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## *Aedes aegypti* Mosquitoes at Nonresidential Sites Might Be Related to Transmission of Dengue Virus in Monterrey, Northeastern Mexico

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**Abstract.** Traditionally the major risk environment for transmission of dengue virus has been assumed to be households. In Mexico, dengue outbreaks continue year after year despite intense control efforts. Nonresidential sites (public and private spaces) infested with *Aedes aegypti* (L.) were evaluated. In total, 141 nonresidential sites were sampled for the presence of potential and active oviposition sites and adult mosquitoes. Eighty percent of the sites were oviposition sites; *Ae. aegypti* adults were recovered at 94.7% of nonresidential sites. Most female *Ae. aegypti*, 21.6 and 10.4, were at schools and recreational sites, respectively. Chi-squared indicated no significant differences in the dengue vector to categories of sample sites ($x^2$ = 17.76, df = 9, $P$ = 0.38). Indoor-use patterns of adult mosquitoes indicated bathrooms and classrooms were preferred resting sites. Reverse transcription polymerase chain reaction (RT-PCR) assay did not identify dengue virus nucleic acids from a group of 221 pools containing 1,521 female *Ae. aegypti*. Daytime human activities; e.g., school and work, synchronize with the bimodal biting pattern of *Ae. aegypti*, increasing the chance of transferring dengue virus.

### Introduction

Infection by dengue virus is a major threat to urban populations in Latin America (Kyle and Harris 2008). It is estimated that 50 million infections occur in tropical and subtropical regions each year (WHO 2009), having remarkable social and economic impact (Peters 1997, Gubler 2002, Guzman and Kouri 2003). Dengue virus is a member of the family Flaviviridae, genus *Flavivirus*. The four serologic types are DENV-1, DENV-2, DENV-3, and DENV-4 (Westaway and Blok 1997), and all can be transmitted to humans by *Aedes aegypti* (L.) and other *Aedes* spp. vectors (Gubler 2004, WHO 2007). *Ae. aegypti* is a mosquito that grows in water-filled containers maintained by human activity or rain (PAHO 1994). Female

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adult *Ae. aegypti* prefer human blood as well as endophilic resting behavior (Yasuno and Tonn 1970, Scott et al. 1993, Ponlawat and Harrington 2005). It is accepted that home environments create a greater risk for transmission of dengue (WHO 2009). For example, 60% of female *Ae. aegypti* rested inside houses in Thailand (Edman et al. 1997, Kittayapong et al. 1997). Later studies and others published elsewhere focused on the human domicile as almost the only targeted area for stopping dengue outbreaks (Gubler and Kuno 1997). WHO (2009) guidelines for dengue prevention and control emphasize the greatest risk for contracting dengue fever and dengue hemorrhagic fever is by infected *Ae. aegypti* sharing human-inhabited houses.

However, despite routine application of controls at residential sites in endemic countries, outbreaks of dengue virus are reported each year. Evidence of the presence of *Ae. aegypti* thriving at nonresidential sites near human settlements has accumulated during the past decade. In Taiwan *Ae. aegypti* were found in schools, vacant land, temples, hospitals, factories, offices, and shops (Hwang and Hsu 1994). In Brazil, ovitraps demonstrated activity of female vectors in dwellings (83.9%); churches, schools, and clubs (6.8%); vacant land (6.4%); and businesses (2.8%) (da Silva et al. 2006). *Ae. aegypti* invading nonresidential sites were surveyed in the Amazonian city of Iquitos, Peru. The authors counted pupae and adults. Public markets yielded as many as 213.1 pupae and 19.3 adults per hectare, while 121.5 pupae and 35.1 adults per hectare were found at ports (Morrison et al. 2006). Movement displacing *Ae. aegypti* to uncontrolled sites could contribute to additional reports of dengue in exposed populations such as students, teachers, and workers. In Mexico, García-Rejón et al. (2011) collected female mosquitoes from different room types including classrooms, offices, and bathrooms and reported 11 (45.8%) of 24 schools positive for dengue virus-infected pools. People at sites with commercial activity and human movement are at risk for infectious bites by *Ae. aegypti*, as found in a slum in Rio de Janeiro, Brazil, with low mosquito densities but high seroprevalence (Honório et al. 2009). Undoubtedly, most nonresidential sites correlate with temporary or permanent human concentrations. Epidemiological relevance for transmission is highlighted especially for day schools and factory shifts. The early morning and evening blood-feeding biorhythm of *Ae. aegypti* synchronizes with learning and labor, thus increasing passage of dengue virus from vectors to humans (Chadee 1988).

Considering that the Mexican government dengue control program focuses activities on households and the need to characterize infesting *Ae. aegypti* nonresidential sites, this research had the followed objectives: identify the presence of dengue vectors by finding oviposition sites (wet or dry containers) and positive oviposition sites as well as adult stages, and determine preferred resting places in the indoor environment at nonresidential areas. In addition, RT-PCR and PCR were used to test female *Ae. aegypti* collected for dengue virus.

## Materials and Methods

**Study Area.** The study site was the metropolitan area of Monterrey with 4,036,112 inhabitants in the second largest industrialized city in Mexico (INEGI 2010a). The climate is hot and dry, with an average temperature of 23°C although during some years, temperatures are hotter than 42°C during the summer and colder than 0°C during winter. Relative humidity averages 62%, with rain during August, September, and October (Gobierno del Estado de Nuevo León 2011).

Dengue has been endemic in the Monterrey area since 1980 when it reemerged in Mexico.  Since then, epidemics have been reported every year. Important nationwide dengue outbreaks have been recorded in recent years; e.g., in 2009, 55,961 cases were confirmed, including 44,565 and 11,396 of dengue fever and dengue hemorrhagic fever, respectively.  During 2010 and 2011, the dengue outbreak in Mexico totaled 30,156 and 12,826 cases, respectively.  Monterrey had 2,249 cases including 2,068 dengue fever and 181 dengue hemorrhagic fever, with two fatalities in 2010, while in 2011 there were only 678 cases with 665 dengue fever and 13 dengue hemorrhagic fever, and in 2012 disease incidence increased to 1,294 cases (1,250 dengue fever and 44 dengue hemorrhagic fever).  Serotype DENV-1 was dominant, with less than 5% of serotype DENV-2 during the 2 years (CENAVECE 2012).

**Collection of Mosquitoes.**  Mosquitoes were collected from nonresidential sites from August 2009 to November 2010 except during the winter (mid-November to mid-March).  The sites were chosen because of environmental characteristics associated with urban ecology of *Ae. aegypti* (Clements 1996), and were in areas with outbreaks of dengue.  Selected sites included schools, churches, tire repair shops, factories, commercial sites, bus stations, junk yards, public libraries, recreational sites, and government buildings.  Preferred resting sites were recorded for individual adult mosquitoes to analyze indoor use-patterns in the sampled environment classified as office, bathroom, classroom (including computer rooms and laboratories), library, cafeteria, workshop, storage rooms, auditorium, other rooms (basement, gymnasium, machine room, etc.), and outdoors (backyard/patio). Adult mosquitoes were collected between 0800 and 1400 hours by using a CDC-style backpack aspirator (Clark et al. 1994) in all rooms, as well as outdoors.

Collection indoors involved aspirations from equipment, furniture, hanging clothes, flags, curtains, blinds, and any dark and humid places where mosquitoes might rest.  Collection outdoors involved aspiration from items in the backyard -- vegetation, pet houses, and other sites.  The length of time spent collecting per site varied with the size and number of rooms and extent of the area, but the overall time ranged from 45 minutes to 2 hours.  The nonresidential sites were georeferenced using a global positioning system receiver (Garmin Inc, Salem, OR). Mosquitoes collected from indoor and outdoor spaces were stored separately and labeled before identification.  The mosquitoes were transported in ice chests with a moist chamber to Laboratorio de Entomologia Medica - Universidad Autonoma de Nuevo Leon and identified to species using identification keys (Carpenter and LaCasse 1955, Darsie and Ward 2005) and a chill table (BioQuip, Rancho Dominguez, CA).  *Ae. aegypti* females were pooled (1-20 per pool) by nonresidential site of collection and stored at -70°C before processing to detect the presence of dengue virus by using RT-PCR.

Male mosquitoes were not studied.  Nonresidential sites were sampled for wet containers and oviposition sites such as flower vases, cans, discarded tires, and 55-gallon metal and plastic drums with larvae and pupae (Fernandez-Salas 2009).  Criteria to define an oviposition site included dry and wet containers (containers that can become positives), while positive oviposition sites had at least one or more larva and/or pupa.  Fourth-instar larvae and adults collected as pupae were identified using taxonomic keys (Ibañez-Bernal and Martinez-Campos 1994, Darsie and Ward 2005).  Containers were categorized into small, medium, large, and atypical (Table 1).  Before the entomological surveys, permission in writing was

requested from managers (chiefs and owners) of nonresidential sites evaluated in the study.


Table 1. Description of the oviposition sites types observed in nonresidential sites in metropolitan area of Monterrey, Nuevo Leon, Mexico.

| Oviposition sites category | Description |
| --- | --- |
| Small | Flower vases, cans (paint, tuna, evaporated milk, drinks, and other food-item cans), potted plants, pot, plate, drinking glass, bowls, bottles (soft-drink, water bottles and alcoholic-beverage), pet dishes and naturals (tree holes, rock holes, leaves and fruit skins). |
| Medium | Tires, drums, washtubs, buckets, toilet tank, sink, ice chests, furniture and car parts. |
| Large | Storage containers, pools, water-storage tanks (≥55-gallon) and well. |
| Atypical | Depressions in floor, holes, ditches, bags or plastic tarpaulins, puddles and others that not be easily classified. |


**Detection of Dengue Virus in *Ae. aegypti*.** Pools of female *Ae. aegypti* (1-20 per pool) were processed using RT-PCR for identification of dengue virus (Lanciotti et al. 1992, García-Rejón et al. 2011). Pooled females were triturated using a tissueruptor (QIAGEN, Valencia, CA) and Eppendorf tubes, in 0.6 ml of cold Minimum Essential Medium containing 2% fetal bovine serum (HyClone, Logan, UT) and anti-fungal and anti-bacterial (100 U/ml of penicillin, 100 g/ml of streptomycin, and 0.25 g/ml of amphotericin B). The resulting suspension was added to QIAshredder columns (QIAGEN, Valencia, CA), and the columns were centrifuged at 14,000 rpm for 3 minutes at 4°C. Immediately after, 300 µl of each sample were transferred to Eppendorf tubes for RNA extraction, and the remaining suspensions were stored at −70°C.

Virus RNA was extracted using the RNeasy kit (QIAGEN, Valencia, CA) following the instructions in the kit. Semi-nested RT-PCR amplifications used the protocol of Lanciotti et al. (1992). Dengue virus RNA was RT-PCR amplified using forward D1 and reverse D2 primers that encompassed a region of the capsid and premembrane genes of all dengue virus serotypes. A second round of semi-nested PCR involving the D1 and D2 and DENV TS1-TS4 serotype-specific primers was used to determine dengue virus serotype. Amplification products were analyzed on a 2% agarose gel (Promega Corp., Madison, WI) containing ethidium bromide.

**Data Analysis.** Statistical analysis was by the Statistical Package for the Social Sciences (SPSS) 19.0 (Chicago, IL). A chi-squared test ($\chi^2$) with a 2 x 2 contingency table was calculated to determine association among oviposition sites, positive oviposition sites, and number of oviposition sites at nonresidential sites. Similar analyses determined the significance of mosquito-preferred resting sites. ANOVA was used to compare the mean number of adult mosquitoes in each category of nonresidential site. Results were considered significant when $P < 0.05$.

## Results

**Survey of Oviposition Sites and Adult *Ae. aegypti* at Nonresidential Sites.** Mosquitoes were surveyed at 141 nonresidential sites: 33 schools (23.4%), 25 churches (17.7%), 10 tire repair shops (7.1%), 10 factories (7.1%), 23 commercial sites (16.3%), three bus stations (2.1%), 12 junk yards (8.5%), seven public libraries (5%), eight recreational sites (5.7%), and 10 government buildings (7.1%) (Table 2). Of the total sites studied, 112 (79.4%) had oviposition sites and 68 (48.2%) were oviposition sites with immature *Ae. aegypti* (positive sites had one or more immature stages). Oviposition sites (wet or dry containers) were found at 100% of the government buildings, tire repair shops, and junk yards, followed by schools (88.8%), recreational sites (75%), churches (72%), factories (70%), commercial businesses (69.6%), bus stations (66.7%), and public libraries (42.9%).

The chi-squared test indicated the presence of wet and dry oviposition sites was independent of nonresidential sites ($x^2$ = 0.38, df = 9, $P$ = 0.3). Most positive oviposition sites were at recreational sites (87.5%) followed by junk yards (75%), factories (60%), commercial businesses (47.8%), schools (45.5%), public libraries (42.9%), tire repair shops and government buildings (both 40%), bus stations (33.3%), and churches (32%) (Table 2). The presence of positive oviposition sites was independent of the type of study site ($x^2$ = 12.571, df = 9, $P$ = 0.183). Data analyses for the container based on water capacity indicated greater frequency in small containers (46.4%), followed by medium (36.4%), and large-sized (13.4%), while atypical containers represented only 3.8% (Table 3). One hundred percent of small containers at bus stations and public libraries were oviposition sites. Medium and large containers were oviposition sites at tire repair shops (50.0 and 31.3%), recreational sites (50.0 and 16.7%), junk yards (36.7 and 20.0%), and schools (39.6 and 13.2%), respectively (Table 3). Statistical analyses indicated no significant relationship between the size of the oviposition site and the nonresidential surveyed site ($x^2$ = 0.012, df = 9, $P$ = 0.01).

Male and female mosquitoes were collected at 133 (94.3%) of the 141 nonresidential sites sampled. *Ae. aegypti* were at 100% of government buildings, commercial businesses, recreational sites, and public libraries (Table 2). This was followed by schools (96.9%), churches (92%), factories (90%), tire repair shops (90%), junk yards (83.3%), and bus stations (66.7%). Schools were sites with many *Ae. aegypti*, with a mean of 51.5 (± 65.0) including 21.6 (± 30.4) females and 30 (± 35.2) males, respectively. Adult mosquitoes were always collected at the rest of the sampled sites with the following mean numbers: recreational sites (22.4 ± 8.6), churches (20.2 ± 17.3), tire repair shops (19.7 ± 25.5), factories 18.1 ± 14.9), public libraries (18.0 ± 14.0), junk yards (15.9 ± 18.6), commercial businesses (15.6 ± 13.9), government buildings (15.4 ± 15.1), and bus stations (3.3 ± 2.9). Overall gender distribution was 57.5% males and 42.5% females at all sampling sites.

**Indoor-use Patterns of *Ae. aegypti* at Nonresidential Sites.** Pooled data from all sampled sites demonstrated that most adult female and male (42.8%) *Ae. aegypti* preferred resting in bathrooms (Table 4). Offices and classrooms were resting sites for 27.8 and 14.3%, respectively. The remaining indoor use by mosquito adults was: storage rooms 6.1%, cafeterias 4.6%, other rooms 2%, libraries 1.5%, workshops 0.5%, and auditoriums 0.4%. Statistical analysis by ANOVA indicated male and female *Ae. aegypti* preferred using classrooms and bathrooms as resting sites at nonresidential sites of Monterrey, Mexico.

Table 2. Summarized Results of the Entomological Surveys at Nonresidential Sites, in the Metropolitan Area of Monterrey, Nuevo Leon, Mexico (August 2009-November 2010). No mosquitoes were positive for dengue virus.

| Sampling site | n | % | Oviposition sites and % | Positive oviposition sites and % | Sites with adult Ae. aegypti and % | Mean total number ± SD adult Ae. aegypti | Mean ± SD female Ae. aegypti | Mean ± SD male Ae. aegypti |
|---|---|---|---|---|---|---|---|---|
| School | 33 | 23.4 | 28 (88.8) | 15 (45.5) | 32 (96.9) | 51.5 ± 65.0 | 21.6 ± 30.4 | 30.0 ± 35.2 |
| Church | 25 | 17.7 | 18 (72) | 8 (32.0) | 23 (92.0) | 20.2 ± 17.3 | 10.0 ± 10.5 | 10.2 ± 7.7 |
| Tire repair shop | 10 | 7.1 | 10 (100) | 4 (40.0) | 9 (90.0) | 19.7 ± 25.5 | 8.6 ± 14.3 | 11.1 ± 11.7 |
| Factory | 10 | 7.1 | 7 (70) | 6 (60.0) | 9 (90.0) | 18.1 ± 14.9 | 6.4 ± 6.3 | 11.7 ± 8.9 |
| Commercial business | 23 | 16.3 | 16 (69.6) | 11 (47.8) | 23 (100.0) | 15.6 ± 13.9 | 5.3 ± 6.2 | 10.3 ± 8.7 |
| Bus station | 3 | 2.1 | 2 (66.7) | 1 (33.3) | 2 (66.7) | 3.3 ± 2.9 | 1.7 ± 1.5 | 10.6 ± 11.5 |
| Junk yard | 12 | 8.5 | 12 (100) | 9 (75.0) | 10 (83.3) | 15.9 ± 18.6 | 5.3 ± 7.1 | 10.6 ± 11.5 |
| Public library | 7 | 5.0 | 3 (42.9) | 3 (42.9) | 7 (100.0) | 18.0 ± 14.0 | 7.1 ± 6.0 | 10.9 ± 8.8 |
| Recreational site | 8 | 5.7 | 6 (75) | 7 (87.5) | 8 (100.0) | 22.4 ± 8.6 | 10.4 ± 6.3 | 12.0 ± 3.9 |
| Government building | 10 | 7.1 | 10 (100) | 4 (40.0) | 10 (100.0) | 15.4 ± 15.1 | 9.6 ± 9.8 | 5.8 ± 5.7 |
| Total | 141 | 100 | 112 (79.4) | 68 (48.2) | 133 (94.3) | | | |

Table 3.  Number (and %) of Oviposition Sites by Size Category Observed in Survey at Nonresidential Sites, in the Metropolitan Area of Monterrey, Nuevo Leon, Mexico (August 2009-November 2010)

| Sampling site | Oviposition site category* | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | Small | | Medium | | Large | | Atypical | |
| School | 24 | (45.3) | 21 | (39.6) | 7 | (13.2) | 1 | (1.3) |
| Church | 17 | (50.0) | 12 | (35.3) | 4 | (11.8) | 1 | (2.9) |
| Tire repair shop | 3 | (18.7) | 8 | (50.0) | 5 | (31.3) | 0 | (0) |
| Factory | 6 | (37.5) | 5 | (31.3) | 2 | (12.5) | 3 | (18.7) |
| Commercial business | 16 | (53.3) | 10 | (33.3) | 2 | (6.7) | 2 | (6.7) |
| Bus station | 2 | (100.0) | 0 | (0) | 0 | (0) | 0 | (0) |
| Junk yard | 12 | (40.0) | 11 | (36.7) | 6 | (20.0) | 1 | (3.3) |
| Public library | 3 | (100.0) | 0 | (0) | 0 | (0) | 0 | (0) |
| Recreational site | 4 | (33.3) | 6 | (50.0) | 2 | (16.7) | 0 | (0) |
| Government building | 10 | (76.9) | 3 | (23.1) | 0 | (0) | 0 | (0) |
| Total | 97 | (46.4) | 76 | (36.4) | 28 | (13.4) | 8 | (3.8) |

*see Table 1


Table 4.  Use of the Indoors by Adult *Aedes aegypti* at Nonresidential Sites in the Metropolitan Area of Monterrey, Nuevo Leon, Mexico (August 2009-November 2010)

| Room | Total females and males | % of total | Female | | Male | |
|---|---|---|---|---|---|---|
| | | | Number | (%) | Number | (%) |
| Bathroom | 1,088 | 42.8 | 430 | 38.5 | 658 | 46.3 |
| Office | 707 | 27.8 | 288 | 25.8 | 419 | 29.4 |
| Classroom | 362 | 14.3 | 185 | 16.6 | 177 | 12.4 |
| Storage | 154 | 6.1 | 87 | 7.8 | 67 | 4.7 |
| Cafeteria | 116 | 4.6 | 66 | 5.9 | 50 | 3.5 |
| Other room | 50 | 2.0 | 28 | 2.5 | 22 | 1.5 |
| Library | 37 | 1.5 | 18 | 1.6 | 19 | 1.3 |
| Workshop | 15 | 0.5 | 9 | 0.8 | 6 | 0.4 |
| Auditorium | 11 | 0.4 | 6 | 0.5 | 5 | 0.4 |
| Total | 2,540 | 100 | 1,117 | 100 | 1,423 | 100 |


**Summary of Mosquito Collection at Nonresidential Sites.**  The 141 nonresidential sites sampled in Monterrey, Mexico produced 41.9% *Ae. aegypti* of the total 8,577 aspirated mosquitoes.  One-hundred and twenty-eight individuals (0.6%) of the potential dengue virus vector *Aedes albopictus* (Skuse) were also collected (Table 5).  As expected, the species with the most mosquitoes collected at nonresidential sites was *Culex quinquefasciatus* (Say) (55.8%).  Other Culicidae collected were *Psorophora cyanescens* (Coquillett) (0.5%) and *Anopheles quadrimaculatus* (Say) (0.1%).

**Dengue Virus Infection in *Ae. aegypti* Females.**  We processed 221 mosquito pools containing 1,532 *Ae. aegypti* females for the presence of DENV RNA.  None of the pools was positive for DENV RNA.  Field infection by dengue fever was not found in *Ae. aegypti* females collected at nonresidential sites during the study period of 2009-2010.

Table 5. Adult Mosquitoes Collected at Nonresidential Sites in the Metropolitan Area of Monterrey, Nuevo Leon, Mexico (August 2009-November 2010)

| Environment and species | Total females and males | Female Number | Female % | Male Number | Male % |
|---|---|---|---|---|---|
| Indoors | | | | | |
| *Aedes aegypti* | 2,540 | 1,110 | 65.8 | 1,430 | 59.8 |
| *Aedes albopictus* | 24 | 8 | 0.5 | 16 | 0.7 |
| *Anopheles quadrimaculatus* | 1 | 0 | 0 | 1 | <0.1 |
| *Culex quinquefasciatus* | 1,511 | 568 | 33.7 | 943 | 39.4 |
| Total | 4,076 | 1,686 | 100 | 2,390 | 100 |
| Outdoor (backyard/patio) | | | | | |
| *Aedes aegypti* | 1,061 | 422 | 28.6 | 639 | 21.1 |
| *Aedes albopictus* | 104 | 48 | 3.3 | 56 | 1.9 |
| *Anopheles quadrimaculatus* | 8 | 4 | 0.3 | 4 | 0.1 |
| *Culex quinquefasciatus* | 3,283 | 968 | 65.6 | 2,315 | 76.5 |
| *Psorophora cyanescens* | 45 | 33 | 2.2 | 12 | 0.4 |
| Total | 4,501 | 1,475 | 100 | 3,026 | 100 |

## Discussion

To date, lack of an efficient tetravalent dengue vaccine has hampered efforts to stop the spread of the disease into endemic developing countries. Currently prevention and control of local and regional outbreaks rely on rapid control of adult vectors and source reduction. However, despite huge amounts of money spent by government dengue control programs, epidemics occur every year. Even when failure of dengue control may be understood as a multifactorial pathogen-host phenomenon, increased control of vector populations would substantially impact morbidity and mortality rates. The household has been taken for granted as the only contact point between *Ae. aegypti* and humans.

The home environment plays a major role in dengue transmission; consequently, control strategies are focused on the endophilic behavior of female *Ae. aegypti*. However, poor policies of trash disposal and expanded urbanization simultaneously have increased the numbers of containers for *Ae. aegypti* oviposition sites. These nonresidential sites are areas of underestimated risk for infectious mosquito bites. Our results showed positive oviposition sites and adult mosquitoes infesting 48.2 and 94.3% of nonresidential sites, characterized as places with temporary human concentrations. School children would be exposed to the blood-feeding habits of hungry female mosquitoes during the morning as recently documented in Yucatan, Mexico (García-Rejón et al. 2011). A change in *Ae. aegypti* sites also has been reported in India where there were container indices of 28.3% in schools and 45.1% in hospitals (Sharma et al. 2001). Similar findings used ovitraps in hospitals in Brazil (Carvalho-Leandro et al. 2010). A study in Iquitos, Peru, sampled schools, factories, ports, public markets, petrol stations, commercial zones, and airports and found pupal and adult *Ae. aegypti* at most sites. Commercial areas, industries, schools, recreational sites, government buildings, and farms had <56 pupae per hectare (Morrison et al. 2006). The container index at 11 airports and six seaports sampled in India had increased over the years at all sites (Gill et al. 2001).

The poikilothermic nature of *Ae. aegypti* correlated with adapted temperature and humidity gradients in bathrooms preferred as resting sites (Clements 1996). The same indoor-use patterns in classrooms were reported in schools in Yucatan, Mexico (García-Rejón et al. 2011). This pattern was expected considering the species prefers human blood, which increases transmission of dengue virus (Scott et al. 1993). In addition to favorable in situ physical variables, concentrations of humans at nonresidential sites are major attractant factors for female *Ae. aegypti*. Daytime shifts of overcrowded workers in factories are a blood source for hematophagous mosquitoes. Similar vector-human contact may be explained for temples, public libraries, commercial shops with open doors, as well as government offices during work-week days. Moreover, sites with people gathering for daytime activities are synchronized with the bimodal, early morning and evening biorhythm of *Ae. aegypti* feeding (Chadee 1988).

Epidemiological rates on the age distributions for 15-45-year olds have shown an increase in dengue fever and dengue hemorrhagic fever in Mexico during the last decade (CENAVECE 2012). The 15-45-year old group, e. g., students and workers, would include people who spend several hours during the day in activities away of home. Under this scenario, transmission of dengue would probably be occurring at some of the nonresidential and/or public sites. Dengue infections in households with few mosquitoes were observed in Rio de Janeiro, Brazil (Honório et al. 2009). The study site was a slum characterized by concentrated intense commercial activity, schools, and a bus station. These authors highlighted the important role in transmission of dengue in public spaces where human movement was intense and possibly more important than in households.

Although the study did not find dengue virus in field-collected *Ae. aegypti* females nor was a sera-survey done, perhaps not found dengue virus in mosquitoes collected, because the study period were scarce rainfall, besides dengue outbreaks were limited. We believe it is only a matter of time to demonstrate that nonresidential sites are contributing to local outbreaks of dengue. Dwelling land encompasses 50% of the Monterrey city surface, leaving the rest to public sites, industrial parks, schools, and vacant lots (INEGI 2010b). However, the misleading concept of "house indices" coined in the policy of Mexico health regulations (NOM-032-SSA2-2010) is interpreted to apply vector control mostly in households and leaving unattended the enormous nonresidential surfaces. Further studies of epidemiology and entomology are required to assess transmission of dengue virus away from households.

## Acknowledgment

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