ORIGINAL ARTICLE

Study of *Aedes aegypti* population with emphasis on the gonotrophic cycle length and identification of arboviruses: implications for vector management in cemeteries

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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[1]. 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[2,3]. Immature stages of *Aedes aegypti* are common in cemeteries, where larvae and pupae are often found inside vases[1,3]. However, few studies have quantified the adult populations and their role in the arboviruses transmission[2]. To the best of our knowledge, there are only two reports of arboviruses identified in mosquitoes collected in cemeteries[4,5]. Therefore, it is important to know the epidemiological importance of cemeteries in areas where dengue, zika and chikungunya viruses are present.
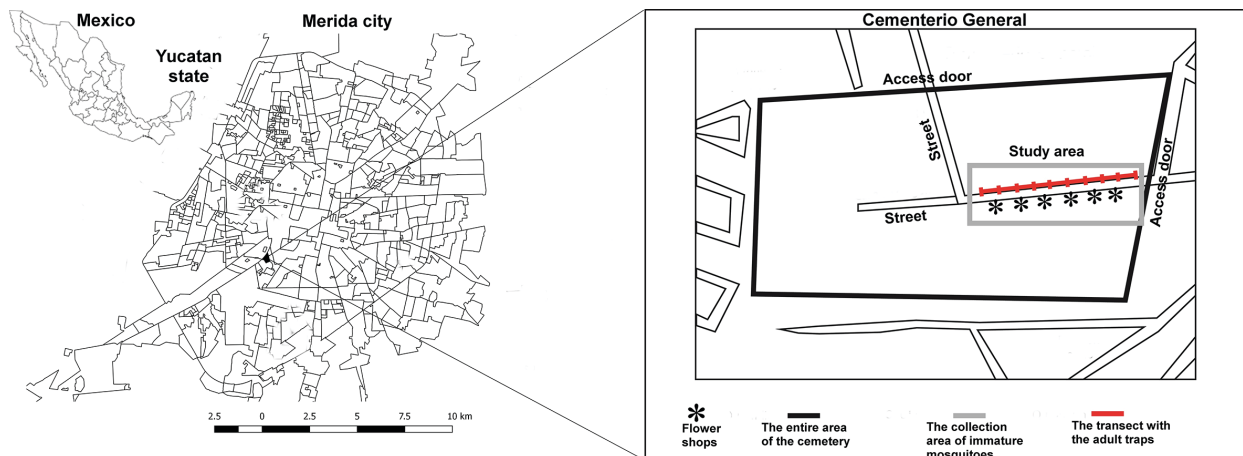
In home environments, survivorship and gonotrophic cycle of *Ae. aegypti* are well-documented[6]. A short time of the gonotrophic cycle of *Ae. aegypti* increases the contact vector-human and thus the risk for arbovirus transmission[7]. Previous studies carried out in houses estimated between 3 to 7 days the gonotrophic cycle of *Ae. aegypti*; the region, season, and temperature affected significantly the cycle[6,8,9]. It has also been observed that mosquitoes can disperse beyond the houses. Previous studies reported engorged *Ae. aegypti* in schools and churches[10,11]. 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[1,12,13]. Cemeteries have also been used for the early detection and monitoring of invasive mosquitoes such as *Aedes albopictus* (Skuse)[14]. There is a growing recognition that cemeteries can also be effective sites for monitoring virus transmitted by mosquitoes[4,5]. In Yucatan State of Southeastern Mexico, dengue, chikungunya and zika viruses co-occur[15,16]. 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 of 27.5 °C. During the dry season, the mean rainfall is 300 mm and the mean temperature is 25.1 °C[10].

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,

Figure 1 - Study area in the cemetery "Cementerio General" of Merida city, Yucatan, Mexico

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[17,18].

Gonotrophic cycle and survival dynamics

Female *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 (freshly 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)[10].

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[19]. *Aedes aegypti* females dissected were stored at -80 °C and assayed for flavivirus RNA.

RNA extraction and RT-PCR

Pools of female adult *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[20]. RT-PCRs were performed in 25 μL reaction volumes containing 2.5 μL of total RNA, 2 μL MgCL2 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®), 0.5 μL of each primer at a concentration of 10 mM. and 16.65 μl ddH2O was added to reach the final volume. Amplification conditions are as follows: an initial denaturation of 95 °C for 1 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™ 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 *Ae. aegypti* presence (larvae, pupae, or both); and 2) a pupal index representing the percentage of containers with *Ae. aegypti* pupae present out of all containers with *Ae. aegypti* immatures presence.

To compare the number of immature and adult of *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 *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) x 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[7] with the formula $M_t = P_u T_{(t-u)}$, where M = the number of parous individuals captured on day t; $T_{(t-u)}$ = the total number of females (nulliparous and parous) captured on day $t-u$; 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 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 1 was obtained by pairing daily P_t data with the corresponding T_t data of 1 day before. The r for day 2 was calculated by pairing daily Pt data with corresponding Tt 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 $Z_t = X - \beta(X_{t-1})$, where $Zt =$ is the filtered time series, $X_t =$ the time series to be filtered, and $\beta =$ 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 $PR =$ the parity rate and $CG =$ 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[24]. The DnaSP v.5.10 software[25] 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% (182/793) 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* (Coquillet) (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

Table 2 - Species composition and abundance of adult mosquitoes collected in a cemetery of Merida Yucatan, from April 2016 to June 2017

Species	Total number adults collected		Blood feeding status		
	Males	Females	Unfed	Fed	Gravid
Rainy season					
Ae. aegypti	1,969	1,471	1,210	121	140
Ae. taeniorhynchus		77	57	4	16
Ae. trivittatus	1	17	10	4	3
Cx. quinquefasciatus	1	3	1	1	1
Subtotal	1,971	1,568	1278	130	160
Dry season					
Ae. aegypti	220	177	117	14	46
Cx. quinquefasciatus	7	14	3	5	6
Subtotal	227	191	120	19	52
Total	2,198	1,759	1,398	149	212

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, $P \geq 0.05$).

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

Table 3 - Correlation indices of the parity rates of *Ae. aegypti* captured in a cemetery from Merida, Yucatan, during the rainy and dry seasons in 2016, by cross-correlation analysis of a time series

Day	Filtered dates of the dry season	Crudes dates of the rainy season
0	0.69	0,02
1	0.03	0,60
2	*0.02	0,56
3	0.00	0,01
4	0.02	0,00
5	*0.38	**0,73
6	0.31	0,11
7	0.14	0,43
8	*0.54	0,22
9	0.20	**0,73
10	0.21	0,55
11	–	0,29
12	–	0,14
13	–	**0,64
14	–	0,07
15	–	0,69

*High correlation coefficient value appearing every three days in dry season. **High correlation coefficient value appearing every four days in rainy season

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

Table 4 - Parity rate of female *Ae. aegypti* captured in cemetery from Merida, Yucatan, during the rainy and dry seasons in 2016

Day	Dry season					Rainy season			
	Dissected	Nulliparous	Parous	Parity rate		Dissected	Nulliparous	Parous	Parity rate
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		36	32	4	0,11
15	4	3	1	0,25		32	13	19	0,59
16	4	2	2	0,50		51	23	28	0,55
17	4	2	2	0,50		66	18	48	0,73
18	3	1	2	0,67		68	25	43	0,63
19	3	1	2	0,67		40	16	24	0,60
Total	86	36	50	0,58		541	209	332	0,61

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[28]. 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[29]. In contrast with this result, in cemeteries from Philippines and Venezuela,

most vases had water and yielded immature mosquitoes during the rainy season[2,28]. 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 *Ae. aegypti*[29-31].

Immature *Ae. aegypti* was found to be the dominant species in the vases. Ninety-two percent of the vases containing larvae and pupae had only *Ae. aegypti*. Nevertheless, *Cx. quinquefasciatus*, *Cx. coronator* and *Cx. nigripalpus* were also found. In cemeteries from Philippines and Argentina, *Ae. aegypti* was found co-inhabiting with *Ae. albopictus* and *Cx. pipiens,* respectively[2,3]. The adaptive features of *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[32]. 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 *Ae. aegypti* populations[12]. Meanwhile, *Toxorhynchites splendens* (Wiedemann) was effective in controlling the larvae of *Ae. albopictus* in Malaysia[13].

In the present study, 80% (1,327/ 1,648) of the *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 *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 *Ae. aegypti* in indoor environments. This may be the result of a closer relationship with human[15]. It should be noted that *Ae. aegypti* display a strong anthropophilia. In houses and schools of Merida city, 57% of the *Ae. aegypti* females were collected as fed, 29% as unfed and 14% as gravid females[11,15]. In churches, 47% of the *Ae. aegypti* females were collected as fed, 34% as unfed and 19% as gravid females[10].

Previous studies on the gonotrophic cycle of *Ae. aegypti* was estimated with human bait and mark-release-recapture experiment[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 *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 *Ae. aegypti*[8]. Our results agree with previous findings in studies conducted in Thailand[33], East Africa[34] and Peru[9]. Additionally, in Thailand, Pant and Yasuno[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[35], Tanzania[36] and Kenya[37]. In Merida city, two studies have estimated the gonotrophic cycle of *Ae. aegypti*. In houses, Rebollar-Tellez *et al.*[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[10].

High values of survival rate increase the potential risk for transmission of pathogens day to day[20]. Under laboratory conditions, the highest survival rate for *Ae. aegypti* females was 84% at 27 °C, reaching 25 days of age[8]. In Mexico, the survivorship for *Ae. aegypti* was estimated by Rebollar-Tellez *et al.*[6] as 0.86. In the cemetery of Merida city, we found a high survival rate (0.83) for *Ae. aegypti*. Previous studies conducted in cemeteries identified arbovirus-infected mosquitoes. For example, La Crosse encephalitis virus-infected *Aedes triseriatus* were collected in cemeteries in Tennessee, USA[4]. In the State of San Luis Potosi, Mexico, ZIKV-infected *Ae. aegypti* were detected in cemeteries[5]. In the present study, DENV-1 RNA and ZIKV RNA were identified in *Ae. aegypti*. It is also the first report of *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[11]. However, our results are similar to the ones from earlier studies performed inside the houses of dengue patients[15,38]. On the other hand, the first report of ZIKV-infected *Ae. aegypti* was from Chiapas, Mexico and the MIR was estimated at 52.49-172.66[39].

We also found evidence of vertical transmission of DENV-1 in nulliparous *Ae. aegypti* females during the gonotrophic cycle. In Mexico, vertical transmission of dengue virus by *Ae. aegypti* and *Ae. albopictus* was reviewed by Ferreira-de-Lima and Lima-Camara[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[40], therefore it should be studied in depth in the cemeteries.

CONFLICT OF INTERESTS

None.

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		2006	Yucatan	Mexico
GQ868539	V8	Dengue		2008	Yucatan	Mexico
GQ868536	V8	Dengue		2008	Yucatan	Mexico
GQ868527	V8	Dengue		2007	Yucatan	Mexico
GQ868509	V8	Dengue		2007	Yucatan	Mexico
GQ868503	V8	Dengue		2007	Yucatan	Mexico
GQ868501	V8	Dengue		2007	Yucatan	Mexico
GQ868499	V8	Dengue		2006	Quintana Roo	Mexico
GQ868498	V8	Dengue		2006	Yucatan	Mexico
GQ199875.	V8	Dengue		2004	Managua	Nicaragua
GQ199873	V8	Dengue		2004	Managua	Nicaragua
GQ199872	V8	Dengue		2004	Managua	Nicaragua
GQ199867	V8	Dengue		2004	Managua	Nicaragua
FJ898433	V8	Dengue		2007	Managua	Nicaragua
FJ873814	V8	Dengue		2005	Managua	Nicaragua
FJ850114	V8	Dengue		2005	Managua	Nicaragua
FJ850113	V8	Dengue		2005	Managua	Nicaragua
KF955408	V9	Dengue		2007	No data	Venezuela
KF955407	V9	Dengue		2005	No data	Venezuela
JN819415	V9	Dengue		2006	Aragua	Venezuela
JN819413	V9	Dengue		2006	Aragua	Venezuela
JN819412	V9	Dengue		2006	Aragua	Venezuela
JN819411	V9	Dengue		2005	Aragua	Venezuela
JN819405	V9	Dengue		2006	Merida	Venezuela
GU131842	V9	Dengue		2007	Aragua	Venezuela
GQ868570	V9	Dengue		2008	Santander	Colombia
GQ868562	V9	Dengue		2005	Santander	Colombia
FJ882579	V9	Dengue		2007	Aragua	Venezuela
FJ873809	V9	Dengue		2007	Aragua	Venezuela
FJ850101	V9	Dengue		2007	Aragua	Venezuela
FJ850100	V9	Dengue		2007	Aragua	Venezuela
FJ850099	V9	Dengue		2007	Aragua	Venezuela
FJ850093	V9	Dengue		2008	No data	Brazil
FJ639824	V9	Dengue		2006	Aragua	Venezuela
FJ639823	V9	Dengue		2006	Aragua	Venezuela
FJ639820	V9	Dengue		2006	Aragua	Venezuela
FJ639818	V9	Dengue		2006	Aragua	Venezuela
FJ639813	V9	Dengue		2005	Aragua	Venezuela
FJ639812	V9	Dengue		2005	Aragua	Venezuela
FJ639802	V9	Dengue		2005	Aragua	Venezuela
FJ639796	V9	Dengue		2005	Aragua	Venezuela
GU056032	V10	Dengue		1998	Aragua	Venezuela
FJ898437	V10	Dengue		2004	Managua	Nicaragua
At present study	V11	Dengue		2016	Yucatan	Mexico
At present study	V1	Zika	Asian	2016	Yucatan	Mexico
MF801426	V2	Zika		2016	No data	Nicaragua
MF801424	V2	Zika		2016	Yucatan	Mexico
MF801423	V2	Zika		2016	Guerrero	Mexico
MF801422	V2	Zika		2016	Guerrero	Mexico
MF801420	V2	Zika		2016	Chiapas	Mexico

Supplemental Table 1 - Database with GenBank accession numbers (cont.)

GenBank accession number	Clave in the study	Virus	Genotype	Collection date	State	Country
MF801418	V2	Zika		2016	Chiapas	Mexico
MF801417	V2	Zika		2016	Chiapas	Mexico
MF801414	V2	Zika		2016	Guerrero	Mexico
MF801413	V2	Zika		2016	Guerrero	Mexico
MF801412	V2	Zika		2016	Guerrero	Mexico
MF801411	V2	Zika		2016	Guerrero	Mexico
MF801410	V2	Zika		2016	Guerrero	Mexico
MF801408	V2	Zika		2016	Chiapas	Mexico
MF801406	V2	Zika		2016	Oaxaca	Mexico
MF801405	V2	Zika		2016	Guerrero	Mexico
MF801403	V2	Zika		2016	Chiapas	Mexico
MF801402	V2	Zika		2016	Chiapas	Mexico
MF801401	V2	Zika		2016	Chiapas	Mexico
MF801400	V2	Zika		2016	Chiapas	Mexico
MF801399	V2	Zika		2016	Chiapas	Mexico
MF801398	V2	Zika		2016	Chiapas	Mexico
MF801396	V2	Zika		2016	Chiapas	Mexico
MF801395	V2	Zika		2016	Chiapas	Mexico
MF801391	V2	Zika		2016	Oaxaca	Mexico
MF801389	V2	Zika		2016	Roatan	Honduras
MF801387	V2	Zika		2016	Roatan	Honduras
MF801386	V2	Zika		2016	Roatan	Honduras
MF801385	V2	Zika		2016	Roatan	Honduras
MF801384	V2	Zika		2016	Roatan	Honduras
MF801383	V2	Zika		2016	No data	Honduras
MF801377	V3	Zika		2016	No data	El Salvador
KX906952	V2	Zika		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		2017	Miami	USA
MF098771	V2	Zika		2017	No data	Russia
MF098770	V2	Zika		2016	No data	Russia
KY927808	V2	Zika		2016	Henan	China
KY765327	V2	Zika		2016	Managua	Nicaragua
KY765326	V2	Zika		2016	Managua	Nicaragua
KY765325	V2	Zika		2016	Managua	Nicaragua
KY765324	V2	Zika		2016	Managua	Nicaragua
KY765323	V2	Zika		2016	Managua	Nicaragua
KY765320	V2	Zika		2016	Managua	Nicaragua
KY785461	V2	Zika		2016	Francisco Morazan	Honduras
KY785457	V2	Zika		2016	Florida	USA
KY785452	V2	Zika		2016	Francisco Morazan	Honduras
KY785442	V2	Zika		2016	Francisco Morazan	Honduras
KY785431	V2	Zika		2016	Francisco Morazan	Honduras
KY785418	V2	Zika		2016	Francisco Morazan	Honduras
KY785414	V2	Zika		2016	Francisco Morazan	Honduras
KY693677	V2	Zika		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, Chiapas	Mexico
KY631493	V2	Zika		2015	Tapachula, 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
V2	0.015		0.011	0.011	0.012	0.012	0.012	0.012	0.012	0.012	0.007
V3	0.03	0.025		0.007	0.005	0.005	0.008	0.007	0.005	0.005	0.013
V4	0.02	0.025	0.01		0.008	0.008	0.008	0.005	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	0.014
V6	0.035	0.03	0.005	0.015	0.01		0.01	0.008	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	0.014
V8	0.025	0.03	0.01	0.005	0.015	0.015	0.015		0.008	0.005	0.014
V9	0.025	0.03	0.005	0.015	0.01	0.01	0.01	0.015		0.007	0.014
V10	0.025	0.03	0.005	0.005	0.01	0.01	0.01	0.005	0.01		0.014
V11	0.025	0.01	0.035	0.035	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		0.008	0.008
V5	0.024	0.006	0.012	0.012		0.008
V6	0.024	0.006	0.012	0.012	0.012	

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)