SEROTYPE-SPECIFIC DIFFERENCES IN CLINICAL MANIFESTATIONS OF DENGUE

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Abstract. Dengue, the most prevalent arthropod-borne viral disease of humans, is caused by four serotypes of
dengue virus (DENV 1–4). Although all four DENV serotypes cause a range of illness, defining precisely which clinical
characteristics are associated with the distinct serotypes has been elusive. A cross-sectional study was conducted on 984
and 313 hospitalized children with confirmed DENV infections during two time periods, respectively, in the same
hospitals in Nicaragua: a 3-year period (1999–2001) when DENV-2 accounted for 96% of the viruses identified, and the
2003 dengue season when DENV-1 predominated (87% of identified serotypes). When the two periods were compared,
more shock (OR 1.91, 95% CI 1.35–2.71) and internal hemorrhage (OR 2.05, CI 1.16–3.78) were observed in the period
when DENV-2 predominated, whereas increased vascular permeability was associated to a greater degree with the
DENV-1 period (OR 2.36, CI 1.80–3.09). Compared with the DENV-2 period, the DENV-1 season was associated with
more hospitalized primary dengue cases (OR 3.86, CI 2.72–5.48) and more primary DENV infections with severe
manifestations (OR 2.93, CI 2.00–4.28). These findings provide new data to characterize the pathogenic potential of
distinct DENV serotypes in human populations.

INTRODUCTION

Dengue virus is the causative agent of dengue fever (DF) and dengue hemorrhagic fever/dengue shock syndrome (DHF/DSS) and consists of four distinct serotypes (DENV 1–4).[1] It is a major cause of morbidity throughout tropical and subtropical regions of the world and continues to spread alarmingly.[2,3] DF is characterized by high fever, headache, retro-orbital pain, myalgia, arthralgia, and rash. The more severe form of disease, DHF, is defined by an increase in vascular permeability ("plasma leakage"), hemorrhagic manifestations, and decreased platelet levels near the time of defervescence.[4] DHF can also progress to DSS, which is associated with hypotension or narrow pulse pressure and clinical signs of shock.[5]

Although the different DENV serotypes can lead to varying clinical and epidemiologic profiles, defining precisely which clinical characteristics are associated with the distinct serotypes has been elusive. Several reports have indicated that DENV-2 and DENV-3 may cause more severe disease than the other serotypes and that DENV-4 is responsible for a milder illness.[6–8] Certain genotypes within particular serotypes have been associated with epidemics of DHF versus classic dengue,[9,10] but no correlation with specific clinical features has been reported.

The prevalence of dengue in the Americas has increased dramatically in recent decades.[11–13] Since its introduction into Nicaragua in 1985[14] until the present, all four DENV serotypes have circulated in the country[15–17]; however, a single serotype predominates in each epidemic. For instance, DENV-3 circulated from 1994 until 1998,[15,18] DENV-2 was the dominant serotype from 1999 to 2002,[17] and DENV-1 became the predominating serotype in 2003 (Balmaseda A and others, unpublished data). The characteristic of DENV circulation in Nicaragua, with a predominant serotype in each

epidemic, allowed us to address the issue of the association of particular DENV serotypes with specific clinical manifestations. We compared detailed clinical characteristics of children with laboratory-confirmed DENV infections who were hospitalized in the same hospitals during the period when DENV-2 dominated (1999–2001) with the 2003 dengue season when DENV-1 was the predominant serotype identified. We found significant differences in the association of the DENV-1 and DENV-2 periods with severe clinical manifestations of dengue as well as with the number of hospitalized primary DENV infections. These results constitute new findings regarding the pathogenic properties of different DENV serotypes in human populations.

MATERIALS AND METHODS

Study population. A cross-sectional study was conducted in the Hospital Infantil Manuel de Jesús Rivera (HIMJR) in the capital city of Managua, Nicaragua, and in the Hospital Escuela Oscar Danilo Rosales Arguello (HEODRA) in the nearby city of León, from January 1999 to December 2001 and again in the HIMJR in Managua from September 2003 to February 2004. These periods represented the 1999–2001 and 2003 dengue seasons, respectively. León is located 100 km from Managua and has the same ethnic composition; both cities have a similar high level of dengue transmission and history of dengue epidemics.[17] The enrollment criteria were the same for both study periods, as follows: hospitalized patients younger than 15 years of age who completed the informed consent and assent process and who presented with acute febrile illness and two or more of the following symptoms: headache, retro-orbital pain, myalgia, arthralgia, rash, and hemorrhagic manifestations. More than 95% of all patients hospitalized in both time periods and who met the enrollment criteria participated in the study. Subjects reflected the gender, age, and ethnic composition of the local pediatric population.

A standardized questionnaire was administered to collect demographic and clinical information, and venous blood was drawn for serological and virological dengue diagnostic tests

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when patients presented to the Infectious Diseases ward, at the time of discharge from the hospital, and when possible 7 days later. The clinical data during hospitalization was prospectively collected using standardized forms that were completed and verified via chart review by physicians and/or health care professionals with experience with dengue. These data entry forms recorded daily temperature, platelet count and hematocrit, hemorrhagic signs, blood pressure, signs of shock, pleural effusion/ascites, and other complications. The same protocols for case management, including fluid interventions, were maintained throughout the study period (1999–2004). Informed consent was obtained from parents or legal representatives authorizing the participation of their children in the study. This study was approved by the University of California Berkeley Committee for the Protection of Human Subjects, the Ethical Review Committee of the Centro Nacional de Diagnóstico y Referencia (CNDR) of the Nicaraguan Ministry of Health, and the Institutional Review Board of the HIMJR.

Definitions. This manuscript focuses on an analysis of severe manifestations of dengue rather than the traditional case definition of DHF/DSS syndrome.[19] Patients who presented with plasma leakage, shock, marked thrombocytopenia, or internal hemorrhage were defined as cases with severe manifestations of dengue. Plasma leakage was defined by hemoconcentration ($\geq$ 20% increase in hematocrit as compared with the value at discharge or a hematocrit 20% above normal for age and sex[15]) or by pleural effusion or ascites observed in ultrasound or x-rays of the thorax and abdominal regions. Shock was defined by hypotension (systolic pressure < 80 mm of Hg for < 5 years of age and < 90 mm of Hg for $\geq$ 5 years of age) or narrow pulse pressure ($\leq$ 20 mm of Hg).[12] Marked thrombocytopenia was defined as a platelet count $\leq$ 50,000/mm^3, a value statistically associated with the presence of additional severe manifestations.[19] Internal hemorrhage included melena, hematemesis, hematuria, and menorrhagia. Cases were considered to be laboratory-confirmed as positive for dengue if 1) DENV was isolated; 2) DENV RNA was detected by reverse transcriptase–polymerase chain reaction; 3) IgM-ELISA was positive (absorbance twice the mean of the negative controls); 4) a fourfold or greater increase in antibody titer as measured by inhibition ELISA was demonstrated in paired acute and convalescent sera; or 5) antibody titer by inhibition ELISA was $\geq$ 2,560 [equivalent to a hemagglutination inhibition (HI) antibody titer of $\geq$ 1,280].[15] Primary infection was defined by an antibody titer by inhibition ELISA of < 20 in acute samples (equivalent to an HI titer of < 10) or < 2,560 in convalescent samples (equivalent to an HI titer of < 1,280). Secondary infection was defined by an antibody titer by inhibition ELISA of $\geq$ 20 in acute samples (equivalent to an HI titer of $\geq$ 10) or $\geq$ 2,560 in convalescent samples (equivalent to an HI titer of $\geq$ 1,280).[15] Samples that did not fit these definitions were classified as indeterminate and were excluded from the analysis. Infants were excluded from analyses of the association of immune status with dengue severity due to the presence of maternal antibodies, which complicate analysis of immune response.

Laboratory methods. Platelet count and hematocrit were obtained using a Sysmex automated counter (Sysmex Corp., Kurashiki City, Japan). The trend over time in each patient's platelet and hematocrit values was examined by reviewing the hospital data collection forms and medical charts to ensure that the values were consistent. IgM antibodies were measured using an antibody capture ELISA[20] that was modified as described in Balmaseda and others.[21] Total antibody levels were measured using an inhibition ELISA[22] that had been previously validated against the HI assay.[15] Viral isolation and RT-PCR detection of viral RNA were performed with sera collected within 5 days since the onset of symptoms. Viral isolation in C6/36 cells and subsequent immunoflourescent detection of viral antigens were performed as described previously.[16] RNA was extracted, reverse transcribed, and amplified using serotype-specific primers directed to the capsid region[23,24] or the NS3 gene[25] with minor modifications.

Statistical analysis. Data was entered and analyzed using Epi-Info (Centers for Disease Control and Prevention, Atlanta, GA). Crude odds ratios and their Cornfield 95% confidence intervals were calculated. χ^2 analysis was used to determine significance using Epi-Info, the Student's t test was used for comparison of means in Excel (Microsoft, Redmond, WA), and Mantel-Haenszel (MH) χ^2 analysis was used to determine significance of a series using STATA (StataCorp LP, College Station, TX) .

RESULTS

Study participants and dengue virus serotypes identified. Between 1999 and 2001, 1,601 children with clinical signs of dengue were hospitalized and enrolled in the study at the HIMJR in Managua and the HEODRA in León, Nicaragua (Table 1). Nine hundred eighty-four (62%) of these suspected cases were laboratory-confirmed as positive for dengue. No significant difference was found with regard to sex, as both male and female children were affected with similar frequency (52% female), and the mean age was 6.85 years old.

TABLE 1
Demographic information about the study population

Age group	1999–2001			2003		
	Hospitalized N	Serologically and/or virologically confirmed dengue cases N (%)*	Female N (%)	Hospitalized N	Serologically and/or virologically confirmed dengue cases N (%)†	Female N (%)
Infants	139	91 (66)	46 (51)	34	27 (82)	16 (57)
1–4 y/o‡	299	149 (50)	77 (52)	83	71 (84)	42 (60)
5–9 y/o	762	498 (65)	273 (55)	141	131 (92)	60 (46)
10–14 y/o	401	246 (61)	117 (48)	99	84 (81)	41 (51)
Total	1,601	984 (62)	513 (52)	357	313 (88)	159 (52)

* 141 (8.8%) samples had indeterminate serology.
† 11 (3.1%) samples had indeterminate serology.
‡ y/o, years old.

TABLE 2
Comparison between statistical analyses in different populations

Variable	All hospitalized patients (Managua and León)	Hospitalized patients (Managua only)	Virologically confirmed (subgroup analysis)*	Risk factor (predominant serotype)
Age†	6.85 years	6.46 years	6.06 years	
Sex (female)†	52%	52%	53%	
Shock	1.91 (1.35–2.71)	2.06 (1.44–2.94)	2.08 (0.76–5.87)	DENV-2
	$P < 0.01$	$P < 0.01$	$P = 0.177$	
Plasma leakage	2.36 (1.80–3.09)	2.11 (1.60–2.79)	2.9 (1.32–6.40)	DENV-1
	$P < 0.01$	$P < 0.01$	$P < 0.01$	
Internal hem	2.05 (1.16–3.78)	2.09 (1.17–3.79)	2.14 (0.40–15.30)	DENV-2
	$P < 0.01$	$P = 0.01$	$P = 0.54$	
Thrombocytopenia	1.11 (0.84–1.46)	1.50 (1.13–2.00)	0.89 (0.39–2.02)	DENV-2
	$P = 0.485$	$P < 0.01$	$P = 0.909$	
Severe manisfestations in 10–14 year old group	5.79 (2.81–12.19)	4.65 (2.20–10.02)	2.85 (1.19–6.96)	DENV-1
	$P < 0.01$	$P < 0.01$	$P = 0.01$	
Petequiae	1.87 (1.43–2.44)	1.91 (1.45–2.52)	0.92 (0.43–1.97)	DENV-1
	$P < 0.01$	$P < 0.01$	$P = 0.951$	
Pos. torniquet test	1.64 (1.26–2.14)	1.94 (1.47–2.55)	0.74 (0.34–1.59)	DENV-1
	$P < 0.01$	$P < 0.01$	$P = 0.511$	
Hematemesis	2.13 (1.04–4.47)	2.23 (1.08–4.73)	2.09 (0.21–50.59)	DENV-2
	$P < 0.05$	$P < 0.05$	$P = 0.854$	
Primary DENV infection: hospitalization	3.86 (2.72–5.48)	3.83 (2.65–5.54)	5.66 (1.64–20.71)	DENV-1
	$P < 0.001$	$P < 0.001$	$P < 0.01$	
Primary DENV infection: severe manifestations	2.93 (2.00–4.28)	2.59 (1.76–3.82)	1.87 (0.62–5.66)	DENV-1
	$P < 0.01$	$P < 0.01$	$P = 0.32$	

* Managua and León.
† 1999–2001.

When hospitalized patients from only the HIMJR were analyzed separately, similar characteristics were observed; 52% were female and the mean age was 6.46. To ensure that no bias was introduced by including the population from León, all analyses were performed in parallel using hospitalized cases in both Managua and León or just hospitalized patients at the HIMJR in Managua, and similar results were obtained (Table 2). In 2003, 357 children were hospitalized, and 313 (88%) of these suspected cases were laboratory-confirmed to be dengue-positive. The difference in percentage of serologically confirmed dengue cases in the two time periods in our study was significant ($P < 0.01$); among laboratory-confirmed dengue cases, there was a statistically significant difference (OR 4.32, 95% CI 3.20–5.85, $P < 0.01$) in the number of IgM-positive cases during the 2003–2004 period compared with the 1999–2001 period (Table 3). This is consistent with results from the national surveillance system (based on IgM positivity), which reported 30% of suspected cases as confirmed positive for dengue in 1999–2001 in comparison with 46% in 2003 ($P < 0.01$) (Balmaseda A, unpublished data).

Between 1999 and 2001, DENV-2 was the dominant serotype identified, accounting for 96% of viruses typed by isolation and immunofluorescence or by RT-PCR. DENV-3 and DENV-4 were also detected in small quantities (1% and 3%, respectively). In 2003, DENV-1 was the predominant sero-

type identified, comprising 87% of serotypes identified. DENV-2 and DENV-4 were also detected to a much lesser extent (6.5% each) (Figure 1; Table 3). In this study, 1999–2001 will be referred to as the period of predominance of DENV-2, while 2003 will correspond to the period when DENV-1 dominated. In terms of virological results, 9.8% and 17.3% were positive by RT-PCR or virus isolation during the 1999–2001 and 2003 periods, respectively. Cases known to be infected with a serotype other than DENV-2 or DENV-1 were excluded from the analysis of 1999–2001 and 2003 data, respectively. To verify that the assumption that the entire population reflected a similar composition of serotypes as the virologically confirmed subsets, subgroup analysis was performed with just the virologically confirmed cases (DENV-2 or DENV-1 only), yielding comparable results for some variables (Table 2). In addition, virologically confirmed cases displayed a similar age and sex distribution as the entire population (Table 2).

In both study periods, children between the ages of 5 and 9 displayed the highest burden of dengue disease (Figure 2A). Slight differences were observed between the two time periods examined in this study in that children 1–4 years of age were more affected when DENV-1 predominated (OR 1.61, CI 1.15–2.25; $P < 0.01$), whereas children 5–9 were statistically more affected when DENV-2 was dominant (OR 1.44, CI

TABLE 3
Positive diagnostic assays for serologically confirmed dengue cases

Study period	Total serologically and/or virologically confirmed	IgM-positive	Fourfold increase in antibody titer	RT-PCR or virus isolation	Serotypes isolated: DENV-1; DENV-2; DENV-3; DENV-4
1999–2001	984 (62%)	824 (51%)	235 (53%)*	96 (9.8)‡	0; 92; 1; 3
2003–2004	313 (88%)	283 (79%)	140 (52%)†	54 (17.3)‡	47; 3; 0; 3; (1 DENV-2 & 4)

* Convalescent samples were obtained from 447 cases.
† Convalescent samples were obtained from 271 cases.
‡ The percentage of virologically confirmed cases was calculated relative to the total number of serologically and/or virologically confirmed cases in each time period.
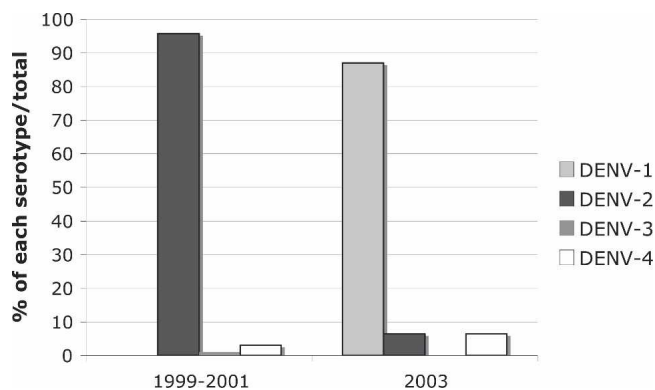
FIGURE 1. Different dominant serotypes in Nicaragua in 1999–2001 versus 2003. Serotype identification was performed by virus isolation and/or RT-PCR on samples from patients suspected of dengue who presented to the HIMJR and HEODRA. The percentage of each serotype was determined in relation to the total number of identified serotypes in each time period (N = 96 for 1999–2001; N = 54 for 2003).
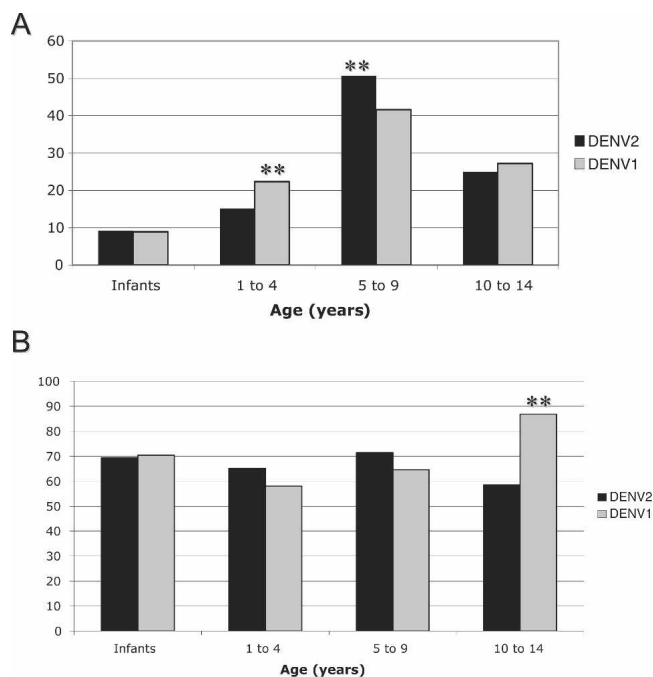

FIGURE 2. Burden of disease and disease severity according to age group. **A**, Age-stratified distribution of dengue disease in 1999–2001 and 2003. The percentage of laboratory-confirmed dengue cases contributed by each age group out of the total number of confirmed dengue cases in hospitalized patients 0–14 years old is indicated. **B**, Frequency of severe manifestations of DHF/DSS according to age group. The percentage of laboratory-confirmed dengue cases in each age group presenting with one or more severe clinical signs associated with DHF/DSS is shown. 1999–2001 is referred to as DENV-2 (dark gray bars), while 2003 is referred to as DENV-1 (light gray bars). Significant differences between the two time periods are designated by asterisks: **P < 0.01. Controlling for age, DENV infection in the DENV-1 period is associated with more severe illness (P < 0.01, MH χ^2). However, most of the difference is due to the 10–14 year age group.

1.10–1.89; P < 0.01). The average age of DENV-infected children was 6.9 years (mode 7) and 6.7 years (mode 6) during the period of circulation of DENV-2 and DENV-1, respectively.

The burden of disease with severe manifestations was evaluated by comparing the percentage of children in each age group who presented with one or more of the critical signs associated with DHF/DSS. Similar percentages of infants and children 1–9 years of age were found to suffer from shock, signs of plasma leakage, internal hemorrhage, and/or marked thrombocytopenia when either DENV-1 or DENV-2 predominated. Interestingly, children between 10 and 14 years of age were more prone to present severe manifestations of dengue during circulation of DENV-1 versus DENV-2 (OR 5.51, 2.67–11.61) (Figure 2B).

DENV-1 and DENV-2 periods are associated with distinct clinical manifestations. To evaluate whether the DENV-1 and DENV-2 periods were equivalently associated with clinical symptoms of dengue or not, the occurrence of the four key signs related to DHF/DSS were assessed; namely, shock, increased vascular permeability (plasma leakage), internal hemorrhage, and marked thrombocytopenia. Shock was defined as hypotension for age or narrow pulse pressure, and plasma leakage was characterized by hemoconcentration, pleural effusion, and/or ascites. Internal hemorrhage was defined by gastrointestinal bleeding (hematemesis and melena), hematuria, and/or menorrhagia. Statistical analysis demonstrated that a platelet count $\leq$ 50,000 per mm^3 increased the risk of developing one or more of the severe manifestations of dengue approximately fourfold.[19] Based on this result, marked thrombocytopenia was defined as a platelet count $\leq$ 50,000/mm^3.

Significantly more shock and internal hemorrhage were observed when DENV-2 predominated (OR 1.91, CI 1.35–2.71; OR 2.05, CI 1.16–3.78, respectively) (Figure 3A). In contrast, plasma leakage was more common in the DENV-1 period (OR 2.36, CI 1.80–3.09). The mean day of presentation to the hospital was 4.0 days since onset of symptoms (mode 4) in 1999–2001 and 4.4 days since symptom onset (mode 4) in 2003; thus, the predominance of shock when DENV-2 was circulating does not appear to be due to presentation at the hospital later in the course of illness. Additionally, there was

no change in case management or fluid replacement protocol from 1999 to 2003. Lastly, when the effect of immune status on the prevalence of severe clinical manifestations was examined by χ^2 analysis in each time period, secondary infection was found to be a risk factor in 1999–2001 (OR 1.70, CI 1.09–2.75; P = 0.01) but not in 2003 (OR 1.31, CI 0.72–2.38; P = 0.42).

To further disaggregate the differences in clinical manifestations caused by distinct serotypes, the age groups were analyzed separately. The predominance of shock associated with DENV-2 was due to increased prevalence of shock in children 1–9 years old in 1999–2001, as infants were equally prone to shock in both time periods (Figure 3B). The association of plasma leakage with DENV-1 was driven primarily by children 10–14 years old. The association of internal hemorrhage with the DENV-2 period can be attributed to the trend of increased frequency of this sign in study participants > 1 year old. The amount of marked thrombocytopenia was similar in the two periods, except for children 10–14 years old, who presented statistically more thrombocytopenia when DENV-1 predominated.

When hemorrhagic manifestations were examined individually, the DENV-2 period tended to be more associated with mucosal or internal bleeding (e.g., hematemesis, melena,
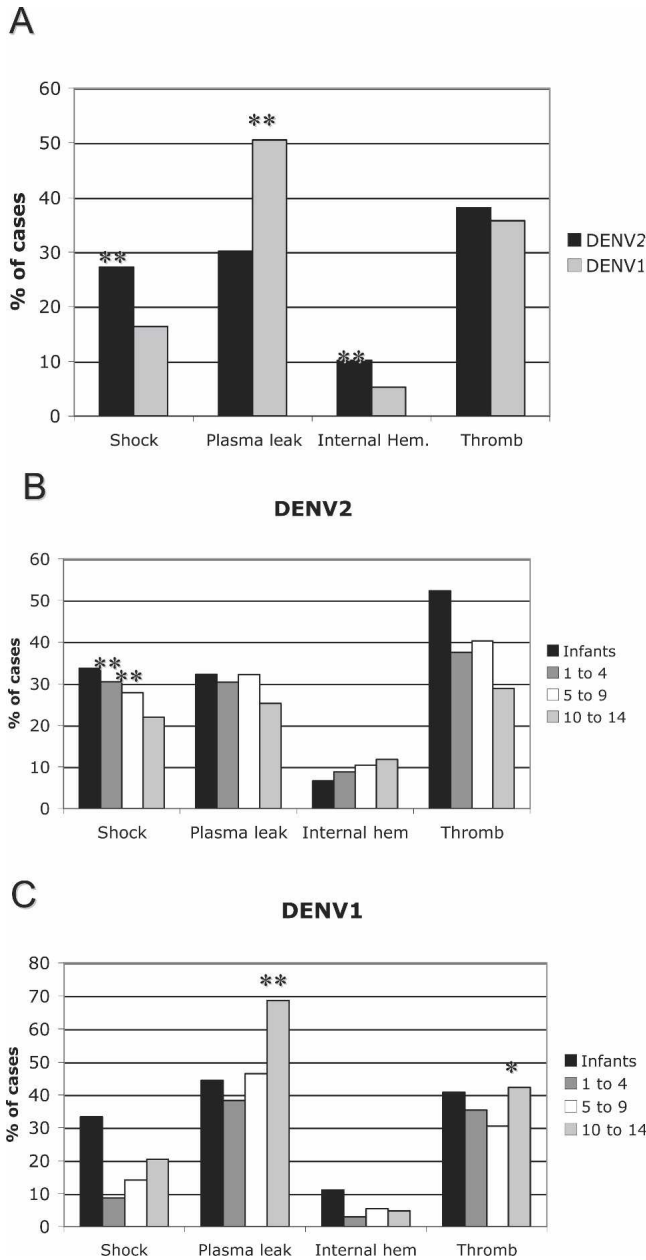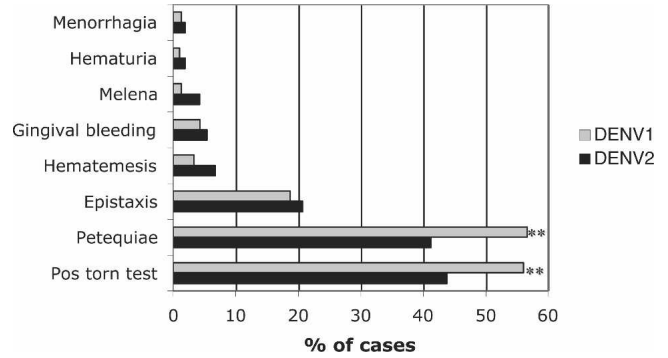
FIGURE 4. Frequency of hemorrhagic manifestations according to DENV serotype. The percentage of laboratory-confirmed dengue cases presenting with hemorrhagic manifestations associated with either the DENV-2 or DENV-1 periods are shown. Significant differences between the manifestations caused by DENV-1 versus DENV-2 are designated by asterisks: **$P < 0.01$.

menorrhagia, gingival bleeding, and epistaxis), whereas the milder signs, such as a positive torniquet test and petequiae, were significantly more associated with the DENV-1 period (Figure 4). In terms of additional signs and symptoms, more discomfort was significantly associated with the DENV-2 period (e.g., arthralgia, retro-orbital pain, chills), as well as melena and hematemesis (Table 4); the latter is consistent with our finding that internal hemorrhage was found more frequently in cases when DENV-2 predominated. The DENV-1 period again demonstrated significantly greater association with milder hemorrhagic signs (e.g., positive torniquet test, petequiae) (Table 4), consistent with the association of plasma leakage with DENV-1 (Figure 3A; Table 2).

The duration of hospitalization was investigated as another marker for disease severity. During the period when DENV-2 was dominant, the average stay in the hospital was 6.3 ± 3.4 days, compared with 3.5 ± 2.4 days when DENV-1 predomi-

FIGURE 3. Differential association of severe clinical manifestations of dengue with distinct DENV serotypes. **A**, Comparison of severe clinical signs of dengue associated with periods when DENV-1 versus DENV-2 predominated. The percentage of cases presenting with one or more of the four clinical manifestations characteristic of DHF/DSS is shown, comparing 1999–2001 to 2003. Significant differences between the clinical signs associated with predominance of DENV-1 versus DENV-2 are designated by asterisks: **$P < 0.01$. Plasma leak, signs of plasma leakage; Internal hem, internal hemorrhage. **B**, Age-stratified association of severe manifestations of dengue with different serotypes. The percentage of patients in each age group presenting with shock, signs of increased vascular permeability, internal hemorrhage, or platelet count $\leq 50,000/mm^3$ (see abbreviations above) was plotted. The upper panel refers to the period when DENV-2 dominated, and the lower panel shows the period when DENV-1 was predominant. As above, *$P < 0.05$; **$P < 0.01$. Controlling for age, dengue in the DENV-2 period is associated with more internal hemorrhage ($P < 0.05$, MH χ^2) and shock ($P < 0.01$, MH χ^2), whereas dengue in the DENV-1 period is associated with more plasma leakage ($P < 0.01$, MH χ^2).

TABLE 4
Symptoms and signs significantly associated with either the DENV-2 or DENV-1 periods

| Symptom/sign	DENV-2 yes (%)	Total No.	DENV-1 yes (%)	Total No.	OR (95% CI)
Arthralgia	568 (59)	960	139 (46)	305	DENV-2 1.79 (1.37–2.34); $P < 0.01$
Retro-orbital pain	542 (57)	957	115 (49)	233	DENV-2 1.34 (1.00–1.80); $P < 0.05$
Chills	462 (49)	948	98 (41)	237	DENV-2 1.35 (1.00–1.82); $P < 0.05$
Melena	41 (4.2)	980	4 (1.3)	305	DENV-2 3.29 (1.12–10.82); $P < 0.05$
Hematemesis	66 (6.7)	980	10 (3.3)	305	DENV-2 2.13 (1.04–4.47); $P < 0.05$
Torniquet test	428 (44)	978	171 (56)	305	DENV-1 1.64 (1.26–2.14); $P < 0.01$
Petequiae	404 (41)	980	173 (57)	305	DENV-1 1.87 (1.43–2.44); $P < 0.01$
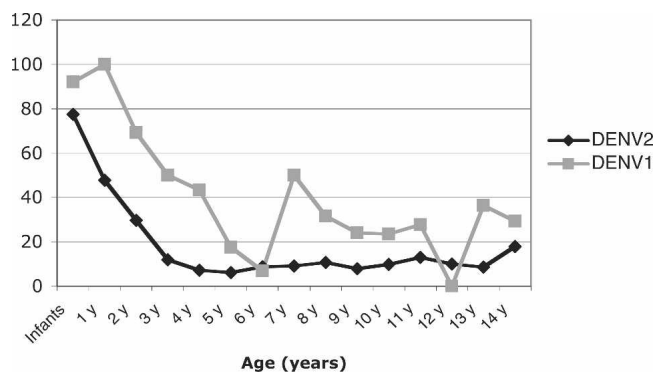
FIGURE 5. Primary DENV infections in hospitalized dengue cases. The percentage of laboratory-confirmed hospitalized dengue cases that experienced a primary DENV infection is presented, stratified by year of age. DENV-2, dark line with diamonds; DENV-1, gray line with squares.

nated ($P < 0.0001$). There were 8 deaths due to dengue in 1999–2001 and 2 in 2003, but there was no difference in the relative proportion of fatalities in the two time periods.

Another difference noted between the two serotypes pertained to frequency of primary DEN infections in hospitalized cases. It was found that primary DENV infection led to hospitalization more frequently during the period of DENV-1 dominance (36.4%) than when DENV-2 was the predominant serotype (14.2%) (Figure 5). The risk for hospitalization with a primary DENV infection was almost four times greater with DENV-1 (OR 3.86, CI 2.72–5.48) and was similar across all age groups. Consistent with this, primary DENV infection was also associated with severe manifestations to a greater extent in the DENV-1 period than the DENV-2 period (OR 2.93, CI 2.00–4.28) (Table 2).

DISCUSSION

An unresolved question in the dengue field has been whether different clinical manifestations of dengue and DHF/DSS can be associated with distinct DENV serotypes. It is well-known that clinical and epidemiologic characteristics can vary according to the circulating serotype[1]; however, few reports have demonstrated a link between distinct serotypes and specific manifestations.[6–8] Here, we focused on the four major clinical signs characteristic of DHF/DSS, because our objective was to determine the association of different serotypes with particular signs and symptoms rather than with a syndrome as a whole. We compared two periods when either DENV-1 or DENV-2 predominated and found that whereas the DENV-1 period was associated to a greater extent with increased vascular permeability, significantly more shock and internal hemorrhage was observed during the period when DENV-2 was dominant. In addition, during the DENV-1 period, primary DENV infection was associated with a ~4× greater risk for hospitalization and a ~3× greater risk for presenting with severe manifestations than during the DENV-2 period. These results demonstrate specific differences in clinical phenotype between serotypes and confirm that DENV serotypes vary in their ability to cause disease in relation to prior DENV exposure.

In contrast to the situation in Southeast Asia where dengue is hyperendemic and all DENV serotypes circulate during the same period of time,[26,27] in Nicaragua and other Latin American countries, a single DENV serotype tends to predominate.[12] For instance, in Nicaragua, DENV-3 caused several epidemics between 1994 and 1998.[15,18] DENV-2 was detected for the first time in Managua in 1998[16] and became the dominant serotype throughout the country until 2002.[17] In 2003, the predominant serotype identified became DENV-1, which had last been detected in Managua in 1990. This epidemiologic situation allowed us to compare the clinical profiles of hospitalized pediatric dengue cases in two time periods in Nicaragua and to associate these findings with the predominant serotype identified. Thus, from 1999 to 2001, 96% of viruses isolated or typed by RT-PCR were DENV-2, and by 2003, 87% of the viruses identified were DENV-1. This differs substantially from circumstances in Thailand, where a study conducted between 1998 and 2000 reported 23% DENV-1, 35% DENV-2, 41% DENV-3, and 1% DENV-4 isolates.[26] From 1983 to 1998, the most prevalent serotype at any given time among patients seen at the Queen Sirikit National Institute of Child Health in Bangkok was 56%.[6]

While the infecting serotype was known for only 10–17% of confirmed dengue cases in our study, virologically confirmed cases were representative of the population of dengue-positive cases in each time period with respect to age and sex. Subgroup analysis of the virologically confirmed cases yielded similar results as analysis of the entire population of hospitalized cases (Table 2). In addition, the relative proportions of each serotype identified in our study closely mirror those reported in the Ministry of Health surveillance data for Managua and León (Balmaseda A, unpublished results). It is true that serotypes that are more difficult to isolate, in particular DENV-4, might be underrepresented. DENV-4, however, is noted to cause more mild disease[6] and in Nicaragua has been less associated with hospitalized cases than the other serotypes (A. Balmaseda, unpublished results). With regard to the inclusion of the hospital in León in 1999–2001, it does not appear to have affected the outcomes of the study, as virtually identical results were obtained when hospitalized cases in Managua and León versus Managua alone were analyzed in parallel (Table 2). Both the population and dengue activity are known to be very similar in Managua and León,[15] the two major cities on the Pacific coast of Nicaragua.

Our results indicate that DENV-2 produced more severe disease than DENV-1, in that while the DENV-1 period was more strongly associated with signs of increased vascular permeability, the DENV-2 period was associated with shock to a greater extent. Because the same clinical treatment and fluid replacement therapy protocols were followed during both time periods and because patients presented to the hospital at very similar times with respect to onset of illness, these findings imply that dengue associated with DENV-2 infection is more refractory to fluid replacement and/or that the pathophysiological responses triggered by DENV-2 are more aggressive. That DENV-2 causes greater disease severity is also supported by our data demonstrating that DENV infection resulted in more serious hemorrhagic manifestations (e.g., hematemesis, melena, hematuria, menorrhagia, epistaxis, and gingival bleeding) during the DENV-2 period, whereas in the DENV-1 period, a higher percentage of the milder hemorrhagic manifestations were observed, such as positive tourniquet test and petequiae, which are indicative of capillary fra-

gility. In terms of the age breakdown, the increased frequency of shock associated with the DENV-2 period derived from an increase in prevalence of shock from ~10% to ~30% in children 1–9 years old. In contrast, children 10 to 14 years old appeared most prone to present signs of increased vascular permeability when DENV-1 predominated. Almost 70% of these children displayed signs of plasma leakage, compared with less than 30% when DENV-2 was predominant; at present, the explanation for this is unclear. The duration of hospitalization was significantly longer during the period of DENV-2 predominance, also supporting the association of DENV-2 with more severe disease; patients presented to the hospital at similar times after onset of symptoms and the discharge criteria were identical during both study periods. While there was no difference in the proportion of fatal cases in the two time periods, there were few fatalities overall and most were due to transfer from other hospitals late in illness when the patient had already deteriorated or was in shock.

It must be kept in mind that the particular sequence of infecting DENV serotypes can also influence the severity of disease.[27–29] For example, differences in clinical manifestations among the various age cohorts in our study could be related to previous exposures to distinct DENV serotypes. Because different serotypes have circulated throughout the past 15 years in Nicaragua, previous infections could very well have influenced the clinical manifestations of the current infection. When all age groups were combined, immune status did not play a statistically significant role in disease severity in 2003, and when the analysis was stratified by age group, again very little difference was noted in severity between primary and secondary DENV infections in this period. However, secondary infection was associated with increased severity of DENV infections in 1999–2001; when stratified by age, this appeared to be driven by children 1 to 4 years old (A. Balmaseda, S.N. Hammond, E. Harris, unpublished results). Analysis of serotypes circulating in Nicaragua since 1985 indicate that the 1–4 age group in 1999–2001 would have exposed primarily to DENV-3; the 5–9 age group to DENV-4 and/or DENV-3; and the 10–14 age group to all three other serotypes. It could be that the DENV-3–DENV-2 sequence was particularly virulent in young children, but no direct proof of this hypothesis exists. An extensive retrospective serological study evaluating neutralizing antibodies to previous infections would best address this question, although this analysis would likely be hampered by the difficulty of ascertaining prior infections due to cross-reacting neutralizing antibody responses in secondary infections.

Several reports articulating differences in disease severity associated with distinct DENV serotypes support our results.[6,8,29,30] In a retrospective study of 25 years of dengue cases in Thailand, Nisalak and others[6] found that DENV-2 was the most frequent serotype isolated from DHF/DSS cases (35%), followed by DENV-3 (31%), DENV-1 (24%), and DENV-4 (10%)—suggesting that DENV-2 causes more severe disease than DENV-1 or DENV-4. In a study of DENV viremia in Thai patients, Vaughn and others[8] showed that DENV-2 was associated with a greater pleural effusion index and with a higher percentage of DHF cases than DENV-1, DENV-3, and DENV-4 in secondary DENV infections. Corwin and others[30] found DENV-1 to be associated with less severe dengue in Indonesia, whereas DENV-3 and to a lesser extent DENV-2 were responsible for more severe disease.

Pereira and others[7] also examined differences in clinical manifestations caused by distinct DENV serotypes in Brazil, but they reported that individuals infected by DENV-3 presented signs indicating more severe disease than either DENV-1 or DENV-2.

Another characteristic that caught our attention was the high percentage of cases hospitalized with primary DENV infection during the DENV-1 period. During three years of DENV-2 predominance (1999–2001), only 14% of hospitalized patients had primary DENV infections (excluding infants). However, when DENV-1 predominated, this profile changed, with 36% of hospitalized dengue cases exhibiting a primary antibody response. Similarly, we found that the DENV-1 period was associated with significantly more primary infections with severe manifestations than was the DENV-2 period. Nisalak and others[6] also reported greater numbers of primary infections with DENV-1 than with other serotypes in their retrospective study of Thai dengue cases, isolating DENV-1 from 44% of primary DHF cases compared with only 4% with DENV-2 isolates. Analysis of the 1997 DENV-2 epidemic in Santiago de Cuba indicated that the great majority of symptomatic DENV-2 infections were associated with a secondary immune response.[31] In their viremia study, Vaughn and others[8] identified primary immune status only among patients with DENV-1 and DENV-3 infections, whereas almost all patients with DENV-2 and DENV-4 infections exhibited a secondary antibody response.[8] Likewise, in the 1998 dengue epidemic in Nicaragua, DENV-3 was associated with greater hospitalization of primary DENV infections.[15] These reports support our findings, and it seems that DENV-1 and DENV-3 can lead to hospitalized illness in primary infections, while individuals experiencing a primary DENV-2 infection tend to exhibit less notable clinical disease. This is consistent with our observation that secondary infection was a risk factor for the presence of severe manifestations of dengue in Nicaragua when DENV-2 was the dominant serotype,[17] but not when DENV-1 or DENV-3[15] predominated.

In conclusion, we have shown that the period of DENV-2 dominance was associated with a more serious clinical profile than the DENV-1 period. Although more evidence of plasma leakage was found when DENV-1 predominated, the DENV-2 period was associated with more shock and more severe hemorrhagic manifestations. When DENV-1 predominated, we observed a higher percentage of laboratory confirmation of dengue, but the clinical manifestations were milder. Also, primary DENV infection in the DENV-1 period led to a significantly larger proportion of hospitalization and of cases with severe manifestations than primary infections in the DENV-2 period. Together, these findings provide new data to better characterize the pathogenic potential of distinct DENV serotypes in human populations.

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