Trends in Patterns of Dengue Transmission over 4 Years in a Pediatric Cohort Study in Nicaragua

Angel Balmaseda, Katherine Standish, Juan Carlos Mercado, Juan Carlos Matute, Yolanda Tellez, Saira Saborio, Samantha N. Hammond, Andrea Nuñez, William Avilés, Matthew R. Henn, Edward C. Holmes, Aubree Gordon, Josefina Coloma, Guillermina Kuan, and Eva Harris

1Departamento de Virología, Centro Nacional de Diagnóstico y Referencia, Ministry of Health, Sustainable Sciences Institute, and Centro de Salud Sócrates Flores Vivas, Managua, Nicaragua; 2Broad Institute of Harvard and Massachusetts Institute of Technology, Cambridge; 3Center for Infectious Disease Dynamics, Department of Biology, The Pennsylvania State University, University Park; 4Fogarty International Center, National Institutes of Health, School of Public Health, University of California, Berkeley

Background. Dengue is the most prevalent mosquito-borne viral disease in humans and a major urban public health problem worldwide.

Methods. A prospective cohort study of ∼3800 children initially aged 2–9 years was established in Managua, Nicaragua, in 2004 to study the natural history of dengue transmission in an urban pediatric population. Blood samples from healthy subjects were collected annually prior to the dengue season, and identification of dengue cases occurred via enhanced passive surveillance at the study health center.

Results. Over the first four years of the study, seroprevalence of anti-dengue virus (DENV) antibodies increased from 22%–40% in the 2-year-old cohort and 90%–95% in the 9-year-old cohort. The incidence of symptomatic dengue cases and the ratio of inapparent to symptomatic DENV infection varied substantially from year to year. The switch in dominant transmission from DENV-1 to DENV-2 was accompanied by an increase in disease severity but, paradoxically, a decrease in transmission. Phylogeographic analysis of full-length DENV-2 sequences revealed strong geographic clustering of dengue cases.

Conclusions. This large-scale cohort study of dengue in the Americas demonstrates year-to-year variation of dengue within a pediatric population, revealing expected patterns in transmission while highlighting the impact of interventions, climate, and viral evolution.

Dengue is a major public health problem worldwide, caused by 4 dengue virus serotypes (DENV-1–4) of the Flavivirus genus. DENV is transmitted principally by Aedes aegypti and Aedes albopictus mosquitoes and causes dengue fever and the more severe dengue hemorrhagic fever/dengue shock syndrome (DHF/DSS) throughout tropical and subtropical regions. Forty million dengue cases are estimated to occur annually worldwide, with 250,000–500,000 hospitalizations and 25,000 fatalities [1]. In the Americas, the incidence, distribution, and severity of dengue have increased dramatically over the past 3 decades, and dengue is now endemic throughout the region [2]. In Nicaragua, the first documented dengue epidemic occurred in 1985 [3]; since 1990, dengue has been endemic, with circulation of all 4 serotypes and tens of thousands of cases each year [4, 5].

A number of multiyear cohort studies have documented incidence and transmission patterns of DENV in Southeast Asia [6–12]. However, in the Americas, few long-term prospective cohort studies have been performed [13, 14]; most have been short-term, spanning 1–2 years [15–17]. Given the variations in DENV...
transmission and disease severity between Southeast Asia and the Americas, a large-scale, cohort study in the Americas is needed to more fully understand the epidemiology of dengue and prepare for possible vaccine and drug trials.

Since 2004, a seroepidemiological cohort study of ∼3800 children aged 2–12 years has been ongoing in Managua, Nicaragua [14], to document the natural history of pediatric dengue transmission, measure symptomatic and inapparent DENV infections, identify circulating DENV serotypes, and maintain a biobank of clinical specimens and viruses. Additionally, this study serves to establish infrastructure for potential dengue vaccine and drug trials. Here, we present the epidemiological observations for 2004–2008, years that encompassed substantial variation in overall DENV infection, the ratio of symptomatic to inapparent infection, disease severity, circulating serotypes, and geographical clustering.

MATERIALS AND METHODS

Study design. This is a community-based prospective seroepidemiological cohort study, with passive surveillance for illnesses among participants at the study health center enhanced by periodic home visits [14]. Participants were observed closely for all illnesses, and children presenting with fever were screened for signs and symptoms of dengue. Those presenting with World Health Organization (WHO) criteria for dengue syndromes and those with undifferentiated fever were evaluated for acute DENV infection using virological, serological, and molecular biological assays. A blood sample was collected annually from all subjects in July-August, prior to the dengue season. The study was initiated in August 2004 and continues to date; this report includes data through July 2008.

Study site and population. The study is being conducted in the catchment area of the Health Center “Sócrates Flores Vivas” (HCSFV), the principal primary care facility serving neighborhoods along Lake Xolotlán in District II in Managua. Children aged 2–9 years were recruited in August-September of 2004, and new 2-year-old children were recruited each year in July [14]. Parents or legal guardians of participants signed informed consent forms, and children aged ≥5 years provided verbal assent. The cohort population varied between 3693 and 3947 (Table 1), with the increase in size attributable to the aging of participants up to 12 years old. This study was approved by the institutional review boards of the Nicaraguan Ministry of Health, the study hospital “Hospital Infantil Manuel de Jesús Rivera” (HIMJR), the University of California, Berkeley, and the International Vaccine Institute.

Symptomatic dengue cases and annual sample collection. To facilitate identification of symptomatic dengue, participants were urged to attend the HCSFV at the onset of fever or illness, where study physicians classified them into 1 of 4 clinical cat-

Table 1. Cohort Characteristics, Infestation, and Precipitation in District II, Managua, Nicaragua, 2004–2008

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<tbody>
<tr>
<td>Total cohort size</td>
<td>3713</td>
<td>3689</td>
<td>3563</td>
<td>3676</td>
</tr>
<tr>
<td><strong>Sex</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Female</td>
<td>1821</td>
<td>1816</td>
<td>1756</td>
<td>1823</td>
</tr>
<tr>
<td>Male</td>
<td>1892</td>
<td>1873</td>
<td>1807</td>
<td>1853</td>
</tr>
<tr>
<td><strong>Age, years</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>2–5</td>
<td>1998</td>
<td>1726</td>
<td>1392</td>
<td>1385</td>
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<tr>
<td>6–9</td>
<td>1715</td>
<td>1674</td>
<td>1569</td>
<td>1578</td>
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<tr>
<td>10–12</td>
<td>0</td>
<td>290</td>
<td>602</td>
<td>713</td>
</tr>
<tr>
<td><strong>Category of initial visit</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>A (Suspected dengue)</td>
<td>210</td>
<td>335</td>
<td>195</td>
<td>233</td>
</tr>
<tr>
<td>B (Undifferentiated febrile illness)</td>
<td>159</td>
<td>267</td>
<td>246</td>
<td>201</td>
</tr>
<tr>
<td>C (Febrile with defined focus)</td>
<td>3916</td>
<td>3500</td>
<td>4565</td>
<td>4074</td>
</tr>
<tr>
<td>D (Afebrile)</td>
<td>4177</td>
<td>5047</td>
<td>5507</td>
<td>5619</td>
</tr>
<tr>
<td><strong>Total initial visits</strong></td>
<td>8462</td>
<td>9149</td>
<td>10,513</td>
<td>10,127</td>
</tr>
<tr>
<td><strong>Aedes aegypti infestation, %</strong></td>
<td></td>
<td></td>
<td></td>
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</tr>
<tr>
<td>House index</td>
<td>13.5</td>
<td>8.8</td>
<td>4.1</td>
<td>7.9</td>
</tr>
<tr>
<td>Block index</td>
<td>9.5</td>
<td>9.0</td>
<td>5.3</td>
<td>10</td>
</tr>
<tr>
<td>Container index</td>
<td>2.3</td>
<td>3.1</td>
<td>1.3</td>
<td>2.6</td>
</tr>
<tr>
<td>Breteau index</td>
<td>24.2</td>
<td>15.8</td>
<td>6</td>
<td>13</td>
</tr>
<tr>
<td>Precipitation, mm</td>
<td>819</td>
<td>1395</td>
<td>683</td>
<td>1412</td>
</tr>
</tbody>
</table>

**NOTE.** Data are no. (%) of patients, unless otherwise indicated.
Table 2. Confirmed Symptomatic and Inapparent Dengue Virus Infections in Cohort, Managua, Nicaragua, 2004–2008

<table>
<thead>
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<tbody>
<tr>
<td>Symptomatic infection&lt;sup&gt;a&lt;/sup&gt;</td>
<td>Total symptomatic cases</td>
<td>17</td>
<td>65</td>
<td>13</td>
<td>64</td>
<td>159</td>
</tr>
<tr>
<td></td>
<td>Category A</td>
<td>15 (88.2)</td>
<td>44 (67.7)</td>
<td>8 (61.5)</td>
<td>51 (79.7)</td>
<td>118 (74)</td>
</tr>
<tr>
<td></td>
<td>Category B</td>
<td>2 (11.8)</td>
<td>21 (32.3)</td>
<td>5 (38.5)</td>
<td>13 (20.3)</td>
<td>41 (26)</td>
</tr>
<tr>
<td></td>
<td>DHF and DSS&lt;sup&gt;b&lt;/sup&gt;</td>
<td>0</td>
<td>0</td>
<td>1 (7.7)</td>
<td>8 (12.5)</td>
<td>9 (6)</td>
</tr>
<tr>
<td></td>
<td>Hospitalizations&lt;sup&gt;c&lt;/sup&gt;</td>
<td>2 (11.8)</td>
<td>2 (3.1)</td>
<td>1 (7.7)</td>
<td>15 (23.4)</td>
<td>20 (13)</td>
</tr>
<tr>
<td>Incidence proportion of DENV infection in the cohort, %&lt;sup&gt;d&lt;/sup&gt;</td>
<td>Symptomatic infections</td>
<td>0.4</td>
<td>1.9</td>
<td>0.4</td>
<td>1.8</td>
<td>1.1</td>
</tr>
<tr>
<td></td>
<td>Total infections</td>
<td>8.6</td>
<td>11.1</td>
<td>5.8</td>
<td>7.3</td>
<td>8.3</td>
</tr>
<tr>
<td>Immune response</td>
<td>Symptomatic DENV infections</td>
<td>10 (70.0)</td>
<td>24 (38.0)</td>
<td>7 (60.0)</td>
<td>19 (32.0)</td>
<td>60 (40.0)</td>
</tr>
<tr>
<td></td>
<td>Inapparent DENV infections</td>
<td>5 (30.0)</td>
<td>40 (62.0)</td>
<td>4 (40.0)</td>
<td>41 (68.0)</td>
<td>90 (60.0)</td>
</tr>
<tr>
<td></td>
<td>Ratio of inapparent to symptomatic DENV infections</td>
<td>18.4:1</td>
<td>5.0:1</td>
<td>16.0:1</td>
<td>3.0:1</td>
<td>6.4:1</td>
</tr>
<tr>
<td></td>
<td>Ratio of primary inapparent to symptomatic DENV infections</td>
<td>8.9:1</td>
<td>5.4:1</td>
<td>11.3:1</td>
<td>5.3:1</td>
<td>6.6:1</td>
</tr>
<tr>
<td></td>
<td>Ratio of secondary inapparent to symptomatic infections</td>
<td>37.4:1</td>
<td>4.7:1</td>
<td>24.3:1</td>
<td>2.0:1</td>
<td>6.2:1</td>
</tr>
<tr>
<td></td>
<td>Ratio of percentage of secondary cases to percentage of secondary DENV infections</td>
<td>0.49</td>
<td>1.06</td>
<td>0.66</td>
<td>1.52</td>
<td>1.03</td>
</tr>
</tbody>
</table>

NOTE. Data are no. (%) of cases, unless otherwise indicated. DENV, dengue virus; DHF, dengue hemorrhagic fever; DSS, dengue shock syndrome.

<sup>a</sup> All confirmed symptomatic DENV infections observed among subjects at the Health Center “Sócrates Flores Vivas.”

<sup>b</sup> Three cases were DSS, and 6 were DHF; no deaths have occurred in the first 4 years of the cohort study as a result of dengue.

<sup>c</sup> Possible dengue cases were referred to the study hospital if they presented any of the following signs of alarm: persistent vomiting; moderate-to-severe dehydration; signs or symptoms of shock; abdominal pain; breathing difficulties; moderate-to-severe hemorrhagic manifestations; neurological manifestations; platelet count, ≤100,000 platelets/μL; hematocrit, ≥20% of normal value.

<sup>d</sup> For incidence proportion, immune status, and ratios, we only included symptomatic cases identified among those subjects who completed the study year and for whom paired annual samples were available (152 of 159 dengue cases among cohort participants identified at the Health Center “Sócrates Flores Vivas”).
seroconversion of DENV-specific immunoglobulin (Ig) M was observed by MAC-ELISA or a ≥4-fold increase in anti-DENV antibody titer was measured using Inhibition ELISA in paired samples. Children with paired annual serum samples demonstrating seroconversion or ≥4-fold increase in DENV-specific antibody titer, but who had not presented to the HCSFV with acute DENV illness were considered to have experienced inapparent DENV infection [14]. Symptomatic dengue cases (henceforth “cases”) were classified as primary infections if the acute-phase antibody titer was <1:10 or if the convalescent-phase antibody titer was <1:2560 and as secondary infection if the titer was ≥1:10 (acute) or ≥1:2560 (convalescent), as determined by Inhibition ELISA. In paired annual samples, seroconversion from a titer of <1:10 to ≥1:10 by Inhibition ELISA was considered primary DENV infection, whereas a ≥4-fold increase in antibody titer was considered secondary infection.

**Phylogeography of DENV-2.** Phylogeographic analysis was performed on 58 DENV-2 sequences. To determine the strength and pattern of spatial structure, we first inferred Bayesian Markov chain Monte Carlo phylogenetic trees utilizing the GTR+Γ4 model of nucleotide substitution, a relaxed molecular clock, a Bayesian skyline coalescent prior, and a total run of 50 million generations, all conducted using the BEAST package [23]. To estimate the strength of geographical clustering, we employed another Bayesian Markov chain Monte Carlo approach (BaTS; [24]), using 1000 replications of the distribution of Bayesian trees inferred above (thereby accounting for phylogenetic uncertainty), with 10% of trees removed as burn-in. This allowed us to compute the association index and parsimony score statistics of clustering strength [24]. We also used a parsimony approach [25] to explore patterns of viral movement between neighborhoods based on the Maximum Clade Credibility tree, which summarizes all the phylogenies produced from BEAST analysis. All DENV-2 sequences were assigned a character state (A–J) reflecting the neighborhood of origin. The minimum number of changes in character state needed to produce the observed distribution of character states on the Maximum Clade Credibility tree was then estimated using the parsimony method in PAUP* [26] (excluding ambiguous changes). To determine the expected number of changes under the null hypothesis of random mixing among neighborhoods, the character states of all isolates were randomized 1000 times. The analysis was then repeated as above.

**Annual precipitation and entomological indices.** Annual precipitation was measured at Managua’s International Airport, which is ~13 km from the HCSFV, and retrieved from the Meteorologic Service of the Nicaraguan Institute of Territorial Studies. Infestation rates of *Ae. aegypti* larvae in District II were collected each year by the Municipal Health Service (SILAIS) of Managua.

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**Figure 1.** A, Number of symptomatic dengue cases, by month and year. 
B, Age-stratified incidence proportion of symptomatic dengue cases among subjects, by age and year. 
C, Age-stratified incidence proportion of total dengue virus (DENV) infection (symptomatic and inapparent), by year.

**Data management and statistical analysis.** All demographic, clinical, and laboratory data were collected using standardized forms and entered into Access databases (Microsoft). Double data entry, frequent quality control queries, and numerous customized informatics tools ensured minimal errors.
RESULTS

Characteristics of study population. Throughout the 4 years, the cohort remained stable with respect to sex and age (Table 1). All 17 neighborhoods served by the HCSFV were represented in the cohort. Compliance with bringing febrile children to the HCSFV was very high, with 97% of persons with possible dengue cases presenting within the first 72 h of symptom onset and 94% providing a convalescent-phase sample. During the study period, 92% of participants attended the HCSFV at least once for a medical consult. There was a 4%–7% loss to follow-up per year (the majority of these had moved and could not be located), and only 2% of participants reported seeking health care providers other than the HCSFV. The number of initial consults averaged 9000–10,000 per year, of which 4.2%–6.6% were categories A and B (Table 1). Confirmed DENV infection among possible cases ranged from 4% to 22% among category A patients and from 1.3% to 7% among category B patients. On average, 26% of laboratory-confirmed dengue cases were category B (Table 2).

Incidence of symptomatic and inapparent DENV infections. Cases were observed primarily during Nicaragua’s dengue season, July–December (Figure 1A). Many more dengue cases were documented in 2005–2006 and 2007–2008, with 1.9% and 1.8% of subjects experiencing symptomatic infection, compared with 0.4% in both 2004–2005 and 2006–2007 (Figure 1A and 1B and Table 2). In the years with higher numbers of cases, older children were more affected (age, ≥9 years) (Figure 1B). Despite greater numbers of symptomatic dengue cases in 2005–2006 and 2007–2008, only in 2005–2006 was there a noticeable increase in the incidence proportion of total DENV infection (11.1%), compared with 8.6%, 5.8%, and 7.3% for 2004–2005, 2006–2007, and 2007–2008, respectively (Figure 1C and Table 2).

The ratio of inapparent to symptomatic DENV infection varied greatly, from 18.4:1 and 16.0:1 in 2004–2005 and 2006–2007, respectively, to 5.0:1 and 3.0:1 in 2005–2006 and 2007–2008, respectively (Figure 2A). In 2005–2006, there were high numbers of both total DENV infections and symptomatic cases; however, in 2007–2008, the number of DENV infections was similar to 2004–2005 and 2006–2007, yet there was a larger proportion of cases. Focusing on 2007–2008, we observe an age-dependent shift in the ratio of inapparent to symptomatic infection, from 4.2:1 in children aged 3–8 years to 1.4:1 for those aged 9–11 years (Figure 2B), reflecting the greater number of cases among older children that year. Severity—both hospitalizations and DHF/DSS cases—also increased markedly in later years. No DHF/DSS cases were documented in the first 2 years, whereas in 2006–2007 and 2007–2008 (Figure 2C) 8%...
and 13%, respectively, of dengue cases were classified as DHF/DSS (Table 2). An increase in hospitalized cases was also observed in 2007–2008, with a total of 15 hospitalizations (23%) (Table 2).

**Primary and secondary DENV infections.** Analysis of immune status yielded expected results: primary cases and infections were more frequent in younger children, whereas secondary cases and infections predominated in older children (Figure 3A and 3B). The ratio of inapparent to symptomatic infections was lower for primary infections in 2004–2005 and 2006–2007 and for secondary infections in 2005–2006 and 2007–2008 (Table 2). We noticed the ratio of percentage of secondary cases to secondary infections increased, especially comparing 2004–2005 to 2007–2008 (Table 2), which could be due to the shift from DENV-1 to DENV-2 (see “Seroprevalence and circulating DENV serotypes” below). When immune status was examined in relation to serotype, DENV-2 was associated with a significantly higher percentage of secondary than primary cases as compared to DENV-1 ($P<.001$) (Figure 3C).

**Seroprevalence and circulating DENV serotypes.** Increasing seroprevalence was observed in the cohort, with an 12.8% overall rise during the study period (Figure 4A). However, the rate of DENV transmission decreased; for instance, 79% seroprevalence was observed in 6-year-old children in 2004–2005, compared with a 47% seroprevalence among 6-year-old children (the 3-year-old cohort) in 2007–2008. Concomitant with the decrease in seroprevalence, the circulating serotype shifted between 2004 and 2008 (Figure 4B). Among the 89.3% of cases with serotype information, DENV-1 prevailed in 2004–2005 (53%) but was almost exclusively replaced by DENV-2 in 2006–2007 (70%) and 2007–2008 (95%). The continued predominance of DENV-2 in later years was accompanied by increasing numbers of symptomatic dengue cases among older children (Figure 1B).

Spatial shifts occurred over the 4 years of the study, as cases clustered geographically according to year and serotype (Figure 5). Phylogeographic analysis revealed strong clustering by neighborhood, using both Bayesian (association index, $P<.001$; parsimony score, $P<.001$) and parsimony ($P<.001$) approaches. In addition, parsimony analysis revealed relatively little viral movement between individual neighborhoods compared to that expected by chance alone. The majority of statistically significant instances of viral movement involved either Santa Ana Norte or Bóer neighborhoods, indicating that viruses move into—or out of—these locations more often than the other neighborhoods studied. In addition, there was phylogenetic evidence for movement between Santa Ana Norte and Santa Ana Sur, Bóer and Monsénor Lezcano, and Bóer and Cuba.

**Climate and entomological indices.** Precipitation was substantially higher in 2005–2006 and 2007–2008 than 2004–2005 and 2006–2007, the latter experiencing the lowest rainfall in the 50 years from which precipitation data is available. *Ae. aegypti* indices in the catchment area of the HCSFV were con-
sistently quite high, except for 2006–2007, when all indices were substantially lower (Table 1).

**DISCUSSION**

Here we present results from the first 4 years of a large-scale prospective study of dengue in Latin America, documenting the natural history of dengue transmission in an urban pediatric population and demonstrating that variations in climate and interventions are associated with annual trends. Understanding the entire spectrum of DENV infection is essential to combating disease, whether via vector control, improved case identification and management, or vaccine and drug development.

This study was characterized by a high degree of participant compliance and comprehensive quality control of data and biological samples [14]. The community-based cohort design was chosen over a school-based design to maximize case capture, as a school-based study conducted previously in Managua was not optimal for dengue surveillance due to numerous healthy absences and frequent school vacations [15]. Others, in Iquitos, Perú, also found that community-based surveillance was more effective than a school-based approach [13], although studies in schoolchildren have been useful in other settings [6, 12, 17, 29]. This study included children from one district of Managua, but as a low- to middle-income area affected by dengue and other infectious diseases, we believe it to be representative of Managua and other Latin American cities.

Indeed, the incidence proportions of DENV infections in our cohort (5.8%-11.1%) fall within the range reported in both Southeast Asian and Latin American pediatric populations. In a similar Nicaraguan pediatric population in 2001–2003, we reported an annual DENV infection incidence of 6%–12% [15], whereas in 2003–2004, during the reintroduction of DENV-1, total infection incidence of 18% was documented in the HCSFV catchment area [30]. In comparison, a prospective study in Maracay, Venezuela, reported 25.8% and 16.9% total infection rates in 2001–2002 and 2002–2003, respectively [17]. Incidence rates of pediatric DENV infection in Southeast Asia fall within the same range: 5.6% in Bangkok, Thailand, in 1980–1981 [29]; an overall annual incidence of 5.8% (2.2%–7.9%) in 1998–2000 in Kamphaeng Phet, Thailand [6]; and 29.2% in Yogyakarta, Indonesia, in 1995–1996 [8].

In terms of clinical manifestations of DENV infection, 74% of symptomatic DENV infection throughout the study period met the WHO case definition for dengue, while the remaining 26% of cases were diagnosed as undifferentiated fever. Undifferentiated fever accounted for the greatest proportion of cases (38.5%) in 2006–2007, which had fewest symptomatic cases. Similar findings have been reported in Vietnam and Pakistan [31, 32]. This implies that dengue surveillance programs are likely underestimating the number of cases, excluding those that present with fever but without characteristic signs and symptoms.

We document substantial year-to-year variation in dengue cases, transmission and serotype circulation. The low numbers of dengue cases in 2004–2005 may be because DENV-1, the dominant serotype that year, had caused many infections the previous year (2003) leaving a smaller susceptible population [4]. In 2005–2006, the dominant serotype switched to DENV-2, which had not circulated widely since 2002, allowing for higher infection rates. With the continued circulation of DENV-2 in 2006–2007 and 2007–2008, we documented an expected decrease in transmission rates. However, while the decrease in incidence proportion of DENV infection from 2005–2006 to 2007–2008 (11.1% to 7.3%) is expected, the large drop in 2006–2007 (5.8%) may be explained by unusually low precipitation and a particularly vigorous vector control campaign that year.
Figure 5. Geographic distribution of symptomatic cases by year and serotype.

(F. Acevedo, personal communication), resulting in exceptionally low *Ae. aegypti* indices (Table 1). Variations were also witnessed in disease by age. Older children presented greater numbers of symptomatic dengue after the introduction of DENV-2, which produces symptomatic disease mainly in secondary infections [33, 34].

DENV-2 caused the majority of symptomatic cases from 2005 to 2008, yet despite decreasing transmission and fewer infections, it produced more severe cases in 2006–2007 and 2007–2008. Sequencing of the complete DENV-2 genome identified two different clades in the cohort; Clade 1 circulated in 2004–5 and 2005–6, while Clade 2—which is associated with DHF/DSS—circulated only during 2006–2007 and 2007–2008 (A. Balmaseda, M. Henn, and E. Harris, unpublished data). The appearance of Clade 2 could explain the increase in severity in later years, as DENV-2 from all of the cohort’s sequenced DHF/DSS cases (7 of 7) were Clade 2, and may account for the higher proportion of symptomatic DENV infections in 2007–2008.

Seroprevalence in this cohort, whose oldest subjects were born in 1995, reflects the recent history of DENV circulation in Nicaragua. DENV-3 caused a large epidemic in 1998, then DENV-2 circulated from the end of 1998 until 2002. Older cohort participants were exposed to DENV-3 and DENV-2 in this phase when both serotypes circulated. In 2003, there was an epidemic caused by DENV-1, which had not circulated for a decade; thus, both younger and older children were susceptible to DENV-1 infection in 2003–4 and the first year of this study (2004–2005). However, since DENV-1—and then, to a greater extent, DENV-2—circulated throughout the study, the rate of DENV transmission started to decline, leading to an accumulation of DENV-susceptible population—exactly what we are currently observing in the fifth year of the study (2008–2009), with the introduction of DENV-3 for the first time since 1998 and a correspondingly high proportion of DENV-3 cases (A. Balmaseda, unpublished).

A detailed phylogeographic analysis of DENV-2 utilizing full-length genome sequences revealed a marked spatial clustering according to neighborhood. A similarly strong phylogeographic spatial structure was recently observed for DENV-1 and DENV-3 sequences in Singapore [35] and previously using classical epidemiological methods in Puerto Rico [36, 37] and Thailand [38, 39]. Hence, despite the population fluidity characterizing many urban environments, DENV spreads within highly localized epidemic foci. Nonetheless, some inter-neighborhood viral traffic was observed, particularly involving Santa Ana Norte and Boer. In some cases, such viral traffic is clearly explained by the spatial proximity of neighborhoods, such as Santa Ana Norte and Santa Ana Sur. In others, it may be explained by mobile populations, for example, Boer is situated...
near numerous Central American regional bus terminals and a seasonal settlement of banana workers from northwestern Nicaragua.

One limitation of our study design is that case capture is dependent on presentation of children to the HCSFV when febrile; thus, nonattendance may lead to misclassification of symptomatic infections as inapparent. However, only 2% of participants reported visiting a health care provider outside the HCSFV, so misclassification is unlikely to significantly affect results. Additionally, secondary infections may be underestimated if a primary infection occurs just after the first annual sample and a second infection occurs just prior to the second sample. Scheduling annual sample collection in July, when few infections occur, limits this type of misclassification. In addition, data is restricted to children 2–12 years of age, but nonetheless represents a broad pediatric age range. Voluntary drop-out and withdrawal hampers the collection of a subset of paired annual samples, but as the annual rate of loss was quite low (4.3%–7.1%), the overall results were not grossly affected. It is possible that there was incomplete capture of inappropriate infections due to undetectable antibody titers in the postinfection annual sample, but more frequent sample collection was not possible in this population. Finally, detailed information about the Ministry of Health intervention in 2006 was unfortunately not available.

The results of this community-based cohort study underscore the year-to-year variation of dengue within a pediatric population, revealing expected patterns in transmission and in clinical manifestations of dengue. Additionally, secondary infections may be underestimated if a primary infection occurs just after the first annual sample and a second infection occurs just prior to the second sample. Scheduling annual sample collection in July, when few infections occur, limits this type of misclassification. In addition, data is restricted to children 2–12 years of age, but nonetheless represents a broad pediatric age range. Voluntary drop-out and withdrawal hampers the collection of a subset of paired annual samples, but as the annual rate of loss was quite low (4.3%–7.1%), the overall results were not grossly affected. It is possible that there was incomplete capture of inappropriate infections due to undetectable antibody titers in the postinfection annual sample, but more frequent sample collection was not possible in this population. Finally, detailed information about the Ministry of Health intervention in 2006 was unfortunately not available.

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References