

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

Angel Balmaseda,<sup>1</sup> Katherine Standish,<sup>2</sup> Juan Carlos Mercado,<sup>1</sup> Juan Carlos Matute,<sup>1</sup> Yolanda Tellez,<sup>1</sup> Saira Saborio,<sup>1</sup> Samantha N. Hammond,<sup>2</sup> Andrea Nuñez,<sup>1</sup> William Avilés,<sup>2</sup> Matthew R. Henn,<sup>4</sup> Edward C. Holmes,<sup>5,6</sup> Aubree Gordon,<sup>7</sup> Josefina Coloma,<sup>7</sup> Guillermina Kuan,<sup>3</sup> and Eva Harris<sup>7</sup>

<sup>1</sup>Departamento de Virología, Centro Nacional de Diagnóstico y Referencia, Ministry of Health, <sup>2</sup>Sustainable Sciences Institute, and <sup>3</sup>Centro de Salud Sócrates Flores Vivas, Managua, Nicaragua; <sup>4</sup>Broad Institute of Harvard and Massachusetts Institute of Technology, Cambridge; <sup>5</sup>Center for Infectious Disease Dynamics, Department of Biology, The Pennsylvania State University, University Park; <sup>6</sup>Fogarty International Center, National Institutes of Health, Bethesda, Maryland; and <sup>7</sup>Division of Infectious Diseases, 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

Received 5 May 2009; accepted 4 August 2009; electronically published 23 November 2009.

Potential conflicts of interest: none reported.

Presented in part: 56th Annual Meeting of the American Society of Tropical Medicine and Hygiene, Philadelphia, Pennsylvania, 4–8 November 2007 (abstract 110); and 5th Network Meeting of the Pediatric Dengue Vaccine Initiative, Montreal, Canada, 5–8 June 2008.

Financial support: The Pediatric Dengue Vaccine Initiative (grant VE-1). Additional support was received from the National Institute of Allergy and Infectious Disease, National Institutes of Health, Department of Health and Human Services, under Contract No. HHSN266200400001C to the Broad Institute.

Reprints or correspondence: Dr Eva Harris, Div of Infectious Diseases, School of Public Health, University of California, Berkeley, 1 Barker Hall, Berkeley, CA 94720-7354 (eharris@berkeley.edu).

The Journal of Infectious Diseases 2010;201:5–14

© 2009 by the Infectious Diseases Society of America. All rights reserved.

0022-1899/2010/20101-0003\$15.00

DOI: 10.1093/infdis/jip252

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

Characteristic	Period			
	2004–2005	2005–2006	2006–2007	2007–2008
Total cohort size	3713	3689	3563	3676
Sex				
Female	1821 (49.0)	1816 (49.2)	1756 (49.3)	1823 (49.6)
Male	1892 (51.0)	1873 (50.8)	1807 (50.7)	1853 (50.4)
Age, years				
2–5	1998 (53.8)	1725 (46.8)	1392 (39.1)	1385 (37.7)
6–9	1715 (46.2)	1674 (45.4)	1569 (44.0)	1578 (42.9)
10–12	0	290 (7.9)	602 (16.9)	713 (19.4)
Category of initial visit				
A (Suspected dengue)	210 (2.5)	335 (3.7)	195 (1.9)	233 (2.3)
B (Undifferentiated febrile illness)	159 (1.9)	267 (2.9)	246 (2.3)	201 (2.0)
C (Febrile with defined focus)	3916 (48.4)	3500 (38.3)	4565 (43.4)	4074 (40.2)
D (Afebrile)	4177 (49.4)	5047 (55.2)	5507 (52.4)	5619 (55.5)
Total initial visits	8462	9149	10,513	10,127
<i>Aedes aegypti</i> infestation, %				
House index	13.5	8.8	4.1	7.9
Block index	9.5	9.0	5.3	10
Container index	2.3	3.1	1.3	2.6
Breteau index	24.2	15.8	6	13
Precipitation, mm	819	1395	683	1412

**NOTE.** Data are no. (%) of patients, unless otherwise indicated.

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 2. Confirmed Symptomatic and Inapparent Dengue Virus Infections in Cohort, Managua, Nicaragua, 2004–2008**

Characteristic	Period				Total
	2004–2005	2005–2006	2006–2007	2007–2008	
<b>Symptomatic infection<sup>a</sup></b>					
Total symptomatic cases	17	65	13	64	159
Category A	15 (88.2)	44 (67.7)	8 (61.5)	51 (79.7)	118 (74)
Category B	2 (11.8)	21 (32.3)	5 (38.5)	13 (20.3)	41 (26)
DHF and DSS <sup>b</sup>	0	0	1 (7.7)	8 (12.5)	9 (6)
Hospitalizations <sup>c</sup>	2 (11.8)	2 (3.1)	1 (7.7)	15 (23.4)	20 (13)
<b>Incidence proportion of DENV infection in the cohort, %<sup>d</sup></b>					
Symptomatic infections	0.4	1.9	0.4	1.8	1.1
Total infections	8.6	11.1	5.8	7.3	8.3
<b>Immune response</b>					
<b>Symptomatic DENV infections</b>					
Primary	10 (70.0)	24 (38.0)	7 (60.0)	19 (32.0)	60 (40.0)
Secondary	5 (30.0)	40 (62.0)	4 (40.0)	41 (68.0)	90 (60.0)
<b>Inapparent DENV infections</b>					
Primary	89 (30.0)	130 (41.0)	79 (40.0)	100 (54.9)	398 (41.8)
Secondary	187 (70.0)	188 (59.0)	97 (60.0)	82 (45.1)	554 (59.2)
Ratio of inapparent to symptomatic DENV infections	18.4:1	5.0:1	16.0:1	3.0:1	6.4:1
Ratio of primary inapparent to symptomatic DENV infections	8.9:1	5.4:1	11.3:1	5.3:1	6.6:1
Ratio of secondary inapparent to symptomatic infections	37.4:1	4.7:1	24.3:1	2.0:1	6.2:1
Ratio of percentage of secondary cases to percentage of secondary DENV infections	0.49	1.06	0.66	1.52	1.03

**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,  $\leq 100,000$  platelets/ $\mu\text{L}$ ; hematocrit,  $\geq 20\%$  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”).

egories (A–D) [14]. Febrile illnesses were classified as those that met the WHO’s dengue case definition (category A) [18], those with undifferentiated fever (category B), or those with an apparent focus other than dengue (category C). Nonfebrile complaints were classified as category D. Category A and B cases (“possible dengue cases”) were followed by study physicians and nurses daily while fever and symptoms persisted, either at the HCSFV or by home visits. A complete blood cell count was determined every other day, and acute- and convalescent-phase blood samples were sent for virological testing at the National Virology Laboratory of the Nicaraguan Ministry of Health. Possible dengue cases presenting signs of alarm were transferred to the HIMJR, where hospital-based study physicians and nurses provided case management and continued data collection. Each July, blood samples were collected from all children to determine DENV infection incidence and seroprevalence through serological testing of paired samples from the current and previous year.

**Sample management and laboratory assays.** Blood sam-

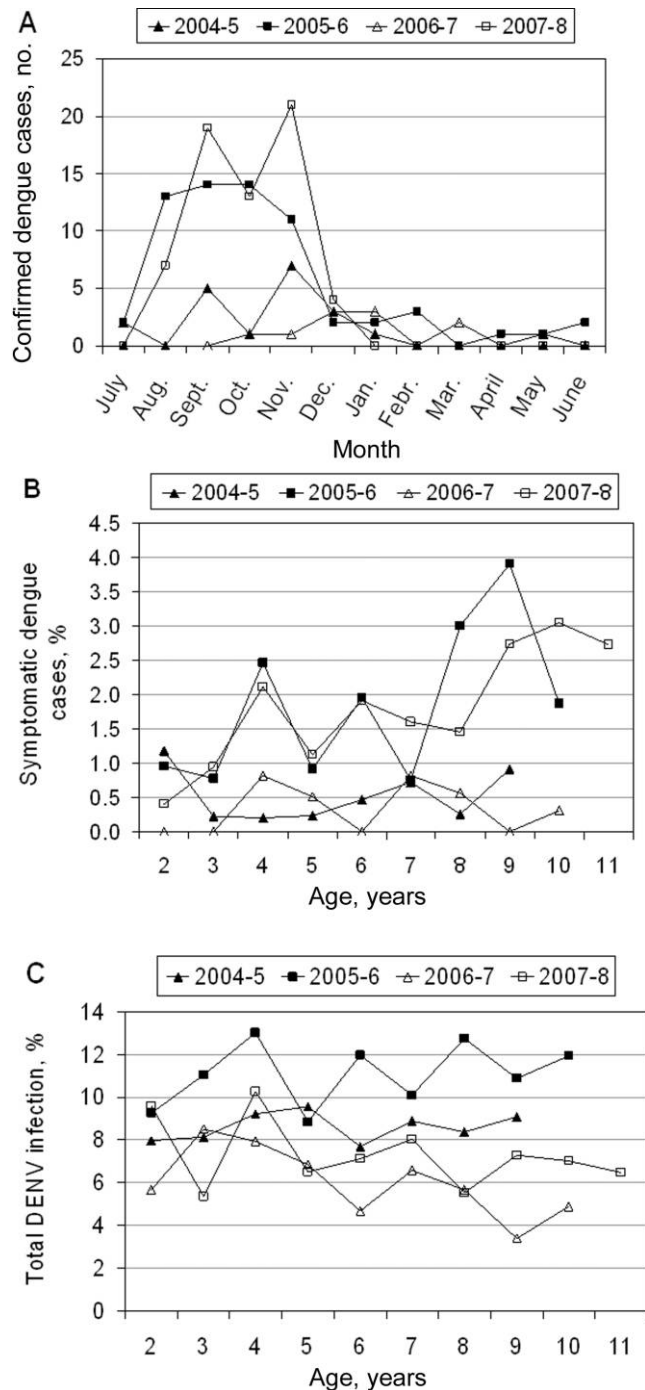
ples from category A and B patients were sent to the National Virology Laboratory of the Nicaraguan Ministry of Health for processing and storage [14]. After separation from red blood cells, acute-phase serum samples were tested for DENV RNA using a nested reverse transcriptase–polymerase chain reaction (RT-PCR) assay that targets the capsid gene and allows both DENV detection and serotyping [19]. Positive samples were confirmed by a second RT-PCR amplification and then viral isolation was attempted by inoculation onto *Ae. albopictus* C6/36 cells [20]. Serotyping after viral isolation was performed by indirect immunofluorescence or, from 2007, by RT-PCR. Acute- and convalescent-phase samples were paired and tested simultaneously for anti-DENV IgM antibodies using an in-house IgM capture ELISA (MAC-ELISA) [21] and for total anti-DENV antibodies by an Inhibition ELISA containing antigens from DENV1–4 [5, 15, 22]. Annual serum samples were processed by Inhibition ELISA.

A possible dengue case was considered positive when DENV RNA was demonstrated by RT-PCR, DENV was isolated, or

seroconversion of DENV-specific immunoglobulin (Ig) M was observed by MAC-ELISA or a  $\geq 4$ -fold increase in anti-DENV antibody titer was measured using Inhibition ELISA in paired samples. Children with paired annual serum samples demonstrating seroconversion or  $\geq 4$ -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  $\geq 1:10$  (acute) or  $\geq 1:2560$  (convalescent), as determined by Inhibition ELISA. In paired annual samples, seroconversion from a titer of  $< 1:10$  to  $\geq 1:10$  by Inhibition ELISA was considered primary DENV infection, whereas a  $\geq 4$ -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+I+ $\Gamma_4$  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  $\sim 13$  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.



**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

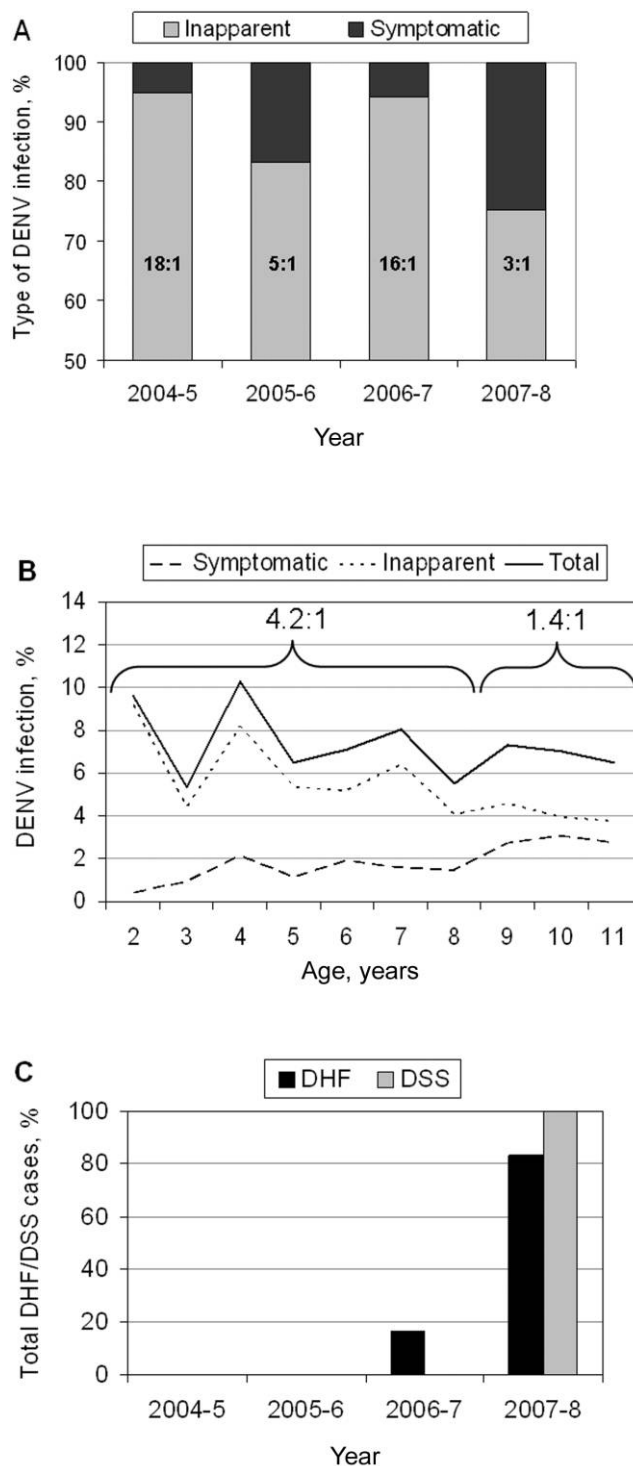
[14, 27, 28]. Statistical analysis was performed in Epi Info 2003 (Centers for Disease Control and Prevention) and Access 2003.

## 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,  $\geq 9$  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%



**Figure 2.** A, Proportion of symptomatic and inapparent dengue virus (DENV) infections, by year. B, Incidence proportion of symptomatic, inapparent and total DENV infections in 2007–2008. Older children had a greater proportion of symptomatic infections than did younger children. C, Percentage of all dengue hemorrhagic fever/dengue shock syndrome (DHF/DSS) cases, by year. There was 1 case of DHF in 2006–2007, and there were 5 cases of DHF and 3 cases of DSS in 2007–2008. The mean age of persons with DHF/DSS was 9.5 years.

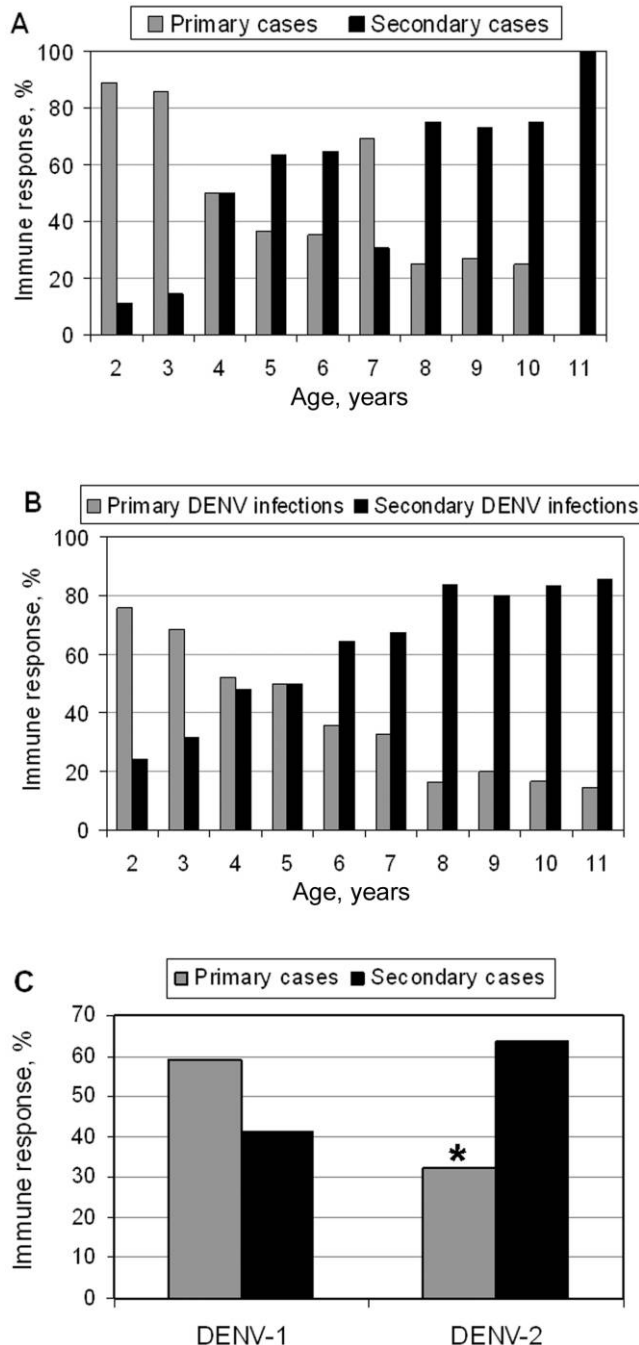
and 13%, respectively, of dengue cases were classified as DHF/DSS (Table 2). An increase in hospitalized cases was also observed in 2007–2008, with a total of 15 hospitalizations (23%) (Table 2).

**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 Monseñor Lezcano, and Bóer and Cuba.

**Climate and entomological indices.** Precipitation was substantially higher in 2005–2006 and 2007–2008 than 2004–2005



**Figure 3.** A, Percentage primary and secondary immune response, by age, among persons with symptomatic dengue cases. B, Percentage primary and secondary immune response, by age, among all persons with symptomatic and inapparent dengue virus (DENV) infections. C, Percentage of primary and secondary cases, by DENV serotype. DENV-2 was associated with a significantly higher percentage of secondary than primary cases, compared with DENV-1. \* $P < .001$ .

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).

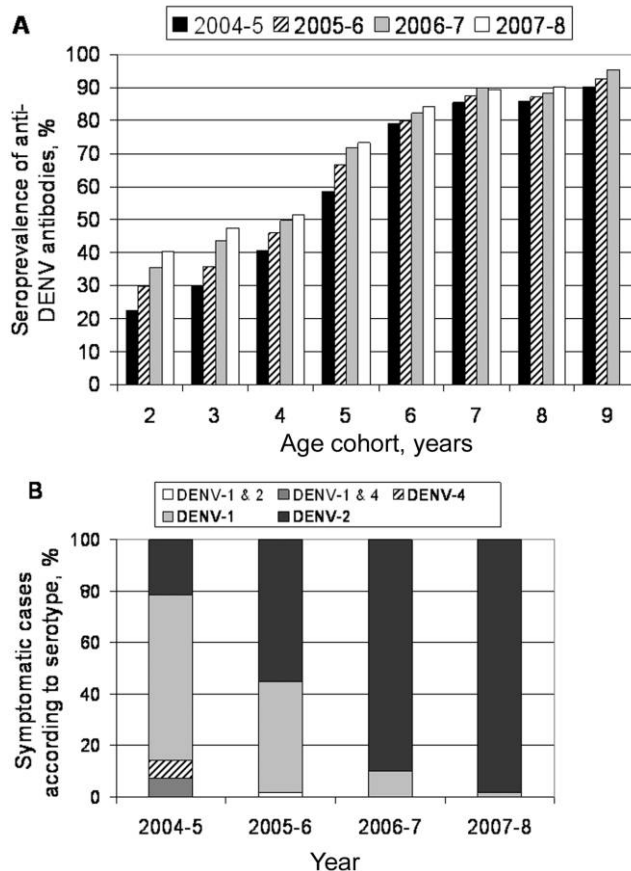
## DISCUSSION

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].

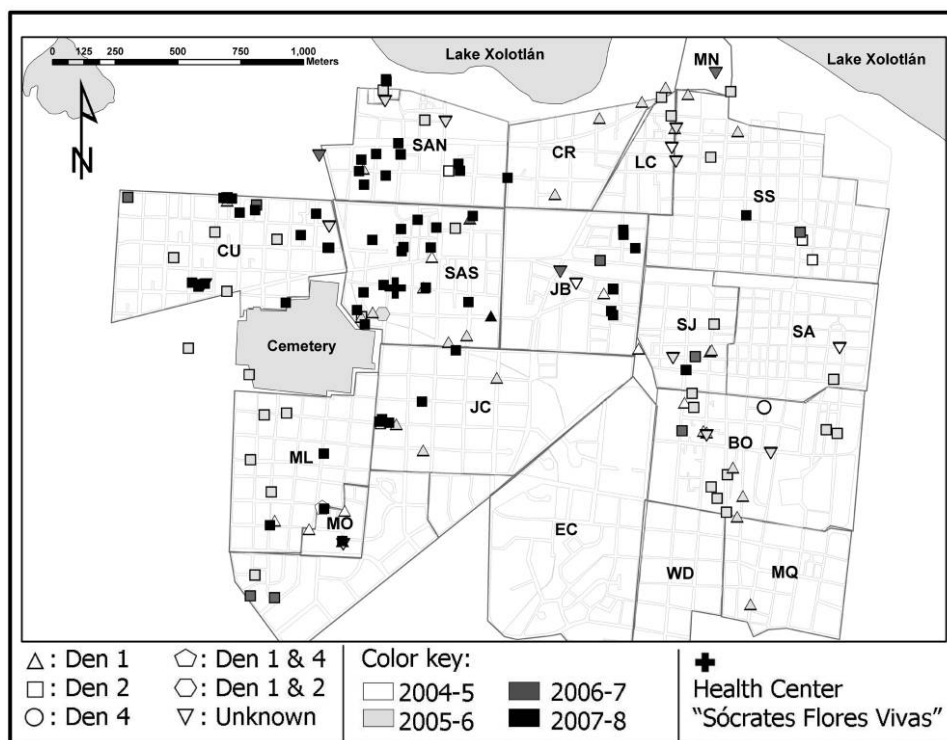
In terms of clinical manifestations of DENV infection, 74% of symptomatic DENV infection throughout the study period met the WHO case definition for dengue, while the remaining 26% of cases were diagnosed as undifferentiated fever. Undifferentiated fever accounted for the greatest proportion of cases (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



**Figure 4.** A, Seroprevalence of anti-dengue virus (DENV) antibodies, by age cohort, beginning with year of enrollment, showing the expected increase in anti-DENV antibodies over time. The 9-year-old cohort has seroprevalence data through 2006–2007, because subjects who turned 12 years old were not reenrolled in 2007 and therefore did not complete the 2007–2008 annual sample. B, Circulating DENV serotypes identified in symptomatic infections by year. There was 100% serotype concordance between reverse transcriptase–polymerase chain reaction and virus isolation findings for samples with positive results of both tests.

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 Bóer. 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, Bóer 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 inapparent 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 infection while highlighting the impact of climate, interventions, and viral evolution. The continuation of this cohort will allow additional insight into these trends as circulating DENV serotypes and subtypes and environmental factors vary. A carefully monitored pediatric cohort not only affords critical data and biological samples to study DENV infection and disease but also enables detailed surveillance of epidemiologic and immunologic trends in the study population necessary for potential vaccine and drug trials.

## Acknowledgments

We thank the phenomenal study team based at the Centro de Salud Sócrates Flores Vivas, the National Virology Laboratory in the Centro Nacional de Diagnóstico y Referencia, and the Hospital Infantil Manuel de Jesús Rivera for their dedication and high-quality work, particularly Magaly Amador, Sonia Arguello, José Ramon Cisneros, Carolina Flores, Nicole Fitzpatrick, Oscar Ortega, Juan Carlos Matute, Berman Moraga, Juan Carlos Mercado, Mirtha Monterrey, Zoila Orozco, Sergio Ojeda, Leonel Perez, Miguel Reyes, Crisanta Rocha, Carlos Romero, Saira Saborio, Leyla Saenz, Nery Sanchez, Sheyla Silva, Yolanda Tellez, and Maria José Vargas. We are especially grateful to Dr. Alcides Gonzalez and Dr. Juan José Amador for their continued support over the years. We thank Patrick Charlebois for his technical support on the comparative bioinformatic analysis of the complete genome data. We are extremely grateful to the study participants and their families.

## References

1. Gibbons RV, Vaughn DW. Dengue: an escalating problem. *BMJ* **2002**; 324:1563–6.
2. Guzman MG, Kouri G. Dengue and dengue hemorrhagic fever in the Americas: lessons and challenges. *J Clin Virol* **2003**; 27:1–13.
3. Kouri G, Valdez M, Arguello L, et al. Dengue epidemic in Nicaragua, 1985. *Rev Inst Med Trop Sao Paulo* **1991**; 33:365–71.
4. Balmaseda A, Hammond S, Pérez L, et al. Serotype-specific differences in clinical manifestations of dengue. *Am J Trop Med Hyg* **2006**; 74:449–56.
5. Harris E, Videz E, Perez L, et al. Clinical, epidemiologic, and virologic features of dengue in the 1998 epidemic in Nicaragua. *Am J Trop Med Hyg* **2000**; 63:5–11.
6. Endy TP, Chunsuttiwat S, Nisalak A, et al. Epidemiology of inapparent and symptomatic acute dengue virus infection: a prospective study of primary school children in Kamphaeng Phet, Thailand. *Am J Epidemiol* **2002**; 156:40–51.
7. Sangkawibha N, Rojanasuphot S, Ahandrik S, et al. Risk factors in dengue shock syndrome: a prospective epidemiologic study in Rayong, Thailand. I. The 1980 outbreak. *Am J Epidemiol* **1984**; 120:653–69.
8. Graham RR, Juffrie M, Tan R, et al. A prospective seroepidemiologic study on dengue in children four to nine years of age in Yogyakarta, Indonesia I. Studies in 1995–1996. *Am J Trop Med Hyg* **1999**; 61:412–9.
9. Porter KR, Beckett CG, Kosasih H, et al. Epidemiology of dengue and dengue hemorrhagic fever in a cohort of adults living in Bandung, West Java, Indonesia. *Am J Trop Med Hyg* **2005**; 72:60–6.
10. Thein S, Aung MM, Shwe TN, et al. Risk factors in dengue shock syndrome. *Am J Trop Med Hyg* **1997**; 56:566–72.
11. Burke DS, Monath TP. Flaviviruses. In: Knipe DM, Howley PM, eds. *Fields virology*. 4th ed. Vol. 1. Philadelphia: Lippincott Williams and Wilkins, **2001**:1043–126.
12. Endy TP, Nisalak A, Chunsuttiwat S, et al. Spatial and temporal circulation of dengue virus serotypes: a prospective study of primary school children in Kamphaeng Phet, Thailand. *Am J Epidemiol* **2002**; 156:52–9.
13. Rocha C, Morrison AC, Forshey BM, et al. Comparison of two active surveillance programs for the detection of clinical dengue cases in Iquitos, Peru. *Am J Trop Med Hyg* **2009**; 80:656–60.
14. Kuan G, Gordon A, Avilés W, et al. The Nicaraguan Pediatric Dengue Cohort Study: study design, methods, use of information technology, and extension to other infectious diseases. *Am J Epidemiol* **2009**; 170: 120–9.
15. Balmaseda A, Hammond SN, Tellez Y, et al. High seroprevalence of antibodies against dengue virus in a prospective study of schoolchildren in Managua, Nicaragua. *Trop Med Int Health* **2006**; 11:935–42.
16. Teixeira MG, Barreto ML, Costa MC, Ferreira LD, Vasconcelos PF, Cairncross S. Dynamics of dengue virus circulation: a silent epidemic in a complex urban area. *Trop Med Int Health* **2002**; 7:757–62.
17. Comach G, Blair PJ, Sierra G, et al. Dengue virus infections in a cohort of schoolchildren from Maracay, Venezuela: a 2-year prospective study. *Vector Borne Zoonotic Dis* **2008**; 9:87–92.
18. World Health Organization. *Dengue haemorrhagic fever: diagnosis, treatment, prevention, and control*. 2nd ed. Geneva: World Health Organization, **1997**.
19. Lanciotti R, Calisher C, Gubler D, Chang G, Vorndam A. Rapid detection and typing of dengue viruses from clinical samples by using reverse transcriptase-polymerase chain reaction. *J Clin Microbiol* **1992**; 30:545–51.
20. Balmaseda A, Sandoval E, Pérez L, Gutiérrez CM, Harris E. Application of molecular typing techniques in the 1998 dengue epidemic in Nicaragua. *Am J Trop Med Hyg* **1999**; 61:893–7.
21. Balmaseda A, Guzman MG, Hammond S, et al. Diagnosis of dengue virus infection by detection of specific immunoglobulin M (IgM) and IgA antibodies in serum and saliva. *Clin Diagn Lab Immunol* **2003**; 10:317–22.

22. Fernandez R, Vasquez S. Serological diagnosis of dengue by an ELISA Inhibition method. *Mem Inst Oswaldo Cruz* **1990**; 85:347–51.
23. Drummond AJ, Rambaut A. BEAST: Bayesian evolutionary analysis by sampling trees. *BMC Evol Biol* **2007**; 7:214.
24. Parker J, Rambaut A, Pybus OG. Correlating viral phenotypes with phylogeny: accounting for phylogenetic uncertainty. *Infect Genet Evol* **2008**; 8:239–46.
25. Slatkin M, Maddison WP. A cladistic measure of gene flow inferred from the phylogenies of alleles. *Genetics* **1989**; 123:603–13.
26. Swofford DL. PAUP\*. Phylogenetic Analysis Using Parsimony (\*and other methods). Version 4. Sunderland, MA: Sinauer Associates, **2003**.
27. Avilés W, Ortega O, Kuan G, Coloma J, Harris E. Integration of information technologies in clinical studies in Nicaragua. *PLoS Med* **2007**; 4:1578–83.
28. Avilés W, Ortega O, Kuan G, Coloma J, Harris E. Quantitative assessment of the benefits of specific information technologies applied to clinical studies in developing countries. *Am J Trop Med Hyg* **2008**; 78: 311–5.
29. Burke DS, Nisalak A, Johnson DE, Scott RM. A prospective study of dengue infections in Bangkok. *Am J Trop Med Hyg* **1988**; 38:172–80.
30. Balmaseda A, Saborio S, Tellez Y, et al. Evaluation of serological markers in serum, filter-paper bloodspots, and saliva for dengue diagnosis and epidemiology studies. *J Clin Virol* **2008**; 43:287–91.
31. Akram DS, Igarashi A, Takasu T. Dengue virus infection among children with undifferentiated fever in Karachi. *Indian J Pediatr* **1998**; 65: 735–40.
32. Phuong HL, de Vries PJ, Nagelkerke N, et al. Acute undifferentiated fever in Binh Thuan province, Vietnam: imprecise clinical diagnosis and irrational pharmaco-therapy. *Trop Med Int Health* **2006**; 11: 869–79.
33. Vaughn DW, Green S, Kalayanarooj S, et al. Dengue viremia titer, antibody response pattern, and virus serotype correlate with disease severity. *J Infect Dis* **2000**; 181:2–9.
34. Nisalak A, Endy TP, Nimmanniya S, et al. Serotype-specific dengue virus circulation and dengue disease in Bangkok, Thailand from 1973–1979. *Am J Trop Med Hyg* **2003**; 68:191–202.
35. Schreiber MJ, Holmes EC, Ong SH, et al. Genomic epidemiology of a dengue virus epidemic in urban Singapore. *J Virol* **2009**; 83:4163–73.
36. Morrison AC, Getis A, Santiago M, Rigau-Perez JG, Reiter P. Exploratory space-time analysis of reported dengue cases during an outbreak in Florida, Puerto Rico, 1991–1992. *Am J Trop Med Hyg* **1998**; 58:287–98.
37. Neff JM, Morris L, Gonzalez-Alcover R, Coleman PH, Lyss SB, Negron H. Dengue fever in a Puerto Rican community. *Am J Epidemiol* **1967**; 86:162–84.
38. Halstead SB, Scanlon JE, Umpaivit P, Udomsakdi S. Dengue and chikungunya virus infection in man in Thailand, 1962–1964. IV. Epidemiologic studies in the Bangkok metropolitan area. *Am J Trop Med Hyg* **1969**; 18:997–1021.
39. Mammen MP, Pimgate C, Koenraadt CJ, et al. Spatial and temporal clustering of dengue virus transmission in Thai villages. *PLoS Med* **2008**; 5:e205.