179

Arbovirus surveillance near the Mexico-U.S. border: co-circulation of dengue virus serotypes 1, 2 and 3, West Nile virus and chikungunya virus in Tamaulipas, northern Mexico

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ABSTRACT

A clinical, serological, and molecular investigation was performed to determine the presence of dengue virus (DENV) and other mosquito-transmitted viruses among residents of the city of Reynosa, Tamaulipas on the Mexico-U.S. border in 2014 to 2016. The sample population consisted of 2355 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 to 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 RT-PCR. Sera from a subset of patients was also tested for chikungunya virus (CHIKV) RNA. A total of 418 (17.7%) patients with suspected dengue had laboratory-confirmed DENV infections, including 82 patients positive for DENV RNA. Three serotypes were detected (DENV-1, DENV-2, and DENV-3). CHIKV RNA was detected in 13 of 34 (38.2%) patients, including five who also contained DENV antigen. Sera from the household cohort were tested for flavivirus-reactive antibodies by IgM and IgG ELISAs using DENV antigen. A total of 217 (62.7%) household participants had flavivirus-reactive antibodies at enrollment and nine flavivirus-naïve individuals seroconverted. Sera from a subset of participants, including all those who seroconverted, were further tested by plaque reduction neutralization test, resulting in the detection of antibodies to DENV-1 and West Nile virus. In summary, we provide evidence for the co-circulation of five medically important arboviruses in Reynosa, Tamaulipas on the Mexico-U.S. border.

INTRODUCTION

Dengue virus (DENV; genus *Flavivirus*, family *Flaviviridae*) and chikungunya virus (CHIKV; genus *Alphavirus*, family *Togaviridae*) are the etiological agents of dengue and chikungunya fever (CHIKF), respectively. Both viruses are transmitted by *Aedes* mosquitoes and endemic in the tropics and subtropics. The co-circulation of DENV and CHIKV presents a clinical and diagnostic challenge in endemic countries because the clinical presentations of these viruses overlap substantially. Dengue is usually characterized by a febrile illness accompanied by headache, arthralgia and myalgia, sometimes with a macular rash. In some cases, DENV infection can result in vascular leakage, hemorrhagic manifestations, thrombocytopenia, and hypotensive shock and progress to organ failure and death. CHIKF is characterized by an acute febrile illness that is often accompanied by severe polyarthralgia, periarticular edema, and a nonspecific macular or maculopapular rash. Complications are rare but include severe organ dysfunction and encephalitis. DENV and CHIKV both occur in Mexico. Other mosquito-borne human pathogens present in Mexico include West Nile virus (WNV), St. Louis encephalitis virus (SLEV) and Zika virus (ZIKV) which are classified in the genus *Flavivirus*. The purpose of this investigation was to determine the presence of DENV, CHIKV and other mosquito-borne pathogens among residents of the city of Reynosa, Tamaulipas on the Mexico-U.S. border in 2014 to 2016.

METHODS

Study area: 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.

Sample population: 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 Secretaria de Salud in Reynosa. To be eligible for inclusion, patients who presented in 2014 or 2015 had to meet the clinical criteria for dengue fever, dengue hemorrhagic fever or dengue shock syndrome, following the guidelines established by the WHO in 1999 for dengue diagnosis. Patients who presented in 2016 were classified using the dengue guidelines established by the WHO in 2009 and therefore, had to meet the clinical criteria for dengue without warning signs, dengue with warning signs or severe dengue. 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. The sample population for the flavivirus seroconversion study consisted of individuals 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.

Quantitative RT-PCR: A subset of DENV NS1-positive sera was randomly selected and tested using a multiplex qRT-PCR designed to detect and differentiate between all four serotypes of DENV. Select sera were also tested for CHIKV RNA by qRT-PCR. Assays were performed using standardized protocols developed by the U.S. Centers for Disease Control and Prevention.

Plaque reduction neutralization tests: Selected sera were assayed by PRNT using DENV-1 to DENV-4, WNV, SLEV and ZIKV. For etiologic diagnosis, the $PRNT_{90}$ antibody titer to the respective virus was required to be at least 4-fold greater than that to the other viruses tested.

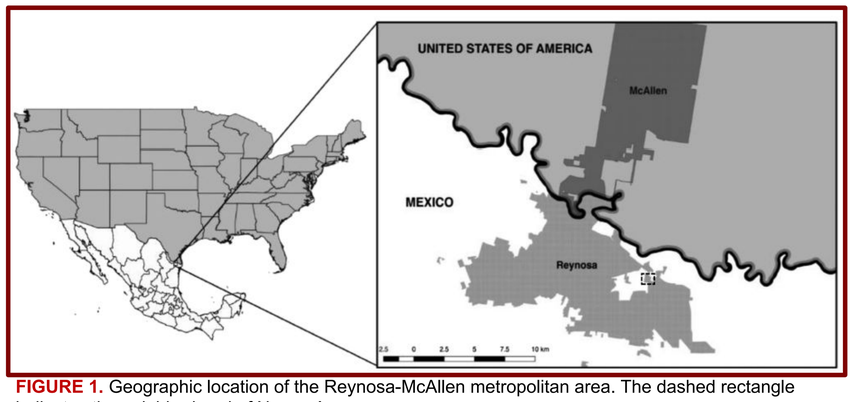


FIGURE 1. Geographic location of the Reynosa-McAllen metropolitan area. The dashed rectangle indicates the neighborhood of Nuevo Amanecer.

RESULTS: Clinical Investigation

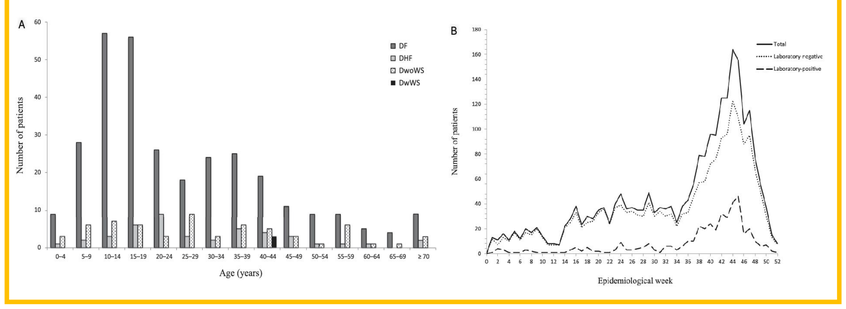
Detection of DENV antigen: A total of 3,012 residents of Reynosa presented with suspected dengue in 2014–2016. Serum was collected from 2,355 (78.2%) patients; the remainder declined to have blood drawn and were excluded from the study. Dengue virus NS1 was detected by ELISA in sera from 418 (17.7%) patients in the sample population (**Table 1**). Confirmed dengue occurred in patients of all age categories, with greatest numbers reported among patients aged 10-19 years (**Figure 2A**). Most of the patients with confirmed dengue presented in epidemiological weeks 38-48 (**Figure 2B**).

TABLE 1: Numbers of suspected and confirmed cases of dengue

	Disease classification					
	DF	DHF	DwoWS	DWWS	SD	Total
Year	*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)

Dengue fever (DF), Dengue hemorrahagic fever (DHF), Dengue without warning signs (DwoWS), Dengue with warning signs (DWWS), Severe dengue (SD). *Thirteen patients with suspected dengue (two, nine, and two patients from 2014, 2015, and 2016, respectively) yielded equivocal teste results.

FIGURE 2. (A) Age characteristics of patients. (B) Weekly incidence of dengue



Detection of DENV RNA: 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 2**). The serotype most commonly detected was DENV-1 (n = 61), followed by DENV-2 (n = 16) and DENV-3 (n = 5). Travel histories were provided for 15 DENV-2 RNA-positive patients, with 13 patients having never left Tamaulipas. Travel histories were also provided for 7 DENV-3 RNA-positive patients, with 6 patients having never left their state of residence.

TABLE 2: Detection of dengue virus RNA in acute sera from dengue patients

				Serotype		
				DENV-1	DENV-2	DENV-3	DENV-4
Year	Disease classification	Total number of patients	Number of patients positive/tested by qRT-PCR	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	–

Dengue virus (DENV), *0

Detection of CHIKV RNA: Sera from select patients (n = 34) who presented in 2015 were also tested for CHIKV RNA by qRT-PCR. Thirteen (38.2%) patients, including five DENV antigen-positive patients, were positive. Sera from three CHIKV RNA-positive patients were further assayed by virus isolation in Vero cell culture and CHIKV was recovered on each occasion. The genome of one isolate, designated CH-R-1950, and structural genes of the other two isolates were sequenced (Genbank accession nos. MG921596, MG822707, and MG822708). The genome sequence of CH-R-1950 was aligned with all other CHIKV sequences in the GenBank database and shown to have highest nucleotide identity (>99.8%) with the genomes of isolates from Chiapas, Nicaragua, and elsewhere in the Americas.

RESULTS: Seroconversion Study

Recruitment of the study population: A longitudinal household-based seroepidemiologic investigation was performed to estimate the seroprevalence of flaviviruses and to identify flavivirus seroconversions among residents in the neighborhood of Nuevo Amanecer. 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. Sera were collected from study participants on each visit, unless they were not home or declined to further participate. Individuals with flavivirus-like symptoms were not purposely excluded from the study, but all subjects were asymptomatic at the time of enrolment.

Serologic findings: All sera were tested by ELISA for flavivirus IgG, and sera collected at enrolment were also tested for flavivirus IgM. A total of 217 (62.7%) study participants were positive for flavivirus IgG at enrolment, including four participants who were also positive for flavivirus IgM. Nine flavivirus-naïve participants seroconverted during the study period (**Table 3**). Sera from the study participants who seroconverted were further tested by PRNT, resulting in the detection of antibodies to DENV-1 and West Nile virus.

TABLE 3: Travel histories, clinical findings, and serologic data for individuals who serovonverted

			Flavivirus IgG				$PRNT_{90}$ titer							
			Date of serum collection											
Human subject	Travel*	Illness†	March 2014§	October 2014	April–May 2015	October 2015‡	DENV-1	DENV-2	DENV-3	DENV-4	SLEV	WNV	ZIKV	PRNT interpretation
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

Dengue virus (DENV), St. Louis encephalitis virus (SLEV), West Nile virus (WNV), Zika virus (ZIKV)

*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

CONCLUSIONS

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 report autochthonous transmission of CHIKV, an *Alphavirus*, in Tamaulipas. Several patients could have been coinfected with CHIKV and DENV because both CHIKV RNA and DENV IgM were detected in their sera. All five 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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