] All individuals seropositive for WNV by PRNT were negative for WNV IgM, suggesting that these infections were not recent.

PRNT, plaque reduction neutralization test; SLEV, St. Louis encephalitis virus.

TABLE 4. SEROLOGIC SUMMARY OF A SUBSET OF INDIVIDUALS WITH ANTIBODIES TO FLAVIVIRUSES

		$PRNT_{90}$ titer						
Sample ID	*Participant type*	*WNV*	*SLEV*	*DENV-1*	*DENV-2*	*DENV-3*	*DENV-4*	*PRNT diagnosis*
B004	Blood donor	20	—[a]	160	40	20	40	DENV-1
B029	Blood donor	320	20	20	—	—	20	WNV
B038	Blood donor	—	—	40	—	—	20	DENV (undetermined serotype)
F100	Febrile patient	640	320	20,480	10,240	2560	10,240	Secondary flavivirus infection
F011	Febrile patient	10,240	640	10,240	10,240	1280	20,480	Secondary flavivirus infection
B034	Blood donor	—	20	320	80	40	80	DENV-1
F160	Febrile patient	20	—	40	320	20	20	DENV-2
F193	Febrile patient	—	—	160	80	—	40	DENV (undetermined serotype)
F047	Febrile patient	20	—	40	160	40	40	DENV-2
F043	Febrile patient	640	20	20	40	—	20	WNV
N 040	Encephalitis	5120	80	10,240	20,480	20,480	20,480	Secondary flavivirus infection
N012	Encephalitis	20	—	160	40	40	40	DENV-1
N017	Encephalitis	20	—	160	320	160	160	DENV (undetermined serotype)
N016	Encephalitis	20	—	80	320	80	80	DENV-2

[a]Less than 20.

findings, we speculate that under-reporting is not the major reason for the low number of WNV cases in northern Mexico.

Flavivirus IgG was detected in the majority of study participants. Of the subset of febrile and encephalitic patients screened by IgG ELISA, 57% and 40%, respectively, were positive for flavivirus IgG. The majority (59%) of asymptomatic blood donors screened for flavivirus IgG were also positive. Taken together, these data suggest that the majority of individuals in northern Mexico have had previous exposure to one or more flaviviruses. The PRNT analysis demonstrated that DENV was responsible for most flavivirus infections. Thus, we propose that the main reason for the absence of an observed outbreak of WNV illness in humans in northern Mexico is that a large proportion of the population has been infected with DENV and is therefore resistant or less susceptible to severe WNV disease. In this regard, laboratory studies have shown that prior immunization of rodents, monkeys, and pigs with heterologous flaviviruses reduces the severity of subsequent WNV infection (Price et al. 1971, Goverdhan et al. 1992, Ilkal et al. 1994, Tesh et al. 2002). The low incidence of WNV illness elsewhere in Mexico, and in Central and South America, could also be attributed to prior exposure to DENV. Indeed, more than 850,000 cases of dengue fever occurred throughout in Latin America in 2007, including 25,000 cases of dengue hemorrhagic fever (Kyle and Harris 2008). However, preexisting immunity to DENV does not account for the apparent lack of WNV-associated equine and avian death in northern Mexico and elsewhere in Latin America because DENV does not usually replicate in nonprimate vertebrates (Thomas et al. 2003). Thus, it is likely that other factors are contributing to the sparse reports of WNV illness in humans and other vertebrates in Latin America.

Of the subset of individuals tested by PRNT, 41% were seropositive for DENV1 and 15% were seropositive for DENV2. These two DENV serotypes were also shown to be responsible for human infections in a household serosurvey conducted in 2004 in the contiguous border cities of Matamoros, Tamaulipas (Mexico), and Brownsville, Texas (Brunkard et al. 2007). Recent DENV infection was identified in 7.3% of Matamoros residents and 2.0% of Brownsville residents. Prior DENV infection was identified in 78% of Matamoros residents and 40% of Brownsville residents. Of the subset ($n = 3$) of individuals further tested by PRNT, two residents from Matamoros were seropositive for DENV-1, and one resident from Brownsville was seropositive for DENV-2. The following year, an outbreak of DENV occurred in the cities of Matamoros and Brownsville (Ramos et al. 2008). Twenty-eight viral isolates were obtained: 27 were DENV-2 and 1 was DENV-1. We also demonstrated that 24% of the individuals tested by PRNT had been infected with an undetermined DENV serotype. For these individuals, the PRNT titer was always highest when DENV-1 or DENV-2 was used for the PRNT analysis. Twenty-two percent of individuals tested by PRNT had secondary flavivirus infections consistent with our findings that multiple flaviviruses (DENV1, DENV2, and WNV) are circulating in northern Mexico. Eight hundred asymptomatic blood donors were serologically assayed for WNV and two (0.25%) were positive. These data indicate that the incidence of WNV infection in the general population in northern Mexico is low. In comparison, 46 (3.1%) of 1505 individuals sampled in 2002 in a household-based seroprevalence survey in Ontario, Canada, were seropositive for WNV (Loeb et al. 2005). In another study, 122 (14.0%) of 869 individuals sampled at a health-fair in Wyoming in 2003 had antibodies to WNV (Murphy et al. 2005). However, it is important to note that different methods were used to recruit study participants in these studies, and that there are limitations associated with using blood donors as a convenience population, most notably selection bias. Indeed, blood donors are potentially more healthy and active than other individuals and therefore could be more likely to participate in outdoor activities that result in increased contact with mosquitoes. Alternatively, because blood donors are potentially more health conscious that other individuals, they could be more inclined to use protective measures to avoid mosquito contact. Household-based serosurveys provide a more accurate means to sample individuals that more closely represent the general population. However, this approach is not always feasible because considerable time, effort, and resources are needed to recruit the study population.

In conclusion, we detected a high seroprevalence of DENV in our study cohort, but did not identify any individuals that met the CDC case definition of WNV. We speculate that preexisting immunity to DENV is the principal reason as to why a major outbreak of WNV illness has not been observed in humans in northern Mexico. Because DENV is endemic in many other regions of Latin America, this phenomenon could be a critical determinant in determining the ultimate geographic distribution and public health importance of WNV in the Americas.

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Disclosure Statement

No Competing financial interests exist.

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