ABSTRACT

*Aedes aegypti* is the vector of the arboviruses causing dengue, chikungunya and zika infections in Mexico. However, its presence in public places has not been fully evaluated. In a cemetery from Merida, Yucatan, Mexico, the productivity of *Ae. aegypti*, the gonotrophic cycle, and the presence of *Ae. aegypti* females infected with arboviruses were evaluated. Immature and adult mosquitoes were inspected every two months between April 2016 to June 2017. For the gonotrophic cycle length, the daily pattern of total and parous female ratio was registered and was analyzed using time series analysis. *Ae. aegypti* females were sorted into pools and assayed for flavivirus RNA by RT-PCR and Sanger sequencing. *Aedes aegypti* immatures represented 82.86% (8,627/10,411) of the collection. In total, 1,648 *Ae. aegypti* females were sorted into 166 pools. Two pools were positive; one for dengue virus (DENV-1) and the other for zika virus (ZIKV). The phylogenetic analysis revealed that the DENV-1 is more closely related to isolates from Brazil. While ZIKV is more closely related to the Asian lineage, which were isolates from Guatemala and Mexico. We report some evidence of vertical transmission of DENV-1 in nulliparous females of *Ae. aegypti*. The gonotrophic cycle was four and three days in the rainy and dry season, respectively. The cemetery of Merida is an important focus of *Ae. aegypti* proliferation, and these environments may play a role in arboviruses transmission; probably limiting the efficacy of attempts to suppress the presence of mosquitoes in domestic environments.

KEYWORDS: Arbovirus. Dengue. Mexico. Daily survival. Zika virus.

INTRODUCTION

Cemeteries are obligatory components of human settlements. In Latin American cultures, cemeteries are important places to honor the deads, and it is common to have a large influx of visitors to cemeteries throughout the year. Previous studies revealed that cemeteries are suitable habitats for mosquitoes due to the great availability of resources such as sugar containing substances, shelter and water-filled vases. Immature stages of *Aedes aegypti* are common in cemeteries, where larvae and pupae are often found inside vases. However, few studies have quantified the adult populations and their role in the arboviruses transmission. To the best of our knowledge, there are only two reports of arboviruses identified in mosquitoes collected in cemeteries. Therefore, it is important to know the epidemiological importance of cemeteries in areas where dengue, zika and chikungunya viruses are present.
In home environments, survivorship and gonotrophic cycle of *Ae. aegypti* are well-documented. A short time of the gonotrophic cycle of *Ae. aegypti* increases the contact vector-human and thus the risk for arbovirus transmission. Previous studies carried out in houses estimated between 3 to 7 days the gonotrophic cycle of *Ae. aegypti*; the region, season, and temperature affected significantly the cycle. It has also been observed that mosquitoes can disperse beyond the houses. Previous studies reported engorged *Ae. aegypti* in schools and churches. Therefore, the vectorial capacity of mosquitoes must be evaluated in cemeteries because they have breeding sites and are near the houses.

Cemeteries have been used to study the mosquito ecology (i.e., productivity, species interaction, competition, composition and temporality), and also in field assays to evaluate biological and chemical mosquito control. Cemeteries have also been used for the early detection and monitoring of invasive mosquitoes such as *Aedes albopictus* (Skuse). There is a growing recognition that cemeteries can also be effective sites for monitoring virus transmitted by mosquitoes. In Yucatan State of Southeastern Mexico, dengue, chikungunya and zika viruses co-occur. Despite this, studies have not been performed to quantify the *Ae. aegypti* population in cemeteries of Merida city and whether they are potential sites for arboviruses transmission. The goals of the study were to determine by season 1) the infestation of breeding sites; 2) abundance of immatures and adults of *Ae. aegypti*; 3) the length of the gonotrophic cycle and the survival rate of *Ae. aegypti*; and 4) the presence of *Ae. aegypti* females infected with arbovirus.

**MATERIALS AND METHODS**

**Study area**

The study was carried out in the “General Cemetery” of Merida city in the Yucatan State of Southeastern Mexico. This cemetery is the oldest and largest (15 hectares), is immersed within a densely populated city. Based on data of the town hall, the cemetery has 25,700 vaults registered as tombs, ossuaries, niches, crypts and mausoleums (http://www.merida.gob.mx/). The area selected for the study is located approximately 300 m from the nearest houses (Figure 1). The cemetery is open to public between 07:00 to 18:00 h.

In Yucatan State, the rainy season extends from May to October and the dry season from November to April. During the rainy season, the mean rainfall is 1,000 mm and the mean temperature is 27.5 °C. During the dry season, the mean rainfall is 300 mm and the mean temperature is 25.1 °C.

**Adult mosquitoes collection**

Adult mosquitoes were collected for three consecutive days in April, June, August, October and December 2016 and in February, April and June 2017. *Aedes aegypti* females were collected using BG-Sentinel traps (Biogents GmbH, Regensburg, Germany) coupled to the attractant BG-Lure (Biogents GmbH, Regensburg, Germany). Inside the cemetery, we chose a transect of 170 m, in which ten traps were placed. The transect was located near the flower shop due to the influx of visitors and presence of cemetery workers (Figure 1). BG-Sentinel traps were placed at every 17 m and were activated between 07:00 and 10:00 h. Female *Ae. aegypti* were sorted into pools of up to 15 and stored at -80 °C until required.

**Sampling of immature mosquitoes**

Mosquitoes were collected into a quadrant of approximately 100 m, where the BG-Sentinel traps were placed. Mosquitoes were removed from vases using nets,
turkey basters and pipettes and placed inside plastic transportation containers labeled according to date, study site and sample identification number. Immature and adult mosquitoes were transported alive to the Laboratory of Arbovirology at Universidad Autonoma of Yucatan and were identified using published identification keys\textsuperscript{17,18}.

Gonotrophic cycle and survival dynamics

Female \textit{Ae. aegypti} were collected using BG-Sentinel traps during 19 consecutive days in the dry (April 20 to May 08) and rainy (September 06 to 24) season in 2016. The blood feeding status (Sella’s stages) was determined by external examination of the abdomen. Insects were then grouped as unfed (the collapsed abdomen and the ovaries occupy one-third of the abdomen), fed (fresher fed, bright red blood and the ovaries occupy two to three segments ventrally; the sub-gravid with dark blood and with great space reduced and ovaries occupy most of abdomen) and gravid (blood completely digested or present only as a black trace or line)\textsuperscript{19}.

To estimate the gonotrophic cycle, all the females were dissected in microscope slides using a drop of 65\% saline solution. They were classified as nulliparous, parous or gravid according to the appearance of the tracheolar system and/or the presence of eggs in the abdomen\textsuperscript{19}. \textit{Aedes aegypti} females dissected were stored at -80 °C and assayed for flavivirus RNA.

RNA extraction and RT-PCR

Pools of female adult \textit{Ae. aegypti} were placed into eppendorf microtubes containing 300 µL of Liebovitz’s L15 medium (Invitrogen, Carlsbad, CA, USA) and mechanically homogenized using sterile pestles. Homogenates were centrifuged at 10,000 × g for 10 min and supernatants were collected. Total RNA was extracted from an aliquot (100 µL) of each supernatant using the RNeasy kit (QIAGEN, Valencia, CA, USA) and tested for flavivirus RNA by reverse transcription-polymerase chain reaction (RT-PCR) using flavivirus-specific primers (cFD2 and FS778) which amplify a 250 nucleotide region of the NS5 gene\textsuperscript{20}. RT-PCRs were performed in 25 µL reaction volumes containing 2.5 µL of total RNA, 2 µL MgCl\textsubscript{2} at a concentration of 25 mM, 2.5 µL of 5 x reaction buffer, 0.2 µL of dNTPs, 0.15 µL Taq polymerase (Invitrogen\textsuperscript{®}), 0.5 µL of each primer at a concentration of 10 mM, and 16.65 µL ddH\textsubscript{2}O was added to reach the final volume. Amplification conditions are as follows: an initial denaturation of 95 °C for \textit{t} minute, followed by 35 cycles each consisting of 1 min at 95 °C, 1.5 min at 75 °C, and 1 min at 72 °C and one cycle of extension for 7 min at 72 °C. Amplicons were visualized on 2\% agarose gels with 0.5 µg/mL of ethidium bromide using a Doc\textsuperscript{™} 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).

Data analysis

Entomological indices were estimated: 1) the percentage of water-filled containers with immature \textit{Ae. aegypti} presence (larvae, pupae, or both); and 2) a pupal index representing the percentage of containers with \textit{Ae. aegypti} pupae present out of all containers with \textit{Ae. aegypti} immatures presence.

To compare the number of immature and adult of \textit{Ae. aegypti} by season, data were submitted to a normality test. A Mann–Whitney U test was used to compare the number of immature and \textit{Ae. aegypti} females by season, because data did not meet the assumptions of normality and homogeneity of variances. The minimum infection rate (MIR) was calculated: (number of positive pools/ total specimens tested) × 1,000. Statistical analysis was performed using the IBM SPSS Statistics version 22 software for Windows (IBM Corporation, Armonk, NY, USA), and results were considered significant when \( P \leq 0.05 \).

The length of the gonotrophic cycle was estimated using a cross-correlation analysis\textsuperscript{7} with the formula \( M_t = P_{t} T_{t+6} \), where \( M \) = the number of parous individuals captured on day \( t \); \( T_{t+6} \) = the total number of females (nulliparous and parous) captured on day \( t+6 \); \( u = \) the length of the gonotrophic cycle; and \( P \) = the survival rate per gonotrophic cycle, calculated from the slope in a regression model. The correlation coefficient \( (r) \) for day 0 represented the correlation between \( P \) and \( T_{t+6} \), data pairs from mosquitoes captured on the same day (15 data pairs). The \( r \) for day was obtained by pairing daily \( P \) data with the corresponding \( T \) data of 1 day before. Likewise, \( r \) for each day was obtained by pairing daily \( P \) data with the corresponding \( T \) data of 1 day before. The \( r \) for day 2 was calculated by pairing daily \( P \) data with corresponding \( T \) data of 2 days before, and so on. It was assumed that a significant \( r \) between the same series expressed a time delay (\( u \)) equivalent to the gonotrophic cycle. The highest correlation coefficient and significance obtained after day 0 (\( u = 0 \)) indicated the number of days per gonotrophic cycle, with descending peaks occurring at multiples of this interval.

To eliminate spurious cross correlations, data were filtered using an autoregressive process with a lag of
1 day, with the formula \( Z_t = X - \beta(X_{t-u}) \), where \( Z_t \) is the filtered time series, \( X_t \) is the time series to be filtered, and \( \beta \) is the estimated auto-regressive parameter\(^{21}\). A significant correlation between 2 filtered time series (\( M_t \) and \( X_{t-u} \) was assumed), and \( r \) corresponded to a lag \( u \) equivalent to the gonotrophic cycle, with regular peaks at the start of each cycle.

Daily survival rates (\( p \)) were calculated from the parity rates using the formula \( p = (PR)^{1/CG} \), where \( PR \) is the parity rate and \( CG \) is the duration of the gonotrophic cycle\(^{22} \).

**Sequence analysis**

Sequences were manually aligned and edited using the BioEdit v.7.0.9\(^{23} \) and the Mega v.7\(^{24} \) softwares. The nucleotide sequences were translated into the corresponding amino acid counterparts using the translation tool of the ExPaSy bioinformatic resource portal (http://web.expasy.org/translate/) and compared to other sequences from the GenBank database using the Basic Local Alignment Search Tool (https://blast.ncbi.nlm.nih.gov/Blast.cgi).

The alignment of the NS5 fragment and amino acid sequences was performed with Mega v.7\(^{25} \). The DnaSP v.5.10 software\(^{26} \) was also used to analyze genetic variants. The similarity and identity were calculated using the MatGat software\(^{26} \). Genetic distances among variants were calculated using the Mega v.7 software\(^{24} \) with 10,000 random permutations.

**RESULTS**

**Immature mosquitoes collection**

The total number of container observations for the entire study was 4,867 (Table 1). Water was detected during 16.29% (793/4,867) of the container observations and 22.95% (1,124/4,867) yielded immatures. In a quadrant of 100 m, the density of positive vases was 112 and 70 during the dry and rainy season, respectively. A total of 10,411 immatures of four species were collected. The most abundant species was *Ae. aegypti* (n = 8,627), followed by *Culex quinquefasciatus* Say (n = 1,663), *Culex nigripalpus* Theobald (n = 69), and *Culex coronator* Dyar and Knab (n = 52).

Immature *Ae. aegypti* represented 82.86% of the collection. Analysis of data at the species level revealed that there was no significant statistical difference between the number of immature *Ae. aegypti* per season (\( Z = -0.142, P \geq 0.05 \)); although two-fold more immatures were collected in the rainy season. A total of 3,014 vases observations were made during the rainy season. Water was detected in 14.56% (439/3,014) of the container observations and 25.51% (112/439) yielded immatures (Table 1). The pupal index was calculated as 47.32% (53/112). During the dry season, 1,853 vases observations were made. Of these, 19.10% (354/1,853) revealed water and 19.77% (70/354) yielded immatures. The pupal index was calculated as 32.85% (23/70).

**Adult mosquitoes collection**

In total, 3,957 adult mosquitoes (2,198 males and 1,759 females) of four species were collected (Table 2). Of the females collected, the most abundant species was *Ae. aegypti* (n = 1,648), followed by *Aedes taeniorhynchus* (Wiedemann) (n = 77), *Aedes trivittatus* (Coquillett) (n = 17), and *Cx. quinquefasciatus* (n = 17).

A significant statistical difference was observed in the median number of *Ae. aegypti* females per season (\( Z = -8.099, P \leq 0.05 \)). Approximately eight-fold more females were collected during the rainy season (n = 1,471) compared to the dry season (n = 177) (Table 2). Of the 1,471 *Ae. aegypti* females collected during the rainy season, 1,210 were identified as unfed, 121 as fed and 140 as gravid (Table 2). In the dry season, 177 *Ae. aegypti* females were collected with 117 identified as unfed, 14 as fed and 46 as gravid.

**Length of *Ae. aegypti* gonotrophic cycle**

There was no significant correlation (\( P \geq 0.05 \)) observed between raw and filtered data in daily changes of parity rates over 19 days in females collected during the dry and rainy season. Following the criteria of Bockarie et al.\(^{27} \), the highest \( r \)-values are considered for the duration of the

**Table 1** - Abundance of *Ae. aegypti* immatures by season in a cemetery from Merida city, Yucatan from April 2016 to June 2017

| Season | Containers | Total number *Ae. aegypti* collected | Entomological index |
|--------|------------|-------------------------------------|---------------------|
|        | Total number examined | Number (%) with water | Larvae | Pupae | Total | % water-filled containers with *Ae. aegypti* immatures present | Pupal index (%) |
| Dry    | 1,853 | 354 (19.10) | 3,084 | 193 | 3,277 | 19.77 | 32.85 |
| Rainy | 3,014 | 439 (14.56) | 4,968 | 382 | 5,350 | 25.51 | 47.32 |
| Total | 4,867 | 793 | 8,052 | 575 | 8,627 | 22.95 | 41.72 |
gonotrophic cycle. During dry season, a high correlation on days 2, 5 and 8 was found, suggesting a gonotrophic cycle of 3 days (Table 3). A daily survival rate of 0.83 and parity rate of 0.58 (Table 4) in a mean temperature of 29.43±2.41°C, 57.16±5.56% HR and 2.03 mm of precipitation were estimated. During the rainy season, a high correlation on days 5, 9 and 13 was found suggesting a gonotrophic cycle of 4 days (Table 3). A daily survival rate of 0.89 and parity rate of 0.61 (Table 4) in a mean temperature of 26.7±1.22 °C, 79.44±5.64% HR and 59.68 mm of precipitation were estimated. The daily survival rate was similar in dry (0.83) and rainy (0.89) seasons, and as a consequence, there was no significant difference between parity rate by season (t = -1.596, d.f. = 36, P ≥ 0.05).

Detection of DENV and ZIKV RNA in Ae. aegypti

Females were sorted into 166 pools and were analyzed for flavivirus RNA by RT-PCR and Sanger sequencing. Two pools were positive. The minimal infection rate (MIR) for female Ae. aegypti was 1.2. One pool contained DENV-1 RNA and the other contained ZIKV RNA. Both pools comprised of mosquitoes collected on day 13 and 17 during the gonotrophic cycle in the rainy season (September 2016). We report some evidence of vertical transmission of DENV-1 in nulliparous females of Ae. aegypti; these females (n = 11) were collected on day 13 in the gonotrophic cycle.

Sequences analyses of DENV-1

The phylogenetic analysis was performed using 94 DENV-1 sequences (Supplemental Table 1). The sequences correspond to a 204 nucleotides region of the NS5 gene. Many sequences were identical to others and therefore considered to represent the same “variant”. There were eleven variants of DENV-1 (designated DENV-1, V1 to V11). The DENV-1 sequence obtained in this study (V11-DENV-1; Mex 2016) has a close phylogenetic relationship with V1-DENV-1 isolates from Brazil in 2015 with 98.5% nucleotide identity and similarity. Alignment of
the deduced amino acid sequences revealed that they have 100% identity and similarity. Likewise, the V11 obtained in this study has a close phylogenetic relationship with V2-DENV-1 identified in Merida, Mexico in 2016 with 99.0% nucleotide identity and similarity (Supplemental Table 1). Alignment of the deduced amino acid sequences revealed that they have 98.5% identity and 100% similarity. The genetic distance Kimura-2 parameter between the V1 and V2 was 0.015, while V11 and V2 was 0.01 (Supplemental Table 2). The most common DENV was V8 (n=46), which was isolated in Mexico, USA, and Nicaragua (Supplemental Table 1).

Sequence analysis of ZIKV

The phylogenetic analysis was performed using 100 ZIKV sequences (Supplemental Table 1). The sequences correspond to a 172 nucleotides region of the NS5 gene. There were six variants of ZIKV (designated V1 to V6). The ZIKV sequence obtained in this study (V1-ZIKV; Mex 2016) has a close phylogenetic relationship with V2-ZIKV (Asian genotype) isolates from Guatemala (2015), Mexico (2015-2016), China (2016), Honduras (2016), Nicaragua (2016), Russia (2016-2017) and USA (2016-2017) with 98.3% nucleotide identity. Alignment of the deduced amino acid sequences revealed that they have 100% identity and similarity. The genetic distance Kimura-2 parameter between the V1 and V2 was 0.018 (Supplemental Table 3). The most common ZIKV was V2 (n=80), followed by V3 (n=17), which was isolated in El Salvador, China, Mexico, Ecuador, Taiwan and Colombia (Supplemental Table 1).

DISCUSSION

The findings of the present study suggest that the Merida city general cemetery is an important focus of Ae. aegypti proliferation. Vases infestation was high in the present study. An average of 15 infested vases was reported in a quadrant of 100 m, while in Venezuela it was 39 per hectare. Another notable result is that the number of larvae and pupae of Ae. aegypti was high in both seasons. The most likely explanation for the high abundance of mosquitoes and frequency of infested vases during the dry season is in part due to the water supplied by human action as occur in houses. In contrast with this result, in cemeteries from Philippines and Venezuela,

| Day | Dry season |          |          | Parity rate |          |          | Parity rate |
|-----|------------|----------|----------|-------------|----------|----------|-------------|
|     | Dissected  | Nulliparous | Parous |            |          |          |             |
| 1   | 9          | 3        | 6        | 0.67        | 8        | 1        | 7           | 0.88        |
| 2   | 3          | 2        | 1        | 0.33        | 13       | 6        | 7           | 0.54        |
| 3   | 8          | 1        | 7        | 0.88        | 7        | 2        | 5           | 0.71        |
| 4   | 4          | 1        | 3        | 0.75        | 16       | 5        | 11          | 0.69        |
| 5   | 4          | 3        | 1        | 0.25        | 27       | 4        | 23          | 0.85        |
| 6   | 6          | 3        | 3        | 0.50        | 8        | 2        | 6           | 0.75        |
| 7   | 2          | 0        | 2        | 1.00        | 18       | 2        | 16          | 0.89        |
| 8   | 6          | 0        | 6        | 1.00        | 6        | 0        | 6           | 1.00        |
| 9   | 4          | 2        | 2        | 0.50        | 12       | 3        | 9           | 0.75        |
| 10  | 7          | 3        | 4        | 0.57        | 27       | 2        | 25          | 0.93        |
| 11  | 3          | 3        | 0        | 0.00        | 27       | 7        | 20          | 0.74        |
| 12  | 4          | 3        | 1        | 0.25        | 24       | 10       | 14          | 0.58        |
| 13  | 5          | 1        | 4        | 0.80        | 55       | 38       | 17          | 0.31        |
| 14  | 3          | 2        | 1        | 0.33        | 32       | 13       | 19          | 0.59        |
| 15  | 4          | 3        | 1        | 0.25        | 51       | 23       | 28          | 0.55        |
| 16  | 4          | 2        | 2        | 0.50        | 66       | 18       | 48          | 0.73        |
| 17  | 4          | 2        | 2        | 0.50        | 68       | 25       | 43          | 0.63        |
| 18  | 3          | 1        | 2        | 0.67        | 40       | 16       | 24          | 0.60        |
| 19  | 3          | 1        | 2        | 0.67        |          |          |             |
| Total | 86       | 36        | 50        | 0.58        | 541      | 209       | 332         | 0.61        |
most vases had water and yielded immature mosquitoes during the rainy season\textsuperscript{22,28}. The results of this study suggest that the heterogeneous urban environment supports a high population of mosquitoes. In addition to the general cemetery, previous studies in Merida have also shown that breeding sites on houses, streets/sidewalks and vacant lots yield high number of immature \textit{Ae. aegypti}\textsuperscript{29-31}.

Immature \textit{Ae. aegypti} was found to be the dominant species in the vases. Ninety-two percent of the vases containing larvae and pupae had only \textit{Ae. aegypti}. Nevertheless, \textit{Cx. quinquefasciatus}, \textit{Cx. coronator} and \textit{Cx. nigripalpus} were also found. In cemeteries from Philippines and Argentina, \textit{Ae. aegypti} was found co-inhabiting with \textit{Ae. albopictus} and \textit{Cx. pipiens}, respectively\textsuperscript{23,29}. The adaptive features of \textit{Ae. aegypti} eggs to enter diapause allowed their reproductive success. The diapause may extend for six months or more, until the eggs get in contact with water in the container again, and then hatching occurs\textsuperscript{22}. In the cemeteries, it is not possible to control the rain factor, therefore, it is important to have a method to control the presence of larvae and pupae in the vases. In a cemetery of Buenos Aires, Argentina, temephos was effective in reducing \textit{Ae. aegypti} populations\textsuperscript{12}. Meanwhile, \textit{Toxorhynchites splendens} (Wiedemann) was effective in controlling the larvae of \textit{Ae. albopictus} in Malaysia\textsuperscript{13}.

In the present study, 80% (1,327/ 1,648) of the \textit{Ae. aegypti} females were classified as unfed. It is possible that the emerged adults fly towards the nearby houses in search of a blood meal. It is necessary to perform studies on the dispersion of \textit{Ae. aegypti} from cemeteries to houses, as this will probably limit the efficacy of attempts to suppress the mosquitoes in domestic environments. In contrast to the cemeteries, it is common to find engorged \textit{Ae. aegypti} in indoor environments. This may be the result of a closer relationship with human\textsuperscript{15}. It should be noted that \textit{Ae. aegypti} display a strong anthropophilia. In houses and schools of Merida city, 57% of the \textit{Ae. aegypti} females were collected as fed, 29% as unfed and 14% as gravid females\textsuperscript{11,15}. In churches, 47% of the \textit{Ae. aegypti} females were collected as fed, 34% as unfed and 19% as gravid females\textsuperscript{10}.

Previous studies on the gonotrophic cycle of \textit{Ae. aegypti} was estimated with human bait and mark-release-recapture experiment\textsuperscript{6,33}. Currently, human bait is not used due to ethical issues, while the second method requires more effort and sometimes has poorer results. We use BG-Sentinel traps and they turned out to be an effective method for surveillance of \textit{Ae. aegypti}. In our study, estimated intervals between two consecutive blood meals were three days during dry season and four days during rainy season. The gonotrophic cycle of three days was affected by high temperatures (29.43 °C) during dry season. Under laboratory conditions, high temperatures are significantly more favorable for shorter gonotrophic cycles of \textit{Ae. aegypti}\textsuperscript{8}. Our results agree with previous findings in studies conducted in Thailand\textsuperscript{34}, East Africa\textsuperscript{34} and Peru\textsuperscript{4}. Additionally, in Thailand, Pant and Yasuno\textsuperscript{35} estimated the gonotrophic cycle of three days during the rainy season, with two days of delay during the dry season. During the rainy season, we estimated a four-day cycle. This result is comparable with the ones from studies performed using the mark-release-recapture method in Thailand\textsuperscript{35}, Tanzania\textsuperscript{36} and Kenya\textsuperscript{37}. In Merida city, two studies have estimated the gonotrophic cycle of \textit{Ae. aegypti}. In houses, Rebollar-Tellez \textit{et al.}\textsuperscript{6} estimated a seven-day cycle, while in churches, the duration of the gonotrophic cycle was similar to the one found in the present study of three and four days during the dry and rainy season, respectively\textsuperscript{10}.

High values of survival rate increase the potential risk for transmission of pathogens day to day\textsuperscript{50}. Under laboratory conditions, the highest survival rate for \textit{Ae. aegypti} females was 84% at 27 °C, reaching 25 days of age\textsuperscript{8}. In Mexico, the survivorship for \textit{Ae. aegypti} was estimated by Rebollar-Tellez \textit{et al.}.\textsuperscript{6} as 0.86. In the cemetery of Merida city, we found a high survival rate (0.83) for \textit{Ae. aegypti}. Previous studies conducted in cemeteries identified arbovirus-infected mosquitoes. For example, La Crosse encephalitis virus-infected \textit{Aedes triseriatus} were collected in cemeteries in Tennessee, USA\textsuperscript{4}. In the State of San Luis Potosi, Mexico, ZIKV-infected \textit{Ae. aegypti} were detected in cemeteries\textsuperscript{5}. In the present study, DENV-1 RNA and ZIKV RNA were identified in \textit{Ae. aegypti}. It is also the first report of \textit{Ae. aegypti} infected with ZIKV RNA in Yucatan State. Notably, the sequence obtained in this study revealed that the viruses are more closely related phylogenetically to DENV and ZIKV from Central and South America (Supplemental Table 1). The MIR in this study was 1.2 which is considerably lower than the 4.6 reported in schools in Merida\textsuperscript{11}. However, our results are similar to the ones from earlier studies performed inside the houses of dengue patients\textsuperscript{35,38}. On the other hand, the first report of ZIKV-infected \textit{Ae. aegypti} was from Chiapas, Mexico and the MIR was estimated at 52.49-172.66\textsuperscript{39}.

We also found evidence of vertical transmission of DENV-1 in nulliparous \textit{Ae. aegypti} females during the gonotrophic cycle. In Mexico, vertical transmission of dengue virus by \textit{Ae. aegypti} and \textit{Ae. albopictus} was reviewed by Ferreira-de-Lima and Lima-Camara\textsuperscript{40}, who mentioned that they occur in Tamaulipas, Oaxaca and Guerrero. Vertical transmission may represent an important strategy for maintaining the circulation of arboviruses in nature\textsuperscript{40}, therefore it should be studied in depth in the cemeteries.
CONFLICT OF INTERESTS

None.

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AUTHORS’ CONTRIBUTIONS

J. Garcia-Rejon and C. M Baak-Baak conceived and designed the study; N. Cigarroa-Toledo and A. Pech-May carried out the phylogenetic analysis; R. C. Cetina-Trejo, L. G. Talavera-Aguilar, and O. M. Torres-Chable carried out the fieldwork and the labwork; A. Ulloa-Garcia, C. Machain-Williams, and J. C. Navarro analyzed the data. All authors contributed for drafted the manuscript, provided critical input regarding the findings and approved the final manuscript.

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**Supplemental Table 1 - Database with GenBank accession numbers**

| GenBank accession number | Clave in the study | Virus | Genotype | Collection date | State      | Country   |
|--------------------------|--------------------|-------|----------|-----------------|------------|-----------|
| KU232287                 | V1                 | Dengue| 1        | 2015            | Pernambuco | Brazil    |
| KU232286                 | V1                 | Dengue| 1        | 2015            | Pernambuco | Brazil    |
| Merida, Mex-2016         | V2                 | Dengue|          | 2016            | Yucatan    | Mexico    |
| KF973475                 | V3                 | Dengue|          | 2012            | No data    | Nicaragua |
| KF973472                 | V3                 | Dengue|          | 2012            | No data    | Nicaragua |
| KF973467                 | V3                 | Dengue|          | 2012            | No data    | Nicaragua |
| KF973466                 | V3                 | Dengue|          | 2012            | No data    | Nicaragua |
| KF973463                 | V3                 | Dengue|          | 2012            | No data    | Nicaragua |
| KF973460                 | V3                 | Dengue|          | 2012            | No data    | Nicaragua |
| KF973458                 | V3                 | Dengue|          | 2012            | No data    | Nicaragua |
| KF973456                 | V3                 | Dengue|          | 2012            | No data    | Nicaragua |
| KF973455                 | V3                 | Dengue|          | 2012            | No data    | Nicaragua |
| KF973454                 | V3                 | Dengue|          | 2012            | No data    | Nicaragua |
| KJ189349                 | V3                 | Dengue|          | 2011            | Yucatan    | Mexico    |
| KJ189348                 | V3                 | Dengue|          | 2011            | Yucatan    | Mexico    |
| GQ199859                 | V3                 | Dengue|          | 2008            | Managua    | Nicaragua |
| KJ189342                 | V4                 | Dengue|          | 2009            | Yucatan    | Mexico    |
| KJ189341                 | V4                 | Dengue|          | 2009            | Yucatan    | Mexico    |
| KF973474                 | V5                 | Dengue|          | 2012            | No data    | Nicaragua |
| KF973473                 | V6                 | Dengue|          | 2012            | No data    | Nicaragua |
| KJ189359                 | V7                 | Dengue|          | 2012            | No data    | Puerto Rico |
| KJ189345                 | V8                 | Dengue|          | 2009            | Yucatan    | Mexico    |
| KJ189343                 | V8                 | Dengue|          | 2009            | Yucatan    | Mexico    |
| KJ189339                 | V8                 | Dengue|          | 2008            | Yucatan    | Mexico    |
| KJ189337                 | V8                 | Dengue|          | 2008            | Yucatan    | Mexico    |
| KJ189333                 | V8                 | Dengue|          | 2008            | Yucatan    | Mexico    |
| KJ189332                 | V8                 | Dengue|          | 2008            | Yucatan    | Mexico    |
| KJ189331                 | V8                 | Dengue|          | 2008            | Yucatan    | Mexico    |
| KJ189321                 | V8                 | Dengue|          | 2007            | Yucatan    | Mexico    |
| KJ189320                 | V8                 | Dengue|          | 2007            | Yucatan    | Mexico    |
| KJ189319                 | V8                 | Dengue|          | 2007            | Yucatan    | Mexico    |
| KJ189318                 | V8                 | Dengue|          | 2007            | Yucatan    | Mexico    |
| KJ189313                 | V8                 | Dengue|          | 2008            | Yucatan    | Mexico    |
| KF955443                 | V8                 | Dengue|          | 2007            | Yucatan    | Mexico    |
| KF955442                 | V8                 | Dengue|          | 2007            | Yucatan    | Mexico    |
| KF955433                 | V8                 | Dengue|          | 2008            | Yucatan    | Mexico    |
| KF955422                 | V8                 | Dengue|          | 2007            | Yucatan    | Mexico    |
| JQ675358                 | V8                 | Dengue|          | 2010            | Florida    | USA       |
| JQ287666                 | V8                 | Dengue|          | 2009            | Managua    | Nicaragua |
| JN819403                 | V8                 | Dengue|          | 2006            | Managua    | Nicaragua |
| JN819402                 | V8                 | Dengue|          | 2005            | Managua    | Nicaragua |
| JF937644                 | V8                 | Dengue|          | 2009            | Managua    | Nicaragua |
| JF937645                 | V8                 | Dengue|          | 2009            | Managua    | Nicaragua |
| HM631855                 | V8                 | Dengue|          | 2007            | Yucatan    | Mexico    |
| GU131976                 | V8                 | Dengue|          | 2007            | Yucatan    | Mexico    |
| GU131968                 | V8                 | Dengue|          | 2007            | Yucatan    | Mexico    |
| GU131966                 | V8                 | Dengue|          | 2007            | Yucatan    | Mexico    |
| GU131964                 | V8                 | Dengue|          | 2007            | Yucatan    | Mexico    |
| GU131961                 | V8                 | Dengue|          | 2007            | Yucatan    | Mexico    |
| GU131960                 | V8                 | Dengue|          | 2007            | Yucatan    | Mexico    |
### Supplemental Table 1 - Database with GenBank accession numbers (cont.)

| GenBank accession number | Clave in the study | Virus | Genotype | Collection date | State | Country   |
|--------------------------|--------------------|-------|----------|-----------------|-------|-----------|
| GU131958                 | V8                 | Dengue| V8       | 2006            | Yucatan | Mexico    |
| GQ868539                 | V8                 | Dengue| V8       | 2008            | Yucatan | Mexico    |
| GQ868536                 | V8                 | Dengue| V8       | 2008            | Yucatan | Mexico    |
| GQ868527                 | V8                 | Dengue| V8       | 2007            | Yucatan | Mexico    |
| GQ868509                 | V8                 | Dengue| V8       | 2007            | Yucatan | Mexico    |
| GQ868503                 | V8                 | Dengue| V8       | 2007            | Yucatan | Mexico    |
| GQ868501                 | V8                 | Dengue| V8       | 2007            | Yucatan | Mexico    |
| GQ868499                 | V8                 | Dengue| V8       | 2006            | Quintana Roo | Mexico |
| GQ868498                 | V8                 | Dengue| V8       | 2006            | Yucatan | Mexico    |
| GQ199875                 | V8                 | Dengue| V8       | 2004            | Managua | Nicaragua |
| GQ199873                 | V8                 | Dengue| V8       | 2004            | Managua | Nicaragua |
| GQ199872                 | V8                 | Dengue| V8       | 2004            | Managua | Nicaragua |
| GQ199867                 | V8                 | Dengue| V8       | 2004            | Managua | Nicaragua |
| FJ898433                 | V8                 | Dengue| V8       | 2005            | Managua | Nicaragua |
| FJ873814                 | V8                 | Dengue| V8       | 2005            | Managua | Nicaragua |
| FJ850114                 | V8                 | Dengue| V8       | 2005            | Managua | Nicaragua |
| FJ850113                 | V8                 | Dengue| V8       | 2005            | Managua | Nicaragua |
| KF955408                 | V9                 | Dengue| V9       | 2007            | No data | Venezuela |
| KF955407                 | V9                 | Dengue| V9       | 2005            | No data | Venezuela |
| JN819415                 | V9                 | Dengue| V9       | 2006            | Aragua   | Venezuela |
| JN819413                 | V9                 | Dengue| V9       | 2006            | Aragua   | Venezuela |
| JN819412                 | V9                 | Dengue| V9       | 2006            | Aragua   | Venezuela |
| JN819411                 | V9                 | Dengue| V9       | 2005            | Aragua   | Venezuela |
| JN819405                 | V9                 | Dengue| V9       | 2006            | Aragua   | Venezuela |
| GU131842                 | V9                 | Dengue| V9       | 2007            | Aragua   | Venezuela |
| GQ868570                 | V9                 | Dengue| V9       | 2008            | Santander | Colombia |
| GQ868562                 | V9                 | Dengue| V9       | 2005            | Santander | Colombia |
| FJ882579                 | V9                 | Dengue| V9       | 2007            | Aragua   | Venezuela |
| FJ873809                 | V9                 | Dengue| V9       | 2007            | Aragua   | Venezuela |
| FJ850101                 | V9                 | Dengue| V9       | 2007            | Aragua   | Venezuela |
| FJ850100                 | V9                 | Dengue| V9       | 2007            | Aragua   | Venezuela |
| FJ850099                 | V9                 | Dengue| V9       | 2007            | Aragua   | Venezuela |
| FJ850093                 | V9                 | Dengue| V9       | 2008            | No data  | Brazil |
| FJ639824                 | V9                 | Dengue| V9       | 2006            | Aragua   | Venezuela |
| FJ639823                 | V9                 | Dengue| V9       | 2006            | Aragua   | Venezuela |
| FJ639820                 | V9                 | Dengue| V9       | 2006            | Aragua   | Venezuela |
| FJ639818                 | V9                 | Dengue| V9       | 2006            | Aragua   | Venezuela |
| FJ639813                 | V9                 | Dengue| V9       | 2005            | Aragua   | Venezuela |
| FJ639812                 | V9                 | Dengue| V9       | 2005            | Aragua   | Venezuela |
| FJ639802                 | V9                 | Dengue| V9       | 2005            | Aragua   | Venezuela |
| FJ639796                 | V9                 | Dengue| V9       | 2005            | Aragua   | Venezuela |
| GU056032                 | V10                | Dengue| V10      | 1998            | Aragua   | Venezuela |
| FJ898437                 | V10                | Dengue| V10      | 2004            | Managua | Nicaragua |
| **At present study**     | V11                | Dengue| V11      | 2016            | Yucatan | Mexico    |
| **At present study**     | V1                 | Zika   | V1       | 2016            | Yucatan | Mexico    |
| MF801426                 | V2                 | Zika   | V2       | 2016            | No data | Nicaragua |
| MF801424                 | V2                 | Zika   | V2       | 2016            | Yucatan | Mexico    |
| MF801423                 | V2                 | Zika   | V2       | 2016            | Guerrero | Mexico |
| MF801422                 | V2                 | Zika   | V2       | 2016            | Guerrero | Mexico |
| MF801420                 | V2                 | Zika   | V2       | 2016            | Chiapas | Mexico |
| GenBank accession number | Clave in the study | Virus | Genotype | Collection date | State     | Country  |
|-------------------------|-------------------|-------|----------|----------------|-----------|----------|
| MF801418                | V2                | Zika  | V2       | 2016           | Chiapas   | Mexico   |
| MF801417                | V2                | Zika  | V2       | 2016           | Chiapas   | Mexico   |
| MF801414                | V2                | Zika  | V2       | 2016           | Guerrero  | Mexico   |
| MF801413                | V2                | Zika  | V2       | 2016           | Guerrero  | Mexico   |
| MF801412                | V2                | Zika  | V2       | 2016           | Guerrero  | Mexico   |
| MF801411                | V2                | Zika  | V2       | 2016           | Guerrero  | Mexico   |
| MF801410                | V2                | Zika  | V2       | 2016           | Chiapas   | Mexico   |
| MF801408                | V2                | Zika  | V2       | 2016           | Chiapas   | Mexico   |
| MF801406                | V2                | Zika  | V2       | 2016           | Oaxaca    | Mexico   |
| MF801405                | V2                | Zika  | V2       | 2016           | Guerrero  | Mexico   |
| MF801403                | V2                | Zika  | V2       | 2016           | Chiapas   | Mexico   |
| MF801402                | V2                | Zika  | V2       | 2016           | Chiapas   | Mexico   |
| MF801401                | V2                | Zika  | V2       | 2016           | Chiapas   | Mexico   |
| MF801400                | V2                | Zika  | V2       | 2016           | Chiapas   | Mexico   |
| MF801399                | V2                | Zika  | V2       | 2016           | Chiapas   | Mexico   |
| MF801398                | V2                | Zika  | V2       | 2016           | Chiapas   | Mexico   |
| MF801396                | V2                | Zika  | V2       | 2016           | Chiapas   | Mexico   |
| MF801395                | V2                | Zika  | V2       | 2016           | Chiapas   | Mexico   |
| MF801391                | V2                | Zika  | V2       | 2016           | Oaxaca    | Mexico   |
| MF801389                | V2                | Zika  | V2       | 2016           | Roatan    | Honduras |
| MF801387                | V2                | Zika  | V2       | 2016           | Roatan    | Honduras |
| MF801386                | V2                | Zika  | V2       | 2016           | Roatan    | Honduras |
| MF801385                | V2                | Zika  | V2       | 2016           | Roatan    | Honduras |
| MF801384                | V2                | Zika  | V2       | 2016           | Roatan    | Honduras |
| MF801383                | V2                | Zika  | V2       | 2016           | No data   | Honduras |
| MF801377                | V3                | Zika  | V3       | 2016           | No data   | El Salvador |
| KX906952                | V2                | Zika  | V2       | 2016           | No data   | Honduras |
| MF593625                | V2                | Zika  | Asian    | 2016           | No data   | China    |
| MF434522                | V2                | Zika  | Asian    | 2016           | Managua   | Nicaragua |
| MF434521                | V2                | Zika  | Asian    | 2016           | Managua   | Nicaragua |
| MF434517                | V2                | Zika  | Asian    | 2016           | Managua   | Nicaragua |
| MF434516                | V2                | Zika  | Asian    | 2016           | Managua   | Nicaragua |
| MF159531                | V2                | Zika  | V2       | 2017           | Miami     | USA      |
| MF098771                | V2                | Zika  | V2       | 2017           | No data   | Russia   |
| MF098770                | V2                | Zika  | V2       | 2016           | No data   | Russia   |
| KY927808                | V2                | Zika  | V2       | 2016           | Henan     | China    |
| KY765327                | V2                | Zika  | V2       | 2016           | Managua   | Nicaragua |
| KY765326                | V2                | Zika  | V2       | 2016           | Managua   | Nicaragua |
| KY765325                | V2                | Zika  | V2       | 2016           | Managua   | Nicaragua |
| KY765324                | V2                | Zika  | V2       | 2016           | Managua   | Nicaragua |
| KY765323                | V2                | Zika  | V2       | 2016           | Managua   | Nicaragua |
| KY765320                | V2                | Zika  | V2       | 2016           | Managua   | Nicaragua |
| KY785461                | V2                | Zika  | V2       | 2016           | Francisco Morazan | Honduras |
| KY785457                | V2                | Zika  | V2       | 2016           | Francisco Morazan | Honduras |
| KY785452                | V2                | Zika  | V2       | 2016           | Francisco Morazan | Honduras |
| KY785442                | V2                | Zika  | V2       | 2016           | Francisco Morazan | Honduras |
| KY785431                | V2                | Zika  | V2       | 2016           | Francisco Morazan | Honduras |
| KY785418                | V2                | Zika  | V2       | 2016           | Francisco Morazan | Honduras |
| KY785414                | V2                | Zika  | V2       | 2016           | Francisco Morazan | Honduras |
| KY693677                | V2                | Zika  | V2       | 2016           | No data   | Honduras |
**Supplemental Table 1 - Database with GenBank accession numbers (cont.)**

| GenBank accession number | Clave in the study | Virus | Genotype | Collection date | State       | Country      |
|--------------------------|--------------------|-------|----------|-----------------|-------------|--------------|
| KY693676                 | V2                 | Zika  |          | 2016            | No data     | Honduras     |
| KY631494                 | V2                 | Zika  |          | 2015            | Tapachula,  | Mexico       |
| KY631493                 | V2                 | Zika  |          | 2015            | Chiapas     | Mexico       |
| KY648934                 | V2                 | Zika  |          | 2016            | Chiapas     | Mexico       |
| KY014327                 | V2                 | Zika  |          | 2016            | Francisco Morazan | Honduras |
| KY014319                 | V2                 | Zika  |          | 2016            | Francisco Morazan | Honduras |
| KY014315                 | V2                 | Zika  |          | 2016            | Francisco Morazan | Honduras |
| KY014312                 | V2                 | Zika  |          | 2016            | Francisco Morazan | Honduras |
| KY014310                 | V2                 | Zika  |          | 2016            | Francisco Morazan | Honduras |
| KY014306                 | V2                 | Zika  |          | 2016            | Francisco Morazan | Honduras |
| KY606274                 | V2                 | Zika  |          | 2016            | Guerrero     | Mexico       |
| KY606273                 | V2                 | Zika  |          | 2016            | Guerrero     | Mexico       |
| KY606272                 | V2                 | Zika  |          | 2016            | Oaxaca       | Mexico       |
| KY606271                 | V2                 | Zika  |          | 2016            | Chiapas      | Mexico       |
| KX421195                 | V2                 | Zika  |          | 2016            | No data      | Nicaragua    |
| KX421194                 | V2                 | Zika  |          | 2016            | No data      | Nicaragua    |
| KY325479                 | V2                 | Zika  |          | 2016            | Florida      | USA          |
| KY325465                 | V2                 | Zika  |          | 2016            | Florida      | USA          |
| KY328289                 | V2                 | Zika  |          | 2016            | No data      | Honduras     |
| KX694534                 | V2                 | Zika  |          | 2016            | No data      | Honduras     |
| KX856011                 | V2                 | Zika  | Asian    | 2016            | Chiapas      | Mexico       |
| KX262887                 | V2                 | Zika  |          | 2016            | No data      | Honduras     |
| KU870645                 | V2                 | Zika  |          | 2016            | No data      | USA          |
| KU501217                 | V2                 | Zika  |          | 2015            | No data      | Guatemala    |
| KU501216                 | V2                 | Zika  |          | 2015            | No data      | Guatemala    |
| MF099651                 | V3                 | Zika  |          | 2016            | Guizhou      | China        |
| MF801421                 | V3                 | Zika  |          | 2016            | Chiapas      | Mexico       |
| MF801419                 | V4                 | Zika  |          | 2016            | Chiapas      | Mexico       |
| MF801397                 | V5                 | Zika  |          | 2016            | Campeche     | Mexico       |
| MF801381                 | V6                 | Zika  |          | 2016            | No data      | Honduras     |
| MF794971                 | V3                 | Zika  |          | 2016            | No data      | Ecuador      |
| MF692778                 | V3                 | Zika  |          | 2016            | No data      | Taiwan       |
| MF574588                 | V3                 | Zika  |          | 2016            | Barranquilla | Colombia     |
| MF574587                 | V3                 | Zika  |          | 2016            | Barranquilla | Colombia     |
| MF574586                 | V3                 | Zika  |          | 2016            | Barranquilla | Colombia     |
| MF574585                 | V3                 | Zika  | Asian    | 2016            | Barranquilla | Colombia     |
| MF574584                 | V3                 | Zika  |          | 2016            | Barranquilla | Colombia     |
| MF574583                 | V3                 | Zika  | Asian    | 2016            | Barranquilla | Colombia     |
| MF574582                 | V3                 | Zika  |          | 2016            | Barranquilla | Colombia     |
| MF574581                 | V3                 | Zika  |          | 2016            | Barranquilla | Colombia     |
| MF574580                 | V3                 | Zika  |          | 2016            | Barranquilla | Colombia     |
| MF574577                 | V3                 | Zika  |          | 2016            | Barranquilla | Colombia     |
| MF574576                 | V3                 | Zika  |          | 2016            | Barranquilla | Colombia     |
| MF574575                 | V3                 | Zika  | Asian    | 2015            | Barranquilla | Colombia     |
| MF574574                 | V3                 | Zika  |          | 2015            | Barranquilla | Colombia     |
**Supplemental Table 2** - Genetic distance (Kimura 2 parameter model) among the different variants of DENV1 (below the diagonal) and the standard error among variants (above the diagonal)

|       | V1   | V2   | V3   | V4   | V5   | V6   | V7   | V8   | V9   | V10  | V11  |
|-------|------|------|------|------|------|------|------|------|------|------|------|
| V1    | 0.008| 0.012| 0.01 | 0.013| 0.013| 0.008| 0.011| 0.011| 0.011| 0.011| 0.011|
| V2    | 0.015| 0.011| 0.011| 0.012| 0.012| 0.012| 0.012| 0.012| 0.012| 0.012| 0.012|
| V3    | 0.03 | 0.025| 0.007| 0.005| 0.005| 0.008| 0.007| 0.005| 0.005| 0.005| 0.013|
| V4    | 0.02 | 0.025| 0.01 | 0.008| 0.008| 0.008| 0.005| 0.008| 0.008| 0.005| 0.013|
| V5    | 0.035| 0.03 | 0.005| 0.015| 0.007| 0.01 | 0.008| 0.007| 0.007| 0.007| 0.014|
| V6    | 0.035| 0.03 | 0.005| 0.015| 0.01  | 0.01 | 0.008| 0.007| 0.007| 0.007| 0.014|
| V7    | 0.015| 0.03 | 0.015| 0.015| 0.02  | 0.02 | 0.008| 0.007| 0.007| 0.007| 0.014|
| V8    | 0.025| 0.03 | 0.01 | 0.005| 0.015| 0.015| 0.015| 0.008| 0.005| 0.005| 0.014|
| V9    | 0.025| 0.03 | 0.005| 0.015| 0.01  | 0.01 | 0.01 | 0.015| 0.007| 0.007| 0.014|
| V10   | 0.025| 0.03 | 0.005| 0.005| 0.01  | 0.01 | 0.01 | 0.005| 0.01  | 0.01 | 0.014|
| V11   | 0.025| 0.03 | 0.035| 0.035| 0.04  | 0.04 | 0.04 | 0.04 | 0.04  | 0.04 | 0.04 |

V1: DENV-1, Brazil (2015); V2: DENV-1, Merida, Mexico (2016); V11-DENV-1, Mexico (2016 - at present study)

**Supplemental Table 3** - Genetic distance (Kimura 2 parameter model) among the different variants of zika virus (below the diagonal) and the standard error among variants (above the diagonal)

|       | V1   | V2   | V3   | V4   | V5   | V6   |
|-------|------|------|------|------|------|------|
| V1    | 0.01 | 0.012| 0.012| 0.012| 0.011|
| V2    | 0.018| 0.006| 0.006| 0.006| 0.006|
| V3    | 0.024| 0.006| 0.008| 0.008| 0.008|
| V4    | 0.024| 0.006| 0.012| 0.012| 0.008|
| V5    | 0.024| 0.006| 0.012| 0.012| 0.008|
| V6    | 0.024| 0.006| 0.012| 0.012| 0.008|

V1: V1ZIKV, Mexico (2016 - at present study); V2: ZIKV, Guatemala (2015), Mexico (2015-2016), China (2016), Honduras (2016), Nicaragua (2016), Russia (2016-2017) and USA (2016-2017)