

Surveillance for Flaviviruses Near the Mexico–U.S. Border: Co-circulation of Dengue Virus Serotypes 1, 2, and 3 and West Nile Virus in Tamaulipas, Northern Mexico, 2014–2016

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Abstract. A clinical, serological, and molecular investigation was performed to determine the presence of dengue virus (DENV) and other flaviviruses among residents of the city of Reynosa, Tamaulipas, on the Mexico–U.S. border in 2014–2016. The sample population consisted of 2,355 patients with suspected dengue, in addition to 346 asymptomatic individuals recruited during a household-based epidemiological investigation designed to identify flavivirus seroconversions. Sera were collected from patients with suspected dengue in the acute phase of illness and from asymptomatic individuals at enrollment and every 5–7 months for 19 months. Sera from suspected dengue patients were tested for DENV antigen by enzyme-linked immunosorbent assay (ELISA), and select antigen-positive sera were further tested using a serotype-specific, quantitative reverse transcription–polymerase chain reaction. Sera from the household cohort were tested for flavivirus-reactive antibodies by immunoglobulin (Ig) M and IgG ELISAs using DENV antigen. A total of 418 (17.7%) patients with suspected dengue had laboratory-confirmed DENV infections, including 82 patients who were positive for DENV RNA. The most frequently detected serotype was DENV-1 (61 patients), followed by DENV-2 (16 patients) and DENV-3 (five patients). A total of 217 (62.7%) asymptomatic individuals had flavivirus-reactive antibodies at enrollment, and nine flavivirus-naïve individuals seroconverted. Sera from a subset of dengue patients and household participants, including all those who seroconverted, were further tested by plaque reduction neutralization test, resulting in the detection of antibodies to DENV-1, DENV-2, and West Nile virus. In summary, we provide evidence for the co-circulation of multiple flaviviruses in Reynosa, Tamaulipas, on the Mexico–U.S. border.

INTRODUCTION

Dengue virus (DENV) is an *Aedes*-transmitted virus that belongs to the genus *Flavivirus* (family *Flaviviridae*). Dengue virus occurs as four serotypes (DENV-1 to DENV-4) and is hyperendemic in the tropics and subtropics.^{1–3} Dengue virus is the most important arthropod-borne virus in terms of its global impact on human health, with more than half of the world’s population living in areas where they are at risk of infection. An estimated 390 million new DENV infections occur worldwide each year, of which 96 million infections produce clinical manifestations.⁴ In Mexico, an estimated 139,000 clinical episodes occur each year.⁵

DENVs are the etiological agents of dengue. The World Health Organization (WHO) previously classified dengue using three disease categories: dengue fever (DF), dengue hemorrhagic fever (DHF), and dengue shock syndrome (DSS).⁶ Dengue fever is an undifferentiated febrile illness, often accompanied by headache, retro-orbital pain, myalgia, arthralgia, rash, and nausea. Dengue hemorrhagic fever is characterized by hemorrhagic manifestations, thrombocytopenia, and hemoconcentration or other evidence of vascular leakage, and can progress to shock and death. Dengue hemorrhagic fever is divided into grades (designated as DHF

grades I–IV) based on disease severity. One limitation of this classification system is that it does not always allow for the reliable identification of severe cases of dengue.^{7,8} To address this concern, WHO revised the guidelines for dengue diagnosis in 2009, with cases now classified as dengue without warning signs (DwoWS), dengue with warning signs (DWWS), and severe dengue (SD).⁹ Dengue without warning signs presents as an acute febrile illness with at least two of the following: nausea/vomiting, rash, aches and pains, leukopenia, and a positive tourniquet test. To fulfill the criteria of DWWS, at least one warning sign must be observed, with warning signs defined as abdominal pain, persistent vomiting, fluid accumulation, mucosal bleeding, lethargy, liver enlargement, and increasing hematocrit with decreasing platelets. Severe dengue is associated with severe plasma leakage, severe bleeding, or severe organ impairment. The Secretariat of Health (Secretaría de Salud; SS) in Mexico incorporated the most recent guidelines into their nationwide dengue surveillance program in 2016.

Other mosquito-transmitted flaviviruses that occur in Mexico include West Nile virus (WNV), St. Louis encephalitis virus (SLEV), and Zika virus (ZIKV), all of which produce clinical presentations that overlap substantially with those of dengue. West Nile virus infections can present as West Nile fever (WNF), a mild febrile illness often accompanied by a variety of nonspecific symptoms such as headache, myalgia, nausea, fatigue, weakness, and vomiting.^{10,11} West Nile virus infection can also lead to West Nile neuroinvasive disease, which manifests as encephalitis, meningitis, or acute flaccid

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paralysis. Clinical SLEV infections are usually characterized by a fever and various other nonspecific symptoms, with severe cases progressing to aseptic meningitis and encephalitis.^{12,13} Symptoms most commonly associated with ZIKV infection are fever, macular or papular rash, arthritis, and arthralgia, but serious manifestations, such as microcephaly and Guillain-Barré syndrome, can occur.^{14,15}

In this study, we performed a clinical, serological, and molecular investigation to increase our understanding of the public health impact of dengue and other flaviviruses in Reynosa, a city in Tamaulipas on the Mexico–U.S. border. There are limited data in the PubMed database on the public health impact of flaviviruses in Reynosa.^{16,17} In the most comprehensive study described to date, 2,706 suspected cases of dengue were reported in Reynosa in 1995, with another 2,052 suspected cases reported elsewhere in Tamaulipas.¹⁷ Dengue virus-2, DENV-3, and DENV-4 were isolated from patients in Reynosa, and DENV-1 was isolated from patients in other locations, demonstrating concurrent circulation of all four serotypes in Tamaulipas in a single season. Other reports have described dengue outbreaks elsewhere on the Tamaulipas–Texas border.^{18–20} The sample population of the present study consisted of 1) patients who presented in January 2014 to December 2016 with suspected dengue at hospitals and clinics of the SS and 2) individuals recruited in 2014 and 2015 during a household-based seroepidemiological survey designed to estimate the seroprevalence of flaviviruses and identify seroconversions in the general population.

MATERIALS AND METHODS

Study area. Study participants were residents of Reynosa, a city in the state of Tamaulipas, northern Mexico, on the Mexico–U.S. border (Figure 1). Reynosa is located in the binational Reynosa–McAllen Metropolitan Area, along with McAllen, a city in Hidalgo County, Texas. According to the 2013 census, Reynosa has a population of 672,183. The official estimated populations of McAllen and Hidalgo County in 2014 are 138,596 and 779,194, respectively. The climate in

Reynosa is semiarid and the mean annual temperature and precipitation are 23.2°C and 532 mm³, respectively.

Sample population and performance sites for the clinical investigation. The sample population for the clinical investigation consisted of patients who presented in January 2014 to December 2016 with suspected dengue at hospitals and clinics of the SS in Reynosa. Seven hospitals that participated in the study are as follows: Hospital General de Reynosa, Hospital Materno Infantil de Reynosa, Hospital Baudelio Villanueva del Instituto de Seguridad y Servicios Sociales de los Trabajadores del Estado (Institute for Social Security and Services for State Workers), Hospital Regional de Pemex, Unidad de Medicina Familiar 33 del IMSS (Instituto Mexicano del Seguro Social; Mexican Social Security Institute), Unidad de Medicina Familiar 40 del IMSS, and Hospital General de Zona 15 del IMSS. Twenty first-level health units from Jurisdiction IV in Reynosa also participated.

To be eligible for inclusion, patients who presented in 2014 or 2015 had to meet the clinical criteria for DF, DHF, or DSS, following the guidelines established by WHO in 1999 for dengue diagnosis.⁶ Dengue hemorrhagic fever patients were not graded by severity. Patients who presented in 2016 were classified using the dengue guidelines established by WHO in 2009 and, therefore, had to meet the clinical criteria for DwoWS, DWWS, or SD.⁹ The most recent dengue disease classifications were not used in Mexico before 2016. Patients who did not live in Reynosa were not included in the sample population.

Five milliliters of whole blood was taken from each patient in the acute phase of illness (defined as 0–5 days post-illness onset).²¹ Blood was extracted by venipuncture without the use of anticoagulant, and sera were collected and stored at 2–8°C. Patients not willing to have blood drawn were excluded from the sample population. Demographic information was documented by health-care providers and select information (age, gender, and sometimes travel histories) was forwarded to us. On most occasions, travel histories were often defined as whether the patient had or had not ever left Tamaulipas. Clinical information was also collected, although we were not

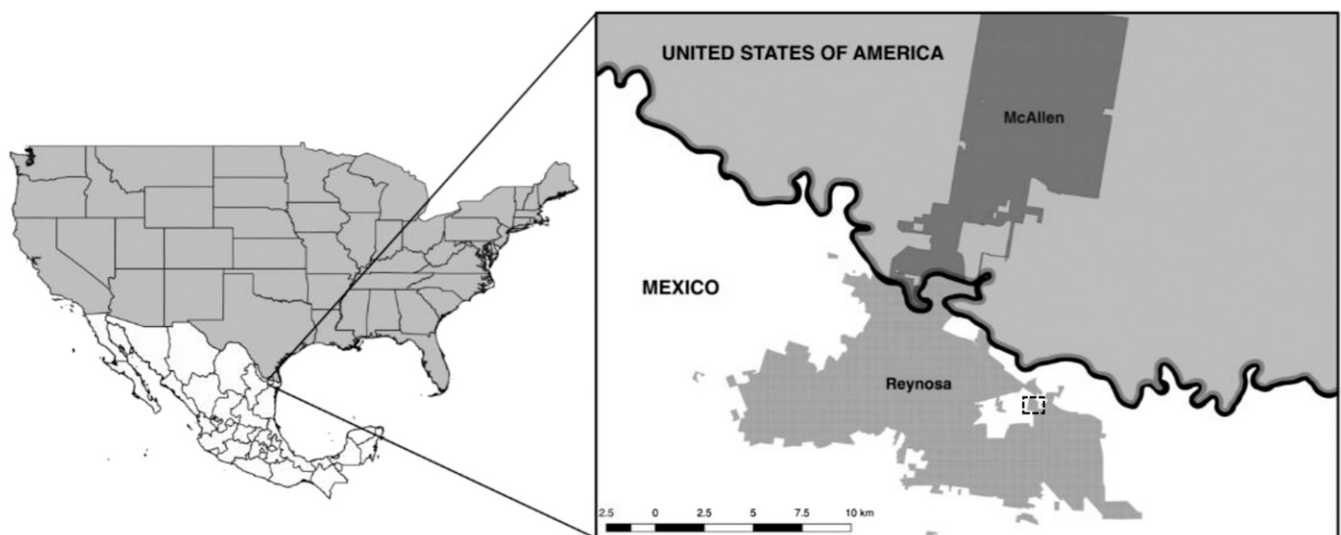


FIGURE 1. Geographic location of the Reynosa–McAllen metropolitan area. The dashed rectangle indicates the neighborhood of Nuevo Amanecer.

provided with information on specific symptoms (only disease classifications and whether the patient had been hospitalized). Informed consent to participate in the study was not required because it is obligatory to report dengue in Mexico. Sera were blind-coded and all identifying information was removed before receipt to protect patient identities. All research protocols were approved by the institutional review boards at the SS in Reynosa, Instituto Politécnico Nacional in Reynosa, Iowa State University, Universidad Autónoma de Yucatán, Universidad Autónoma de Ciudad Juárez, and Instituto Politécnico Nacional in the Ciudad de México.

Sample population for the seroconversion study. The sample population consisted of individuals recruited during a household survey designed to detect evidence of flavivirus exposure and seroconversions. The study cohort was selected using a convenient sampling method. Individuals with flavivirus-like symptoms were not purposely excluded from the study, but all subjects were asymptomatic at the time of enrollment. The study was performed in the neighborhood of Nuevo Amanecer in Reynosa (Figure 1). Each house was visited on four occasions: March 2014, October 2014, April or May 2015, and October 2015. Sera were collected from study participants on each visit, unless they were not home or declined to further participate. Every participant was assigned a unique and randomly generated four-letter identification code. Sera collections were performed by registered nurses.

Written consent to participate was obtained from each subject after a full explanation of the study. If the person was < 18 years of age, written consent was obtained from a parent or legal guardian. Individuals aged 8–18 years were asked to assent. Adults who declined to sign the consent form and children who did not assent were not included in the study. Children < 2 years of age were ineligible for inclusion. One person from each household was asked to complete a questionnaire to obtain demographic information and other pertinent information (i.e., availability of air-conditioning). Data were handled confidentially and anonymously. All research protocols were approved by the institutional review boards at the participating universities.

Enzyme-linked immunosorbent assays. Sera from all patients with suspected dengue were tested for DENV non-structural protein 1 (NS1) by enzyme-linked immunosorbent assay (ELISA) using the Panbio Dengue Early ELISA (Inverness Medical Innovations Ltd., Australia [formerly Panbio Ltd.]) or Platelia™ Dengue NS1 Antigen ELISA (BioRad, Hercules, CA) assays. Both assays have been approved for use by the SS for dengue diagnosis.²² Sera were tested at a dilution of 1:2. When the Panbio Dengue Early ELISA was used, index values were calculated following the manufacturer's instructions, with index values < 0.9, between 0.9 and 1.1, and > 1.1 considered to be negative, equivocal, and positive, respectively. When the Platelia Dengue NS1 Antigen ELISA was used, sample ratios were calculated following established protocols, with ratios < 0.5, between 0.5 and 1.0, and > 1.0 considered to be negative, equivocal, and positive, respectively. If DENV NS1 was detected, the patient was considered to have a laboratory-confirmed DENV infection. Nonstructural protein 1 ELISAs were performed in the Laboratorio Estatal de Salud Pública de Tamaulipas (State Laboratory of Public Health of Tamaulipas) and diagnostic findings were forwarded to health-care providers.

Sera from all participants sampled in the household survey were tested for flavivirus-specific immunoglobulin (Ig) M and

IgG using the DENV Detect™ IgM Capture ELISA (Inbios International Inc., Seattle, WA) and DENV Detect IgG ELISA (Inbios International Inc.), respectively. For both assays, immune status ratios (ISRs) were calculated following the manufacturer's instructions, with negative, equivocal, and positive results defined as those that generated ISR values of ≤ 1.65 , > 1.65 and < 2.84 , and ≥ 2.84 , respectively. Immunoglobulin M and IgG ELISAs were performed at the Centro de Biotecnología Genómica del Instituto Politécnico Nacional in Reynosa, Tamaulipas.

Quantitative reverse transcription–polymerase chain reaction. A subset of DENV NS1-positive sera was randomly selected and an aliquot of each was sent to the National Institute for Diagnosis and Epidemiological Reference (INDRE) in Mexico City. Total RNA was extracted using the QIAamp viral RNA extraction kit (Qiagen, Valencia, CA) and tested using a multiplex, quantitative reverse transcription–polymerase chain reaction (qRT-PCR) designed to detect and differentiate between all four serotypes of DENV. Assays were performed using standardized protocols developed by the U.S. Centers for Disease Control and Prevention and routinely used by the SS for dengue diagnosis.^{22,23} Samples that yielded quantitation cycle (Cq) values ≤ 35 and ≥ 39 were considered to be positive and negative, respectively, for DENV RNA. Samples that produced Cq values > 35 but < 39 were repeated in duplicate and, if the same result was obtained, the sample was considered to be positive.

Plaque reduction neutralization tests. Sera were assayed by plaque reduction neutralization test (PRNT), following standard protocols.²⁴ Plaque reduction neutralization tests were performed using DENV-1 (strain Hawaii), DENV-2 (strain NGC), DENV-3 (strain H-87), DENV-4 (strain 241), SLEV (strain TBH-28), WNV (strain NY99-35261-11), and ZIKV (strain PRVABC59). Viruses 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, CO). Plaque reduction neutralization tests were performed in six-well plates using African green monkey kidney (Vero) cells. Sera were tested in the presence of 8% labile serum factor.²⁵ Sera were tested at a starting dilution of 1:40 and titers were expressed as the reciprocal of serum dilutions yielding $\geq 90\%$ reduction in the number of plaques (PRNT₉₀). For etiologic diagnosis, the PRNT₉₀ antibody titer to the respective virus was required to be at least 4-fold greater than that of the other flaviviruses tested. If the PRNT₉₀ titers for two or more viruses were $\geq 1,280$, the individual was considered to have a secondary flavivirus infection. Plaque reduction neutralization tests were performed at Iowa State University.

RESULTS

Clinical investigation. A total of 3,012 residents of Reynosa presented with suspected dengue in 2014–2016. Serum was collected from 2,355 (78.2%) patients; the remainder declined to have blood drawn and were excluded from the sample population (Table 1). Dengue virus NS1 was detected by ELISA in sera from 418 (17.7%) patients in the sample population. Most patients with confirmed dengue presented in 2014 (218 cases), followed by 2015 and 2016 (134 and 66 cases, respectively). There were 309 confirmed cases of DF (183 cases in 2014 and 126 cases in 2015) and 43 confirmed

TABLE 1
Numbers of suspected and confirmed cases of dengue in Reynosa, Tamaulipas, 2014–2016

Year	Disease classification					Total
	DF	DHF	DwoWS	DWWS	SD	
	*Number (%) of confirmed/suspected cases					
2014	183/703 (26.0)	35/58 (60.3)	NA	NA	NA	218/761 (28.6)
2015	126/1,064 (11.8)	8/26 (30.8)	NA	NA	NA	134/1,090 (12.3)
2016	NA	NA	63/491 (12.8)	3/6 (50.0)	0/7 (0.0)	66/504 (13.1)
Total	309/1,767 (17.5)	43/84 (51.2)	63/491 (12.8)	3/6 (50.0)	0/7 (0.0)	418/2,355 (17.7)

DF = dengue fever; DHF = dengue hemorrhagic fever; DwoWS = dengue without warning signs; DWWS = dengue with warning signs; NA = not applicable; SD = severe dengue.
 * Thirteen patients with suspected dengue (two, nine, and two patients from 2014, 2015, and 2016, respectively) yielded equivocal test results. Another 657 patients with suspected dengue (96, 37, and 524 patients from 2014, 2015, and 2016, respectively) were excluded from the sample population because acute sera were not collected and/or diagnostic testing was not performed.

cases of DHF (35 cases in 2014 and eight cases in 2015). There were no suspected or confirmed cases of DSS. In 2016, which is when the most recent dengue classifications were adopted by the SS, there were 63 confirmed cases of DwoWS and three confirmed cases of DWWS. There were no confirmed cases of SD. Thirteen patients (two, nine, and two patients from 2014, 2015, and 2016, respectively) yielded equivocal ELISA results. Thirty-five patients were hospitalized, including 18 patients with confirmed dengue. Hospitalized patients with confirmed dengue presented in 2014 (17 patients) and 2015 (one patient), and all survived. The dengue patients hospitalized in 2014 were diagnosed with DHF (16 patients) and DF (one patient), and DENV-1 RNA was detected in one DHF patient. The dengue patient hospitalized in 2015 was diagnosed with DHF and was positive for DENV-2 RNA.

The study population consisted of 1,005 (42.7%) males and 1,350 (57.3%) females. There were 174 males (41.6%) and 244 females (58.4%) with confirmed dengue. The ages of patients with suspected and confirmed dengue ranged from < 1 to 98 and 1 to 87 years, respectively. Confirmed dengue occurred in patients of all age categories, with greatest numbers reported among patients aged 10–14 and 15–19 years (Table 2). Confirmed DF and DwoWS also occurred in patients of all age categories, and DHF occurred in patients of most age categories (Figure 2). The three cases of DWWS occurred in 40- to 44-year-old patients. Most of the patients with confirmed dengue presented in epidemiological weeks (EWs) 38–48, and cases peaked in EW 45 (Figure 3A). Similar findings were

observed for patients without laboratory-confirmed dengue. Cases of confirmed DF, DHF, DwoWS, and DWWS also peaked late in the year (Figure 3B).

Sera from 85 patients with confirmed dengue were tested for DENV RNA by serotype-specific qRT-PCR. Viral RNA was detected in 82 patients (Table 3). The serotype most commonly detected was DENV-1 (*N* = 61), followed by DENV-2 (*N* = 16) and DENV-3 (*N* = 5). Dengue virus-4 RNA was not identified in any patient. The most common serotype in 2014 was DENV-1 (55 of 59 patients). The most common serotype in 2015 and 2016 was DENV-2 (11 of 20 and four of six patients, respectively). Dengue virus-1 RNA was not detected in any patients in 2016. Travel histories were provided for 15 DENV-2 RNA-positive patients, with 11 of 12 patients from 2015 and two of three patients from 2016 having never left Tamaulipas. Travel histories were also provided for seven DENV-3 RNA-positive patients, with all three patients from 2014, one of two patients from 2015, and both patients from 2016 having never left their state of residence.

Sera from a subset of patients with confirmed dengue (*N* = 67) were tested by PRNT for antibodies to selected flaviviruses (data not shown). Fourteen patients were seropositive for DENV-1 and three patients were seropositive for DENV-2. Another 13 patients had secondary flavivirus infections, 33 patients had antibodies to an undetermined flavivirus, and three patients had no detectable neutralizing antibodies. Antibodies to DENV-4, SLEV, WNV, or ZIKV were not identified.

TABLE 2
Age characteristics of confirmed and suspected dengue cases in Reynosa, Tamaulipas, 2014–2016

Ages (years)	Number (%) of confirmed/suspected cases			
	2014	2015	2016	All years
5	9/67 (13.4)	1/47 (2.1)	3/31 (9.7)	13/145 (9.0)
5–9	21/132 (15.9)	9/104 (8.7)	6/55 (10.9)	36/291 (12.4)
10–14	44/153 (28.8)	16/158 (10.1)	7/70 (10.0)	67/381 (17.6)
15–19	38/110 (34.5)	24/141 (17.0)	6/59 (10.2)	68/310 (21.9)
20–24	26/48 (54.2)	9/83 (10.8)	3/30 (10.0)	38/161 (23.6)
25–29	13/44 (29.5)	8/64 (12.5)	9/41 (22.0)	30/149 (20.1)
30–34	13/41 (31.7)	13/89 (14.6)	3/39 (7.7)	29/169 (17.2)
35–39	20/51 (39.2)	10/100 (10.0)	6/39 (15.4)	36/190 (18.9)
40–44	13/39 (33.3)	10/85 (11.8)	8/39 (20.5)	31/163 (19.0)
45–49	8/23 (34.8)	6/53 (11.3)	3/30 (10.0)	17/106 (16.0)
50–54	3/19 (15.8)	7/59 (11.9)	1/24 (4.2)	11/102 (10.8)
55–59	3/13 (23.1)	7/36 (19.4)	6/18 (33.3)	16/67 (23.9)
60–64	3/7 (42.9)	3/22 (13.6)	1/8 (12.5)	7/37 (18.9)
65–69	0/5 (0.0)	4/17 (23.5)	1/8 (12.5)	5/30 (16.7)
≥ 70	4/9 (44.4)	7/31 (22.6)	3/12 (25.0)	14/52 (26.9)
Not provided	0/0 (0.0)	0/1 (0.0)	0/1 (0.0)	0/2 (0.0)
Total	218/761 (28.6)	134/1,090 (12.3)	66/504 (13.1)	418/2,355 (17.7)

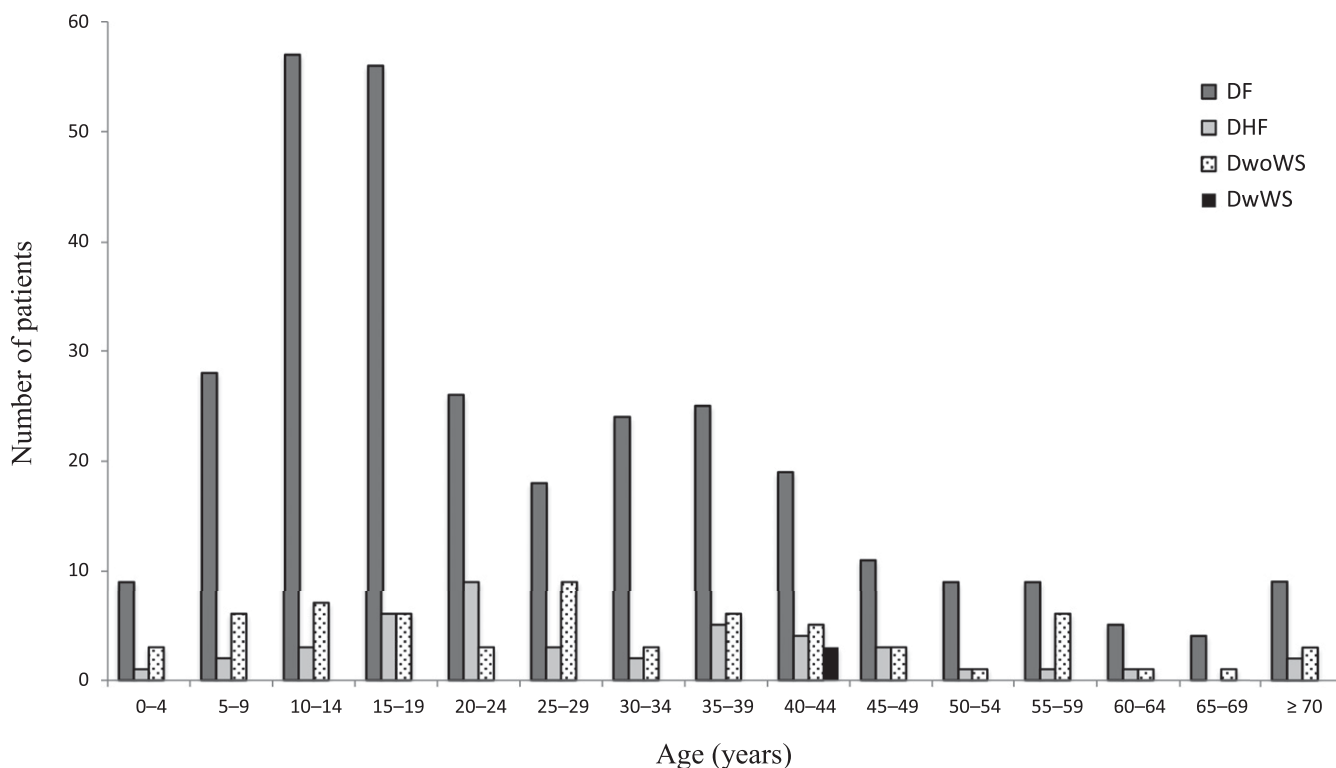


FIGURE 2. Age characteristics of patients from the hospital-based clinical investigation. Numbers of patients with confirmed dengue fever, dengue hemorrhagic fever, dengue without warning signs, and dengue with warning signs according to age.

Seroconversion study. The sample population consisted of 346 study participants from 114 houses. Another six houses were also visited but the residents declined to participate. Each participating household was visited on four occasions: March 2014, October 2014, April–May 2015, and October 2015. The mean number of study participants in each house was 3.0, with a range of 1–10, and none had symptoms consistent with a flavivirus infection at enrollment. Ages of the study participants ranged from 3 to 95 years. There were 143 males and 203 females. Sera were collected from 118 participants on all four occasions and from 61, 71, and 96 participants on three, two, or one occasion, respectively. Air-conditioning was available in 24 (21.1%) households.

All sera were tested by ELISA for flavivirus IgG, and sera collected at enrollment were also tested for flavivirus IgM. A total of 217 (62.7%) study participants were positive for flavivirus IgG at enrollment, including four participants who were also positive for flavivirus IgM. Seroprevalence was lowest among study participants < 20 years of age (Table 4). The male to female ratios for participants with and without flavivirus IgG at enrollment were similar (0.71 and 0.68, respectively).

Nine flavivirus-naïve participants seroconverted during the study period (Table 5). Flavivirus IgG was first detected in sera collected in October 2014 (three subjects), April or May 2015 (two subjects), and October 2015 (four subjects). The mean age of participants who seroconverted was 23.7 years, with a range of 8–50 years. There were four males and five females. Seven individuals who seroconverted had never left Tamaulipas, whereas the other two individuals (denoted as MDRR and MLMM) had recently traveled. MDRR had visited family in Veracruz, eastern Mexico, and MLMM had traveled to Nuevo Leon, northern Mexico, during the study period.

Sera from the study participants who seroconverted were further tested by PRNT (Table 5). Of the seven study participants who had never traveled, three individuals were seropositive for WNV, one individual was seropositive for DENV-1, two individuals were seropositive for an undetermined flavivirus, and one individual had no detectable neutralizing antibodies. The individual seropositive for DENV-1, a 31-year-old female denoted as SACL, developed symptoms consistent with DF. One individual seropositive for WNV, a 15-year-old female denoted as IFCF, developed symptoms consistent with WNF. MDRR and MLMM, the two individuals who had recently traveled, were seropositive for WNV and DENV-1, respectively.

Sera from the four study participants with flavivirus IgM and IgG at baseline were tested by PRNT (Table 6). Two participants were seropositive for DENV-1, whereas one each had antibodies to DENV-2 and an undetermined flavivirus. The two participants seropositive for DENV-1 and the participant with antibodies to an undetermined flavivirus had never traveled outside of Tamaulipas. Sera from five study participants with flavivirus IgG, but not IgM, at baseline were randomly selected and also tested by PRNT. Two participants had antibodies to DENV-2, whereas one each had antibodies to DENV-1, WNV, and an undetermined flavivirus. The participants seropositive for WNV and an undetermined flavivirus, in addition to one of the participants seropositive for DENV-2, had never traveled outside of Tamaulipas.

DISCUSSION

We provide evidence of autochthonous transmission of multiple flaviviruses in northern Mexico. In the household-based

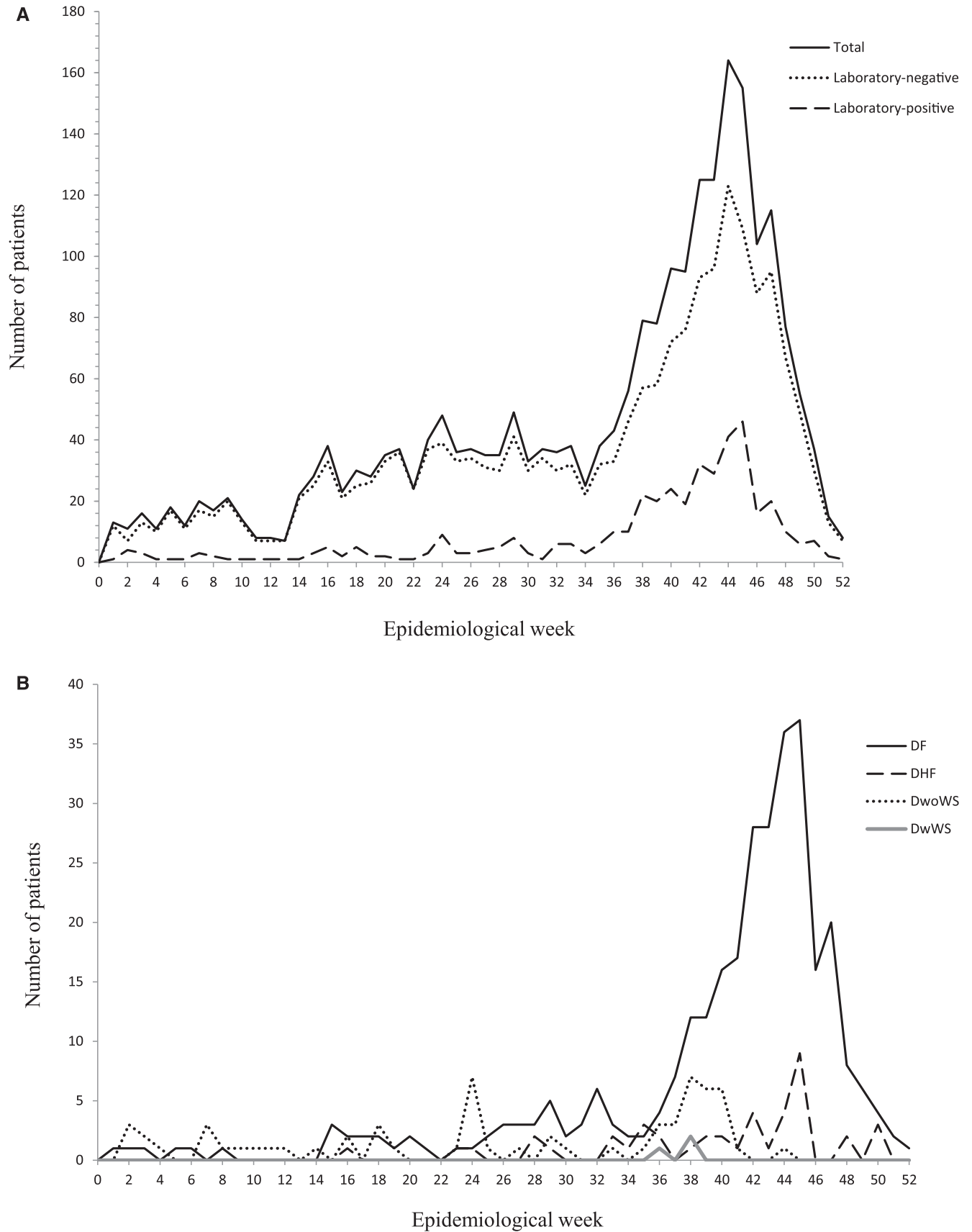


FIGURE 3. Weekly incidence of dengue in Reynosa, Tamaulipas, in 2014–2016. (A) Numbers of patients with suspected dengue and with or without laboratory-confirmed dengue according to epidemiological week. (B) Numbers of patients with confirmed dengue fever, dengue hemorrhagic fever, dengue without warning signs, and dengue with warning signs according to epidemiological week.

TABLE 3
Detection of dengue virus RNA in acute sera from dengue patients in Reynosa, Tamaulipas, 2014–2016

Year	Disease classification	Total number of patients	Number of patients positive/tested by qRT-PCR	Serotype			
				DENV-1	DENV-2	DENV-3	DENV-4
				Number of qRT-PCR–positive patients			
2014	DF	183	53/53	51	–*	2	–
	DHF	35	6/6	4	1	1	–
2015	DF	126	14/17	6	8	–	–
	DHF	8	3/3	–	3	–	–
2016	DwoWS	63	6/6	–	4	2	–
	DWWS	3	–	–	–	–	–
	SD	–	–	–	–	–	–
Total		418	82/85	61	16	5	–

DENV = dengue virus; DF = dengue fever; DHF = dengue hemorrhagic fever; DwoWS = dengue without warning signs; DWWS = dengue with warning signs; qRT-PCR = quantitative reverse transcription–polymerase chain reaction; SD = severe dengue.

*0.

investigation, antibodies to DENV-1, DENV-2, and WNV were detected in study participants who had never traveled outside of Tamaulipas, consistent with local virus transmission. The detection of flavivirus IgM in study participants who had never left Tamaulipas suggests that some infections were recent. The detection of antibodies to DENV-1 and WNV in participants who seroconverted and had not recently traveled provides further evidence of recent autochthonous virus transmission. Dengue virus-1, DENV-2, and DENV-3 RNA were identified in dengue patients who presented at hospitals and clinics of the SS. Although information on travel histories was not provided for most cases, several patients positive for DENV-2 and DENV-3 RNA had never traveled. Several local cases of DENV-2 and DENV-3 occurred in 2015, the same year that the DENV-1 and WNV seroconversions were identified. In addition, we recently detected several cases of non–travel-acquired chikungunya virus (CHIKV; genus *Alphavirus*, family *Togaviridae*) in Tamaulipas in 2015.²⁶ Taken together, we provide evidence for the co-circulation of five arboviruses of medical concern in a single season in Tamaulipas, northern Mexico.

A total of 418 (17.7%) patients with suspected dengue had laboratory-confirmed DENV infections, although this is likely an underestimate because DENV NS1-negative patients were not tested for flavivirus IgM which presumably would have resulted in the identification of additional dengue cases. Eighty-two patients had DENV RNA. The majority (74.4%) was positive for DENV-1; the remainder had been infected with DENV-2 (19.5%) and DENV-3 (6.1%). Dengue virus-1 was the dominant serotype in Nuevo Leon, northern Mexico, in 2010.²⁷ Dengue virus-1 was also the dominant serotype in southern Texas in 2013.²⁰ However, our data should be interpreted with caution if used to predict the identity of the dominant serotype in Tamaulipas. First, travel histories were not obtained for most patients and, therefore, some infections may have been acquired elsewhere. Second, much higher numbers of patients were tested for DENV RNA in 2014 ($N = 59$) compared with 2015 ($N = 20$) and 2016 ($N = 6$). Although most DENV RNA-positive patients had been infected with DENV-1, the serotype most frequently detected in both 2015 and 2016 was DENV-2, and there was no evidence of DENV-1 in 2016. These findings could indicate that serotype displacement had occurred. The phenomenon of serotype displacement is well documented. A review of the nationwide surveillance data from 2000 to 2011 revealed that DENV-2 was the dominant

serotype in Mexico in 2000–2005, followed by DENV-1 in 2006–2010, with both serotypes detected in similar proportions in 2011.²⁸

Simultaneous circulation of all four DENV serotypes has been reported in Tamaulipas. Dengue virus-2, DENV-3, and DENV-4 were isolated from patients in Reynosa in 1995, with DENV-1 recovered from patients elsewhere in Tamaulipas that same year.¹⁷ Concurrent circulation of all four serotypes has also been reported in South America and Asia.^{29–32} We provide evidence for the co-circulation of three serotypes. Dengue virus-4 was not detected, although nationwide surveillance data collected in 2000–2011 revealed that DENV-4 is the least common serotype in Mexico.²⁸ Many other studies also report the concurrent circulation of three serotypes.^{33–37}

Dengue cases peaked in September to November. Other studies performed in northern Mexico report similar findings.^{17,27,38,39} Most confirmed cases of dengue identified in southern Texas in 2013 also occurred during these months.²⁰ The dengue season coincides with peak mosquito abundance which is dependent on climatic conditions favorable for mosquito breeding. Fifty-eight percent of patients with confirmed dengue were female. Other studies performed on the Mexico–U.S. border also report dengue in slightly more females than males, although it is important to note that often slightly more females than males also present with suspected dengue.^{19,20,38,39} In this regard, 57% of patients in our entire

TABLE 4
Age characteristics of the household cohort

Ages (years)	Number (%) positive for flavivirus IgG at baseline/total number tested
< 5	1/5 (20.0)
5–9	6/25 (24.0)
10–14	16/37 (43.2)
15–19	16/43 (37.2)
20–24	17/35 (48.6)
25–29	14/17 (82.4)
30–34	14/24 (58.3)
35–39	16/21 (76.2)
40–44	30/36 (83.3)
45–49	18/24 (75.0)
50–54	16/18 (88.9)
55–59	19/22 (86.4)
60–64	10/11 (90.1)
65–69	7/8 (87.5)
≥ 70	17/20 (85.0)
Total	217/346 (62.7)

TABLE 5
Travel histories, clinical findings, and serologic data for study participants who seroconverted

Human subject	Travel*	Illness†	Flavivirus IgG				PRNT ₉₀ titer							PRNT interpretation
			Date of serum collection				DENV-1	DENV-2	DENV-3	DENV-4	SLEV	WNV	ZIKV	
			March 2014§	October 2014	April–May 2015	October 2015‡								
SACL	No	Yes	–	NT¶	+#	+	80	–**	–	–	–	–	–	DENV-1
MBCO	No	No	–	+	+	+	80	80	–	–	–	–	–	Flavivirus
AMGO	No	No	–	+	NT	+	–	–	–	–	40	640	–	WNV
MDRR	Yes	No	–	+	+	+	–	–	–	–	–	160	–	WNV
MLMM	Yes	No	–	–	+	+	640	–	160	–	–	–	–	DENV-1
MLLN	No	No	–	–	–	+	–	–	–	–	–	640	–	WNV
YFVO	No	No	–	–	–	+	–	–	–	–	–	–	–	Negative
IFCF	No	Yes	–	–	–	+	–	–	–	–	–	320	–	WNV
FCLG	No	No	–	–	–	+	–	40	–	–	–	–	–	Flavivirus

DENV-1 = dengue virus 1; DENV-2 = dengue virus 2; DENV-3 = dengue virus 3; DENV-4 = dengue virus 4; IgG = immunoglobulin G; PRNT = plaque reduction neutralization test; SLEV = St. Louis encephalitis virus; WNV = West Nile virus; ZIKV = Zika virus.
 * Defined as study participants who have or have never left Tamaulipas.
 † SACL and IFCF developed symptoms consistent with DF and WNF, respectively.
 ‡ Most PRNTs were performed using sera collected in October 2015.
 § Sera collected at baseline (March 2014) were also tested for flavivirus IgM but all were negative.
 || Negative.
 ¶ Not tested (study participant unavailable for serum collection).
 # Positive.
 ** < 40.

sample population were female. Dengue cases peaked during childhood; almost one-third of cases were 10–19 years old, and lowest numbers occurred in those older than 50 years of age. A similar age-group distribution pattern has been reported elsewhere in Mexico.^{27,40,41} Most of the patients with suspected dengue did not have laboratory-confirmed DENV infections, as observed in other dengue studies performed in Mexico.^{27,40,42} Taken together, the epidemiological characteristics of dengue in Reynosa in 2014–2016 are similar to those reported in many other dengue investigations.

Reynosa is located within the binational Reynosa–McAllen Metropolitan Area, along with McAllen, a city in Hidalgo County, Texas. The number of dengue cases identified in our study greatly exceeds the number of dengue cases reported in Hidalgo County over the same time interval according to data released by the U.S. Centers for Disease Control and Prevention. Two dengue cases (both imported) occurred in Hidalgo County in 2014, with no dengue cases reported in 2015 and 2016.⁴³ Five dengue cases (all imported) occurred in Texas counties located in the lower Rio Grande Valley in

2014–2016.⁴³ This is in sharp contrast to the 418 dengue cases identified in our hospital-based investigation.

Other studies performed at the Mexico–U.S. border have also reported a much higher incidence of dengue in Mexico compared with the United States.^{17–19,39,44,45} One example is a household-based seroepidemiologic study performed in 2005 in the contiguous border cities of Matamoros, Tamaulipas, and Brownsville, Texas.¹⁸ The study population consisted of 132 participants from 111 households in Matamoros and 141 participants from 118 households in Brownsville. Dengue virus-reactive antibodies were detected by ELISA in 101 (76.5%) participants from Matamoros and 49 (39.2%) participants from Brownsville. Forty-two (31.7%) participants from Matamoros and six (3.7%) participants from Brownsville had recent infections. Environmental and socioeconomic factors that affect mosquito–human contact, such as air-conditioning, appear to be the primary reasons for these differences.^{18,44} Air-conditioning was available in 29% of participating households in Matamoros and 85% of participating households in Brownsville.¹⁸ In another household-based study,

TABLE 6
Serologic findings for select participants from the household-based investigation, including all with flavivirus IgM at baseline

Human subject	Travel*	Illness	Baseline ELISA data†		PRNT ₉₀ titer‡							PRNT interpretation
			IgM	IgG	DENV-1	DENV-2	DENV-3	DENV-4	SLEV	WNV	ZIKV	
BROO	No	No	+§	+	≥ 1,280	160	160	40	–	40	–	DENV-1
GAPG	No	No	+	+	640	–	80	–	–	320	–	Flavivirus
MMPO	No	No	+	+	640	160	160	40	–	–	–	DENV-1
ADTO	Yes	No	+	+	–	80	–	–	–	–	–	DENV-2
GMJO	Yes	No	–¶	+	640	–	80	40	–	40	–	DENV-1
CDPG	No	No	–	+	–	160	40	–	–	–	80	Flavivirus
MILO	No	No	–	+	160	≥ 1,280	40	–	–	–	–	DENV-2
CAZF	Yes	No	–	+	–	≥ 1,280	80	–	40	320	–	DENV-2
MTR0	No	No	–	+	160	160	160	40	80	≥ 1,280	–	WNV

DENV-1 = dengue virus 1; DENV-2 = dengue virus 2; DENV-3 = dengue virus 3; DENV-4 = dengue virus 4; ELISA = enzyme-linked immunosorbent assay; IgG = immunoglobulin G; IgM = immunoglobulin M; PRNT = plaque reduction neutralization test; SLEV = St. Louis encephalitis virus; WNV = West Nile virus; ZIKV = Zika virus.
 * Defined as study participants who have or have never left Tamaulipas.
 † All baseline sera were collected in March 2014, except for GAPG with their baseline serum collected in October 2014.
 ‡ All PRNTs were performed using sera collected in October 2015.
 § Positive.
 || < 40.
 ¶ Negative.

air-conditioning was available in 24% and 82% of participating households in Nuevo Laredo, Tamaulipas, and Laredo, Texas, respectively.⁴⁴ In all, 21% of households in our study were air-conditioned. Other factors associated with an increased risk of infection are the presence of mosquito breeding sites, nonuse of mosquito repellents, smaller lot sizes, and low family income.^{18,44–46}

Three WNV seroconversions were identified in the household-based investigation, and one study participant developed clinical manifestations consistent with WNV. However, the case cannot be conclusively linked to WNV because a precise date of symptom onset was not obtained. There have been no documented cases of WNV in Tamaulipas, although two cases have occurred in the neighboring state of Nuevo Leon.^{47,48} The first evidence of WNV in Tamaulipas was provided in a serological investigation that identified antibodies to WNV in resident birds in Tamaulipas in 2013.⁴⁹ Evidence of WNV infection has also been detected in mosquitoes and horses elsewhere in northern Mexico.^{47,50–54} Unlike dengue, the incidence of WNV disease is much lower in Mexico compared with that in the United States, potentially because preexisting immunity to DENV protects against WNV disease.^{10,42}

Antibodies to SLEV or ZIKV were not detected in any participants in the household- or hospital-based investigations, although these findings are not unexpected. St. Louis encephalitis virus is not a common cause of infection in humans in Mexico.¹² Zika virus was not present in Mexico for most of the study period, with the first case detected in November 2015 according to WHO.⁵⁵ The first confirmed case of ZIKV in Tamaulipas occurred in June 2016 in the municipality of Madero (S. D. Carmona, unpublished data). The first confirmed case of ZIKV in Reynosa occurred in August 2016, with a total of 125 confirmed cases reported in Reynosa as of EW 14 of 2018.

In summary, we provide evidence for the concurrent circulation of four flaviviruses, DENV-1, DENV-2, DENV-3, and WNV, in Tamaulipas, northern Mexico. In addition, we recently reported autochthonous transmission of CHIKV, an *Alphavirus*, in Tamaulipas.²⁶ All of the aforementioned viruses were associated with human disease in the study area, demonstrating the important need to continue performing vigilant arbovirus surveillance and diagnosis on the Mexico–U.S. border.

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