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Risks of Dengue Secondary Infective Biting Associated with *Aedes aegypti* in Home Environments in Monterrey, Mexico

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Abstract. Secondary dengue virus infections are a major risk for developing dengue hemorrhagic fever. Recent exposure to infectious bites of *Aedes aegypti* (L.) females in previously diagnosed dengue cases fulfills the epidemiological model of dengue hemorrhagic fever. A study was comprised of 357 (89.2%) dengue and 43 (10.8%) dengue hemorrhagic fever cases confirmed by laboratory tests and clinical manifestations. An entomological survey was done in homes and backyards. Concurrently, a questionnaire was used to assess the impact of health-promotion campaigns through knowledge of the vector and its epidemiological role. Seventy-six (28.4%) of the 268 (67.0%) total wet or dry oviposition sites were positive for the presence of larvae or pupae, while adult *Ae. aegypti* were found in 32 (8.0%). One hundred thirty-two (33%) householders who formerly had dengue fever or dengue hemorrhagic fever had knowledge of either larval or adult dengue vector stages. According to gender distribution, 145 (36.2%) and 14 (3.5%) of the males confirmed with cases of dengue and dengue hemorrhagic fever lived in houses with 17.9 and 2% of the *Ae. aegypti* larval and pupal habitats. Houses with females who had dengue and dengue hemorrhagic fever were 212 (53%) and 29 (7.3%), with containers with immature *Ae. aegypti* in 19.4 and 7%, respectively. Lack of sustainability of government-targeted health education campaigns is the major problem for involving communities in prevention and control of dengue.

Introduction

Fifty million dengue infections occur annually, and approximately 2.5 billion people live in dengue-endemic countries (WHO 2008). Four antigenically related

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serotypes (DENV-1-4) have the same transmission cycles and cause similar diseases. Infection with one serotype confers long-term protection to that serotype but no cross protection to other serotypes (WHO 1999). Clinical manifestations range from asymptomatic infection to dengue fever, an acute febrile disease with headache, muscle ache, and joint pain, to dengue hemorrhagic fever, a severe illness characterized by increased vascular permeability, which may lead to shock and death (Monath 1994, Kalayanarooj and Nimmannitya 2004). *Aedes aegypti* (L.) (Diptera: Culicidae), a domestic daytime-biting mosquito, is the major vector for DENV in Mexico (Garcia-Rejon et al. 2008). The landing periodicity of female *Ae. aegypti* shows a bimodal, morning-evening blood-feeding biorhythm (Chadee 1988). The mosquito is adaptable, and in urban environments deposit eggs in numerous artificial containers filled with clean water (such as tires, vases, animal water dishes, water storage tanks, and cans) (Gubler 1988, Scott et al. 2000).

Dengue epidemics are maintained consistently every year despite vector control measures based on insecticide treatments including ultra-low volume spraying to target adult mosquito populations and temephos applied to oviposition sites to impede maturation of the aquatic stages of the mosquito (PAHO 1994). Increased production of solid waste coupled with inadequate refuse collection services have contributed to failure of most Latin American countries to control *Ae. aegypti* in recent years (Gubler 1989). A common approach to this problem on the part of vector-control personnel has been to organize periodic “clean-up campaigns”, especially just before, or at the beginning of, the rainy season. Health-promotion campaigns through “Patio y techo limpios” (backyard and roof clean) media messages stimulate homeowners in Mexico to remove trash containers in backyards. However, dengue health education indicates the effectiveness of establishing knowledge of dengue recognition, prevention, and control methods, but does not necessarily change behavior (Khun and Manderson 2007). Partial results are frequently obtained in the community health education approach because even with adequate knowledge, people may resist household or personal practices to control the vector and see such as actions by government rather than personal responsibility (Whiteford 2000, Perez-Guerra et al. 2005). Lack of sustainability is the major problem for dengue health education, and consequently after a period of time, households become infested with *Ae. aegypti* (Lloyd et al. 1992). Important from the DENV-transmission standpoint are dwellings where dengue fever or dengue hemorrhagic fever cases were reported in the past but where both old and new oviposition sites have been maintained. Hypothetically, if a person infected in the past were bitten again by infected mosquitoes in his home environment, the chance of developing dengue hemorrhagic fever would greatly increase. DENV reinfection by any of the four serotypes is the immunological response of a host in acquiring dengue hemorrhagic fever (Halstead 2008).

We investigated household environments of individuals with previously confirmed dengue fever and dengue hemorrhagic fever. The household environment of this seropositive population was explored to evaluate the risk of receiving secondary infectious bites and therefore developing potential dengue hemorrhagic fever manifestations. The specific aims of this study were to determine the association of *Ae. aegypti* mosquitoes in houses and to assess knowledge of dengue vector recognition.

Materials and Methods

The metropolitan area of Monterrey was the study site. It is the second-largest industrialized city in Mexico and in the northeast, bordering with Texas, USA. The urban metropolitan area encompasses seven municipalities, with Monterrey the state capital and about 4 million inhabitants. Annual climate is hot and dry, with an average temperature of 23°C, although in some years it reaches 40°C during the summer and colder than 0°C in winter. Relative humidity averages 62%, with rain during August, September, and October. The average annual rainfall is about 500 mm. Located at an altitude of 537 m, the city is surrounded by mountains and foothills (Gobierno del Estado de Nuevo Leon 2011).

The study population was 400 households of individuals who experienced clinical dengue fever (89.2%) and dengue hemorrhagic fever (10.8%) during 2010. Addresses and names were provided by the State Laboratory of Nuevo Leon Health Department. Diagnostic testing for confirmed dengue cases was distributed as: 242 (60.5%) IgM-capture ELISA, 107 (26.8%) NS1, and 51 (12.8%) IgG-capture ELISA. The Health Department classified the cases according to clinical manifestations (WHO 2009) as: 357 (89.2%) dengue fever and 43 (10.8%) dengue hemorrhagic fever. A sample of blood from a patient was sent to the State Laboratory for testing at the time dengue was suspected. During the same patient visit, vector control personnel promptly applied hand-held ultra-low volume spray and 1.0% temephos to control larval and adult mosquitoes (NOM-032-SSA2-2010) in each of the 400 households. In addition, health educational information to promote behavioral change to identify dengue illness and vectors as well as eliminate larval habitats was given to the inhabitants of the house.

A team of entomologists visited each household to conduct a cross-sectional entomological survey during September through December 2011, which corresponds to the dengue season in Monterrey. Larval *Ae. aegypti* were sampled, and their presence or absence confirmed in all potential wet or dry oviposition sites containing standing water, and were recorded as larval densities. Sampling included frequently found domestic and backyard larval habitats such as flower vases, empty cans, discarded tires, and 55-gallon metal or plastic drums. Adult mosquitoes were collected using a CDC-style backpack aspirator (Clark et al. 1994). Collections were done between 0800 and 1400 hours in all rooms; 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 was 20-30 minutes. Indoor collection included aspiration on furniture, behind curtains, and in dark and humid places. Aspiration in the backyard included various stored items and vegetation. All larvae, pupae, and adult mosquitoes were transported separately to the Medical Entomological Laboratory at the University of Nuevo Leon for identification of species with the aid of stereoscope and microscope (Darsie and Ward 2005). Pupae were left to emerge as adults for identification and enumeration by species and gender. Also, at this first visit, a household questionnaire was administered to assess knowledge of adult and immature vector stages. Demographic data related to gender and age also were recorded.

Prior consent of homeowners was given before conducting entomological surveys. Parents provided permission for their children to participate and gave consent verbally before commencing the questionnaire.

Two-by-two contingency tables were used to evaluate the risk of former confirmed dengue fever and dengue hemorrhagic fever cases of being exposed in

their home environment to potential secondary infectious mosquito bites. The chi-square (χ^2) test was used to evaluate the dichotomous nature (presence or absence) of the study design (Zar 1999). Entomological and questionnaire data were individually analyzed for households with dengue fever or dengue hemorrhagic fever. Association between dengue classifications and adult presence and larval oviposition sites was calculated for statistical significance. Recognition of *Ae. aegypti* by home dwellers who experienced disease was also determined and tested by similar chi-square analysis. The two latter statistical associations were analyzed after dividing by gender, individuals in households with dengue fever or dengue hemorrhagic fever. A stratified age-interval analysis was used to correlate exposure of cases to vector populations. The data obtained were examined using the Statistical Package for the Social Science (SPSS) 19.0 (Chicago, IL).

Results

The sample size was 400 households of patients with confirmed dengue clinical illness and laboratory serology and molecular positive tests a year before. Patients enrolled in the study were 357 (89.2%) individuals classified as having had dengue fever and 43 (10.8%) dengue hemorrhagic fever. The surveyed households were predominantly in low- and middle-socioeconomic urban and suburban areas.

Pooled numbers of houses where individuals with former cases of dengue fever or dengue hemorrhagic fever were living indicated 268 (67.0%) with wet or dry *Ae. aegypti* oviposition sites (Table 1). Statistically, the presence and absence of former oviposition sites with the manifestation of dengue fever or dengue hemorrhagic fever were not significantly dependent ($\chi^2 = 0.931$, $df = 1$, $P = 0.335$). Seventy-six (28.4%) of the 268 (67.0%) total wet or dry oviposition sites were positive for the presence of larvae or pupae. However, households with dengue fever or dengue hemorrhagic fever did not have significantly more active larval habitats ($\chi^2 = 0.555$, $df = 1$, $P = 0.456$; $\chi^2 = 0.793$, $df = 1$, $P = 0.373$, respectively).

Table 1. Amounts of Oviposition Sites for *Ae. aegypti* in the Home Environments of People Who had Dengue

Dengue classification ^a	n	Wet or dry oviposition sites		Larval or pupal oviposition sites	
		Present	Absent	Present	Absent
DF	357	242 67.8%	115 32.2%	67 18.8%	175 49.0%
DHF	43	26 60.5%	17 39.5%	9 21.0%	17 39.5%
Total	400	268 67.0%	132 33.0%	76 28.4%	192 71.6%

^aDF = dengue fever, DHF = dengue hemorrhagic fever

In 32 (8.0%) of dengue pooled households, adult *Ae. aegypti* mosquitoes were aspirated during the study period although numbers were not large enough to demonstrate significant dependence ($\chi^2 = 2.320$, $df = 1$, $P = 0.128$) (Table 2). One hundred thirty-two (33%) of householders who formerly had dengue fever or dengue hemorrhagic fever had knowledge of either larval or adult dengue vector stages. As suspected, inadequate knowledge of the *Ae. aegypti* vector was not significantly associated with experience of either dengue fever or dengue hemorrhagic fever ($\chi^2 = 1.711$, $df = 1$, $P = 0.191$).

Table 2. Number of Households with and Knowledge of *Ae. aegypti* among Individuals Who had Dengue

Dengue classification ^a	n	Number of houses with adult <i>Ae. aegypti</i>				Knowledge of vector			
		Present		Absent		Present		Absent	
DF	357	26	7.3%	331	92.7%	114	32.0%	243	68.0%
DHF	43	6	14.0%	37	86.0%	18	41.9%	25	58.1%
Total	400	32	8.0%	368	92.0%	132	33.0%	268	67.0%

^aDF = dengue fever, DHF = dengue hemorrhagic fever

By gender distribution, 145 (36.2%) and 14 (3.5%) of males had confirmed cases of dengue and dengue hemorrhagic fever during 2010 (Table 3). Houses with females who had dengue and dengue hemorrhagic fever were 212 (53%) and 29 (7.3%), respectively. Wet or dry oviposition sites were found in home environments of 98 (67.6%) and 7 (50.0%) of males who had dengue and dengue hemorrhagic fever, respectively. Similarly, 144 (68.0%) and 19 (65.5%) of females were found with dengue and dengue hemorrhagic fever, respectively, in households with mosquito oviposition sites. However, the presence of larval containers in houses where individuals with dengue and dengue hemorrhagic fever were living was not significantly related to male or female sex ($\chi^2 = 1.041$, $df = 1$, $P = 0.308$). *Ae. aegypti* oviposition sites were found in households with 26 (17.9%) and 2 (14.3%) males and 41 (19.4%) and 7 (24.1%) females with dengue and dengue hemorrhagic fever, respectively. Computed 2-x-2 contingency tables did not significantly associate this pair of dichotomous variables ($\chi^2 = 0.243$, $df = 1$, $P = 0.622$). Of epidemiologically importance, more houses with adult *Ae. aegypti* mosquitoes were recorded for males and females with dengue fever; i. e., 10 (6.9%) and 16 (7.5%), respectively.

Table 3. Amounts of Oviposition Sites for *Ae. aegypti* in the Home Environments of Individuals by Gender Who had Dengue

Gender ^a	n	Wet or dry oviposition sites		Larval or pupal oviposition sites					
		Present	Absent	Present	Absent				
Male (DF)	145	98	67.6%	47	32.4%	26	17.9%	72	49.7%
Male (DHF)	14	7	50.0%	7	50.0%	2	14.3%	5	35.7%
Female (DF)	212	144	68.0%	68	32.0%	41	19.4%	103	48.6%
Female (DHF)	29	19	65.5%	10	34.5%	7	24.1%	12	41.4%
Total	400	268	67.0%	132	33.0%	76	28.4%	192	71.6%

^aDF = dengue fever, DHF = dengue hemorrhagic fever

Fewer adult mosquitoes were found in houses of males and females who had dengue hemorrhagic fever; i.e., 3 (21.4%) and 3 (10.3%), respectively. Statistically, the presence of *Ae. aegypti* was independent of being found in houses inhabited by men ($\chi^2 = 0.018$, $df = 1$, $P = 0.892$) or women ($\chi^2 = 0.011$, $df = 1$, $P = 0.916$). Only 54 (37.2%) of males who suffered dengue fever were able to

recognize the dengue vector, while 3 (21.4%) with experience of dengue hemorrhagic fever could identify it (Table 4). On the other hand, 62 (29.2%) of women who had had dengue had knowledge of *Ae. aegypti* while 13 (44.8%) of women with dengue hemorrhagic fever recognized it as the arthropod vector. Lack of knowledge of the major DENV-transmission route poses potential risks for secondary infectious biting of people in households with mosquitoes because the null hypothesis was not rejected for men ($\chi^2 = 0.705$, $df = 1$, $P = 0.401$) or women ($\chi^2 = 0.969$, $df = 1$, $P = 0.325$).

Table 4. Number of Households with and Knowledge of *Ae. aegypti* among Individuals Who had Dengue

Gender ^a	n	Number of houses with adult <i>Ae. aegypti</i>				Knowledge of vector			
		Present		Absent		Present		Absent	
Male (DF)	145	10	6.9%	135	93.1%	54	37.2%	91	62.8%
Male (DHF)	14	3	21.4%	11	78.6%	3	21.4%	11	78.6%
Female (DF)	212	16	7.5%	196	92.5%	62	29.2%	150	70.8%
Female (DHF)	29	3	10.3%	26	89.7%	13	44.8%	16	55.2%
Total	400	32	8.0%	368	92.0%	132	33.0%	268	67.0%

^aDF = dengue fever, DHF = dengue hemorrhagic fever

Table 5 shows results of four age groups and the parameters studied. Age intervals were defined based on exposure risk and school and employment activities, individuals with dengue fever and dengue hemorrhagic fever as well as male and female data were pooled in advance. Water-filled or dry oviposition sites were recorded for 92 (73.0%) households where 15-29 year olds lived. Houses owned by people aged 45 years or older totaled 23 (21.7%) with larval or pupal oviposition sites, while those with 1-14 year olds accounted for 8 (10.2%). Interestingly houses with the youngest group showed the fewest number of active *Ae. aegypti* oviposition sites.

Table 5. Amounts of Oviposition Sites for *Ae. aegypti* in the Home Environments of Individuals by Age Who had Dengue

Age (years)	n	Wet or dry oviposition sites				Larval or pupal oviposition sites			
		Present		Absent		Present		Absent	
1 - 14	78	48	61.5%	30	38.5%	8	10.2%	40	51.3%
15 - 29	127	92	73.0%	35	27.0%	30	24.0%	62	49.0%
30 - 44	89	54	61.0%	35	39.0%	15	17.0%	39	44.0%
45 or older	106	74	69.8%	32	30.2%	23	21.7%	51	48.1%
Total	400	268	67.0%	132	33.0%	76	28.4%	192	71.6%

Adult mosquitoes were collected in less than 7.0% of households with all the four age-stratified groups. In addition, more 15-29 year olds (64 or 50.0%) recognized the dengue mosquito than did individuals in other age groups (Table 6). Less than 30.0% of individuals in the other age groups recognized *Ae. aegypti* as the vector of dengue.

Table 6. Number of Houses with and Knowledge of *Ae. aegypti* by Age of Individuals Who had Dengue

Age (years)	n	Number of houses with adult <i>Ae. aegypti</i>				Knowledge of the vector			
		Present		Absent		Present		Absent	
1 - 14	78	9	11.5%	69	88.5%	18	23.0%	60	77.0%
15 - 29	127	9	7.0%	118	93.0%	64	50.0%	63	50.0%
30 - 44	89	6	6.7%	83	93.3%	27	30.3%	62	69.7%
45 or older	106	8	7.5%	98	92.5%	23	21.7%	83	78.3%
Total	400	32	8.0%	368	92.0%	132	33.0%	268	67.0%

Discussion

Once a person has experienced dengue in his/her life, the risk increases for developing dengue hemorrhagic fever/dengue shock syndrome. This is because dengue hemorrhagic fever is associated with secondary-type dengue infections and the occurrence of the immune-enhancement phenomenon, which is a major risk factor (Monath 1994, Halstead 2008). Many causes are related to sustaining such an increase in the frequency of secondary reinfection and dengue hemorrhagic fever in recent decades; i.e., demographic, cultural, environmental, and political changes that have been extensively reviewed (Monath 1994, Kuno 1995, Gubler 1998). However, the entomological risk factor or secondary DENV infectious biting has been inadequately studied in dengue hemorrhagic fever epidemiology. Our research demonstrated that people who had had confirmed dengue fever or dengue hemorrhagic fever 1 year before were exposed to a new mosquito generation thriving in their household environments. Surprisingly, although it was a dry year in Monterrey, Mexico, 28.4% of homes maintained active larval habitats (Table 1) and 8.0% adult *Ae. aegypti* populations (Table 2). Clearly, these latter results indicated that dengue health and promotion educational programs produced partial effectiveness in establishing adequate knowledge of dengue recognition, prevention, and control methods and low impact in changing household behavior (Khun and Manderson 2007). Ineffectiveness of community-based programs is explained in part because translation of the knowledge to practice varies in targeted health educational campaigns (Lloyd et al. 1992, Rosenbaum et al. 1995). For example, in Yucatan, Mexico, the Breteau index remained the same in the intervention group, while it increased significantly in the comparison group (Lloyd et al. 1992). Dwellers responded to prevention-source-reduction messages discarding small trash-like containers and allowed large water-filled larval habitats. Moreover, lack of sustainability remains a major obstacle for educational control efforts as documented in Puntarenas, Costa Rica, a city with 10 years of mosquito control

history. A cross-sectional entomological survey showed that 83.0% of 99 locations had habitats positive for mosquito larvae in the wet season and 5.1% of 508 in the dry season (Troyo et al. 2008).

We found that most (67.0%) of pooled householders with dengue fever or dengue hemorrhagic fever did not associate a mosquito bite with the cause of their dangerous and unpleasant disease. Thus, 68.0% of enrolled dengue householders would lack preventative behavior to avoid secondary potential infectious bites by *Ae. aegypti* (Table 2) and so develop severe dengue. Similarly, 58.1% of individuals with old cases of dengue hemorrhagic fever would ignore the role of the mosquito with high probabilities of receiving tertiary infectious bites. Statistically, vector knowledge of our study population was calculated to be independent for previously confirmed cases of dengue and dengue hemorrhagic fever.

Ae. aegypti reinfestations of treated household and non-residential premises in Mexico after a short period of time are not surprising. Field operational failures and poor knowledge translation of education and promotion health campaigns contribute to patchy vector reduction. For example, larval source control is attempted before the rainy season in house-by-house distribution of 1.0% temephos granules. As expected, adult mosquitoes from non-treated houses fly to the indoors and backyards of treated houses. Locked doors and closed windows do not allow penetration of insecticide mists during early evening ultra-low volume, vehicle-mounted spraying which, as a result, is judged as ineffective (Gubler and Kuno 1997). Furthermore, after more than two decades of *Ae. aegypti* control in Monterrey, a common, flawed interpretation resulting from health education campaigns is to consider dengue (disease) instead of mosquito (vector) biting as the major route of DENV transmission.

Cases of dengue hemorrhagic fever have dramatically increased in the last decade and extended endemic countries (Gubler 1998). Accepting secondary infections by either more lethal serotypes or genotypes as the main risk factor, this virological theory led us to understand that the bulk of previous dengue fever epidemiological statistics will contribute to future dengue hemorrhagic fever statistics. If the release of tetravalent dengue vaccine and pharmaceutical anti-virus drugs are delayed, current dengue fever:dengue hemorrhagic fever epidemiological proportions will approach each other. This research using a group of 400 households inhabited for 1 year with confirmed dengue fever and dengue hemorrhagic fever cases have generated results that correlate entomological factors as an additional explanation for secondary DENV infections. Clearly, stronger achievements in vector population reduction through improved chemical control and health education methods are urgently needed to lower dengue morbidity and mortality statistics.

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