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Mosquito Infestation and Dengue Virus Infection in *Aedes aegypti* Females in Schools in Mérida, México

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Abstract. We determined abundance of *Aedes aegypti* mosquitoes and presence of dengue virus (DENV) in females collected from schools in Mérida, México, during 2008 and 2009. Backpack aspiration from 24 schools produced 468 females of *Ae. aegypti* and 1,676 females of another human biter, *Culex quinquefasciatus*. *Ae. aegypti* females were collected most commonly from classrooms followed by offices and bathrooms. Of these females, 24.7% were freshly fed. Examination of 118 pools of *Ae. aegypti* females (total of 415 females) for presence of DENV RNA produced 19 positive pools (16.1%). DENV-infected pools were detected from 11 (45.8%) of 24 schools and came from different room types, including classrooms, offices, and bathrooms. The overall rate of DENV infection per 100 *Ae. aegypti* females was 4.8. We conclude that schools in Mérida present a risk environment for students, teachers, and other personnel to be exposed to mosquitoes and bites of DENV-infected *Ae. aegypti* females.

INTRODUCTION

The arbovirus vector *Aedes aegypti*, which transmits viruses causing important human diseases including dengue, yellow fever, and chikungunya, is closely associated with indoor and peridomestic environments.[1] Immatures can be found in a wide range of containers located indoors (vases, flower pots, etc.) or in backyards or other peridomestic settings (bottles, cans, buckets, tires, drums, water storage tanks, etc.).[2] The female, which almost exclusively bites humans,[3–5] most commonly feeds and rests indoors and if there are larval development sites available indoors, may not even venture outside to lay eggs.[6]

Indoor and peridomestic use patterns of the adult stage of *Ae. aegypti* have been examined in numerous studies (reviewed by García-Rejón and others[7]). Home environments have been shown to harbor large numbers of *Ae. aegypti* females.[5,8–10] Furthermore, dengue virus (DENV)-infected *Ae. aegypti* females were reported in the home environment in Southeast Asia[11–20] and the Americas.[7,21–27] For example, we recently showed that DENV-infected *Ae. aegypti* females are commonly found in the homes of laboratory-confirmed dengue patients in Mérida, México, up to 4 weeks after onset of symptoms.[7]

Although it is well-understood that the home is an important risk environment for exposure to *Ae. aegypti* females and DENV, there is a lack of knowledge of the potential epidemiological significance of other indoor environments where people congregate, such as schools, work places, hospitals, and business areas. Schools have commonly been associated with presence and sometimes with high abundance of *Ae. aegypti* immatures,[28–36] but few published studies have presented data on indoor infestation of schools with *Ae. aegypti* adults.[21,34,37] In the Americas, *Ae. aegypti* females were collected from 63% of examined schools in Valle del Cauca State, Colombia, and abundances of *Ae. aegypti* adults on school premises in Iquitos, Peru, reached 6.1/ha in the winter.[21,34] In contrast, a study from Thailand reported collection of very few *Ae. aegypti* adults from schools (total catch of eight *Ae. aegypti* females from 11 schools), with none of the females testing positive for DENV RNA.[37]

We present here a follow-up study to our previous work on DENV-infected *Ae. aegypti* females in homes in Mérida, and we now shift the focus to the potentially very important but poorly studied indoor environment of the school. The goals of the study were to (1) document infestation of schools by *Ae. aegypti* and other mosquitoes, (2) determine use patterns by key mosquito species of different room types in the schools, and (3) show the presence of DENV-infected *Ae. aegypti* females in schools.

MATERIALS AND METHODS

Study environment. Studies were conducted in the city of Mérida (population ~ 800,000) in the Yucatan peninsula of southern México. The flat and low Yucatan peninsula (elevation range = 0–250 m above sea level) has a bedrock dominated by limestone and is characterized by a subtropical climate. Mean monthly maximum temperatures in Mérida range from 29°C in December to 34°C in July, and the majority of the rainfall occurs from May to October, with a peak from June to September (data from Comision Nacional del Agua weather station at the Mérida airport). *Ae. aegypti* adults and dengue cases may occur throughout the year in Mérida, but mosquito abundance and numbers of dengue cases typically peak from July to October.[7,38]

Mosquito collection. Adult mosquitoes were collected from 24 schools in the southern part of Mérida from October 2008 to December 2009. Schools included 5 kindergartens, 14 elementary schools, 2 junior high schools, 2 high schools, and 1 college. Twenty-one schools were sampled during October 13–29, 2008, and 18 schools were sampled again during December 2–13, 2008. Three additional schools were sampled in early December of 2008. All 24 schools were then sampled again from November 25 to December 11, 2009. School locations were georeferenced using a global positioning system (GPS) receiver (Garmin, Salem, OR), and mosquito collection environments were classified as follows: bathroom, classroom (including computer rooms and laboratories), office, storage room, other room (including libraries, workshops, kitchens, etc), and outdoors.

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Mosquitoes were collected from the schools during 0800–1500 hours using Centers for Disease Control and Prevention (CDC)-style backpack aspirators.[8] Collected mosquitoes were separated by school, date, and collection environment. Indoor collection included aspiration from furniture, behind hanging clothes and curtains, and from dark and humid places where mosquitoes can be found resting. Outdoor collection focused primarily on areas directly adjacent to the school building. The length of time spent actively collecting per school varied with school size, but the overall time typically was in the range of 45 minutes to 1 hour. Mosquitoes were identified to species using stereo microscopes and published identification keys.[39,40]

Blood feeding status of females (Sella's stages) was determined by external examination of the abdomen.[41] Sella's stages include I (unfed: collapsed abdomen and ovaries occupying one-third of the abdomen), II (freshly fed: bright red blood and ovaries occupying two to three segments ventrally and four segments dorsally), III–IV (half-gravid: dark red blood and ovaries occupying four to five segments ventrally and six segments dorsally), V (sub-gravid: blood greatly reduced and dark in color and ovaries occupying most of abdomen), and VI–VII (gravid: blood completely digested or present only as a black trace or line). *Ae. aegypti* females were pooled by school, date, and collection environment and stored at −70°C before processing for presence of DENV by reverse transcription polymerase chain reaction (RT-PCR).

DENV detection from *Ae. aegypti* female pools. We processed 118 mosquito pools containing 415 *Ae. aegypti* females (range per pool = 1–13 females with the exception of four pools containing 14, 18, 18, and 31 females, respectively) for DENV identification by RT-PCR. Pooled females were triturated using sterile pestles and Eppendorf tubes in 0.6 mL cold minimum essential medium (MEM) containing 2% fetal bovine serum (FBS) (HyClone, Logan, UT) and antibacterial and antifungal antibiotics (100 U/mL penicillin, 100 μg/mL streptomycin, 0.25 μg/mL 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. Thereafter, 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). This was followed by RT-PCR–based DENV amplification using primers targeting the NS3 gene.[42] A second round of semi-nested PCR including the upstream consensus primer and DENV 1–4 serotype-specific primers[43] was used to determine DENV serotype. Amplification products were visualized on a 2% LE agarose gel (Promega Corp., Madison, WI) containing ethidium bromide.

Data analysis. Statistical analyses were carried out using the JMP statistical package.[44] Specific tests used are indicated in the text. Results were considered significant when $P < 0.05$.

RESULTS

Summary of mosquito collections in the schools. Collections of adult mosquitoes from Mérida schools from October 2008 to December 2009 produced a total of 7,964 specimens, including 6,033 *Culex quinquefasciatus*, 1,175 *Ae. aegypti*, 746 *Ae. taeniorhynchus*, 5 *Ae. trivittatus*, and 5 *Cx. interrogator* (Table 1). These collections included 468 females of *Ae. aegypti* and 1,676 females of another important human biter, *Cx. quinquefasciatus* (Table 1).

The percentage of schools from which *Ae. aegypti* females were collected was consistently high, ranging from 85.7% in December 2008 to 87.5% in November to December 2009 and 100% in October 2008 (Table 1). A similar pattern was seen for *Cx. quinquefasciatus* females, which were collected from 90.4% of schools in October 2008 and 100% of schools in December 2008 and November to December 2009. Most schools produced only a few *Ae. aegypti* females on a given sampling occasion (Table 2). However, 10 or more *Ae. aegypti* females were collected from a single school on 16 occasions (including from kindergartens, elementary schools, and junior high schools), with one school yielding as many as 39 females. With regards to the more abundant *Cx. quinquefasciatus*,

TABLE 1
Summary of mosquito collections from schools in Mérida during 2008 and 2009

	Females				Males			
Time period and species	No. collected	Percentage of total females for period	Range for individual schools	Percentage of schools with females	No. collected	Percentage of total males for period	Range for individual schools	Percentage of schools with males
October 2008*								
Aedes aegypti	217	32.6	1–32	100	322	28.1	1–39	100
Aedes taeniorhynchus	129	19.4	0–52	66.7	71	6.2	0–54	28.6
Aedes trivittatus	1	0.2	0–1	4.8	0	0	0	0
Culex interrogator	0	0	0	0	0	0	0	0
Culex quinquefasciatus	318	47.8	0–62	90.4	751	65.6	0–212	90.4
December 2008*								
Aedes aegypti	66	12.3	0–15	85.7	82	5.1	0–43	61.9
Aedes taeniorhynchus	6	1.1	0–3	14.3	0	0	0	0
Aedes trivittatus	0	0	0	0	0	0	0	0
Culex interrogator	0	0	0	0	0	0	0	0
Culex quinquefasciatus	466	86.6	1–144	100	1,515	94.9	0–566	90.5
November to December 2009*								
Aedes aegypti	185	12.0	0–39	87.5	303	12.2	0–136	95.8
Aedes taeniorhynchus	457	29.6	0–72	100	83	3.4	0–712	45.8
Aedes trivittatus	4	0.3	0–3	8.3	0	0	0	0
Culex interrogator	5	0.3	0–3	8.3	0	0	0	0
Culex quinquefasciatus	892	57.8	0–393	100	2,091	84.4	0–28	100

*Numbers of schools sampled were 21 in October 2008, 21 in December 2008, and 24 in November and December 2009.

TABLE 2
Numbers of *Ae. aegypti* and *Cx. quinquefasciatus* collected from schools in Mérida during 2008 and 2009

	October 2008				December 2008				November to December 2009			
	Ae. aegypti		*Cx. quinquefasciatus*		*Ae. aegypti*		*Cx. quinquefasciatus*		*Ae. aegypti*		*Cx. quinquefasciatus*	
School*	Females	Males	Females	Males	Females	Males	Females	Males	Females	Males	Females	Males
1 (H)	7	20	33	121	3	3	144	411	8	3	17	81
2 (K)	10	22	4	15	1	2	6	20	1	2	2	10
3 (K)	1	2	12	18	1	0	7	6	0	0	5	8
4 (E)	2	17	9	6	NS†	NS	NS	NS	1	1	3	9
5 (E)	9	10	53	212	0	0	4	8	6	9	13	83
6 (E)	6	5	0	3	2	0	2	0	18	1	4	10
7 (K)	15	28	2	3	2	5	2	8	0	4	2	4
8 (H)	1	4	3	16	NS	NS	NS	NS	2	5	12	10
9 (E)	2	2	4	17	0	0	4	7	2	6	23	133
10 (J)	15	29	18	35	3	0	18	34	12	5	7	16
11 (E)	28	39	7	3	10	43	10	33	7	1	17	43
12 (E)	2	1	51	32	1	0	5	5	5	3	130	551
13 (K–J)	22	33	26	52	1	6	18	51	39	55	34	48
14 (E)	9	17	4	41	5	3	8	14	9	13	19	44
15 (C)	2	7	62	108	5	1	118	566	21	13	393	712
16 (K)	2	3	0	0	0	0	1	0	4	8	11	23
17 (E)	8	13	11	36	NS	NS	NS	NS	1	3	15	12
18 (K)	18	26	8	14	2	7	42	108	3	2	6	13
19 (E)	32	22	1	0	5	5	2	8	5	10	14	60
20 (E)	8	10	4	7	5	3	6	14	6	3	17	29
21 (J)	18	12	6	12	15	1	16	60	12	15	28	89
22 (E)	NS	NS	NS	NS	1	1	6	8	1	2	12	29
23 (E)	NS	NS	NS	NS	3	2	22	17	22	136	91	52
24 (E)	NS	NS	NS	NS	1	0	25	137	0	3	17	22
Total	217	322	318	751	66	82	466	1,515	185	303	892	2,091

* School type denoted in parenthesis. K = kindergarten; E = elementary school; J = junior high school; H = high school; C = college.
† School was not sampled for mosquitoes for this time period.

20 or more females were collected from a single school on 16 occasions (including from kindergartens, elementary schools, and junior high schools), and a single school produced as many as 393 females (Table 2).

Indoor-use patterns of *Ae. aegypti* and *Cx. quinquefasciatus*. *Ae. aegypti* females were collected most commonly from classrooms ($N = 292$) followed by offices ($N = 65$), bathrooms ($N = 56$), storage rooms ($N = 36$), and other rooms ($N = 16$) (Figure 1). Outdoor collections produced three *Ae. aegypti* females. A similar indoor-use pattern was observed for *Ae. aegypti* males, with the exception that males were more abundant in bathrooms (Figure 1). *Cx. quinquefasciatus* females were collected most commonly from classrooms ($N = 647$) followed by bathrooms ($N = 559$), offices ($N = 157$), other rooms ($N = 127$), and storage rooms ($N = 113$) (Figure 2). Outdoor collections produced 73 *Cx. quinquefasciatus* females. A similar indoor-use pattern was seen for *Cx. quinquefasciatus*

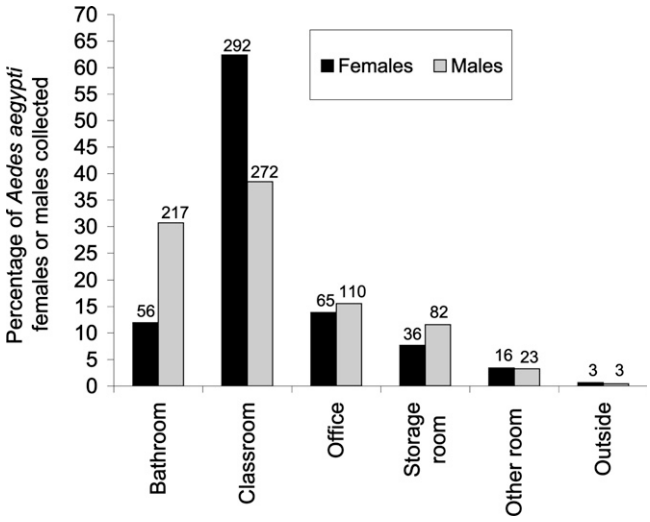

FIGURE 1. Percentages of *Ae. aegypti* females or males collected from different environments in Mérida schools during 2008 and 2009. Numbers above the bars indicate the total number collected by sex and environment.

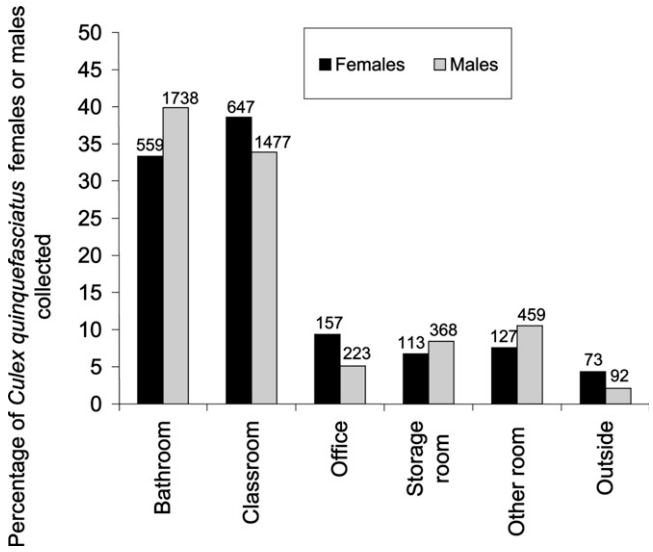

FIGURE 2. Percentages of *Cx. quinquefasciatus* females or males collected from different environments in Mérida schools during 2008 and 2009. Numbers above the bars indicate the total number collected by sex and environment.

males, with most specimens collected from bathrooms and classrooms (Figure 2).

The percentage of *Ae. aegypti* females collected by room type compared with *Cx. quinquefasciatus* females was higher for classrooms (62.4% versus 38.6%; contingency table analysis likelihood ratio: $\chi^2 = 83.80$, degrees of freedom [df] = 1, $P < 0.001$) and offices (13.9% versus 9.4%; $\chi^2 = 7.57$, df = 1, $P = 0.006$) but lower for bathrooms (12.0% versus 33.4%; $\chi^2 = 92.96$, df = 1, $P < 0.001$), other room types (3.4% versus 7.6%; $\chi^2 = 11.70$, df = 1, $P < 0.001$), and the outside environment (0.6% versus 4.4%; $\chi^2 = 20.33$, df = 1, $P < 0.001$). No significant difference was found for storage rooms (7.7% versus 6.7%; $\chi^2 = 0.50$, df = 1, $P = 0.48$).

Feeding status of *Ae. aegypti* and *Cx. quinquefasciatus* females. Collections included females of *Ae. aegypti* and *Cx. quinquefasciatus* with different blood-feeding status. For *Ae. aegypti*, we recorded 137 unfed females (29.9%; Sella's stage I), 113 freshly fed females (24.7%; Sella's stage II), 93 half-gravid females (20.3%; Sella's stages III–IV), 52 sub-gravid females (11.3%; Sella's stage V), and 63 gravid females (13.8%; Sella's stages VI–VII). For *Cx. quinquefasciatus*, we collected 1,027 unfed females (70.5%; Sella's stage I), 64 freshly fed females (4.4%; Sella's stage II), 79 half-gravid females (5.4%; Sella's stages III–IV), 39 sub-gravid females (2.7%; Sella's stage V), and 247 gravid females (17.0%; Sella's stages VI–VII).

Notably, *Ae. aegypti* females collected from the schools were more than two times as likely to have fed compared with *Cx. quinquefasciatus* females (70.1% versus 29.5% of females classified as Sella's stages II–VII, respectively; $\chi^2 = 238.82$, df = 1, $P < 0.001$). Additional more detailed analyses showed that *Ae. aegypti* females compared with *Cx. quinquefasciatus* females were more likely to be freshly fed (24.7% versus 4.4%; $\chi^2 = 143.04$, df = 1, $P < 0.001$), half-gravid (20.3% versus 5.4%; $\chi^2 = 80.64$, df = 1, $P < 0.001$), or sub-gravid (11.3% versus 2.7%; $\chi^2 = 48.57$, df = 1, $P < 0.001$), whereas there was no significant difference for gravid females (13.8% versus 17.0%; $\chi^2 = 2.83$, df = 1, $P = 0.09$).

Dengue virus infection in *Ae. aegypti* females. We examined a total of 118 pools of *Ae. aegypti* females comprising a total of 415 females for presence of DENV RNA (Table 3). This produced 19 positive pools: nine from October 2008, one from December 2008, and nine from November to December 2009. The percentage of DENV-positive pools ranged from 20.9% in November to December 2009 to 3.7% in December 2008 (Table 3). When comparing the 18 schools that were sampled during each time period, we found that the DENV infection rate in *Ae. aegypti* females was highest in November to December 2009 (6.1 per 100 females) followed by October 2008 (4.7 per 100 females) and December 2008 (1.9 per 100 females) (Table 4).

Determination of DENV serotypes present in positive pools indicated a shift from DENV-1 in 2008 to DENV-2 and DENV-3 in 2009. In 2008, 9 of 10 positive pools contained DENV-1 (the remaining pool contained DENV-4). In 2009, we commonly recorded multiple serotypes from a single pool. Of the nine positive pools from 2009, four contained DENV-1, whereas all nine pools contained DENV-2 and eight pools contained DENV-3.

DENV-infected pools were detected from 11 (45.8%) of the 24 examined schools, including two kindergartens, five elementary schools, one junior high school, one high school, one college, and one school including kindergarten to junior high (Table 3). Furthermore, DENV-infected pools came from a variety of different school room types, including classrooms ($N = 6$), offices ($N = 5$), storage rooms ($N = 3$), other room

TABLE 3
Detection of DENV RNA from *Ae. aegypti* females collected from schools in Mérida during 2008 and 2009

School*	October 2008			December 2008			November and December 2009		
	Total females tested	No. of pools tested	No. of DENV-positive pools (%)	Total females tested	No. of pools tested	No. of DENV-positive pools (%)	Total females tested	No. of pools tested	No. of DENV-positive pools (%)
1 (H)	7	3	2 (66.7)	2	2	0 (0)	8	3	0 (0)
2 (K)	10	2	2 (100)	1	1	0 (0)	1	1	0 (0)
3 (K)	1	1	0 (0)	1	1	0 (0)	NT†	NT	NT
4 (E)	2	1	0 (0)	NS‡	NS	NS	1	1	0 (0)
5 (E)	9	2	2 (100)	NT	NT	NT	6	2	0 (0)
6 (E)	6	1	0 (0)	2	1	0 (0)	18	1	0 (0)
7 (K)	15	2	2 (100)	1	1	0 (0)	NT	NT	NT
8 (H)	1	1	0 (0)	NS	NS	NS	2	1	0 (0)
9 (E)	2	2	0 (0)	NT	NT	NT	2	1	0 (0)
10 (J)	15	3	0 (0)	3	1	0 (0)	12	5	3 (60.0)
11 (E)	28	4	0 (0)	10	3	0 (0)	7	3	1 (33.3)
12 (E)	2	2	0 (0)	1	1	0 (0)	5	2	1 (50.0)
13 (K–J)	22	4	1 (25.0)	1	1	0 (0)	NT	NT	NT
14 (E)	9	3	0 (0)	5	1	0 (0)	9	2	0 (0)
15 (C)	2	2	0 (0)	5	3	1 (33.3)	21	6	0 (0)
16 (K)	2	2	0 (0)	NT	NT	NT	4	2	0 (0)
17 (E)	8	3	0 (0)	NS	NS	NS	1	1	0 (0)
18 (K)	18	2	0 (0)	2	1	0 (0)	3	2	0 (0)
19 (E)	32	2	0 (0)	5	1	0 (0)	5	3	2 (66.7)
20 (E)	8	3	0 (0)	5	3	0 (0)	6	2	0 (0)
21 (J)	18	3	0 (0)	7	2	0 (0)	8	0	0 (0)
22 (E)	NS	NS	NS	1	1	0 (0)	1	1	0 (0)
23 (E)	NS	NS	NS	3	2	0 (0)	22	3	2 (66.7)
24 (E)	NS	NS	NS	1	1	0 (0)	NT	NT	NT
Total	217	48	9 (18.8)	56	27	1 (3.7)	142	43	9 (20.9)

* School type denoted in parenthesis. K = kindergarten; E = elementary school; J = junior high school; H = high school; C = college.
† No *Ae. aegypti* females were tested from this school and time period.
‡ School was not sampled for mosquitoes for this time period.

TABLE 4

Minimum infection rates and maximum likelihood estimates for infection rates of DENV for *Ae. aegypti* females collected from schools in Mérida that were included for all three sampling periods during 2008 and 2009

Time period	No. of females examined	No. of pools examined	No. of DENV-positive pools (%)	DENV infection rate per 100 females	
				MIR*	MLE (95% CI)†
October 2008	206	43	9 (20.9)	4.4	4.7 (2.5–8.3)
December 2008	51	23	1 (4.3)	2.0	1.9 (0.1–8.8)
November and December 2009	115	36	7 (19.4)	6.1	6.1 (2.9–11.1)
Total for 2008 and 2009	372	102	17 (16.7)	4.6	4.8 (3.0–7.3)

Data were based on 18 schools that were examined for all three time periods during 2008–2009.
* MIR = minimum infection rate per 100 females based on the assumption of a single infected female per infected pool.[64]
† MLE = Bias-corrected maximum likelihood estimate for infection rate per 100 females calculated with the Excel Add-In PooledInfRate, version 3.0[64,65]; 95% CI = 95% confidence interval.

types (N = 3), and bathrooms (N = 2) (Figure 3). The percentages of DENV-positive pools for different room types were 8.3% for bathrooms, 10.7% for classrooms, 25.0% for storage rooms, 29.4% for offices, and 37.5% for other room types (including libraries, kitchens, etc). However, because of the limited sample sizes, there were no significant differences among these room types.

DISCUSSION

The published literature contains minimal information about infestation of schools with adult *Ae. aegypti* and the risk for exposure to DENV-infected *Ae. aegypti* females in this important indoor environment where children congregate. To close this knowledge gap, we assessed entomological risk factors in schools in Mérida, México, to determine the potential epidemiological significance of schools for DENV transmission. Our study shows that students, teachers, and other personnel in Mérida schools are at risk for exposure to human-biting mosquitoes, especially *Ae. aegypti* and *Cx. quinquefasciatus*, as well as exposure to bites by DENV-infected *Ae. aegypti* females. Backpack aspiration of individual schools commonly produced > 10 *Ae. aegypti* females, and the overall rate of DENV infection per 100 *Ae. aegypti* females was as high as 4.8. Furthermore, DENV-infected pools of *Ae. aegypti* females came from a variety of different school room types, including classrooms, offices, storage rooms, and bathrooms. Thus, schools may serve as transmission nodes for DENV in Mérida, contributing to virus dispersal in the city during dengue outbreaks.

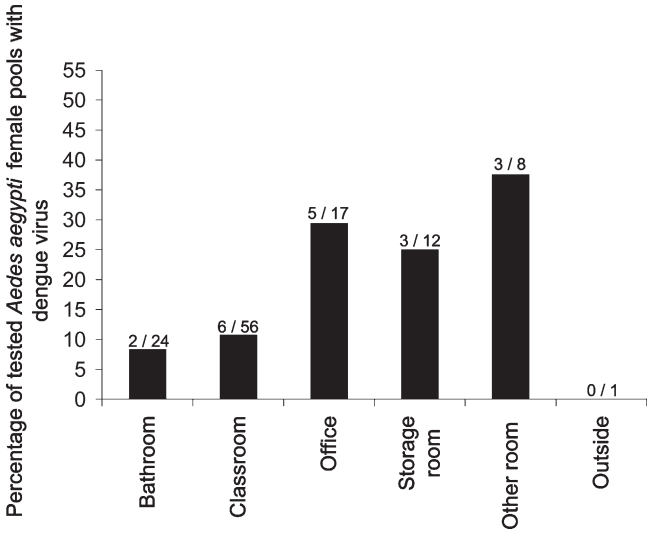

FIGURE 3. Percentages of tested pools of *Ae. aegypti* females with dengue virus RNA from different environments in Mérida schools during 2008 and 2009. Numbers above the bars indicate numbers of positive pools per total tested pools.

One weakness of the study was that *Ae. aegypti* females were examined for presence of DENV RNA in pooled samples. To determine rates of infected mosquitoes (those containing DENV) as well as potentially infectious mosquitoes (those with disseminated DENV infections), follow-up studies could assay individual mosquitoes and include assessment of virus dissemination to the head/salivary glands. Another issue that needs to be addressed in future studies is an abundance measure for adult mosquitoes in the schools. In the case of homes, which are readily sampled *in toto* with backpack aspirators, abundance of adults per examined home is a reasonable abundance measure. Schools, however, may differ dramatically in size and contain room types with distinct uses, such as classrooms, offices, and storage rooms. Based on the results of this study, perhaps the most appropriate abundance measure would be adults per examined classroom.

Schools in Mérida were infested with multiple species of human-biting mosquitoes that are capable of transmitting a wide range of pathogens. As expected from a previous study focusing on infestation of homes in Mérida by adult mosquitoes,[7] the species most commonly collected from Mérida schools was *Cx. quinquefasciatus* followed by *Ae. aegypti* and *Ae. taeniorhynchus*. With regards to pathogen transmission, *Ae. aegypti* is the primary vector of DENV in Latin America and also is capable of transmitting the viruses causing yellow fever and chikungunya if these viruses emerge/reemerge in this part of the world.[45–47] *Cx. quinquefasciatus* readily bites humans and is capable of transmitting several pathogens, including arboviruses (e.g., West Nile virus, St. Louis encephalitis virus, and Japanese encephalitis virus) and parasites (*Wuchereria bancrofti* that causes filariasis).[48–51] In the specific case of Mérida, *Cx. quinquefasciatus* may, in addition to its role as a nuisance biter of humans,[52] contribute as an enzootic vector in mosquito–bird West Nile virus transmission cycles and potentially, also as a bridging vector to humans of this virus. Furthermore, a novel flavivirus called T'Ho virus with as yet unknown pathogenicity to humans recently was detected from *Cx. quinquefasciatus* in Mérida.[53] *Ae. taeniorhynchus* feeds on mammals, occasionally including humans, and is capable of transmitting West Nile virus and eastern equine encephalitis virus.[50,54–56] As a side note, this mosquito also is considered an important vector of heartworm, *Dirofilaria immitis*, to dogs in Mérida.[57]

Both *Ae. aegypti* and *Cx. quinquefasciatus* females were collected from a variety of indoor environments in the schools, including classrooms, offices, bathrooms, and storage rooms. Perhaps the most notable result was that these two species appeared to exhibit different room type preferences. *Ae. aegypti* females were more prevalent, relative to the total numbers collected by species in the schools, in classrooms and offices, whereas *Cx. quinquefasciatus* females were more prevalent in bathrooms and the outdoor environment. Together with previous studies from home environments showing a preference of *Ae. aegypti* females for bedrooms,[7,58–61] this underscores the importance of a detailed understanding of indoor-use patterns by key mosquito vector species in different indoor environments to most effectively implement indoor-targeted control measures.

Because *Ae. aegypti* females feed almost exclusively on humans,[4,5] it was not surprising to find that most females collected from the schools, which provide an abundance of human hosts, had fed previously and that one-quarter of the females contained a fresh blood meal. In contrast, the majority of *Cx. quinquefasciatus* females were unfed, and very few (< 5%) contained a fresh blood meal. We speculate that *Cx. quinquefasciatus* may, in part, use the indoor school environment as a resting place when they are not actively seeking blood meals from their favored avian hosts. This is supported by a recent study from Mérida showing that *Cx. quinquefasciatus* females collected from peridomestic environments had fed predominantly on birds (accounting for 82% of blood meals) and only rarely on humans (7%).[52]

To the best of our knowledge, this is the first study to show that DENV-infected *Ae. aegypti* females can occur commonly in school environments. We found that (1) the overall rate of DENV infection per 100 *Ae. aegypti* females during the study period was as high as 4.8, (2) DENV-infected pools of *Ae. aegypti* females were detected from 11 (45.8%) of the 24 examined schools, and (3) DENV-infected females originated from a variety of different school environments, including classrooms, offices, storage rooms, and bathrooms. Our data on infected mosquitoes also indicated a shift in DENV serotypes circulating in the schools from DENV-1 during October–December 2008 to DENV-2 and DENV-3 during November–December 2009. Data for dengue patients in the Yucatan similarly showed an increase in the frequency of DENV-2 infections relative to DENV-1 from 2008 to 2009 (Loroño-Pino MA and Farfán-Ale JA, unpublished data).

Taken together, our results indicate that schools may play an important role in DENV transmission dynamics in Mérida. Schools may serve as DENV transmission nodes during dengue outbreaks, because both children and adults (teachers and other school staff) congregate in the schools on a regular basis and if they are infected with DENV in the schools, then can disperse the virus to their homes. The spatial range of this dispersal mechanism likely differs by school type. Kindergartens and elementary schools tend to have small recruitment areas and therefore will contribute primarily to local DENV dispersal by infected humans, whereas high schools and especially colleges have larger recruitment areas and thus, may contribute to DENV dispersal by infected humans at a larger spatial scale within a city.

Furthermore, some aspects of dengue virus infection dynamics in humans and the feeding behavior of *Ae. aegypti* females conspire to make the school a potentially very effective DENV transmission environment. First, humans may be infectious to feeding mosquitoes before the onset of fever, which typically occurs 2–7 days after the DENV infection event.[62] Second, *Ae. aegypti* females are nervous feeders that frequently take multiple blood meals from different human hosts to engorge sufficiently to produce an egg batch.[63] This provides a scenario where infectious children or adults are present in schools and where the biting habits of *Ae. aegypti* increases the likelihood of these infectious individuals being bitten. In addition, the propensity of *Ae. aegypti* to take multiple blood meals increases the likelihood that an infected and infectious mosquito will transmit DENV to more than one person (for example, children are easy targets in a classroom).

In conclusion, our study underscores the critical need for additional studies on the potential role of schools for DENV transmission in dengue endemic areas and highlights the need for improved indoor control of *Ae. aegypti* in schools in Latin America.

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