Dengue fever is considered as one of the most important vector-borne viral disease in the world. This arboviral disease is caused by an RNA virus of the family Flaviviridae; genus Flavivirus. Other arboviruses such as chikungunya (CHIKV, Togaviridae, Alphavirus) and Zika (ZIKV, Flaviviridae, Flavivirus) viruses expanded their distribution from their origin in Africa to America, increasing the burden of mosquito-borne diseases in the region. The co-circulation of these viruses is leading cause of morbidity among susceptible populations in Mexico. These viruses are transmitted to humans through the bites of infected female Aedes (Stegomyia) aegypti species. Commonly, A. aegypti acquires the viruses while feeding on the blood of an infected human. One recent estimate indicates occurrence of 390 million of dengue infections per year, of which nearly 100 million manifest clinically, with any level of disease severity. Furthermore, the public health impact of chikungunya and Zika has increased dramatically over the last years. Outbreaks of CHIKV are characterized by rapid spread, which are symptomatic in 72–93% of infected persons. While, the Zika virus causes birth defects in babies born to some infected pregnant women. Aedes aegypti display a strong anthropophilia, females feeding almost exclusively on human hosts. Therefore, it is important to study the blood meal digestion process of Ae. aegypti in longitudinal studies as it may provide insight into seasonal patterns on feeding behaviour which might influence the dynamics of arboviruses transmission.

There are limited studies documenting the sources of blood meal in Ae. aegypti in public sites, like catholic churches. These are buildings used for religious activities and are important places for the Latin American culture. In Mexico, there are approximately 7,739 catholic churches, where every day hundreds of people congregate. In Yucatan State, there are 143 catholic churches, with 78 in the Merida city. At present, there is limited knowledge of the vectorial capacity of Ae. aegypti in public sites. The purpose of this study was to determine the blood meal digestion status (seasonally) and the source of blood meal of Ae. aegypti caught in catholic churches from Merida city, Yucatan.

The study was carried out in Merida City (population 892,363; census 2015) in the Yucatan Peninsula of southeastern Mexico. Merida has a distinct rainy (May–October) and a dry season (January–April). During the rainy season, the mean rainfall is 1000 mm and mean temperature is 27.54 °C. However, during the dry season, the mean rainfall is 300 mm and mean temperature is 25.14 °C.

The catholic churches are usual places for the congregation of people for religious cult (including men, women, and children). Three areas with high transmission of dengue virus were selected for the study: the neighborhoods of San Jose Tecoh (20°55'8.76"N, 89°37'29.86"W), Bojorquez (20°58'32.89"N, 89°39'0.44"W) and Vergel III (20°57'15.38"N, 89°34'43.8"W). The churches (n = 3) are similar in size (approximately 10,000 m²), close to markets and are located between 8 and 10 km from each other within the Merida City.

Females of Ae. aegypti were caught between 0800–1300 hrs from September 2015 to December 2016, using a backpack aspirator (Prokopack Aspirator®, model 1419, John W. Hock company). Each church was inspected for resting adults once every week, and the mosquitoes were
captured by direct aspiration while resting on the furniture, hanging clothes, curtains, and dark and humid places. The central area of the church (nave) was designated as indoor. Outdoor collections focused primarily on garden areas and fences. The length of time spent in active collection of adult mosquitoes per church ranged from 1 to 2 h.

Mosquitoes caught were transported alive to the laboratory of Arbovirologia at Universidad Autonoma de Yucatan, Yucatan and were identified to species level using stereo microscopes and published identification keys.

The blood meal digestion status (Sella’s stages) was determined by external examination of the abdomen. Sella’s stages include seven scales: I (unfed; with collapsed abdomen and ovaries occupying one third of the abdomen), II (freshly fed; with bright red blood and ovaries occupying two to three segments ventrally and four dorsally), III–IV (half-gravid; with dark red blood and ovaries occupying four to five segments ventrally and six dorsally), V (sub-gravid; with blood greatly reduced and dark in color and ovaries occupying most of abdomen), and VI–VII ( gravid; with blood completely digested or present only as a black trace or line).

For blood meal identification, abdomens were dissected and removed from freshly fed female mosquitoes and individually placed into 1.5 ml centrifuge (Eppendorf) tubes. Abdomens were manually homogenized in 300 μl fetal bovine serum (2%). DNA was isolated using a DNeasy Blood and Tissue Kit (Qiagen, Valencia, CA) by following the manufacturer’s protocols. After centrifugation, the supernatant was removed and stored at –80°C until analysis.

A portion of the mitochondrial cytochrome b gene (228 base pairs) was amplified by PCR using human-specific primers [5’TTCGCCGCGTATGAGCTGGAG TCC-3’ (forward) and 5’TATGCGGGAAAACGCCATATCG-3’ (reverse)]11. Briefly, a 25 μl PCR volume was prepared containing 2.5 μl of extracted template DNA, 2.5 μl buffer 5x, 2 μl MgCl₂ (25 mM), 0.2 μl of dNTP’s, 0.15 μl Taq polymerase, and 1 μl primer (10 mM).

Thermal cycling conditions consisted of incubation at 94°C for 2 min, 35 cycles at 94°C for 30 sec, 70°C for 30 sec, and 72°C for 30 sec, followed by a final elongation at 72°C for 10 min. Negative (water) and positive controls (human blood) were included in each PCR. Ten μl samples of PCR products were analyzed using a 2% agarose gel ethidium bromide staining and visualized on Doc™ XR+ Gel Documentation System.

Samples that did not amplify with the human specific primers were then tested by a PCR targeted to cytochrome b gene of avian (508 base pairs) and mammalian sequences (772 base pairs)13. PCR products were purified by using the Zymoclean Gel DNA recovery kit Cat (D4008), and sequenced by using a 3500xL genetic analyzer (Applied Biosystems, Foster City, CA, USA). Sequences were entered into the Basic Local Alignment Search Tool available at the National Center for Biotechnology Information database (http://blast.ncbi.nlm.nih.gov/Blast.cgi) to identify the blood meal source as described by Barrera et al.14.

A Mann-Whitney U-test was performed to compare the number of female Ae. aegypti by season, as the data did not meet the assumptions of normality and homogeneity of variances. A correspondence analysis was used to address the combined effects of the room types on blood meal digestion of female Ae. aegypti. Statistical analysis was performed using the IBM SPSS Statistics version 22 software for Windows (IBM Corporation, Armonk, NY), and results were considered significant when \( p \leq 0.05 \).

In total 1380 female Ae. aegypti were caught. A significant statistical difference was observed in the number of females caught per season (\( p \leq 0.05 \)). In the rainy season, 1178 females were caught, which were majorly unfed (n = 437), followed by freshly fed (n = 258) and gravid females (n = 205). In the dry season, 202 females were caught, which primarily included unfed populations (n = 89); remaining counts were almost similar for freshly fed (n = 34), gravid (n = 34), and half gravid females (n = 31, Table 1).

In both season, maximum numbers of female Ae. aegypti were caught from indoors (n = 930). In the rainy season, unfed (n = 304) and freshly fed (n = 178) females were more abundant in indoor (nave), as compared to other room types (storage room, offices, bedroom, bathroom, classroom and kitchen.). Dry season collection commonly included unfed (n = 39), but with similar number of freshly fed (n = 15), half gravid (n = 18) and gravid (n = 178) females.

Correspondence analysis showed the importance of room types on blood meal digestion status (\( p \leq 0.05 \)). A higher percent of unfed females was observed in kitchen (92.31%) and outdoor (71.88%). The majority of Ae. aegypti females collected in the indoor were unfed females (36.88%), followed by freshly fed (20.75%) and gravid (16.77%, Table 1).

A total of 78.90% (228/292) freshly fed Ae. aegypti females were processed, and the remaining were used for detection of arbovirus (data not shown). Out of 228 Ae. aegypti examined, 222 samples produced amplification products from the human-specific primers (Fig. 1). Six of them failed to identify human blood in the first test. Those samples that did not amplify were then used as a template in a universal avian and mammalian-specific primers. Six
PCR products were sequenced and BLAST analysis was performed to compare them with other sequences in the GenBank database. One sequence was closely related to *Homo sapiens* with 97% nucleotide identity. The sequences of the remaining five samples did not match to other sequences in the GenBank database; probably DNA was damaged or purification was not good enough.

The results indicate that churches are highly suitable settings/environments for presence of *Ae. aegypti* in Merida City. These sites have several characteristics that make them potentially important sources for *Ae. aegypti*, such as, location near residential premises, presence of big gardens, and several rooms that might serve as adult harborage sites. It is worth noting that a large influx of visitors occurs in the churches, who act as source of blood meal for mosquitoes and also influences the transmission dynamics of arboviruses. It is suggested that these nonresidential urban environments, should be considered for inclusion in mosquito surveillance and control efforts.

In the present study, the room type impacted in the mosquito collection. Females *Ae. aegypti* were commonly collected from indoors of churches, i.e. nave and office areas, which are precisely the room type where people meet and spend the majority of their time. These results agree with earlier findings from the houses and schools. In Merida city, infestation of female *Ae. aegypti* has been reported highest for bedrooms and living/dining room of homes. Meanwhile, in the schools from Colombia and Mexico, *Ae. aegypti* were collected predominantly from classrooms and offices.

In this study, females *Ae. aegypti* were found at least five times higher in number in rainy season compared to dry season. Peak numbers of females *Ae. aegypti* during the rainy season have been reported previously from Yucatan State. Notably, high abundance of *Ae. aegypti* is associated with human dengue and chikungunya cases. In this season, there is increase in abundance of water-filled containers in Merida City. In contrast, a high percent of sub-gravid and gravid females is indicator of older mosquito populations.

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Recently, it was estimated that females of *Ae. aegypti* caught from churches at Merida City completed their oogenic development (reached to gravid status), in

<table>
<thead>
<tr>
<th>Room types</th>
<th>Fed</th>
<th>Freshly fed</th>
<th>Half gravid</th>
<th>Sub gravid</th>
<th>Gravid</th>
<th>Frequency of human blood identified</th>
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<tbody>
<tr>
<td>rainy season</td>
<td></td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>Indoor*</td>
<td>304 (36.36)</td>
<td>178 (21.29)</td>
<td>122 (14.59)</td>
<td>94 (11.24)</td>
<td>138 (16.51)</td>
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<tr>
<td>Storage room</td>
<td>36 (48.65)</td>
<td>13 (15.77)</td>
<td>6 (8.11)</td>
<td>3 (4.05)</td>
<td>16 (21.62)</td>
<td>8</td>
</tr>
<tr>
<td>Offices</td>
<td>17 (19.54)</td>
<td>31 (35.63)</td>
<td>14 (16.09)</td>
<td>13 (14.94)</td>
<td>12 (13.79)</td>
<td>17</td>
</tr>
<tr>
<td>Bedroom</td>
<td>15 (41.67)</td>
<td>9 (25)</td>
<td>3 (8.33)</td>
<td>6 (16.67)</td>
<td>3 (8.33)</td>
<td>8</td>
</tr>
<tr>
<td>Bathroom</td>
<td>1 (9.09)</td>
<td>8 (72.73)</td>
<td>1 (9.09)</td>
<td>0 (0)</td>
<td>1 (9.09)</td>
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<tr>
<td>Classroom</td>
<td>22 (38.60)</td>
<td>11 (19.30)</td>
<td>7 (12.28)</td>
<td>2 (3.51)</td>
<td>15 (26.32)</td>
<td>10</td>
</tr>
<tr>
<td>Outdoor**</td>
<td>42 (54.55)</td>
<td>8 (10.39)</td>
<td>4 (5.19)</td>
<td>3 (3.90)</td>
<td>20 (25.97)</td>
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<td>0 (0)</td>
<td>0 (0)</td>
<td>0 (0)</td>
<td></td>
</tr>
<tr>
<td>dry season</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Indoor*</td>
<td>39 (41.49)</td>
<td>15 (15.96)</td>
<td>18 (19.15)</td>
<td>4 (4.26)</td>
<td>18 (19.15)</td>
<td>15</td>
</tr>
<tr>
<td>Storage room</td>
<td>7 (58.33)</td>
<td>2 (16.67)</td>
<td>1 (8.33)</td>
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<td>1 (8.33)</td>
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</tr>
<tr>
<td>Offices</td>
<td>21 (31.34)</td>
<td>16 (23.88)</td>
<td>10 (14.93)</td>
<td>8 (11.94)</td>
<td>12 (17.91)</td>
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<tr>
<td>Bathroom</td>
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<td>0 (0)</td>
<td>0 (0)</td>
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<td>0</td>
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<tr>
<td>Classroom</td>
<td>4 (57.14)</td>
<td>1 (14.29)</td>
<td>1 (14.29)</td>
<td>0 (0)</td>
<td>1 (14.29)</td>
<td>1</td>
</tr>
<tr>
<td>Outdoor**</td>
<td>4 (57.14)</td>
<td>0 (0)</td>
<td>0 (0)</td>
<td>1 (14.29)</td>
<td>2 (28.57)</td>
<td>0</td>
</tr>
<tr>
<td>Kitchen</td>
<td>12 (92.31)</td>
<td>0 (0)</td>
<td>1 (7.69)</td>
<td>0 (0)</td>
<td>0 (0)</td>
<td>0</td>
</tr>
<tr>
<td>Grand total</td>
<td>526 (38.12)</td>
<td>292 (21.16)</td>
<td>188 (13.62)</td>
<td>135 (9.78)</td>
<td>239 (17.32)</td>
<td>223</td>
</tr>
</tbody>
</table>

*The inside of the church (nave) was designated as indoor; **The garden of the church was designated as outdoor.*

**Table 1. Blood meal digestion status of *Ae. aegypti* caught in churches from Merida, Yucatan, and frequency of human blood identified**

**Fig. 1:** Analysis of blood meal using human-specific primers (cytochrome b gene) on DNA extracted from the *Ae. aegypti* caught in churches of Merida City, Yucatan. Lane 1–DNA molecular weight marker; Lanes 2, 4–8, and 10–*Ae. aegypti* females fed on human blood (228 bp product); Lane 14–Negative control; and Lane 15–Positive control (human blood).
minimum four days in the rainy season and three days in the dry season.

All females of *Ae. aegypti*, tested for source of blood meal were identified with human blood. This can be due to non-availability of other hosts like dogs, cats, and chickens in the churches. It is consistent with other studies, where it has been observed to feed largely on humans and less frequently on dogs, cats and chickens. In fact, *Ae. aegypti* is adapted to live in close proximity to humans, often resting and blood feeding within human dwellings. We expanded on these findings by demonstrating that churches are highly suitable environments for *Ae. aegypti* and represents potential risk for transmission of the arboviruses among the human population that visit the churches of the Merida City. It may be noted that, *Ae. albopictus* (Skuse) is considered as a secondary vector of human arboviruses in America (i.e. dengue, chikungunya and Zika viruses), because it is thought to preferentially feed on animals rather than humans. In this regard, early studies carried out in United States reported that few *Ae. albopictus* feed on humans; however, most fed on an array of hosts including dogs, cats, cow, deer, rabbits and chickens. At present, *Ae. albopictus* is not registered in Yucatan State, though it is present in other areas of Mexico.

The limitation of this study was that a subset of mosquitoes (fresher fed) was used to determine the host preference and multiple meals were not estimated in the *Ae. aegypti* mosquitoes. However, according to other studies *Ae. aegypti* almost exclusively fed on humans (~90%)6-7. The information generated in this study can be used for studies of vectorial capacity in non-residential areas because human biting rate is an essential parameter for the transmission of viruses.

**Conflict of interest**

There is no conflict of interest among the authors in the publication of this article.

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