RISK FACTORS FOR INFECTION DURING A SEVERE DENGUE OUTBREAK IN EL SALVADOR IN 2000

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Abstract. In 2000, El Salvador experienced a large dengue-2 virus epidemic with many severe cases. A seroepidemiologic survey was conducted in 106 randomly selected households (501 residents) in an affected community (Las Pampitas). The frequency of recent infection, documented by the presence of IgM antibodies or high-titer IgG antibodies to dengue virus, was estimated to be 9.8% (95% confidence interval [CI] = 5.8–13.7), of which at least 44% were secondary infections. The number of containers positive for Aedes mosquito larvae or pupae per 100 premises (Breteau Index) was 62 and the frequency of positive premises (House Index) was 36%; 33% (35 of 106) of the informants reported having taken action against mosquito larval habitats and 82% (87 of 106) reported having taken actions against adult mosquitoes. Recent infection was associated with the presence in the home environment of mosquito infested discarded cans (odds ratio [OR] = 4.30, 95% CI = 2.54–7.28), infested discarded plastic containers (OR = 3.98, 95% CI = 1.05–15.05), and discarded tire casings (OR = 2.57, 95% CI = 1.09–6.04). The population attributable fractions associated with these factors were 4%, 13%, and 31%, respectively. Our data suggest that targeted community cleanup campaigns, particularly those directed at discarded tires and solid waste, are likely to have the greatest impact on reducing the risk of dengue infection.

INTRODUCTION

Dengue is an acute viral disease caused by any one of four dengue viruses (DEN-1, -2, -3, and -4) and transmitted by Aedes mosquitoes.1,2 El Salvador eliminated Ae. aegypti during the yellow fever eradication campaigns of the 1950s and 1960s, but the mosquito was reintroduced in 1965.3,4 The first case of dengue, recorded in 1980, marked the beginning of an era of epidemics every 2–4 years. The epidemic of 2000, with 33,648; Department of San Salvador) had the highest incidence of laboratory confirmed dengue in the country (1.1 cases/1,000 persons). Las Pampitas (area = 0.45 km², population = 944), the community that reported the first dengue cases in Aguilares, was selected for the study. Systematic mosquito control efforts were implemented prior to the survey in the municipality, including Las Pampitas, in the form of public education announcements, ultra low-volume application of insecticides from spray trucks, and distribution of free larvicide (temephos) granules at the municipal health center.

Statistical considerations. A simple random sample of 107 homes was generated from a list of lots in the community. Data were analyzed using Epi-Info version (CDC, Atlanta, GA), SAS version 8.2 (SAS Institute, Cary, NC), and SASS-callable SUDAAN (RTI, Research Triangle Park, NC) software. For analyses at the individual level, responses were weighted by the inverse of the proportion of responders in the house to account for different household sizes and within-household participation rates. Furthermore, correlation among responses within households, as induced by the sampling design, was incorporated in computation of standard errors for confidence intervals (CIs) and P values. Therefore, both weighted percentages and raw numbers are provided in the results. Responses on the household level were a simple random sample and required no such weighting or adjustment, so standard methods for CI and P value computation were used. Logistic regression was used to investigate the effect (measured by odds ratios), of potential risk factors for recent infection. Individual response weighting (as described earlier in this report) and clustering within households was incorporated for coefficient estimation, model selection, and computation of CIs.

To test for clustering of recent infections within households, the chi-square-type statistic $\chi^2 = \sum_{i=1}^{h} (U_i - E_i)^2/E_i$, where $h$ is the number of households, was used; significance was determined using a Monte Carlo approximation to the randomization distribution of $\chi^2$. Values for the expected number of recent positive individuals in each household ($E_i$) were computed under the null hypothesis of no within-household clustering, based on the number of house-
hold members providing blood samples and using the observed rate of recent infections (9.8%, see Results). The $P$ value of the test was computed based on 9,999 realizations of $\chi^2$ under the null hypothesis.

**Household surveys.** Health educators administered standardized questionnaires, and physicians or nurses obtained blood samples, while MSPAS vector control personnel inspected premises for *Aedes* mosquito breeding sites. If no one was home at the time a house was first contacted, a repeat attempt was made later in the day or the next day; if that contact failed, the non-enrolled house immediately to the right of the original house was selected. One adult per household was asked questions about water storage; knowledge about dengue (transmission, clinical manifestations, mosquito control actions); the number of residents; and each resident’s age, sex, occupation, history of chronic disease, and symptoms in the two months before the survey. All residents were asked to provide finger stick blood samples. Two filter paper disks (1.27 cm in diameter) were saturated with blood, air-dried, sealed in a plastic bag, and refrigerated until tested. Only results from fully saturated filter paper disks were used for the analysis because partially saturated disks could give false-negative serologic results if the quantity of serum eluted was not sufficient for testing. The MSPAS vector control personnel used a premise inspection work sheet with predefined container types to determine their frequency and how many were infested with mosquito larvae. No specific identification of *Ae. aegypti* or *Ae. albopictus* was carried out. All questionnaire and premise inspection variables were included in the univariate analysis.

**Laboratory methods.** Serum samples were tested at the CDC Dengue Branch in San Juan, Puerto Rico for IgM antibody to dengue virus with an antibody-capture, enzyme-linked immunosorbent assay (MAC-ELISA). Samples were also tested for IgG antibody to dengue virus with an IgG-ELISA to determine whether the infection was primary or secondary. Because the measurement of IgM antibody may fail to diagnose about 5% of secondary dengue infections, testing by IgG-ELISA was also used to detect anamnestic IgG antibody responses to dengue virus indicative of recent secondary infections. All samples were tested in duplicate; if results were discrepant, testing was repeated.

**Case definitions.** Persons with recent dengue infection were IgM positive or had an IgG antibody titer $\geq$163,840. Persons with previous dengue infections were identified by the presence of low-titered IgG antibodies to dengue virus. Dengue-negative persons showed no detectable antibodies to dengue virus. A case of dengue-like illness (DLI) was based on the MSPAS case definition for surveillance (a person reporting having had in the two months prior to the survey fever and two or more of the following symptoms: headache, retro-orbital pain, bone and joint pains, chills, vomiting, rash, or hemorrhagic manifestations).

The incidence of DLI was defined as the proportion of survey participants who fulfilled the DLI case definition. The incidence of recent dengue infection in the two months prior to the survey was defined as the proportion of persons with evidence of recent dengue infection among the persons who provided an adequate sample for testing. The prevalence of IgG antibody was defined as the proportion of persons with detectable IgG antibody (any titer) to dengue virus among the persons who provided an adequate sample for testing.

The population-attributable fraction for a specific exposure was calculated as one minus the ratio of the incidence rate among the unexposed divided by the incidence rate in the total population.

The study plan was reviewed by MSPAS officials and the Human Subjects Coordinator at the National Center for Infectious Diseases, CDC, and determined to be a public health response that did not require further human subjects review. Interviewers ensured participants were informed about the investigation’s purpose, what participation involved, and that participation was voluntary, and that they had consented to participation.

**RESULTS**

In 2000, 3,248 cases of dengue with a laboratory diagnosis (0.5 cases per 1,000 persons) (Figure 1) were reported in El Salvador. Of these cases, 411 were reported as dengue hemorrhagic fever (DHF), and 26 died. For the municipality of Aguilarles, the attack rate was 1.8 per 1,000, and, in Las Pampitas, where the serosurvey was conducted, the rate was 6.4 per 1,000 (MSPAS surveillance data).

The participation rate in household interviews was 99% (106 of 107), which covered 39% (106 of 273) of the homes in the community. Entomologic surveys were completed in 99% (105) of the participating households. The 106 participating households had 501 inhabitants (53% of the population of Las Pampitas), and adequate blood samples were obtained from 74% (373).

**Household characteristics.** Of the informants, 69% (73) were the mother of the household, 22% (23) were the father, and 10% (9) were other household members. The median household size was five (range = 1–13). The majority (77%, 82 of 106) of the homes were constructed of mixed brick, concrete, and iron; 13% (14) of adobe; 5% (5) of sheet metal; and 5% (5) of cardboard and pasteboard. The community lacked piped water, but some houses had wells. Informants reported water was stored in 92% (97 of 106) of households, with some houses using more than one type of water storage container. The most frequent type of water storage containers mentioned by informants were concrete tanks (74%, 72 of 97), barrels (36%, 35), and a variety of others (33%, 32), such as buckets and pails. When asked about practices for protecting the stored water, only 31% (30 of 97) reported covering their water storage containers, with 7% (5 of 72) covering concrete tanks, 26% (9 of 35) covering barrels, and 66% (21

![Study Period](image)

**FIGURE 1.** Laboratory-positive dengue and dengue hemorrhagic fever cases in El Salvador, by week of laboratory diagnosis.
A total of 647 potential mosquito breeding containers were identified on the premises of 105 participating households, and 10% (66) were positive for \textit{Aedes} larvae or pupae. The median number of positive breeding containers per house was five (range = 1–40, interquartile range = 4–8). The number of positive containers per 100 premises (Breteau Index) was 62 and the frequency of premises positive for larvae or pupae (House Index) was 36%. Most (88%, 93 of 106) informants indicated that they had noticed mosquitoes in their homes in the previous two months.

Eight percent (8 of 106) of the informants reported that someone in their household had dengue within the previous two months, and 98% (104 of 106) believed that dengue was a disease that could be fatal. Eighty-five percent (90 of 106) of the informants said that mosquitoes transmit dengue, and 23% (21 of 90) reported taking actions that would eliminate larval habitats. When asked what potential measures could be taken to prevent dengue transmission, 69% (73 of 106) mentioned actions against larval habitats (i.e., putting larvicide in water containers, washing containers that store water, destroying containers that might hold water) and 45% (48 of 106) mentioned actions against adult mosquitoes (i.e., using smoke to repel mosquitoes, insecticide, mosquito coils, or mosquito nets). In contrast, when asked what actions they had taken to control dengue transmission, 33% (35 of 106) of informants reported having taken actions against larval habitats and 82% (87 of 106) having taken actions against adult mosquitoes.

\textbf{Individual characteristics.} The median age of residents included in the survey was 18 years (range = 27 days to 100 years), and half were females (49.5%, 248 of 501). Among persons more than six years old, 24% (90 of 380) were reported not having attended school. The estimate of the incidence of DLI was 10.5% (95% CI = 7.2–13.9; raw numbers = 53 of 501 persons), with highest incidence among those 60–69 years old (33%; 95% CI = 11–54; raw numbers = 6 of 18 persons) (Figure 2).

Among the 373 persons who provided adequate blood samples, the median age was 19 years, and 51.2% (191 of 373) were females. Children (0–9 years old) were less likely to provide adequate samples than persons more than nine years old (68.8%; 95% CI = 60.9–76.0 versus 86.6%; 95% CI = 82.5–90.0). Adequate blood samples were obtained from at least one person in 103 of the 106 participating households. The estimated incidence of laboratory-diagnosed recent dengue infection in Las Pampitas was 9.8% (95% CI = 5.8–13.7) compared with 10.5% for symptom-based DLI. Among households, 24% included at least one person with a recent infection, and the permutation test indicated \(P = 0.0002\) that there was clustering of cases within households. Thirty-six persons (age range = 3 months to 56 years, median = 12 years) had experienced a recent dengue infection, of which 56% (20) were diagnosed by IgM positivity only, 17% (6) by secondary IgG response only, and 28% (10) by both IgM positivity and secondary IgG response. Incidence of recent dengue differed by age (although without statistical significance), with the highest incidence age groups as follows: 0–9 years old = 13.7% (95% CI = 5.4–21.9), 10–19 years old = 13.4% (95% CI = 5.3–21.5), and 50–59 years old = 10.5% (95% CI = 0–24.7) (Figure 2). The incidence was 10.8% (95% CI = 4.9–16.8) for males and 8.8% (95% CI = 4.4–13.1) for females.

All 20 recent cases diagnosed by IgM positivity only also had low IgG titers, while 95.6% (95% CI = 93.3–97.9) of residents were positive for IgG antibodies. The seroprevalence of IgG did not vary significantly by 10-year age group (Figure 2). The proportion of secondary infections among recent cases was at least 44% (16 cases with secondary IgG titers), but may have been as high as 100% (those 16 cases plus the 20 with IgM positivity and low IgG titers). Two of the three infants tested (age range = 27 days to 4 months) and 78% (7 of 9) of children 12–23 months old were IgG positive. The mothers of the three infants were IgG positive. Neutralization tests were performed on the remaining serum samples (7 of the 8) from the children one year of age or less with low titers of IgG and no IgM antibodies to dengue virus.12 One had only DEN-2 neutralizing antibody, indicating previous infection with that serotype. Two cases were suggestive of previous DEN-2 infection, and the rest were uninterpretable. These results suggest that three (43%) of these seven children with negative IgM results and low IgG titers might have had recent dengue infection.

Of 42 residents who met the DLI case definition and provided adequate samples, 14% (raw numbers = 6 of 42) had a recent infection (95% CI = 2.7–25.6). The DLI was not significantly associated with recent dengue infection, neither in the entire sample nor in age-specific groups. Only 43% (raw numbers = 15 of 36) of the persons with recent infection reported any symptoms in the previous two months. No individual symptom was significantly associated with recent dengue infection.

\textbf{Factors associated with recent infection.} Using univariate analysis, we found that the following five factors were significantly associated with recent dengue infection in Las Pampitas: discarded containers, discarded tire casings, unspecified types of discarded containers, infested discarded plastic containers, and infested discarded cans (Table 1). Other factors significantly associated with illness were not considered further because there were too few observations of houses or persons for meaningful interpretation. The presence of discarded containers was not further included as a factor in the multivariate logistic regression analysis because it is a broad category that covers all the other factors. Using backward selection of the four remaining variables representing biologically and/or epidemiologically plausible risk factors for dengue, we found that three factors were significantly associated

\begin{figure}[h]
\centering
\includegraphics[width=0.5\textwidth]{fig2.png}
\caption{Comparison of the proportions of survey participants with IgG antibodies, recent infection, and dengue-like illness, by age group (in years) in Las Pampitas, Aguilares, El Salvador in 2000.}
\end{figure}
Factors associated with recent dengue infection by univariate analysis in Las Pampitas, Aguilares, El Salvador, 2000

<table>
<thead>
<tr>
<th>Factors</th>
<th>Cases (n = 36) with risk factor (%)</th>
<th>Non-cases (n = 337) with risk factor (%)</th>
<th>Odds ratio*</th>
<th>95% Confidence interval</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Discarded containers</td>
<td>32 (86.5)</td>
<td>202 (59.9)</td>
<td>4.28</td>
<td>1.32, 13.90</td>
<td>0.016</td>
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<tr>
<td>Discarded tire casings</td>
<td>17 (45.9)</td>
<td>70 (20.5)</td>
<td>3.30</td>
<td>1.38, 7.93</td>
<td>0.0079</td>
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<tr>
<td>Unspecified discarded containers</td>
<td>19 (52.8)</td>
<td>93 (27.3)</td>
<td>2.98</td>
<td>1.25, 7.11</td>
<td>0.0142</td>
</tr>
<tr>
<td>Infested discarded plastic containers</td>
<td>6 (16.2)</td>
<td>10 (2.9)</td>
<td>6.43</td>
<td>1.80, 23.00</td>
<td>0.0046</td>
</tr>
<tr>
<td>Infested discarded cans</td>
<td>2 (5.4)</td>
<td>5 (1.5)</td>
<td>3.85</td>
<td>1.47, 10.12</td>
<td>0.0067</td>
</tr>
</tbody>
</table>

* Weighted odds ratios account for household clustering.

with recent dengue infection: infested discarded cans, infested discarded plastic containers, and discarded tire casings on the premises (Table 2). The population-attributable fractions associated with each factor were 4% for infested discarded cans, 13% for infested discarded plastic containers, and 31% for discarded tire casings. These population-attributable fractions reflect the frequency of the incriminated breeding sites in the surveyed households. In particular, 17 discarded cans were distributed among 11% (12 of 105) of the houses and four of them were infested and distributed among 17% (2 of 12) of these houses; 47 discarded plastic containers were distributed among 25% (26 of 105) of the houses and seven of them were infested and distributed among 19% (5 of 26) of these houses; and 46 discarded tire casings were distributed among 26% (27 of 105) of the houses and 13 of them were infested and distributed among 22% (6 of 27) of these houses.

**DISCUSSION**

Our survey was conducted when only one-sixth of the cases had been reported in the community of Las Pampitas (reported incidence at that time was 1.1 per 1,000 persons), and likely provides an underestimate of incidence. Even so, our estimate of 98 per 1,000 was 15-fold higher than the incidence of clinical illness documented by the dengue surveillance system in Las Pampitas by the end of the epidemic, which was 6.4 per 1,000. Persons living in houses with infested discarded cans, infested discarded plastic containers, or discarded tire casings were more likely to have recent infection. Poor source reduction and solid waste disposal programs are contributing to high *Ae. aegypti* densities and dengue illness in Central America. In Las Pampitas, attributable risk calculations indicated that removal of tire casings would have the greatest public health impact. Tire casings are a solid waste item that has long been recognized as an important oviposition site for *Ae. aegypti* and *Ae. albopictus*. In the last two decades, several studies have investigated risk factors for dengue in affected communities, such as lower income neighborhoods, wood house construction, and the number of *Ae. aegypti* per person per house. Lack of a community potable water supply has been associated with increased larval indices. Identified dengue risk factors vary greatly depending on the location of the investigation, the circulating virus serotype and strain, human population density and previous exposure to specific serotypes, ambient temperature, humidity, and availability of oviposition sites. The minimum threshold of dengue vectors required to support the transmission of dengue has not been determined.

In Las Pampitas we found high levels of *Aedes* mosquito larval infestation, as indicated by the Breteau (62), House (36%), and Container (10%) indices. The high larval indices can be partially explained by community resources, knowledge, attitudes, and practices identified by the survey. Almost all (98%) of the 106 informants believed dengue could be fatal, most (85%) knew that it was transmitted by mosquitoes, and a majority (69%) mentioned possible actions against the aquatic stage of the mosquito as a way to control transmission. However, only one-third (33%) of the informants said they actually took action against the aquatic stage of the mosquito, and only 37% of the 97 persons who reported storing water reported covering their water storage containers. In contrast, 82% of respondents indicated taking action against the adult mosquito. Therefore, the study community had a high awareness about the characteristics and severity of dengue, but showed a lack of action towards preventing the emergence of the adult mosquito by their reported behaviors and the results of the entomologic survey. Based on the preliminary findings of this investigation, the MSPAS refocused its community education efforts towards eliminating the mosquito's aquatic stage.

The experience in Las Pampitas, if representative of other affected populations in the country, might explain why 12.7% (411 of 3,248) of the laboratory confirmed cases during the 2000 epidemic progressed to DHF. There was circulation of a virus strain with high epidemic potential (i.e., the DEN-2 Asian genotype strain of virus) and high prevalence of serologic markers of previous infection, both of which are important risk factors for severe dengue. The seroprevalence of IgG (96%, similarly high for all age groups) indicates intense dengue activity in Las Pampitas in previous years. During the 1990s, the MSPAS surveillance system identified all four dengue serotypes circulating in the country at different times, there were large epidemics in 1995 (DEN-3 and DEN-4) and 1998 (DEN-3), and there may have been underreporting of local outbreaks. The difficulty of accurate clinical diagnosis of dengue fever has been demonstrated in previous investigations, and reiterated in our survey, which found a low
14% of cases of dengue hemorrhagic fever have been described,
and 43% of recently infected patients. The accuracy of serologic tests for dengue is dependent on the interval between disease onset and blood sample collection, and the dynamics of antibody production and titer change. Optimal detection of recent cases requires serologic sampling at two or three points (early, middle, and late) in the epidemic. The high IgG seropositivity (78%) in one-year-old children and the identification of only DEN-2 neutralizing antibodies in those children suggests a very recent high incidence of dengue. It is therefore likely that IgM antibody had waned to the undetectable level and IgG had become detectable in some cases by the time of our survey, and they would have been considered previous infections.

Another study limitation is the necessary reliance on participant recall. Nevertheless, this study demonstrated the importance of solid waste in promoting the dengue epidemic in Las Pampitas. Although the community was knowledgeable about dengue and how to control the mosquito, reported actions and entomologic surveys suggest that the community's efforts on eliminating the larval stage of the mosquito were insufficient. Our data suggest that targeted community cleanup campaigns, particularly those directed at discarded tires and solid waste, are likely to have the greatest impact on reducing the risk of dengue infection in Las Pampitas and other similar communities throughout El Salvador.

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REFERENCES


