Dengue Virus–Infected Aedes aegypti in the Home Environment

Julian Garcia-Rejon, Maria Alba Loroño-Pino, Jose Arturo Farfan-Ale, Luis Flores-Flores, Elsy Del Pilar Rosales-Paredes, Nubia Rivero-Cardenas, Rosario Najar-Vazquez, Salvador Gomez-Carro, Victor Lira-Zumbardo, Pedro Gonzalez-Martinez, Saul Lozano-Fuentes, Darwin Elizondo-Quiroga, Barry J. Beaty, and Lars Eisen*

Laboratorio de Arbovirología, Centro de Investigaciones Regionales Dr. Hideyo Noguchi, Universidad Autónoma de Yucatán, Merida, Yucatan, Mexico; Servicios de Salud de Yucatán, Merida, Yucatan, Mexico; Department of Microbiology, Immunology and Pathology, Colorado State University, Fort Collins, Colorado

Abstract. We determined abundance of Aedes aegypti mosquitoes and presence of dengue virus (DENV) in females collected from premises of laboratory-confirmed dengue patients over a 12-month period (March 2007 to February 2008) in Merida, Mexico. Backpack aspiration from 880 premises produced 1,836 females and 1,292 males indoors (predominantly from bedrooms) and 102 females and 108 males from patios/backyards. The mean weekly indoor catch rate per home peaked at 7.8 females in late August. Outdoor abundances of larvae or pupae were not predictive of female abundance inside the home. DENV-infected Ae. aegypti females were recovered from 34 premises. Collection of DENV-infected females from homes of dengue patients up to 27 days after the onset of symptoms (median, 14 days) shows the usefulness of indoor insecticide application in homes of suspected dengue patients to prevent their homes from becoming sources for dispersal of DENV by persons visiting and being bitten by infected mosquitoes.

INTRODUCTION

Dengue virus (DENV), which is transmitted primarily by the yellow fever mosquito Aedes aegypti, is the most common cause of arboviral disease in tropical and subtropical areas of the world. More than 50 million cases of dengue fever and 500,000 cases of the more severe dengue hemorrhagic fever are estimated to occur each year. In the Americas, a resurgence of Ae. aegypti after the cessation of a large-scale eradication program in the 1970s and the introduction and co-circulation of new DENV serotypes and genotypes have resulted in dengue becoming hyperendemic in Mexico, Central America, and parts of South America and the Caribbean. The dengue situation in the Americas has become critical in recent years, with both rising case numbers and increasing ratios of severe dengue hemorrhagic fever (DHF) to milder dengue fever (DF).

In the absence of a vaccine against DENV, control of the mosquito vector is the primary option for prevention and control of dengue outbreaks. Currently used control methods for Ae. aegypti, which include environmental sanitation and source reduction to destroy container-inhabiting immature mosquitoes and space spraying in and around homes and ultra-low-volume (ULV) spraying from ground vehicles or airplanes to decimate adults, have not proven adequate to effectively prevent and control dengue outbreaks. In the case of control targeting adults, ULV spraying from vehicles has been questioned because insecticides broadcast by this method may fail to reach the indoor home environment, which is suspected to account for a large proportion of DENV transmission. Some of the early successes with ULV spraying likely resulted from spraying being conducted in areas with open housing structures allowing for effective insecticide penetration and often not be replicated in environments where closed housing structures provide refuge for the mosquitoes from insecticides broadcast outdoors using handheld or vehicle-mounted sprayers.

Numerous studies in different parts of the world have determined abundance of adult Ae. aegypti in the home environment by means of landing counts, resting boxes, or active mosquito collection by handheld aspirators or backpack aspirators. Seasonal peak abundances reportedly reached 5–7 mosquitoes per house in Manila, Philippines, 15 per house in the Dominican Republic, and >30 per house in Puerto Rico and Thailand. However, few studies have evaluated how commonly mosquitoes are found in different parts or rooms of the home. This critical information to facilitate indoor control of adult Ae. aegypti through space spraying, indoor residual spraying, or use of insecticide-treated materials, and additional studies from areas with differing housing characteristics are needed to gain a better understanding of how the mosquito uses the indoor environment.

Studies showing that DENV-infected Ae. aegypti do occur in or around the home environment have predominantly originated from Asia with few studies from the Americas. Two of the studies from the Americas focused specifically on the homes of patients with suspected dengue. This approach provides an intriguing opportunity to link household-level data for DENV in mosquitoes and humans.

We present here the results of a collaboration between Universidad Autonoma de Yucatan, Servicios de Salud de Yucatan and Colorado State University with the primary aims to 1) determine abundances of Ae. aegypti and other mosquitoes in the homes of laboratory-confirmed dengue patients in Merida, Yucatan, Mexico, over a 12-month period; 2) elucidate how commonly Ae. aegypti and other mosquitoes are found in different types of rooms; and 3) show the presence of DENV-infected Ae. aegypti from the homes of dengue patients.

MATERIALS AND METHODS

Study environment. Studies were conducted in the city of Merida (population of ≈800,000) in the Yucatan peninsula of southern Mexico. The flat and low Yucatan peninsula (eleva-
A second round of semi-nested PCR including NS3–kit (QIAGEN, Valencia, CA). This was followed by RT-PCR was extracted from patient serum samples using the RNeasy kit. Lack of exposure to DENV. Values of < 0.10 were considered to indicate lack of exposure to DENV. Values of 0.10–0.19 were classified as inconclusive, and values of > 0.19 were indicative of DENV infection.

Examination of dengue patient premises in conjunction with implementation of vector control measures by Servicios de Salud de Yucatan (SSY) was made possible by a close collaboration between UADY and SSY. Our collection efforts did not delay or in any other way interfere with normal SSY mosquito control activities. Team leaders from UADY entomology teams identified themselves to the household, provided an explanation for the visit, and thereafter asked for permission to collect mosquitoes in and around the house. Houses were georeferenced using a GPS receiver (Garmin, Salem, OR). All houses had electricity and running water and were one-story buildings constructed from cement. Backyard sizes were variable, as was the amount of vegetation in the backyards. Rooms were classified based on their main use as follows: kitchen, bathroom, living room, dining room, bedroom, laundry room, storage room, and other rooms.

Mosquitoes were collected using CDC style backpack aspirators. Collections were conducted from 0800 to 1500 hours and included all rooms, as well as the patio and other parts of the backyard. Indoor collection included aspiration from furniture, behind hanging clothes and curtains, and from dark and humid places where mosquitoes can be found resting. Aspiration in the backyard included the patio, and mosquitoes also were collected from pet houses, sundry things stored in the backyard, and vegetation. The length of time spent collecting per premise varied with size and number of rooms and the extent of the backyard, but the overall time typically was in the 20-minute range. Mosquito collections from different rooms or from the backyard/patio were stored separately before identification.

Mosquitoes were identified to species at LA-UADY using stereo microscopes and published identification keys. Blood feeding status of females (Sella’s stages) was determined by external examination of the abdomen (WHO 1975). Sella’s stages include 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). Ae. aegypti females were pooled by home of collection and stored at −70°C before processing for presence of DENV by RT-PCR.

We also conducted surveys for immature mosquitoes (larvae, pupae) following routine surveillance methodology used by SSY. This included inspection of common mosquito development sites inside the home and in the backyard/patio. Containers were classified with regard to presence of water, and the numbers of larvae or pupae in water-filled containers were counted. Sub-samples of larvae and pupae were identified using stereo microscopes and published identification keys.

DENV serotypes were identified as follows. Virus RNA was extracted from patient serum samples using the RNeasy kit (QIAGEN, Valencia, CA). This was followed by RT-PCR–based DENV amplification using primers targeting the NS3 gene. A second round of semi-nested PCR including the upstream consensus primer and DENV 1–4 serotype–specific primers was used to determine DENV serotype. Amplification products were visualized on a 2% LE agarose gel (Promega, Madison, WI) containing ethidium bromide.

Confirmation of dengue cases. Mosquitoes were collected from the homes of dengue patients in Merida from March 2007 (Epidemiologic Week 10) to February 2008 (Epidemiologic Week 9). This included 880 dengue cases that were confirmed by Laboratorio Estatal de Referencia Epidemiologica (Epidemiological Reference Laboratory of Yucatan State) using IgM-capture ELISA or by Laboratorio de Arbovirologia at Universidad Autonoma de Yucatan (LA-UADY) using IgM-capture ELISA and reverse transcriptase-polymerase chain reaction (RT-PCR). At LA-UADY, the IgM capture ELISA was performed using Nunc-Immuno F8 Maxisorp Modules (Nunc, Roskilde, Denmark), and the wells of the modules were washed with phosphate-buffered saline (PBS; pH 7.2) before each step. Wells were coated with goat anti-human IgM antibodies ( Biosource International, Camarillo, CA) diluted in carbonate buffer (pH 9.6) and incubated at 4°C overnight. This was followed by blocking with bovine serum albumin (BSA; Sigma Chemical, St. Louis, MO) for 15 minutes at room temperature. Serum samples, positive controls (high and low titers), and negative controls were diluted 1:40 in PBS (pH 7.2) with 0.5% BSA-PBS and added to the wells. After 2 hours of incubation at 37°C, pooled antigen from DENV serotypes 1–4 produced in culture of C6/36 mosquito cells was added. Plates were incubated overnight at 4°C. Thereafter, horseradish peroxidase–conjugated mouse anti-flavivirus (MAb 6B6C-1; CDC, Fort Collins, CO) diluted in 0.5% skim milk and PBS was added to each well, and plates were incubated for 1 hour at 37°C. Finally, ABTS peroxidase substrate system (KPL Laboratories, Gaithersburg, MD) was added, and after 30 minutes at 37°C and 2 hours at room temperature, the absorbance was determined using a microplate reader at 405 nm (Bio-Rad Laboratories, Hercules, CA). Absorbance values of 0.20 or higher were considered indicative of DENV infection, values of 0.10–0.19 were classified as inconclusive, and values of < 0.10 were considered to indicate lack of exposure to DENV.

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DENV detection from Ae. aegypti. We processed 336 mosquito pools containing 1,938 Ae. aegypti females (range per pool, 1–30 females, with the exception of two pools containing 47 and 82 females, respectively) for DENV identification by RT-PCR. These 336 pools originated from 335 different pre-
mises; two pools were tested from one premise. Pooled fe-
males were triturated, using sterile pestles and Eppendorf
 tubes, in 0.6 mL of cold medium Minimum Essential Medium
Eagle containing 2% fetal bovine serum (FBS; HyClone, Lo-
gan, UT) and anti-bacterial and anti-fungal antibiotics (100
U/mL of penicillin, 100 μg/mL of streptomycin, and 0.25 μg/
 mL of amphotericin B). The resulting suspension was added
to QIAshredder columns (QIAGEN), and the columns were
centrifuged at 14,000 rpm for 3 minutes at 4°C. Thereafter,
300 μL of each sample was transferred to Eppendorf tubes for
RNA extraction, and the remaining suspensions were stored
at −70°C. Extraction and amplification of viral RNA from
mosquito pools and determination of DENV serotype fol-
lowed the methodology described above for human samples.

Data analysis. Dengue cases were assigned to epidemio-
logic week by date of onset. A weekly Entomological Risk
Index (ERI) was calculated as mean weekly indoor abun-
dance of *Ae. aegypti* females × weekly proportion of female
pools testing positive for DENV. Statistical analyses were
carried out using the JMP statistical package, and results
were considered significant when *P* < 0.05. Specific tests used
are indicated in the text.

RESULTS

Dengue cases in Merida during the study period and out-
come of house visits. There were 1,310 serologically con-
firmed dengue cases in Merida during the March 2007 to
February 2008 study period; weekly case numbers ranged
from 0 to 95, with a distinct peak during Epidemiologic
Weeks 35–48 from late August to late November (Figure 1).
During the 12-month study period, 1,243 dengue patient
homes were visited. We were given permission to access 880
(70.8%) of the homes, whereas access was denied for 50
homes (4%), and no one was home at the time of the visit in
the remaining 313 (25.2%).

Collection of immatures. Among the 880 premises accessed
and examined, 190 (21.6%) produced immatures. Larvae of
*Ae. aegypti* were collected from 179 (20.3%) of the homes and
pupae from 93 (10.6%; Table 1). Immatures recorded from
containers on the patio and in the backyard were predomi-
nantly *Ae. aegypti* (2,279 larvae and 488 pupae), followed by
*Culex quinquefasciatus* (178 larvae and 29 pupae; Table 1).
Other species encountered included *Cx. coronator, Cx. lacta-
tor*, *Cx. interrogator*, and *Ochlerotatus trivittatus*. Indoor col-
lection of *Ae. aegypti* immatures yielded only 45 larvae and 15
pupae and thus was far less productive than outdoor collec-
tion (Table 1).

Summary data for adult mosquito collection. Among the
880 premises accessed and examined, 471 (53.5%) produced
adult mosquitoes and 395 (44.9%) produced *Ae. aegypti*. Fe-
nale *Ae. aegypti* were collected from 37.7% of the homes and
males from 31.8% (Table 2). The most commonly collected
mosquito in the indoor environment was *Culex quinquefasciatus*
(2,641 females and 2,076 males), followed by *Ae. aegypti*
(1,836 females and 1,292 males; Table 2). Other species re-
corded indoors included *Oc. taeniorhynchus* and *Oc. trivittat-
ts*. Numbers of specimens collected inside individual homes
ranged from 0 to 83 for *Ae. aegypti* females, 0 to 48 for *Ae.
aegypti* males, 0 to 59 for *Culex quinquefasciatus* females, and 0
to 189 for *Culex quinquefasciatus* males. Collection on the patio
and in the backyard yielded far lower total numbers of *Ae.
aegypti* (102 females and 108 males) or *Culex quinquefasciatus*
(135 females and 237 males) compared with indoor collection
(Table 2).

Use of the indoor home environment by adult *Ae. aegypti*
and *Cx. quinquefasciatus*. *Ae. aegypti* females were collected
most commonly from bedrooms (60.3% of 1,836 females),
followed by living/dining rooms (18.4%), kitchens (7.5%),
bathrooms (6.6%), and storage rooms (3.9%; Table 3). The
intradomicile use pattern of *Ae. aegypti* males was similar to
that for the females with bedrooms (51.9% of 1,292 males).
### Table 1

Immatures collected from dengue patient premises in Merida, Yucatan, Mexico, from March 2007 to February 2008

<table>
<thead>
<tr>
<th>Environment and species</th>
<th>Larvae</th>
<th></th>
<th>Pupae</th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Total number</td>
<td>Percent of total</td>
<td>Range for individual homes</td>
<td>Number (%) of homes with larvae*</td>
<td>Total number</td>
</tr>
<tr>
<td>Indoors</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>Aedes aegypti</em></td>
<td>45</td>
<td>91.8</td>
<td>7–19</td>
<td>9 (1.0)</td>
<td>15</td>
</tr>
<tr>
<td><em>Culex quinquefasciatus</em></td>
<td>4</td>
<td>8.2</td>
<td>0–4</td>
<td>1 (0.1)</td>
<td>0</td>
</tr>
<tr>
<td><em>Culex coronator</em></td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td><em>Culex lactator</em></td>
<td>13</td>
<td>14.8</td>
<td>0–11</td>
<td>34 (3.1)</td>
<td>26</td>
</tr>
<tr>
<td><em>Ochlerotatus trivittatus</em></td>
<td>2</td>
<td>0.2</td>
<td>0–1</td>
<td>1 (0.1)</td>
<td>0</td>
</tr>
<tr>
<td>Total</td>
<td>49</td>
<td>100</td>
<td></td>
<td></td>
<td>15</td>
</tr>
<tr>
<td>Backyard/patio</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>Aedes aegypti</em></td>
<td>2,279</td>
<td>91.3</td>
<td>14–45</td>
<td>179 (20.3)</td>
<td>488</td>
</tr>
<tr>
<td><em>Culex quinquefasciatus</em></td>
<td>178</td>
<td>7.1</td>
<td>10–15</td>
<td>27 (3.1)</td>
<td>29</td>
</tr>
<tr>
<td><em>Culex coronator</em></td>
<td>25</td>
<td>1.0</td>
<td>5–8</td>
<td>5 (0.6)</td>
<td>7</td>
</tr>
<tr>
<td><em>Culex lactator</em></td>
<td>12</td>
<td>0.5</td>
<td>0–4</td>
<td>4 (0.5)</td>
<td>0</td>
</tr>
<tr>
<td><em>Culex interrogator</em></td>
<td>2</td>
<td>0.1</td>
<td>0–2</td>
<td>1 (0.1)</td>
<td>0</td>
</tr>
<tr>
<td><em>Ochlerotatus trivittatus</em></td>
<td>1</td>
<td>0.0</td>
<td>0–1</td>
<td>1 (0.1)</td>
<td>0</td>
</tr>
<tr>
<td>Total</td>
<td>2,497</td>
<td>100</td>
<td></td>
<td></td>
<td>524</td>
</tr>
</tbody>
</table>

* Based on examination of 880 homes.

### Table 2

Adult mosquitoes collected from dengue patient premises in Merida, Yucatan, Mexico, from March 2007 to February 2008

<table>
<thead>
<tr>
<th>Environment and species</th>
<th>Females</th>
<th></th>
<th>Males</th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Number collected</td>
<td>Percent of total</td>
<td>Range for individual homes</td>
<td>Number (%) of homes with females*</td>
<td>Number collected</td>
</tr>
<tr>
<td>Indoors</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>Aedes aegypti</em></td>
<td>1,836</td>
<td>39.7</td>
<td>0–83</td>
<td>332 (37.7)</td>
<td>1,292</td>
</tr>
<tr>
<td><em>Culex quinquefasciatus</em></td>
<td>2,641</td>
<td>57.1</td>
<td>0–59</td>
<td>312 (35.5)</td>
<td>2,076</td>
</tr>
<tr>
<td><em>Ochlerotatus taeniorhynchus</em></td>
<td>147</td>
<td>3.2</td>
<td>0–11</td>
<td>64 (7.3)</td>
<td>14</td>
</tr>
<tr>
<td><em>Ochlerotatus trivittatus</em></td>
<td>3</td>
<td>&lt; 0.1</td>
<td>0–3</td>
<td>3 (0.3)</td>
<td>0</td>
</tr>
<tr>
<td>Total</td>
<td>4,627</td>
<td>100</td>
<td></td>
<td></td>
<td>3,382</td>
</tr>
<tr>
<td>Backyard/patio</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>Aedes aegypti</em></td>
<td>102</td>
<td>35.0</td>
<td>0–21</td>
<td>33 (3.8)</td>
<td>108</td>
</tr>
<tr>
<td><em>Culex quinquefasciatus</em></td>
<td>135</td>
<td>46.2</td>
<td>0–14</td>
<td>34 (3.9)</td>
<td>237</td>
</tr>
<tr>
<td><em>Ochlerotatus taeniorhynchus</em></td>
<td>50</td>
<td>17.1</td>
<td>0–10</td>
<td>10 (1.1)</td>
<td>20</td>
</tr>
<tr>
<td><em>Ochlerotatus trivittatus</em></td>
<td>5</td>
<td>1.7</td>
<td>0–3</td>
<td>3 (0.3)</td>
<td>5</td>
</tr>
<tr>
<td>Total</td>
<td>292</td>
<td>100</td>
<td></td>
<td></td>
<td>370</td>
</tr>
</tbody>
</table>

* Based on examination of 880 homes.

### Table 3

Use of the indoor home environment by adult *Ae. aegypti* and *Cx. quinquefasciatus* in dengue patient homes in Merida, Yucatan, Mexico, from March 2007 to February 2008

<table>
<thead>
<tr>
<th>Room</th>
<th>Number collected</th>
<th>Percent of total</th>
<th>Range for individual homes</th>
<th>Number (%) of homes with females in room type*</th>
<th>Number collected</th>
<th>Percent of total</th>
<th>Range for individual homes</th>
<th>Number (%) of homes with males in room type*</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Aedes aegypti</em></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Bedroom</td>
<td>1,107</td>
<td>60.3</td>
<td>0–40</td>
<td>218 (24.8)</td>
<td>670</td>
<td>51.9</td>
<td>0–48</td>
<td>181 (20.6)</td>
</tr>
<tr>
<td>Living/dining room</td>
<td>337</td>
<td>18.4</td>
<td>0–11</td>
<td>100 (11.4)</td>
<td>296</td>
<td>22.9</td>
<td>0–30</td>
<td>77 (8.8)</td>
</tr>
<tr>
<td>Kitchen</td>
<td>137</td>
<td>7.5</td>
<td>0–7</td>
<td>44 (5.0)</td>
<td>110</td>
<td>8.5</td>
<td>0–28</td>
<td>38 (4.3)</td>
</tr>
<tr>
<td>Bathroom</td>
<td>121</td>
<td>6.6</td>
<td>0–7</td>
<td>48 (5.5)</td>
<td>61</td>
<td>4.7</td>
<td>0–11</td>
<td>26 (3.0)</td>
</tr>
<tr>
<td>Storage room</td>
<td>71</td>
<td>3.9</td>
<td>0–6</td>
<td>12 (1.4)</td>
<td>66</td>
<td>5.1</td>
<td>0–21</td>
<td>4 (0.5)</td>
</tr>
<tr>
<td>Other rooms</td>
<td>54</td>
<td>2.9</td>
<td>0–19</td>
<td>8 (0.9)</td>
<td>66</td>
<td>5.1</td>
<td>0–29</td>
<td>10 (1.1)</td>
</tr>
<tr>
<td>Laundry room</td>
<td>9</td>
<td>0.5</td>
<td>0–2</td>
<td>6 (0.7)</td>
<td>23</td>
<td>1.8</td>
<td>0–6</td>
<td>10 (1.1)</td>
</tr>
<tr>
<td>Total</td>
<td>1,836</td>
<td>100</td>
<td></td>
<td></td>
<td>1,292</td>
<td>100</td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>Culex quinquefasciatus</em></td>
<td>1,388</td>
<td>52.6</td>
<td>0–35</td>
<td>194 (22.0)</td>
<td>716</td>
<td>34.5</td>
<td>0–83</td>
<td>129 (14.7)</td>
</tr>
<tr>
<td>Bedroom</td>
<td>436</td>
<td>16.2</td>
<td>0–39</td>
<td>85 (9.7)</td>
<td>262</td>
<td>12.6</td>
<td>0–26</td>
<td>57 (6.5)</td>
</tr>
<tr>
<td>Living/dining room</td>
<td>287</td>
<td>10.9</td>
<td>0–51</td>
<td>36 (4.1)</td>
<td>259</td>
<td>12.5</td>
<td>0–67</td>
<td>36 (4.1)</td>
</tr>
<tr>
<td>Kitchen</td>
<td>225</td>
<td>8.8</td>
<td>0–59</td>
<td>14 (1.6)</td>
<td>432</td>
<td>20.8</td>
<td>0–189</td>
<td>16 (1.8)</td>
</tr>
<tr>
<td>Bathroom</td>
<td>172</td>
<td>6.5</td>
<td>0–6</td>
<td>52 (5.9)</td>
<td>161</td>
<td>7.8</td>
<td>0–38</td>
<td>38 (4.3)</td>
</tr>
<tr>
<td>Other rooms</td>
<td>85</td>
<td>3.2</td>
<td>0–13</td>
<td>12 (1.4)</td>
<td>168</td>
<td>8.1</td>
<td>0–50</td>
<td>12 (1.4)</td>
</tr>
<tr>
<td>Laundry room</td>
<td>48</td>
<td>1.8</td>
<td>0–5</td>
<td>11 (1.3)</td>
<td>78</td>
<td>3.8</td>
<td>0–27</td>
<td>11 (1.3)</td>
</tr>
<tr>
<td>Total</td>
<td>2,641</td>
<td>100</td>
<td></td>
<td></td>
<td>2,076</td>
<td>100</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

* Based on examination of 880 homes.
and living/dining rooms (22.9%) accounting for > 75% of specimens collected. In the case of *Cx. quinquefasciatus*, females also were collected most commonly in bedrooms (52.6% of 2,641 females; Table 3). Furthermore, *Ae. aegypti* females and *Cx. quinquefasciatus* females were collected in bedrooms in 24.8% and 22%, respectively, of the examined homes (Table 3).

We also determined whether blood feeding status (Sella’s stages) influences use by *Ae. aegypti* females of different room types (Table 4). This appears not to be the case. For the four room types producing > 100 females (bedroom, living/dining room, kitchen, bathroom), we found similar distributions of females of different blood feeding status (Table 4). For example, percentages of females in these room types that were unfed ranged from 28.1% to 29.2% (contingency table analysis; \( \chi^2 = 0.06, df = 3, P = 0.99 \)). Furthermore, percentages of freshly fed females ranged from 24.1% to 33.1% (\( \chi^2 = 2.97, df = 3, P = 0.40 \)) and gravid ones from 9.9% to 10.7% (\( \chi^2 = 0.21, df = 3, P = 0.98 \)). We also found that percentages of females collected in different room types were stable across Sella’s stages (Table 4). For example, regardless of their blood feeding status, females were found most commonly in bedrooms (53.1–64.1% across Sella’s stages) and living/dining rooms (17.4–21.6%; \( \chi^2 = 9.77, df = 5, P = 0.08 \) and \( \chi^2 = 1.82, df = 5, P = 0.87 \), respectively).

The only notable exception with respect to room use by feeding status was that storage rooms harbored elevated numbers of gravid females; storage rooms accounted for 14.0% of 2,641 females; Table 3). Furthermore, unlike all other room types, storage rooms yielded more gravid than unfed females (\( N = 29 \) and 20, respectively; Table 4). Finally, blood feeding status differed for *Ae. aegypti* females collected indoors versus outdoors (Table 4). Most notably, females collected on the patio or in the backyard were, in relation to those collected indoors, more commonly unfed (58.8% versus 29.8%; \( P < 0.001 \)).

**Relationships between outdoor presence or abundance of immature and indoor abundance of adult *Ae. aegypti***

For the 347 homes examined during the July–November peak activity period of *Ae. aegypti*, we examined the relationships between outdoor presence or abundance of immatures (based on total counts of observed larvae or pupae) and indoor abundance of females. The indoor abundance of females was similar for homes with versus without larvae present outdoors (Wilcoxon rank sum test with \( \chi^2 \) approximation; \( \chi^2 = 2.64, df = 1, P = 0.10 \)) and for homes with versus without pupae present outdoors (\( \chi^2 = 1.06, df = 1, P = 0.30 \)). Furthermore, there were no significant correlations with indoor abundance of females for outdoor abundance of either larvae (Spearman rank correlation; \( \rho_s = -0.050, N = 347, P = 0.35 \)) or pupae (\( \rho_s = -0.045, N = 347, P = 0.40 \)). Strong correlations were recorded for abundance of larvae and pupae outdoors (\( \rho_s = 0.724, N = 347, P < 0.001 \)) and for abundance of females and males indoors (\( \rho_s = 0.599, N = 347, P < 0.001 \)).

**DENV infection in *Ae. aegypti* females from dengue patient premises.** A total of 1,938 *Ae. aegypti* females collected from the premises of 335 dengue patients during March 2007 to February 2008 were pooled (total of 336 pools) and tested for presence of DENV by RT-PCR (Tables 5 and 6). DENV-positive pools were recorded from July to August (18.2% of 33 pools), September (17.6% of 74 pools), October (6.7% of...
105 pools), and November–December (8.6% of 93 pools; Table 5). None of 20 pools from March to June 2007 or 11 pools from January to February 2008 were positive for DENV. Monthly minimum DENV infection rates of females (based on the assumption of one infected female per positive pool) ranged from 0% to 4%, and the minimum DENV infection rate for the full 12-month period was 1.8% (Table 5).

More detailed information for 34 premises yielding DENV-
infected mosquito pools is provided in Table 6. Positive mosquito pools were infected with DENV-1 (N = 24), DENV-2 (N = 8), or DENV-3 (N = 2), and the serotype matched with the dengue patient’s serotype in all five cases where serotype-specific information was available for the dengue patient. The number of days elapsed from onset of symptoms in the dengue patient to collection of DENV-infected mosquitoes ranged from 4 to 27, with one half of the collections being conducted at least 14 days after the onset of symptoms. Of the 34 DENV-positive pools, 29 included only females from the indoor home environment, 4 included females from both the indoor and outdoor environments, and 1 single pool consisted only of females collected outdoors (Table 6). Furthermore, 12 DENV-positive pools included only females collected from the bedroom.

Seasonal pattern of dengue cases in relation to temperature, rainfall, abundance of *Ae. aegypti* females, and ERI. The weekly pattern from March 2007 to February 2008 for dengue cases is compared with weekly patterns for mean indoor abundance of *Ae. aegypti* females, rainfall, and mean temperature in Figure 1. The mean weekly indoor catch rate peaked at 7.8 females per home (late August), and weekly numbers of dengue cases commonly exceeded 80 in September and October. The time lag between the peaks for abundance of *Ae. aegypti* and for dengue cases was in the 2- to 4-week range. Future studies using fixed homes to determine longitudinal patterns in mosquito abundance are needed to explore the nature of this relationship. The observed patterns indicate that variation in rainfall, rather than temperature, is the main driver for seasonal changes in abundance of *Ae. aegypti* and, ultimately, dengue cases in Merida (Figure 1).

In Figure 2, we compare weekly patterns from March 2007 to February 2008 for dengue cases, proportion of *Ae. aegypti* female pools testing positive for DENV, and ERI (mean weekly indoor abundance of *Ae. aegypti* females × weekly proportion of female pools testing positive for DENV). Peak ERI seems to precede the peak in human dengue cases by several weeks to a month. Notably, the rebound and small peak in dengue cases during Epidemiologic Weeks 52 to 1 was foreshadowed by small peaks for proportion of DENV-positive female pools and ERI in Epidemiologic Week 50.

**DISCUSSION**

The main findings of this study are that 1) the combination of backpack aspiration in the indoor environment and subsequent detection of DENV by RT-PCR in collected *Ae. aegypti* females is a productive but currently underused strategy to address the critically important but poorly understood issue of where and when people are at risk for exposure to DENV-infected mosquitoes; 2) outdoor abundances of larvae or pupae were not predictive of abundance of *Ae. aegypti* females inside the home; 3) *Ae. aegypti* and *Cx. quinquefasciatus* were collected predominantly from bedrooms during indoor collections; and 4) DENV-infected *Ae. aegypti* females were collected from homes of dengue patients up to 27 days after the onset of symptoms (range, 4–27 days; median, 14 days), which shows the usefulness of indoor application of insecticides in the homes of suspected dengue patients to prevent their homes from becoming sources for dispersal of DENV by persons visiting and being bitten by infected mosquitoes.

Peridomestic and intradomicile use patterns of *Ae. aegypti* and *Cx. quinquefasciatus*. Homes in Merida commonly harbored two species of mosquitoes that have been implicated in transmission of a wide range of mosquito-borne pathogens: *Ae. aegypti*, which is a principal vector of DENV and yellow fever and chikungunya viruses, and *Cx. quinquefasciatus*, which is a principal vector of the parasitic filarial worms causing lymphatic filariasis and a vector of West Nile virus. Protecting the indoor environment from these mosquitoes in the Americas is important not only for control of dengue but also to minimize the risk of future outbreaks of the other pathogens they are capable of transmitting.

**FIGURE 2.** Weekly patterns for number of laboratory-confirmed dengue cases, proportion of *Ae. aegypti* female pools testing positive for DENV, and Entomological Risk Index (mean weekly indoor abundance of *Ae. aegypti* females × weekly proportion of female pools testing positive for DENV) from March 2007 to February 2008 in Merida, Yucatan, Mexico. This figure appears in color at www.ajtmh.org.
Our study yielded several important insights into peri-domestic and intradomicile use patterns by *Ae. aegypti* and *Cx. quinquefasciatus*. First, our data showed that activity by *Ae. aegypti* adults can occur throughout the year in Merida. However, lower temperatures and infrequent rainfall from December to May seem to suppress development of immatures and activity of adults compared with the June–November period, which is characterized by higher temperatures and consistent rainfall, leading to accelerated development times for immatures and a greater abundance of water-filled containers for females to deposit their eggs in. Longitudinal studies are needed to more definitively characterize the relationships between temperature, rainfall, and *Ae. aegypti* activity in Merida.

Second, despite the fact that backpack aspiration likely yields less than one half of the *Ae. aegypti* present in the indoor home environment, we found this method to be adequate to collect large numbers of mosquitoes for testing of presence of DENV. Indeed, we collected as many as 83 *Ae. aegypti* females in a single dengue patient home and a total of 1,938 females over the full study period. Other studies also have reported successful use of backpack aspiration to collect large numbers of *Ae. aegypti* from the indoor environment. It is perhaps time to revisit the potential for using backpack aspiration in routine indoor surveillance for *Ae. aegypti*; although houses were sampled opportunistically in this study, we found an intriguing seasonal pattern of mosquito abundance. Future studies where fixed sentinel homes spread throughout Merida are visited and backpack aspirated at regular intervals, as previously done with good results in Colombia and Singapore, would provide valuable information regarding the potential for using this methodology in combination with RT-PCR–based detection of DENV to track seasonal changes in 1) indoor mosquito abundance; 2) infection rates of females with DENV; and 3) an ERI combining data for abundance of females and DENV infection.

Third, we found outdoor presence and abundance of larvae or pupae to be poor predictors of abundance of *Ae. aegypti* females inside the home. This agrees with other studies from the Americas. The strength of the association between outdoor abundance of immatures and indoor abundance of adults undoubtedly is influenced by housing characteristics, e.g., construction features such as open eaves that allow the mosquito to enter the home or use of air conditioners to cool the home without the need for opening windows and doors. Our finding from Merida highlights the need to conduct local studies before making the assumption that outdoor abundance of immatures can be used to estimate risk of exposure to *Ae. aegypti* in the indoor home environment.

Fourth, we found that bedrooms accounted for 60% of all collected *Ae. aegypti* females and 53% of *Cx. quinquefasciatus* females. Similar results have been reported for *Ae. aegypti* females in studies from Panama and Malaysia. Furthermore, *Ae. aegypti* was commonly collected from bedrooms in Puerto Rico. This intriguing finding has important implications for indoor control of *Ae. aegypti*; special emphasis should be placed on the bedroom during indoor spray activities and the common use of the bedroom by the mosquito may provide an opportunity for application of insecticides through novel routes specifically targeting this room type. We must caution, however, that additional studies are needed to more exhaustively explore the use of the intradomicile environment by *Ae. aegypti* and to rule out the possibility that the apparent importance of the bedroom is not an artifact of backpack aspiration being more effective in bedrooms relative to other room types.

Fifth, we collected few adult *Ae. aegypti* outdoors and only a single DENV-infected pool consisted of females collected exclusively outdoors. This could, however, have resulted from backpack aspiration being more effective for indoor compared with outdoor mosquito collection. To gain a better understanding of risk of exposure to *Ae. aegypti* inside the home versus in the backyard, studies are needed that combine backpack aspiration with use of traps for host-seeking mosquitoes such as the BG-Sentinel. Finally, our finding that females collected on the patio or in the backyard were, in relation to those collected indoors, more likely to be uneled agrees with results from Monterrey in northern Mexico.

**DENV-infected mosquitoes in the home environment.** Although presence of DENV-infected *Ae. aegypti* females in the home environment has been shown previously from Southeast Asia and South America, ours is the first study specifically targeting the homes of large numbers of laboratory confirmed dengue patients within 1–4 weeks of onset of symptoms. This unique study was made possible by a close collaboration between Universidad Autonoma de Yucatan and Servicios de Salud de Yucatan and a rapid turn-around time for laboratory confirmation of suspected dengue cases at Laboratorio de Arbovirolgia at Universidad Autonoma de Yucatan and Laboratorio Estatal de Referencia Epidemiologica. Testing of 336 pools of female *Ae. aegypti* from 335 dengue patient premises produced 34 (10.1%) pools positive for DENV by RT-PCR, with 29 of 34 positive pools including only females collected in the indoor home environment and 12 pools exclusively containing females collected from the bedroom. Similar results for testing of *Ae. aegypti* pools for presence of DENV by RT-PCR have been reported from dengue-endemic areas in South America, with 12.7% of 292 pools testing positive in Valle del Cauca State, Colombia, 17.1% of 82 pools testing positive in the city of Manaus, Brazil, and 18% of 296 pools testing positive in the city of Maracay, Venezuela.

DENV-positive mosquito pools were recorded each month during July–December 2007, whereas no positive pools were found during March–June 2007 or January–February 2008. The latter probably resulted, in part, from that low numbers of pools (*N* = 31) and females (*N* = 103) were tested during March–June 2007 and January–February 2008. On the other hand, testing of 100 females in 11 pools in July yielded 4 positive pools and testing of 126 females in 32 pools in December yielded 3 positive pools. The simplest explanation is perhaps that increasing mosquito abundances in late summer and fall leads to intensified intradomicile transmission of DENV with multiple persons commonly being infected within a single home; this, in turn, may have resulted in that female mosquitoes collected from dengue patient homes from July to December were more likely to have fed on a DENV-infected person than those collected from March to June or January to February, when mosquito abundances were lower. Prospective studies where the DENV infection status of all members of a household is determined are needed to test this hypothesis.

DENV-1 predominated among the infected mosquito pools, followed by DENV-2 and DENV-3. We found a match
with dengue patient DENV serotype for all five cases where serotype-specific information was available for the dengue patient. Isolation and genetic analysis of the dengue viruses are underway. This information will be presented as part of a larger study on serologic and genetic variability of DENV in the Yucatan peninsula.

**Implications for operational vector control.** The results of the study have important implications for operational vector control activities in Merida. First, our data on intradomicile use patterns of *Ae. aegypti* show that indoor interventions such as fogging with insecticide should place special emphasis on bedrooms and living/dining rooms. Second, our finding that DENV-infected *Ae. aegypti* females were collected from homes of dengue patients up to 27 days after the onset of symptoms demonstrates the usefulness of indoor insecticide application in the homes of suspected or laboratory-confirmed dengue patients to prevent their homes from becoming sources for dispersal of DENV by persons visiting and being bitten by infected mosquitoes.

Our results, together with those of recent studies from the Americas showing that use of insecticide-treated materials (ITMs) can protect the home from *Ae. aegypti* and DENV exposure, suggest that the following dengue prevention and control strategies are well suited for operational implementation in the Americas:

1. **Proactive integrated vector management with ITMs to create a “Casa Segura” safe house** are deployed together with targeted source reduction.

2. **Reactive control where the homes of suspected dengue patients (based on clinical diagnosis) and surrounding homes promptly are equipped with ITMs and treated by indoor fogging with insecticide.** Further empirical studies are needed to determine the optimal size of the perimeter for implementation of control measures around a dengue case home.

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Authors’ addresses: Julian Garcia-Rejon, Maria Alba Loroño-Pino, Jose Arturo Farfan-Alc, Luis Flores-Flores, Elsy Del Pilar Rosado-Paredes, and Nubia Rivero-Cardenas, Laboratorio de Arbovirologia, Centro de Investigaciones Regionales Dr. Hideto Noguchi, Universidad Autonoma de Yucatan, Av. Itza No. 490 x 59, Centro, Merida, Yucatan, México CP 97000. Rosario Najera-Vazquez, Salvador Gomez-Carro, Victor Lira-Zumbardo, and Pedro Gonzalez-Martinez, Servicios de Salud de Yucatan, Calle 72 #463 por 53 y 55, Centro, Merida, Yucatan, México CP 97000. Suil Lozano-Fuentes, Darwin Elizondo-Quiroga, Barry J. Beaty, and Lars Eisen, Department of Microbiology, Immunology, and Pathology, 1600 Campus Delivery, Colorado State University, Fort Collins, CO 80523, E-mail: lars.eisen@colostate.edu.

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Aedes aegypti and Dengue Virus in the Home


