HUMAN AND MOSQUITO INFECTIONS BY DENGUE VIRUSES DURING AND AFTER EPIDEMICS IN A DENGUE–ENDEMIC REGION OF COLOMBIA

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Abstract. We conducted a study in a dengue–endemic area of Colombia to evaluate the dynamics of transmission of dengue viruses during and after epidemics. Information was simultaneously gathered about occurrence of infection in humans and mosquitoes every three months in four cities with endemic transmission. Viral isolation was confirmed in 6.7% of the persons and most were asymptomatic. Adult mosquito and larvae house indexes were not found associated with increased burden of disease. The only entomologic indicator related to dengue infection in humans was the pooled infection rate of mosquitoes. Aedes aegypti infection rates showed significant differences between the epidemic (10.68, 95% confidence interval [CI] = 7.04–15.62) and after epidemic periods of the study (6.15, 95% CI = 3.46–10.19). In addition, Ae. albopictus were also infected with dengue viruses. Increases in mosquito infection rates were associated with increases in human infection rates in the following trimester.

INTRODUCTION

Dengue is a major threat to public health in many regions of the tropics. The disease is a large burden to communities and health services that are severely affected during epidemics. The estimated number of cases of classic dengue fever worldwide is estimated to be 50–100 million. In addition, there are hundreds of thousands of cases of dengue hemorrhagic fever (DHF), the severe form of the disease.1 In Colombia, dengue re-emerged in the early 1970s, became endemic, and has caused periodic outbreaks with increasing numbers of cases of severe DHF; major outbreaks were reported in 1991, 1994, 1998, and 2001.2

Absence of an effective vaccine has lead to focusing control efforts on mosquito source reduction and active surveillance of disease for early detection of epidemic events. The development of these early warning systems requires detailed knowledge of dynamics of infection in humans and mosquitoes between and within epidemics, and the study of factors potentially influencing the origins of epidemics. Unfortunately, this process is poorly studied in many regions of the world.

Dengue virus serotypes DEN-1, DEN-2, and DEN-4 have been reported to circulate endemically in most areas of Colombia where disease is reported; DEN-3 was re-introduced into Colombia in 2001 after being absent for more than two decades.2,3 The geographic distribution of DEN-3 and the potential impact of this virus serotype on human morbidity has not been examined in this country.

Aedes aegypti is the main vector of dengue in Colombia, and vector density indexes correlate poorly with disease incidence.3,5 Furthermore, there is uncertainty about the potential effect on transmission of Ae. albopictus that was first introduced in the Amazon region of Colombia in 19986 and later on found on the Pacific coast in the city of Buenaventura.7

Most dengue infections in humans are asymptomatic and silent circulation of dengue virus among human and mosquitoes has been reported during interepidemic periods of the disease.8,9 Therefore, understanding the dynamics of dengue transmission before outbreaks may offer a window of opportunity for prevention. Surveillance of dengue transmission in Colombia is made based in a passive system through the notification of disease cases in humans and the survey for variations in Aedes larvae indexes. There is no information in the occurrence of silent transmission of dengue before epidemics, and control measurements are mainly imposed after an outbreak has already been established.

We conducted a prospective ecologic study in a dengue–endemic area of Colombia to evaluate the dynamics of infection by dengue viruses in humans and mosquitoes during and after epidemics, focusing on the relationship between mosquito infection rates, frequency of dengue infections in humans, and circulating dengue serotypes.

MATERIALS AND METHODS

Study area. The study was conducted in the state of Valle del Cauca in southwestern Colombia. This region has a tropical climate and two rainy seasons, usually from April to July and from September to December. Re-emergence of dengue fever in this region was reported in the 1970s and the first serologically confirmed outbreaks of the disease occurred in 1971. Since that time, the disease has shown a similar trend in other regions in the Americas with periodic outbreaks. The first cases of DHF were observed in 1989, and there has been an increasing trend in reports of the severe form of the disease; by 2001 more than 400 cases of DHF were reported in Valle del Cauca.2

We focused our attention on four cities in Valle del Cauca: Cali, Palmira, Tuluá, and Buenaventura. These four cities represented two different areas of Valle del Cauca. Cali, Palmira and Tuluá in the south central part of the state are located in the Cauca River valley, a tropical dry forest, and share similar conditions of altitude (approximately 900 meters above sea level), average maximum temperature (28°C), relative humidity (85%), and average rainfall (1,000–2,000 mm per year).10 Most of the inhabitants of these three cities are Mestizos. Buenaventura is located in a humid tropical forest zone on the Pacific coast. It is at the sea level, and has an average maximum temperature of approximately 32°C,
relative humidity > 90% and an annual rainfall of 8,000–12,000 mm. More than 70% of the population of Buenaventura is Afro-Colombian.

**Study design.** Reported cases of dengue from the state surveillance system were used to describe conditions of transmission before and during the prospective study. Information for the study was collected every three months between November 2002 and March 2004 in the four cities included (total = six visits). Neighborhoods in all cities were randomly selected among those with the highest numbers of dengue cases in the previous year. Information was simultaneously gathered at every visit about occurrence of infection in humans and mosquitoes.

**Infection in humans.** For evaluation of infection in humans, middle schools located in the study neighborhoods were invited to participate. Informed consent was obtained from parents or legal guardians of minors, and ethical clearance was attained from the Institutional Review Board of the Universidad del Valle School of Health. Based on the rosters of registered students, a random sample of 100–150 persons per city visit was selected. A blood sample was obtained from selected persons for viral isolation and serologic analysis, and information was collected with a questionnaire that included data on demographic variables, clinical symptoms of dengue-like disease, and previous episodes of disease. Infection in humans was diagnosed by virus isolation using the C636 cell line of *Ae. albopictus* and by immunofluorescent analysis using serotype-specific monoclonal antibodies (kindly provided by the Centers for Disease Control and Prevention [CDC], San Juan PR). Serologic evidence of recent infection was determined by detection of viral-specific IgM by an antigen capture enzyme-linked immunosorbent assay. All persons positive for viral isolation who met clinical criteria of the disease were defined as cases of symptomatic infection, and those who did not develop symptoms of disease, mainly fever, were defined as asymptomatic cases.

**Infection in mosquitoes.** Collection of adult mosquitoes and larvae in the same neighborhoods was carried using a multi-stage sampling technique in randomly selected blocks around middle schools included in the study and subsequently selected households. Mosquitoes were captured in both houses and schools using oral aspirators and entomologic nets for a standardized period of 15 minutes per household. Third- and fourth-stage larvae were collected from water containers inside the households.

After species identification, adult *Aedes* mosquitoes were processed according to sex and site of collection into pools ranging from 1 to 30 mosquitoes. These pools were frozen at −70°C for dengue virus detection by reverse transcription–polymerase chain reaction (RT-PCR). Viral RNA was detected by RT-PCR from cephalo-thorax of female *Aedes* mosquitoes according to procedures previously described. The RNA from each pool of mosquitoes was extracted using Trizol (Gibco-BRL, Gathersburg, MD) and cDNA was prepared by reverse transcription of approximately 1 μg of RNA using avian myeloblastosis virus reverse transcriptase (Promega, Madison, WI) and a reverse primer. Amplification of cDNA was conducted by a nested PCR using specific primers in the first round and primers specific for each of the four dengue serotypes in the second round. A glutamine synthetase mRNA that is constitutively expressed in *Aedes* was amplified by RT-PCR using 5’ and 3’ primers GGA CTA AGA GAA GAT GGC and GAG CTT GAT AGG TTG AGC, respectively (GenBank accession no. AF004351) and used as an internal control for RNA extraction.

**Statistical analysis.** Determinants of infection in humans at individual level were evaluated by simple and multiple logistic regressions. We evaluated the association between IgM antibodies and viral isolation with demographic variables (age, sex, and ethnicity), presence of symptoms, and previous episodes of the disease.

The proportion of households and schools with mosquitoes (adult and larvae) were estimated and the results were stratified by species, city, and visit number. Binomial confidence intervals (CIs) were calculated for all estimates and their trends were described. Comparisons were made between a period of high reported occurrence of dengue (epidemic) and a period of low incidence of dengue (after an epidemic).

The pooled infection rate of mosquito infection by dengue virus was calculated using maximum likelihood estimation (MLE) methods for unequal pool sizes. These methods estimate the infection rate and are more accurate and robust than the widely used minimum infection rate (MIR), which estimates the lower bound of the infection rate. The MLE for different pool sizes is iterative and requires computer implementation. We calculated point intervals and 95% CIs using the application provided by Dr. Brad Biggerstaff (CDC; PooledInfRate version 2.0 available from www.cdc.gov.gov/ncidod/dvbid/westnile/software.htm). We used bias-corrected MLE methods for point estimations and the skewness-corrected function to compute asymptotic confidence limits. We stratified mosquito infection rates by city and visit, and determined their association with the corresponding incidence of viral infection found in humans by linear regression.

**RESULTS**

Between 1996 and 2003, two outbreaks of dengue were observed in the study area in Colombia (Figure 1). The four cities included in the study contributed approximately 60% (34,821 of 58,076) of the total number of cases reported in Valle del Cauca during this period. The study began in 2002.
during a major dengue outbreak. We observed a significant increase in the number of cases of DHF and continued collecting data during a period of lower incidence of the disease. Cities included in the study showed a similar trend during the study period (Figure 2). The first three visits were conducted during the epidemic period, and subsequent visits (visits 4–6) were carried out in a period with a lower incidence of dengue after the epidemic.

Infections in humans. During the six visits in the four cities, 3,189 students between 7 and 20 years of age were examined. Sixty-six percent (2,109/3,189) were females, 90.7% were Mestizos, and 19.3% were Afro-Colombians, mostly from Buenaventura. Table 1 summarizes the number of persons enrolled per visit, some descriptive variables, and the main reported symptoms of dengue-like disease. No differences in presence of fever, rash, or hemorrhage were found between the first three visits (i.e., the epidemic) and the subsequent period.

Viral isolation was confirmed in 6.7% (215 of 3,189) of the persons tested. Most were asymptomatic and disease was documented only in 14% of the infected persons. However, infection was significantly associated with the presence of a skin rash (odds Ratio [OR] = 1.9, 95% CI = 1.2–3.1). In addition, infection was more frequent in Afro-Colombians (OR = 1.3, 95% CI = 1.0–1.8). No differences were found by sex or age for distribution of virus isolation.

We observed by detection of specific IgM that in contrast to viral isolation, only 1.9% (61 of 3,189) of the persons examined had developed an immune response against dengue virus during the study period. Although most (44 of 61, 72.1%) IgM-positive persons were negative for viral isolation, the presence of dengue-specific IgM was associated with fever (OR = 2.5, 95% CI = 1.2–5.3) in the week immediately before the test was performed, suggesting they were probable cases of dengue.

The virus serotype was not identified in 49 (22.8%) of the 215 samples in which dengue viruses were isolated. The remaining 166 samples had the following serotype distribution: DEN-1 (30 of 166, 18.1%), DEN-2 (75 of 166, 45.2%), DEN-3 (13 of 166, 7.8%), and DEN-4 (48 of 166, 28.9%). The frequency of dengue infections was not associated with disease incidence during and after the outbreak (6.1% versus 7.5%, respectively, Pearson’s $\chi^2 P = 0.10$). However, re-emerging serotype 3 and viral serotype 2 almost exclusively circulated during the last three visits of the study after the outbreak (visits 4–6).

Infections in mosquitoes. A total of the 4,739 households and 82 schools were visited during the six sequential visits for

<table>
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<th>No.</th>
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<th>Rash (%)</th>
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entomologic surveys. Table 2 summarizes the number of inspected households and the corresponding findings for adult mosquitoes. An average of 53.9% of the households contained adult mosquitoes and more than half of the mosquitoes were female *Ae. aegypti* (i.e., 38.6% of the households). Adult *Ae. albopictus* mosquitoes were found only in Buenaventura, although at a lower level of infestation (10.5% of the households had female *Ae. albopictus*). The corresponding indexes for schools were higher than those for households. An average of 85.4% of the schools had adult mosquitoes compared with 53.9% of the households (Pearson’s χ² = 32.1, *P* < 0.001) and 63.4% had female *Ae. aegypti* (Pearson’s χ² = 20.9, *P* < 0.001). In Buenaventura, 37.3% of the schools had female *Ae. albopictus* (Pearson’s χ² = 19.2, *P* < 0.001). The average proportion of houses with female *Ae. aegypti* mosquitoes during the first three visits (epidemic period) was not different from the proportion observed during the subsequent three visits of the study (38.6% versus 38.5%, respectively).

In addition, 9.6% of the households examined in all cities had larvae of *Ae. aegypti*, and *Ae. albopictus* was collected only in Buenaventura (household index [HI] = 8.0%). Table 3 summarizes HI values by city and visit. There were no differences in larva indexes between the epidemic (visits 1–3) and post-epidemic (visits 4–6) periods of observation (9.3% versus 9.8%, respectively; *P* = 0.539).

A total of 4,964 female mosquitoes composed of 4,628 *Ae. aegypti* and 336 *Ae. albopictus* were grouped by place (neighborhoods) and visit number into 322 pools (292 pools of *Ae. aegypti* and 30 pools of *Ae. albopictus*). Among *Ae. aegypti* pools, only 7 (2.4%) contained 1 mosquito, 88 (30.1%) contained 2–10 mosquitoes, 96 (32.9%) contained 11–20 mosquitoes, and 101 (34.6%) contained 21–30 mosquitoes. Among *Ae. albopictus* pools, the corresponding numbers of pools by size were 4 (13.3%) with 1 mosquito, 14 (46.7%) with 2–10, 3 (10%) with 11–20, and 9 (30%) with 21–30.

Thirty-seven (12.7%) of 292 pools of *Ae. aegypti* were positive for dengue virus by RT-PCR. This resulted in an overall pooled infection rate of 8.50 per 1,000 mosquitoes (95% CI = 6.11–11.56). Two-thirds of the positive pools (*n* = 24) were captured during the epidemic period, and the corresponding infection rates showed significant differences between the two periods of the study (epidemic = 10.68, 95% CI = 7.04–15.62 and non-epidemic = 6.15, 95% CI = 3.46–10.19). Figure 3 shows the trend of pooled infection rate during the study, which showed a decrease from 11.74 to 3.56 infected mosquitoes per 1,000.

Figure 4 shows the occurrence of dengue infection in mosquitoes and humans by city during the six visits during the study. Changes in the pooled infection rate among mosquitoes generally preceded by one visit (trimester) changes in the incidence of dengue virus isolation among humans. The association was evaluated by linear regression in all cities. Although this association showed only borderline significance (*P* = 0.08), it suggests that a 1 per 1,000 increase in mosquito infections is associated with a subsequent increase of approximately 3 dengue isolations in humans per 1,000 persons.

In addition, consistent with our findings in human infections, all four serotypes of dengue viruses were also detected in mosquitoes. The frequency of viral types was 29.7% of the positive pools were positive for DEN-1, 32.4% for DEN-2; 24.3% for DEN-3, and 10.8% for DEN-4. One pool (2.7%) with 26 mosquitoes was positive for more than one serotype (DEN-1 and DEN-3). *Aedes albopictus* was infected with DEN-1 and DEN-2 in two infected pools (2 of 30, 6.7% of the pools were positive) containing 25 and 1 mosquitoes, respectively. The pooled infection rate for *Ae. albopictus* in Buenaventura was 6.1 per 1,000 mosquitoes (95% CI = 1.10–19.57).

**DISCUSSION**

In this prospective study of dengue infection, we studied the dynamics of transmission in mosquitoes and humans, including the occurrence of symptomatic and asymptomatic infections in young persons (7–20 years of age). Adult and lar-

### Table 2

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<th>% positive</th>
<th>No. of households inspected</th>
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<th>No. of households inspected</th>
<th>% positive</th>
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<tr>
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<td>Ae. albopictus</td>
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### Table 3

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* Data not available at this visit.
Vae house indexes were not found associated with increased burden of disease. The only entomologic indicator related with dengue infection in humans was the pooled infection rate of mosquitoes.

In contrast to calculations of the lower bound of infection in mosquitoes using the MIR, we used MLE methods to estimate pooled infection rates. These methods do not require the assumption that when a pool is positive only one mosquito in that pool is positive. Our results are consistent with previous studies suggesting that virologic surveillance by RT-PCR for detecting infected Aedes mosquitoes in the field may serve as an early warning system for dengue outbreaks.5,17,18 We found that increases in mosquito infection rates were associated with increases in human infection rates in the following trimester.

Given that viral infections in mosquitoes during periods of low notification of disease were found mostly associated with silent circulation of virus in humans (i.e., persons with asymptomatic infections), it can be hypothesized that these infections may be the source of subsequent outbreaks. It would be beneficial to help to understand continuous virus circulation between observed outbreaks. Studies in Brazil provided evidence about silent circulation of viruses undetected by the official surveillance system.6 Other studies have suggested that the dynamic interaction between host, vector, and parasite populations offers a parsimonious explanation for the interepidemic periods of disease.19

We described a positive association between dengue infection and Afro-Colombian ethnicity, in spite of a low recorded incidence of dengue in Buenaventura, where most students in this ethnic group were enrolled. A relationship between ethnicity and genetics of human populations and DHF/dengue shock syndrome has been reported.20 High transmission of dengue in Haiti was observed, although DHF cases were not recorded, suggesting the existence of human genes that moderate clinical expression of dengue infection in individuals of African origin.21

Data from this study confirm the re-emergence of dengue virus serotype 3 and the natural infection of a new vector in this region. DEN-3 was absent from this area of Colombia for almost three decades.2,3 This is the first study to report its endemic circulation (November 2002–March 2004) in southwestern Colombia after its re-emergence in the northeastern region of this country in 2001.2

![Graph](image)

**Figure 3.** Overall trend of mosquito pooled infection rates and 95% confidence intervals by study visit in four cities in Valle del Cauca, in Colombia, November 2002–March 2004.

**Figure 4.** Pooled infection rate in mosquitoes (mosq.) and dengue-virus isolation rate in humans by city and visit (every three months) in four cities in Valle de Cauca, Colombia, November 2002–March 2004. B/tura = Buenaventura.
The presence of *Ae. albopictus*, which was recently introduced into this area of Colombia, has caused concerns regarding its vectorial capacity for dengue transmission. This mosquito species was infected with DEN-1 and DEN-2, although it is still locally restricted to the Pacific coast region in Buenaventura. Therefore, it is important to develop continued surveillance and control efforts to deter the spread of *Ae. albopictus* to other regions of the country.

The implementation of an early warning program for dengue would benefit from an understanding of dynamics of dengue transmission during and after outbreaks. Effective control requires knowledge of *Aedes* ecology as well as understanding factors that regulate patterns of transmission. We have provided evidence that the pooled infection rate in mosquitoes should be included in comprehensive entomologic surveillance systems.

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