

Entomological and virological surveillance for dengue virus in churches in Merida, Mexico

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ABSTRACT

This study was designed to assess whether churches in endemic dengue districts in Merida, Mexico provide suitable breeding habitats for mosquitoes and are potential sites for dengue virus (DENV) transmission. Churches were inspected for immature and adult mosquitoes once every week from November 2015 to October 2016. A total of 10,997 immatures of five species were collected. The most abundant species were *Aedes aegypti* (6,051) and *Culex quinquefasciatus* (3,018). The most common source of immature *Ae. aegypti* were buckets followed by disposable containers. Adult collections yielded 21,226 mosquitoes of nine species. The most common species were *Cx. quinquefasciatus* (15,215) and *Ae. aegypti* (3,902). *Aedes aegypti* were found all year long. Female *Ae. aegypti* (1,380) were sorted into pools (166) and assayed for flavivirus RNA by RT-PCR and Sanger sequencing. Two pools were positive for DENV (DENV-1 and 2). In conclusion, we demonstrated that some churches in Merida are infested with mosquitoes all year long and they potentially serve as sites for DENV transmission and should therefore be considered for inclusion in mosquito and arboviruses control and surveillance efforts.

KEYWORDS: *Aedes aegypti*. Flavivirus. Mosquitoes. Dengue.

INTRODUCTION

Dengue virus (DENV; Flaviviridae, *Flavivirus*) represents an enormous problem in terms of human morbidity and economic burden. Globally, DENV is responsible for approximately 1.14 million disability-adjusted life-years annually¹. In Mexico, an estimated 139,000 symptomatic DENV infections occur each year, with a mean of 65 disability-adjusted life years per million individuals². There are four serotypes of DENV (DENV-1 to 4). The serotypes are genetically distant from each other and can be further divided into different genotypes³. Some serotypes are more virulent than others and have been associated with increased disease severity (e.g., DENV-2 American/Asian genotype)⁴. The introduction of distinct viral genotypes can trigger epidemics; thus, it is important that sequencing studies are performed so that viruses are identified and critical information on virus emergence and persistence is acquired⁵.

DENV is primarily transmitted to humans through the bite of *Aedes aegypti*³ infected females which feed almost exclusively on humans and are commonly found in households and other sites where people spend much of their time⁶. DENV is

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hyperendemic in Yucatan State and elsewhere in the Yucatan Peninsula in Mexico^{6,7}, and chikungunya virus (CHIKV) also circulates in this region⁸. Previous studies performed inside the houses in Merida, Yucatan State, resulted in the detection of DENV- and CHIKV-infected *Ae. aegypti*⁶⁻⁸ females. Virus-infected mosquitoes have also been detected in frequently visited public locations. For example, DENV-infected *Ae. aegypti* were collected in schools in Merida⁹. In the State of San Luis Potosi, ZIKV-infected *Ae. aegypti* were detected in cemeteries¹⁰. It is therefore important to monitor public sites for potential arboviruses transmission.

Churches regularly receive a large influx of visitors, both locals and tourists, who could potentially become infected during their visits and introduce the virus into new areas. We have previously revealed that engorged *Ae. aegypti* often occur inside churches in Merida¹¹. Despite this, studies have not been performed to determine whether churches located in neighborhoods with a high incidence of DENV provide suitable breeding habitats for immature mosquitoes and are potential sites for virus transmission. To address these gaps in our knowledge, longitudinal entomological and virological surveillance were performed in selected churches in three endemic dengue districts of Merida. Churches were inspected regularly for the presence of mosquitoes breeding sites, the abundance and species composition of immature and adult mosquitoes were determined, and *Ae. aegypti* females were assayed for evidence of DENV infection.

MATERIAL AND METHODS

Study sites

Merida (population ~ 800,000) is located in the Yucatan Peninsula of Mexico. The city has a distinct rainy (May–October) season and a dry season (November–April). In the rainy season, the mean rainfall is 1,000 mm and the mean temperature is 27.5 °C. In the dry season, the mean rainfall is 300 mm and the mean temperature is 25.1 °C⁶.

The studied sites were located in three neighborhoods where high DENV transmission has been previously reported^{6,7,9}. The largest church in each neighborhood was selected based on the assumption that it would have more mosquitoes breeding sites and a higher abundance of immature and adult mosquitoes than other churches in the neighborhood. Church leaders provided unlimited access to the entire area, including rooms, offices, kitchens, storage rooms and gardens. It is estimated that each church is visited by at least 900 individuals each week (300 and 600 individuals on weekdays and weekends, respectively), although these numbers are higher when there

are important religious holidays, festivities or celebrations (personal communication from church authorities). The neighborhoods are San Jose Tecoh, Bojorquez and Vergel III, located in Southern, Central and Eastern Merida, respectively. The first two churches are located approximately 20 m from the nearest houses. The church in Vergel III is located approximately 80 m from the nearest house. The churches are similar in size (~ 10,000 m²), close to markets and 8 to 10 km apart from each other (Figure 1).

Sampling of immature mosquitoes

Mosquitoes were collected at each church once a week from November 2015 to October 2016. Collections were made both indoor and outdoor. Outdoor collections were primarily focused on garden areas and parking lots on church grounds. Containers were classified following the methodology of Garcia-Rejon *et al.*¹². Immatures were removed from water-holding containers using nets, turkey basters and pipettes and placed inside plastic transportation containers labeled according to date, study site and sample identification number. Mosquitoes were individually removed from transportation containers and counted in a white tray. Immatures were allowed to emerge, then adults were identified to species^{11,12}.

Adult mosquito collections

Adult mosquitoes were collected between 8 a.m. and 1 p.m. using a backpack-mounted aspirator (Prokopack Aspirator®, model 1419, John W. Hock Company). Each church was inspected for resting adults once every week. Indoor collections focused primarily on furniture, hanging clothes, curtains as well as dark and humid places. Outdoor collections focused primarily on garden areas and fences. The amount of time actively spent searching for resting mosquitoes at each site was 1-2 h per visit. Both adults and immatures were transported alive to the laboratory of *Arbovirologia* at *Universidad Autónoma de Yucatán* and identified according to species using stereomicroscopes and a published identification key¹³. Female *Ae. aegypti* were sorted into pools of up to 15 specimens and stored at -80 °C until required.

Mosquitoes homogenizations, RNA extractions and RT-PCR

Pools of female adult *Ae. aegypti* were placed into eppendorf tubes containing 300 µL of Liebovitz's L15 medium (Invitrogen, Carlsbad, CA, USA) and mechanically homogenized using sterile pestles. Homogenates were

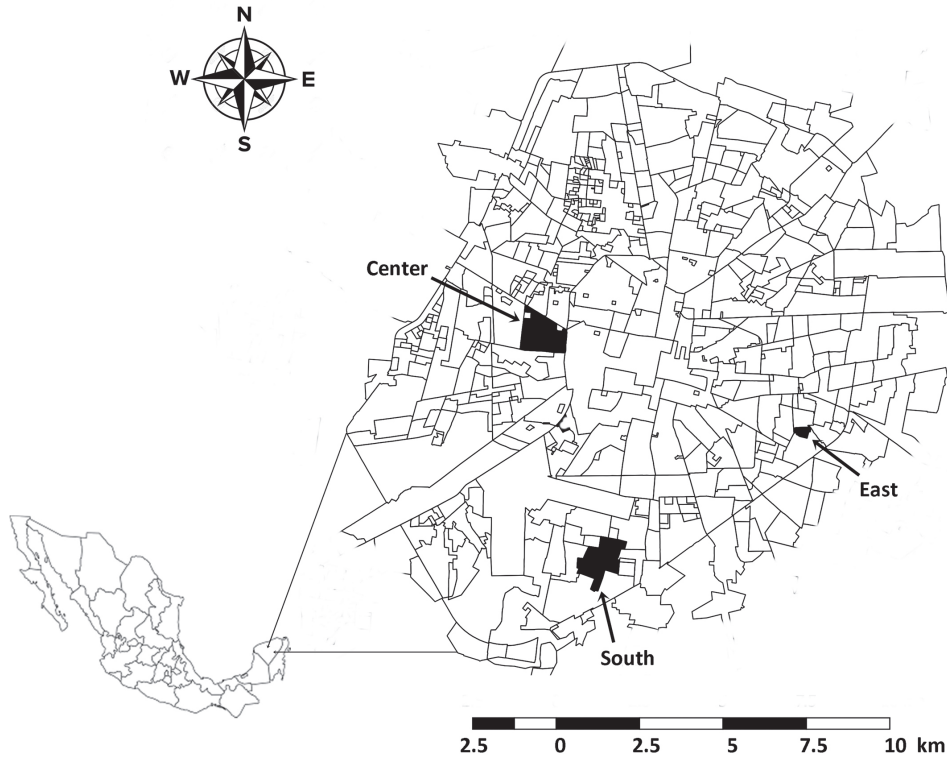


Figure 1 - Location of study sites in Merida, Yucatan, Mexico. Catholic churches were located in three neighborhoods: Bojorquez (Center), San Jose Tecoh (South) and Vergel-III (East).

centrifuged at $10,000 \times g$ for 10 min. and supernatants were collected. Total RNA was extracted from an aliquot (100 μL) of each supernatant using an RNeasy kit (Qiagen, Valencia, CA, USA) and tested for flavivirus RNA by reverse transcription-polymerase chain reaction (RT-PCR) using flavivirus-specific primers (MAMD, cFD2 and FS778) which amplify an ~ 250 nt region of the NS5 gene¹⁴. RT-PCRs were performed in 25 μL reaction volumes containing 2.5 μL of total RNA, 2 μL MgCl_2 at a concentration of 25mM, 2.5 μL of 5 x reaction buffer, 0.2 μL of dNTPs, 0.5 U of Taq polymerase (Invitrogen), 0.5 μL of each primer at a concentration of 10 mM and 16.15 μL sterile distilled water. Total RNA extracted from DENV-2-infected cells was used as the positive control after reverse transcription. As the negative control, molecular biology grade water was used instead of total RNA. Amplification conditions are as follows: an initial denaturation of 95°C for 1 minute, followed by 35 cycles, each one consisting of 1 min at 95 °C, 1.5 min at 75 °C, and 1 min at 72 °C and then a final extension for 7 min at 72 °C. Amplicons were visualized on 2% agarose gels with 0.5 $\mu\text{g}/\text{mL}$ ethidium bromide using a Doc™ XR+ Gel Documentation System. RT-PCR products were purified using the Zymoclean DNA recovery kit Cat (Zymo Research, Irvine, CA, USA) and sequenced using a 3500xL DNA sequencer (Applied Biosystems, Foster City, CA, USA). Sequences were

manually aligned and edited using the Bioedit v.7.0.9 software¹⁵ and the Mega v.7 software¹⁶.

Statistical analysis

Entomological indices estimated in this study are as follows: 1) the container index, defined as the percentage of water-filled containers with immature *Ae. aegypti* and 2) the pupal index, defined as the percentage of containers with *Ae. aegypti* pupae when compared to containers with *Ae. aegypti* immatures (larvae and/or pupae). A Mann-Whitney U test was used to compare the number of female *Ae. aegypti* by season because the data did not meet the assumptions of normality and homogeneity of variances. The statistical analysis was performed using the IBM SPSS Statistics version 22 software for Windows (IBM Corporation, Armonk, NY) and results were considered significant when $P \leq 0.05$.

RESULTS

Immature mosquito collections

The total number of containers observed in the entire study was 2,918 (Table 1). This tally is based on the assumption that one container can be counted more than

Table 1 - Collections of *Ae. aegypti* immatures in churches of Merida, Yucatan by season and container type, from November 2015 to October 2016.

Type of container	Total n° container observations/with water	N° water-filled containers with immatures		Water (L) per container (mean±SD)	Total n° of immatures	
		Larvae and pupae	^b Pupae		Larvae	Pupae
Rainy season						
Buckets	242/129	25	14	4.18±4.38	2,898	211
Disposable containers	1,099/379	13	7	0.80±4.39	580	30
^a Larger containers	97/35	13	4	1.81±3.32	922	25
Vases	57/34	1	1	1.80±0.0	40	8
Flower pots	172/38	1	1	1.15±0.0	115	5
Tires	11/2	1	0	0.35±0.0	88	0
Discarded toilet	6/2	0	0	2.82±1.44	0	0
Storm water-drains	68/45	1	0	78.33±153.57	88	0
Subtotal	1,752/664	55	27	2.63±4.18	4,731	279
Dry season						
Buckets	205/72	6	2	7±6.04	785	33
Disposable containers	692/31	0	0	0.46±0.83	0	0
^a Larger containers	52/8	4	1	3.30±1.56	220	3
Vases	3/3	0	0	0.81±0.44	0	0
Flower pots	157/15	0	0	0.95±0.80	0	0
Tires	2/0	0	0	0	0	0
Storm water-drains	55/11	0	0	21.82±18.34	0	0
Subtotal	1,166/140	10	3	5.15±4.61	1,005	36
Total	2,918/804	65	30	3.02±4.31	5,736	315

^aWith a capacity >5 liters; ^bNumber of containers with pupae

once (i.e. one count for every visit in which the container was observed). Water was detected during 27.5% (804/2,918) of the container observations and 8.1% (65/804) yielded immatures. A total of 10,997 immatures of five species were collected (Table 2). The most abundant species was *Ae. aegypti* (6,051) followed by *Culex quinquefasciatus* Say (3,018), *Culex nigripalpus* Theobald (1,894), *Culex thriambus* Dyar (26), and *Culex interrogator* Dyar and Knab (8).

Seven-fold more immatures were collected in the rainy season (9,628) compared to the dry season (1,369, Table 2). Analysis of the data at the species level also revealed that immature *Ae. aegypti* were more common in the rainy season. A total of 1,758 container observations were made in the rainy season. Water was detected in 37.8% (664/1,758) of the container observations and 8.2% (55/664) yielded immatures (Table 1). The pupal index was calculated as 49.1% (27/55). In the dry season, 1,166 container observations were made. Of these, 12.0% (140/1,166) revealed water and 7.1% (10/140) yielded immatures. The pupal index was calculated as 30.0% (3/10) (Table 1). Buckets were the most common source of *Ae. aegypti* during both seasons and accounted for 64.9% (3,927/6,051) of those collected. Disposable containers and

Table 2 - Abundance and species composition of immature mosquitoes collected in churches of Merida, Yucatan, from November 2015 to October 2016.

Mosquito species	Church	Total n° of immatures collected	
		Larvae	Pupae
Rainy season			
<i>Aedes aegypti</i>	Vergel III	4,257	242
<i>Culex quinquefasciatus</i>	Vergel III	2,648	68
<i>Culex nigripalpus</i>	Vergel III	1,847	47
<i>Culex interrogator</i>	Vergel III	8	0
<i>Aedes aegypti</i>	San Jose Tecoh	67	8
<i>Aedes aegypti</i>	Bojorquez	407	29
Subtotal		9,234	394
Dry season			
<i>Aedes aegypti</i>	Vergel III	994	9
<i>Culex quinquefasciatus</i>	Vergel III	291	11
<i>Culex thriambus</i>	Vergel III	26	0
<i>Aedes aegypti</i>	San Jose Tecoh	11	27
Subtotal		1,322	47
Total		10,556	441

larger containers were the next most common sources of *Ae. aegypti* at both times of the year. *Ae. aegypti* were present in every container that held immatures.

When collections were grouped by church, 95.0% (10,448/10,997) of mosquito immatures were collected in Vergel III. Five species were collected in this church. The most abundant species in Vergel III was *Ae. aegypti* (5,502) followed by *Cx. quinquefasciatus* (3,018). Sixty breeding sites of *Ae. aegypti* were registered in Vergel III, with 28 buckets, 16 larger containers and 13 disposable containers yielding 3453, 993 and 755 immatures, respectively. The pupal index was calculated as 43.3% (26/60). In Bojorquez and San Jose Tecoh, one and four breeding sites were registered, respectively.

Adult mosquito collections

In total, 21,226 adult mosquitoes (13,518 males and 7,708 females) of nine species were collected (Table 3). Of the females collected, the most abundant species was *Cx. quinquefasciatus* (4,686) followed by *Ae. aegypti* (1,380), *Aedes taeniorhynchus* (Wiedemann) (1,172) and *Cx. nigripalpus* (363) which together accounted 98.6% (7,601/7,708) of the adults. *Cx. quinquefasciatus* was common during both the rainy and dry seasons whereas the other three species were far more abundant in the rainy season.

Significant statistical difference in the number of *Ae. aegypti* females per season was observed ($Z=-0.57$,

Table 3 - Abundance and species composition of adult mosquitoes collected in churches of Merida, Yucatan from November 2015 to October 2016.

Species	Church	Total nº of adults collected		Blood feeding status		
		Males	Females	Unfed	Fed	Gravid
Rainy season						
<i>Ae. aegypti</i>	Vergel-III	2,036	1,029	375	474	180
<i>Ae. taeniorhynchus</i>	Vergel-III	272	1,039	410	280	349
<i>Ae. trivittatus</i>	Vergel-III	0	32	9	5	18
<i>Cx. coronator</i>	Vergel-III	0	1	1	0	0
<i>Cx. interrogator</i>	Vergel-III	21	67	53	5	9
<i>Cx. nigripalpus</i>	Vergel-III	129	321	185	12	124
<i>Cx. quinquefasciatus</i>	Vergel-III	4,766	1,813	974	505	334
<i>Cx. stigmatosoma</i>	Vergel-III	0	1	0	1	0
<i>Ps. ferox</i>	Vergel-III	0	3	1	1	1
<i>Ae. aegypti</i>	San Jose Tecoh	7	26	10	10	6
<i>Cx. quinquefasciatus</i>	San Jose Tecoh	87	33	14	7	12
<i>Ae. taeniorhynchus</i>	San Jose Tecoh	0	6	4	2	0
<i>Ae. aegypti</i>	Bojorquez	193	123	52	52	19
<i>Cx. quinquefasciatus</i>	Bojorquez	184	119	55	47	17
<i>Ae. taeniorhynchus</i>	Bojorquez	34	115	69	38	8
<i>Ae. trivittatus</i>	Bojorquez	0	3	2	0	1
Subtotal		7,729	4,731	2,214	1,439	1,078
Dry season						
<i>Ae. aegypti</i>	Vergel-III	281	187	79	76	32
<i>Ae. taeniorhynchus</i>	Vergel-III	9	12	6	2	4
<i>Cx. nigripalpus</i>	Vergel-III	2	42	35	2	5
<i>Cx. quinquefasciatus</i>	Vergel-III	5,421	2,680	1,925	609	146
<i>Ae. aegypti</i>	San Jose Tecoh	2	7	5	1	1
<i>Cx. quinquefasciatus</i>	San Jose Tecoh	47	23	10	7	6
<i>Ae. aegypti</i>	Bojorquez	3	8	5	2	1
<i>Cx. quinquefasciatus</i>	Bojorquez	24	18	10	3	5
Subtotal		5,789	2,977	2,075	702	200
Total		13,518	7,708	4,289	2,141	1,278

$p=0.001$). Approximately six-fold more *Ae. aegypti* females were collected in the rainy season compared to the dry season (Table 3). Of the 1,178 *Ae. aegypti* females collected during the rainy season, 536 were identified as fed, 437 as unfed and 205 as gravid (Table 4). In the dry season, 202 *Ae. aegypti* females were collected with 89 identified as unfed, 79 as fed and 34 as gravid.

Vergel III was the most common source of adult mosquitoes; 95% (12,937 males and 7,227 females) of adults of nine species were collected (Table 3). Of the 7,227 females collected, the most abundant species was *Cx. quinquefasciatus* (4,493) followed by *Ae. aegypti* (1,216), *Ae. taeniorhynchus* (1,051), *Cx. nigripalpus* (363), *Cx. interrogator* (67), *Ae. trivittatus* (32), *Cx. thriambus* (26), *Ps. ferox* (3), *Cx. coronator* (1) and *Cx. stigmatosoma* (1). Eighty-eight percent (1,216/1,380) of *Ae. aegypti* females was collected in this site.

Detection of DENV in *Ae. aegypti*

Adult *Ae. aegypti* females were sorted into 166 pools and analyzed for flavivirus RNA by RT-PCR and Sanger sequencing. Two (1.2%) pools were positive (Table 4). One pool contained DENV-1 and the other contained DENV-2 (GenBank accession number KU232287 and KJ189370, respectively). The DENV minimal infection rate (MIR) for *Ae. aegypti* females (expressed as the number of positive mosquito pools per 1,000 mosquitoes tested) was 1.5. Both DENV-positive pools were collected inside the church in Vergel III. The DENV-1-positive pool consisted of mosquitoes collected in a storage room. The DENV-2-positive pool consisted of mosquitoes collected inside the nave, the main area of the church where the congregation

is held. Both pools comprised of mosquitoes were collected in October 2016.

DISCUSSION

Vergel III represented a higher potential entomological risk than the other investigated churches. The pupal index in this church over the entire study period was 43.3%, peaking at 47.0% in the rainy season. Pupae are considered a better proxy than other life stages for estimating mosquitoes abundance¹⁷. Based on the operative criteria of the Mexican Ministry of Health, a pupal index greater than 5% is regarded as an emergency level status that requires immediate action (i.e. treatment or elimination of breeding sites)¹⁸. Adult *Ae. aegypti* were also more abundant at Vergel III compared to the other churches in this study. It has previously been reported that greater mosquitoes abundance increases the risk of human DENV transmission⁷. Both pools of DENV-infected *Ae. aegypti* were detected in the church of Vergel III. Similarly, in Iquitos, Peru, abundance of *Ae. aegypti* females were better proxies for DENV risk¹⁹.

DENV-infected *Ae. aegypti* have been detected inside schools and houses in Merida^{6,7,9}. Water-filled containers are common in Merida and contribute to the high abundance of mosquitoes^{12,20}. It should also be noted that *Ae. aegypti* with Ile 1,016 and Cys 1,534 mutations in their voltage gated sodium channel gene have been detected in Merida. These mutations are associated with pyrethroid resistance^{21,22}. Thus, increased vector control is required in this region. One way to achieve this goal is through increased community intervention. In this study, we educated church workers on mosquitoes (i.e. their life cycle and role in arbovirus transmission) and

Table 4 - Detection of dengue virus RNA in adult *Ae. aegypti* females in churches of Merida from November 2015 to October 2016.

Area within Merida city	Neighborhoods	N° total females	N° total unfed	N° total fed	N° total gravid	N° of pools tested	N° of positive pools for DENV
Rainy season							
East	Vergel-III	1,029	375	474	180	125	2
South	San Jose Tecoh	26	10	10	6	3	0
Center	Bojorquez	123	52	52	19	14	0
Subtotal		1,178	437	536	205	142	2
Dry season							
East	Vergel-III	187	79	76	32	22	0
South	San Jose Tecoh	7	5	1	1	1	0
Center	Bojorquez	8	5	2	1	1	0
Subtotal		202	89	79	34	24	0
Total		1,380	526	615	239	166	2

recommended that church visitors should use personal protection against mosquitoes (e.g. repellents).

Environmental conditions may influence the mosquitoes population size. High populations of *Ae. aegypti* in the rainy season have been previously reported in Yucatan State^{6,9,20}. Seven-fold more immature *Ae. aegypti* were collected in the rainy season compared to the dry season presumably due to an increase in the number of water-filled containers. A high number of breeding sites and immature *Ae. aegypti* were identified in Vergel III. Buckets followed by disposable containers were the most common breeding sites for *Ae. aegypti* and most were filled by rain water. Disposable containers were also common breeding sites for *Ae. aegypti* in and around houses in Merida^{6,12}. Previous studies in Merida have also shown that tires and flower pots are common breeding sites in vacant lots and parking lots, respectively²⁰. Storm-water drains along streets and sidewalks harbor many species of mosquitoes throughout the year²³.

It is important to highlight two characteristics of Vergel III: 88% of *Ae. aegypti* females were collected in this church and it is located approximately 80 m from the nearest house. We speculate that most *Ae. aegypti* in Vergel III had not traveled there from the houses. *Aedes aegypti* has a limited flight range; it is generally accepted that females do not fly more than 50-100 m in their entire lifetime²⁴ and that they remain close to human hosts and oviposition sites^{12,20}. Therefore, humans potentially contribute more than the vector for the movement of DENV to new areas. To the best of our knowledge, this is the first study to detect DENV-infected *Ae. aegypti* in church environments. Mosquito-based surveillance for arboviruses can be used to detect viruses prior to the occurrence of outbreaks as human infections are often asymptomatic^{2,3}. The DENV MIR in this study was 1.5 which is considerably lower than the 4.6 reported in Merida schools⁹. However, our results are similar to earlier studies performed inside the houses of dengue patients^{6,7}.

Nine mosquito species were collected in Vergel III, and the most common species was *Ae. aegypti*, consistent with previous studies performed in Merida. Diversity of mosquito species represented 17.3% (9/52) of mosquito fauna identified in Yucatan State²⁵. Other mosquito species involved in arbovirus disease transmission were also identified including *Cx. interrogator*, *Cx. quinquefasciatus*, *Cx. nigripalpus*, *Cx. stigmatosoma* and *Cx. thriambus* which are competent vectors of West Nile virus and St. Louis encephalitis virus^{26,27}. West Nile virus has been detected in *Cx. quinquefasciatus* in Nuevo Leon, Northern Mexico²⁸ and was abundant in the present study. West Nile virus has also been detected in *Cx. nigripalpus* and

Cx. interrogator in Chiapas, Southern Mexico²⁷. The alphavirus, Venezuelan equine encephalitis virus, was detected in *Cx. coronator* in Veracruz State²⁹. Several orthobunyaviruses, including Cache Valley virus were isolated from *Ae. taeniorhynchus* in Yucatan State³⁰. It is therefore important that mosquito control efforts in Merida do not focus only on *Ae. aegypti*.

Our study provides additional evidence that public sites harbor infected disease vectors. Previous studies performed in Merida identified DENV-infected *Ae. aegypti* in schools⁹ and a cemetery³¹. DENV- and ZIKV-infected *Ae. aegypti* and *Ae. albopictus* were identified in cemeteries in San Luis Potosi in Central-Northern Mexico¹⁰. In Belo Horizonte, Brazil, both DENV and ZIKV were detected in *Ae. aegypti* collected at a university³². Furthermore, La Crosse virus was detected in *Aedes triseriatus* in cemeteries in Tennessee, USA³³. However, it is important to note that our study has several limitations. For example, a small number of churches were surveyed and those from areas with low arbovirus transmission were not included. Therefore, it is not known if our findings are typical for churches in Merida or elsewhere in Mexico. Nevertheless, the findings from this study and previous reports demonstrate the need to monitor public sites to control disease transmission.

CONFLICT OF INTERESTS

None

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AUTHORS' CONTRIBUTIONS

Carlos M. Baak-Baak and Julian García-Rejón conceived and designed the study. Nohemi Cigarroa-Toledo and Angelica Pech-May carried out the phylogenetic analysis. Guadalupe A. Cruz-Escalona, Rosa C. Cetina-Trejo, Julio C. Tzuc-Dzul, Lourdes G. Talavera-Aguilar, and Suemy Flores-Ruiz carried out the fieldwork and the labwork and analysis and interpretation of these data. Oswaldo M. Torres-Chable and Carlos Machain-Williams drafted the

manuscript. Bradley J. Blitvich and Jorge Méndez-Galván critically revised the manuscript for intellectual content. All authors read and approved the final manuscript.

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