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CLINICAL, EPIDEMIOLOGIC, AND VIROLOGIC FEATURES OF DENGUE IN THE 1998 EPIDEMIC IN NICARAGUA

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Abstract. From July to December 1998, a hospital- and health center-based surveillance system for dengue was established at selected sites in Nicaragua to better define the epidemiology of this disease. Demographic and clinical information as well as clinical laboratory results were obtained, and virus isolation, reverse transcriptase–polymerase chain reaction, and serologic assays were performed. World Health Organization criteria were used to classify disease severity; however, a number of patients presented with signs of shock in the absence of thrombocytopenia or hemoconcentration. Therefore, a new category was designated as ''dengue with signs associated with shock'' (DSAS). Of 1,027 patients enrolled in the study, 614 (60%) were laboratory-confirmed as positive cases; of these, 268 (44%) were classified as dengue fever (DF); 267 (43%) as DF with hemorrhagic manifestations (DFHem); 40 (7%) as dengue hemorrhagic fever (DHF); 20 (3%) as dengue shock syndrome (DSS); and 17 (3%) as DSAS. Interestingly, secondary infection was not significantly correlated with DHF/DSS, in contrast to previous studies in Southeast Asia. DEN-3 was responsible for the majority of cases, with a minority due to DEN-2; both serotypes contributed to severe disease. As evidenced by the analysis of this epidemic, the epidemiology of dengue can differ according to geographic region and viral serotype.

INTRODUCTION

Dengue fever (DF) and dengue hemorrhagic fever/dengue shock syndrome (DHF/DSS) are the most important mosquito-borne viral diseases affecting humans worldwide and constitute a major public health problem in tropical and subtropical regions. The four serotypes of dengue virus (DEN), a member of the *Flaviviridae* family of positive-sense single-stranded RNA viruses, cause a broad spectrum of clinical manifestations in humans ranging from the acute febrile illness DF to the life-threatening DHF/DSS. Dengue fever is a self-limited though debilitating disease characterized by headache, retro-orbital pain, myalgia, arthralgia, rash, and in some cases hemorrhagic manifestations; DHF is defined by hemorrhagic signs, thrombocytopenia, and hemoconcentration or other evidence of vascular leakage, and can progress to shock (DSS) and death. The exact mechanism of DHF/DSS is poorly understood, but it is thought to involve immunopathologic processes associated with sequential infections with different serotypes.[1,2] Viral virulence factors as well as genetic and acquired host factors may also be determinants of disease severity. It is estimated that 2.5 billion people are at risk for dengue infection, of which nearly 100 million people contract dengue fever annually and over 250,000 progress to DHF/DSS.[3]

Over the last 20 years, DF and DHF/DSS have spread dramatically throughout Latin America,[4,5] and dengue has recently emerged as one of the main public health problems in Nicaragua. Periodic epidemics affect thousands of people, and hundreds of cases of dengue are reported every year, particularly during the rainy season when the density of the mosquito vector, *Aedes aegypti*, increases. The entomologic indices of *Ae. aegypti* infestation in Nicaragua are high; in the areas studied in this report, an average house index of 12.5 and 7.7 and Breteau index of 18.6 and 13.9 were recorded in 1997 and 1998, respectively (Programa Nacional de Control de Dengue, Nicaraguan Ministry of Health, unpublished data). In 1985, the first recorded epidemic of dengue in the country occurred, with 17,000 cases including seven deaths, and was attributed to DEN-1 and DEN-2.[6] After that, sporadic cases were observed until 1990, when the introduction of serotype 4 resulted in more than 4,000 notified cases. At the end of 1994 and during the rainy season of 1995, more than 20,000 cases of dengue were reported; the majority were caused by DEN-3,[7,8] with several cases of DEN-2 infection.[8] During the next two years, the incidence of dengue was relatively low; however, the number of cases increased abruptly in the beginning of 1998 and remained high throughout the year, with the epidemic peaking in September. The dominant serotype was DEN-3; however, DEN-2 was identified in several cases starting in July in the capital and had spread to other provinces by the end of the year.[9] Increased DEN-2 transmission was documented in 1999, along with the reappearance of DEN-4.[10] Here we report the results of a hospital- and health center-based study in Nicaragua in which we define the characteristics of the 1998 dengue epidemic.

POPULATION, MATERIALS, AND METHODS

Study population. Nicaragua has a population of approximately 5 million; 2.5 million people live in the five departments of the Pacific region where this study was conducted. Hospitals and health centers in the urban centers of these departments were selected. Participating hospitals included the 336-bed Hospital Escuela Oscar Danilo Rosales Argüello ''HEODRA'' teaching hospital in León, serving a population of 375,000, and the 221-bed Hospital Infantil Manuel de

Jesus Rivera ''La Mascota,'' a pediatric reference hospital in Managua, serving a population of 1.2 million. The participating health centers, run by the Ministry of Health, included Centro de Salud (C/S) Morazan (Managua), C/S Francisco Buitrago (Managua), C/S Silvia Ferrufino (Managua), C/S La Paz Centro (León), C/S Monimbo (Masaya), C/S Policlínico (Matagalpa), and C/S Nandaime (Granada), which serve a combined population of 560,000 people. A cross-sectional study was conducted from July 1 to December 31, 1998, with enrollment criteria consisting of an acute febrile illness and two or more of the following symptoms and signs: headache, retro-orbital pain, myalgias, arthralgia, rash, and hemorrhagic manifestations. Patients presenting to the participating hospitals and health centers who met the above criteria were invited to participate in the study after giving informed consent; this study was approved by the University of California Berkeley Committee for the Protection of Human Subjects (#99-4-38) and the Institutional Review Committee of the Centro Nacional de Diagnóstico y Referencia (CNDR) of the Nicaraguan Ministry of Health (#99-04). Subjects included both sexes and all ages and ethnicities, as reflected in the local population. A standardized questionnaire was administered to collect demographic and clinical information, and venous blood was drawn; a convalescent serum specimen was obtained when possible (15% of cases). The clinical evolution of hospitalized patients was documented by chart review using a standardized data-entry form.

Definitions. The World Health Organization (WHO) grading system was used to classify patients infected with dengue virus.[11] Dengue fever was divided into classic dengue fever (DF) and dengue fever with hemorrhagic manifestations (DFHem). Dengue hemorrhagic fever (DHF) was defined as fever with hemorrhagic manifestations, thrombocytopenia, and hemoconcentration or other signs of plasma leakage (equivalent to WHO classification DHF grades I and II). Dengue shock syndrome (DSS) was defined using DHF criteria plus either hypotension for age (systolic pressure $<$ 80 mm Hg for those $<$ 5 years of age and $<$ 90 mm Hg for those $\geq$ 5 years of age)[11] or narrow pulse pressure ($<$ 20 mm Hg) in the presence of clinical signs of shock, e.g., slow capillary filling, cold clammy skin (equivalent to DHF grades III and IV). An additional classification was designated ''dengue with signs associated with shock'' (DSAS) when hypotension for age or narrow pulse pressure plus clinical signs of shock were present in the absence of thrombocytopenia or hemoconcentration. Severe dengue was defined as DHF, DSS, or DSAS. Thrombocytopenia was defined as a platelet count $\leq$ 100,000/mm^3, and hemoconcentration as a 20% increase in hematocrit (compared to the stabilized hematocrit at hospital discharge) or a hematocrit 20% above normal for age and sex ($>$ 42 in children $\leq$ 18 years of age; $>$ 45 for females and $>$ 50 for males $>$ 18 years old). Since data were not available on the normal hematocrit by age and sex for the Nicaraguan population, an elevated hematocrit was defined as 20% above the mean values for the United States;[12] this should result in a conservative estimate for elevated hematocrit since the Nicaraguan population is likely to have a lower baseline hematocrit than that in the United States due to malnutrition. Cases were considered laboratory-confirmed if: 1) dengue virus was isolated; 2) viral RNA was demonstrated by reverse transcriptase–polymerase chain reaction (RT-PCR); 3) an IgM–enzyme-linked immunosorbent assay (ELISA) was positive (absorbance twice the mean of the negative controls); 4) a four-fold increase in antibody titer 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 $\geq$ 1,280). Primary infection was defined by an antibody titer by inhibition ELISA $<$ 20 in acute samples (equivalent to an HI titer $<$ 10) or $<$ 2,560 in convalescent samples (equivalent to a HI titer $<$ 1,280). Secondary infection was defined by an antibody titer by inhibition ELISA $\geq$ 20 in acute samples (equivalent to an HI titer $\geq$ 10) or $\geq$ 2,560 in convalescent samples (equivalent to an HI titer $\geq$ 1,280).[13] Specimens that did not fit this definition were classified as indeterminate and were excluded from analysis (~7%).

Laboratory methods. Platelet count was determined by the Neubauer method[14] and hematocrit was obtained by manual centrifugation or by using the Sysmex automated counter (Sysmex Corp., Kurashiki City, Japan) at the associated clinical laboratories. The automated system was validated against the manual method (Alonso RE, unpublished data), and controls were used routinely to standardize measurements. Periodic training in clinical hematologic methods is conducted by the Clinical Chemistry Division of the CNDR. In hospitalized patients, hematologic analysis was conducted at least once per day, and the values were recorded in the hospital data collection form. 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 and were not the result of laboratory error. IgM antibodies were measured using an antibody capture ELISA. Briefly, the standard IgM-antigen capture (MAC)-ELISA[15] was modified to decrease the time required for the assay by reducing fixation and incubation times through increasing the temperature. The modified ELISA was validated against the standard MAC-ELISA, resulting in a sensitivity of 98.5% and a specificity of 97.6% (Balmaseda A, Sandoval E, Pérez L, Gutierrez CM, Videa E, Téllez Y, and Gonzalez A, unpublished data). Total antibody levels were measured using an inhibition ELISA[16] that had been previously validated against the HI assay,[17] resulting in values that were approximately one dilution higher than HI titers (Balmaseda A, Téllez L, unpublished data). In addition, a subset of specimens from this study was analyzed by both HI and inhibition ELISA, and comparable results were obtained. Viral isolation and RT-PCR detection of viral RNA were performed with sera collected within five days of the onset of symptoms. Viral isolation in C6/36 cells and subsequent immunofluorescent detection of viral antigens were performed as described previously.[9] The RNA was extracted, reverse-transcribed, and amplified using serotype-specific primers directed to the capsid region[8] or to the nonstructural 3 (NS3) gene[18] with minor modifications.

Statistical analysis. Data were entered and analyzed using Epi-Info (Centers for Disease Control and Prevention, Atlanta, GA). Crude odds ratios (ORs) and their Cornfield 95% confidence intervals (CIs) were calculated. The analysis

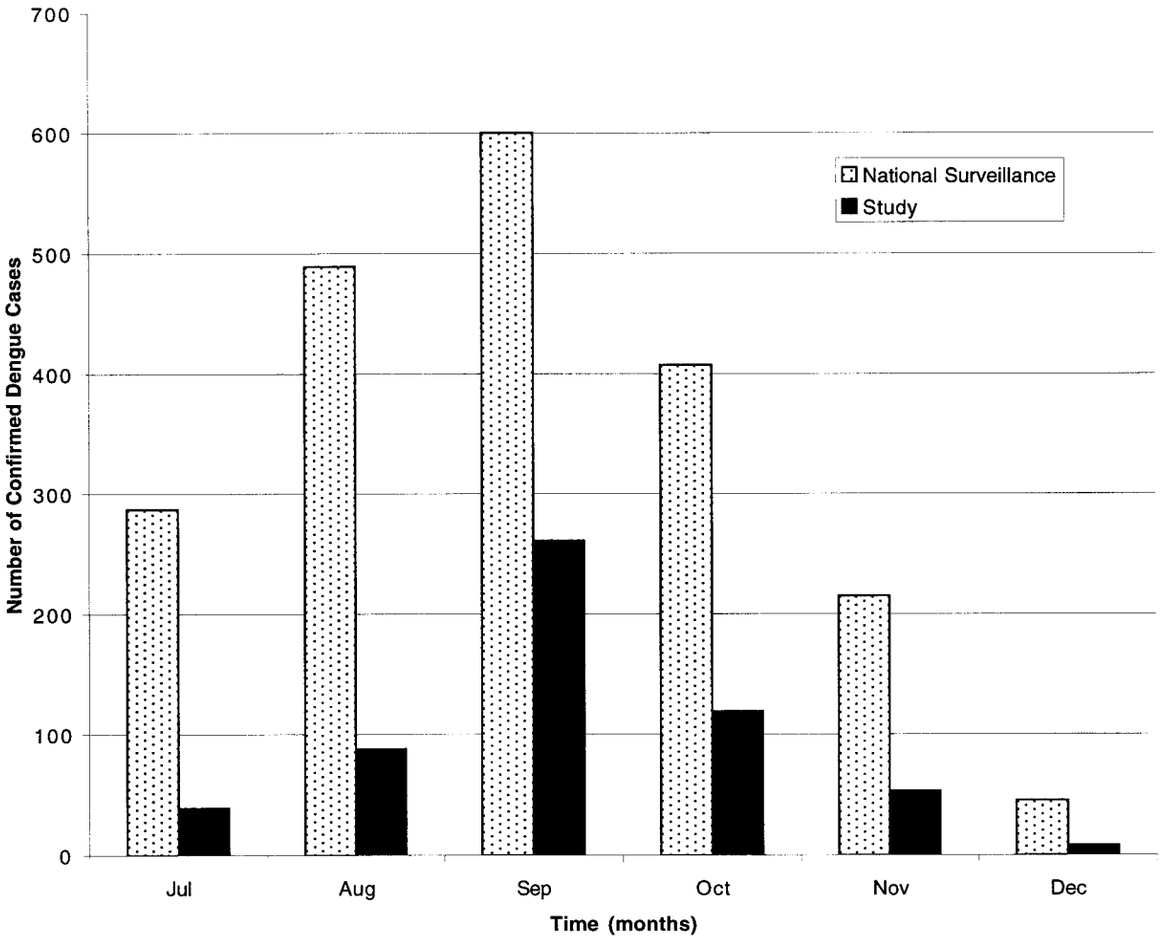

FIGURE 1. Confirmed dengue cases in the 1998 epidemic in Nicaragua. The number of laboratory-confirmed dengue cases is plotted month by month from July to December 1998. **Stippled bars** show cases included in the national surveillance program and **black bars** show cases included in this study.

of variance test was used for comparison of means and proportions.

RESULTS

Demographic information. The number of dengue cases confirmed during the study (July 1 through December 31, 1998) was representative of the cases reported by the national surveillance system, with the peak of the epidemic occurring in September (Figure 1). Of 1,027 patients enrolled in the study, 614 (60%) were laboratory-confirmed for dengue. Demographic information is given in Table 1, with laboratory-confirmed cases consisting of approximately equal numbers of males and females (52% and 48%, respectively). The majority of the cases (398; 65%) were less than 15 years of age, with 120 (20%) in the range of 0.1–4 years old, 154 (25%) 5–9 years old, and 124 (20%) 10–14 years old; however, it must be taken into consideration that 46% of the cases in this study came from a pediatric hospital. Seven hundred seventy-five (75%) of the patients were seen at hospitals and 252 (25%) were seen at health centers participating in the study, which were located in the major cities (Managua, León, Granada, Masaya, and Matagalpa) in the Pacific region of Nicaragua.

Clinical data. Cases were classified according to the WHO guidelines,[11] as summarized in the Population, Materials, and Methods. Hospitalized cases were graded based on clinical evolution as determined from chart review. In classifying dengue cases, we found that a number of patients with signs of shock did not fit the definition of DHF/DSS; nonetheless, we considered these to be severe cases and designated a new category, ''dengue with signs associated with shock'' (DSAS). These patients presented with hypotension (88%) or narrow pulse pressure (18%) in the presence of clinical signs of shock (e.g., poor capillary filling, cold clammy skin, cold extremities, lethargy) in the absence of thrombocytopenia or hemoconcentration. Of the 614 confirmed cases, 268 (44%) presented with DF, 267 (43%) with DFHem, 40 (7%) with DHF, 20 (3%) with DSS, and 17 (3%) with DSAS (Table 1); there was one fatality. Slightly greater than half of the DHF/DSS cases were in males (33; 55%), and the majority were in children less than 15 years of age (45; 75%). Although more than half (57%) of the hospitalized patients were from a pediatric hospital, 15 (25%) of

TABLE 1
Demographic data on study participants and distribution of laboratory-confirmed cases according to disease severity*

	Enrolled No. (%)	Laboratory-confirmed No. (%)	DF No. (%)	DFHem No. (%)	DHF/DSS No. (%)	DSAS No. (%)
Total	1,027	614 (60)	268 (44)	267 (43)	60 (10)	17 (3)
Sex						
M	491 (48)	316 (52)	123 (46)	131 (49)	33 (55)	6 (35)
F	535 (52)	297 (48)	145 (54)	136 (51)	27 (45)	11 (65)
Age (yr)						
0.1–4	220 (21)	120 (20)	53 (20)	55 (20)	11 (18)	1 (6)
5–9	220 (21)	154 (25)	60 (22)	63 (24)	20 (33)	12 (65)
10–14	198 (20)	124 (20)	47 (18)	59 (22)	14 (23)	4 (70)
>15	389 (38)	216 (35)	108 (40)	91 (34)	15 (25)	0 (0)
Mean age (yr)	15.5	15.0	16.7	14.6	11.7	7.6
Range	0–84	0–84	0–84	0–70	0–56	3–18
Source						
Hospital	775 (75)	483 (79)	183 (70)	222 (84)	60 (100)	17 (100)
Health Center	252 (25)	128 (21)	79 (30)	43 (16)	0 (0)	0 (0)
Hospitalized	474 (49)	328 (56)	91 (34)	167 (63)	56 (95)	17 (100)
Mean duration of hospitalization†	5.7	5.8	4.7	6.0	6.1	6.4

* DF = dengue fever; DFHem = DF with hemorrhagic manifestations; DHF/DSS = dengue hemorrhagic fever/dengue shock syndrome; DSAS = dengue with signs associated with shock.
† Information about the duration of hospitalization was available from 198 (41%) of all hospitalized study participants, 186 (57%) of hospitalized laboratory-confirmed cases, 33 (36%) of hospitalized DF cases, 88 (53%) of hospitalized DFHem cases, 48 (85%) of hospitalized DHF/DSS cases, and 17 (100%) of hospitalized DSAS cases.

DHF/DSS were found in patients 15 years or older (mean = 20.4 years). The mean age was 16.7 years for DF cases, 14.6 years for DFHem cases, 11.7 years for DHF/DSS cases, and 7.6 years for DSAS cases. A summary of the clinical symptoms and signs in patients stratified by age (< 15 versus ≥ 15 years of age) is given in Table 2; in general, older adolescents and adults reported more symptoms except for rash, vomiting, and hemorrhagic manifestations. Overall, the rates of hospitalization were quite high, with 91 (34%) of DF patients hospitalized; 167 (63%) of DFHem cases; 56 (95%) of DHF/DSS cases, and 17 (100%) of DSAS patients. In terms of the length of hospitalization, data were available for between 36% and 100% of the hospitalized patients, depending on disease classification (Table 1). The mean and median duration, respectively, was 4.7 and 4 days for DF, 6.0 and 5 days for DFHem, 5.9 and 5 for DHF, 6.4 and 6 days for DSS, and 6.4 and 6 days for DSAS.

As is often the situation, a large percentage of patients with clinical suspicion of classic dengue fever were not laboratory-confirmed; of 508 patients clinically diagnosed as DF and 395 initially diagnosed as DFHem, 268 (53%) and 267 (68%) were laboratory-confirmed, respectively. Clinical diagnosis was more accurate in severe dengue cases; of 51 DHF, 40 (78%) were laboratory-confirmed; of 24 DSS, 20 (83%) were confirmed, and of 19 DSAS, 17 (90%) were confirmed. For serologic diagnosis, in addition to an IgM-capture ELISA, total antibodies were measured by inhibition ELISA (similar to the hemagglutination inhibition assay). In addition to allowing classification into primary and secondary infections, this assay detected high antibody titers in 24 (25%) of 96 specimens taken more than five days since onset of symptoms that had produced a negative IgM ELISA result.

Epidemiology. Cases were classified as primary, secondary, or indeterminate infections depending on antibody titer, as measured by the inhibition ELISA (see Population, Materials, and Methods). In general, the majority (66%) of cases were secondary, increasing with age from 57% in 0.1–14 year-olds to 74% in those >15 years old. A similar analysis conducted only with patients with paired sera resulted in comparable numbers of secondary infections (65%). The mean age in secondary cases was an average of 4.5 years higher than in primary cases (Table 3). When disease sever-

TABLE 2
Frequency of symptoms and signs

Symptoms	<15 years No. (%)	≥15 years No. (%)
Fever	362 (92)	212 (99)
Headache	305 (77)	194 (91)
Rash	245 (62)	120 (56)
Myalgia	217 (55)	178 (83)
Retro-orbital pain	216 (55)	178 (83)
Vomiting	217 (55)	93 (44)
Arthralgia	206 (52)	175 (82)
Abdominal pain	181 (46)	102 (48)
Petechiae	151 (38)	75 (35)
Tourniquet test	148 (37)	70 (33)
Epistaxis	66 (17)	23 (11)
Diarrhea	67 (17)	31 (15)
Melena	14 (4)	9 (4)
Hepatomegaly	11 (3)	15 (7)

TABLE 3
Disease severity and mean age according to immune status*

	Primary No. (%)	Secondary† No. (%)	OR (95% CI); *P* value
Disease classification			
DF	85 (32)	170 (64)	0.70 (0.48, 1.02); <0.01
DFHem	71 (27)	183 (68)	1.11 (0.76, 1.63); <1
DHF/DSS	9 (15)	43 (72)	2.11 (0.96, 4.77); <0.1
DSAS	2 (12)	9 (53)	1.99 (0.40, 13.48); <1
Mean age (y)			
DF	13.4	18.8	<0.05
DFHem	11.5	15.6	<0.05
DHF/DSS	7.2	13.0	<0.05
DSAS	6.0	8.3	NS

* OR = odds ratio; CI = confidence interval; NS = not significant. For definitions of other abbreviations, see Table 1.
† The remaining cases were classified as indeterminate.

TABLE 4
Distribution of serotypes with respect to disease severity*

	DEN-2	DEN-3
DF	4	63
DFHem	5	34
DHF	1	7
DSS	1	3
DSAS	1	3
Total	12	110

* For definitions of abbreviations, see Table 1.

ity was analyzed with respect to immune status (Table 3), 9 (15%) of the DHF/DSS cases were found to be in primary infections, a higher percentage than is usually reported. Secondary infection was not associated with DHF/DSS (OR = 2.11, 95% CI = 0.96, 4.77), as shown in Table 3. Two other ''primary'' DHF/DSS cases were in infants between six and eight months of age, a period where if the mother had been seropositive, the maternal antibodies could have waned to enhancing levels, thus imitating a secondary infection;[19] since serologic information about the mother was not available, the immune status of these cases was classified as indeterminate. The DHF/DSS cases with primary infections were examined to determine whether there were any distinguishing epidemiological features. Eight (89%) of the primary DHF/DSS cases were in males; while the association of male sex with primary DHF/DSS was significant (OR = 8.84; 95% CI = 1.07, 196, $P < 0.05$), this may be inaccurate due to the small numbers and must be interpreted with caution. No other basic epidemiologic information available about these cases indicated any correlation due to age or location.

Virologic data. Viral serotype was identified in 122 (20%) of the confirmed cases; of these, 110 (90%) were DEN-3 and 12 (10%) were DEN-2. Both serotypes were associated with severe disease, as shown in Table 4.

DISCUSSION

The epidemiology of dengue in Central America has not been well-described, although the disease has been spreading alarmingly in the region for the past 15 years. We report the results of a hospital- and health center–based study of dengue during the 1998 epidemic in Nicaragua. This study allowed careful disease classification based on accurate clinical information and laboratory results, which led to a better understanding of the distribution of disease severity and to the description of a new disease category. Determination of patients' immune status revealed that the strong association of secondary infection with severe disease often described in other regions was not observed in this study. Earlier investigations of dengue in Nicaragua were on a smaller scale and were not analyzed on site;[6,7] this is the first epidemiologic study of dengue conducted entirely in-country.

In discussing age in relation to disease severity, it is important to point out that our study population was biased towards younger age since one of the two participating hospitals was a pediatric hospital, which was the source of 57% of hospitalized cases. Thus, in our study 65% of the laboratory-confirmed cases were less than 15 years old, whereas nationally 40% of dengue cases were less than 15 years old (Campo LA, Acevedo F, Amador JJ, unpublished data). The mean age of DHF/DSS cases (11.7 years old) was lower than that of classic dengue cases (16.7 years old), but not as low as that reported in Southeast Asia, where dengue is almost exclusively a pediatric disease with a modal age at hospitalization of 4–6 years.[11,20] Fifteen (25%) of the DHF/DSS cases were in patients 15 years of age or older; other studies of dengue in the Americas also report similar findings.[21–24] Presumably, this is because in the Americas dengue is not yet hyperendemic, with all four serotypes circulating concurrently, as it is in Southeast Asia; thus, older adolescents and adults are still susceptible to new serotypes of virus.[25] In Nicaragua, dengue has maintained an endemic-epidemic pattern, with each epidemic associated with a dominant serotype and generally no more than two serotypes co-circulating. As expected, the mean age of primary infections was lower than that of secondary infections. While studies in Asia and the Caribbean have found females to be slightly more at risk for DSS and death,[26,27] we found a small majority (55%) of DHF/DSS cases in males, similar to reports in Puerto Rico.[23]

Careful classification of cases according to the WHO scheme[11] resulted in the identification of a clinical picture (DSAS) that appeared to be severe but did not comply with the criteria for DHF/DSS. These patients manifested signs of shock without the presence of both hemoconcentration and thrombocytopenia. Some patients fit the description of DSAS upon admission; others, who appeared to be progressing towards DHF/DSS, were resuscitated with intravenous fluids and presumably did not manifest hemoconcentration due to the intervention. Other investigators have also reported severe disease due to dengue that did not conform to the WHO classification of DHF/DSS.[28,29] The DSAS cases clearly contribute to the economic burden of the disease, since all were hospitalized and the length of hospitalization was equal to that of DSS. This phenomenon needs to be further examined, and we continued the study during the 1999 dengue epidemic to further evaluate this condition.

Interestingly, in this study, secondary infection was not significantly associated with either DHF/DSS or DSAS. This contrasts with previous community-based prospective studies in children conducted in Southeast Asia, where the OR or relative risk range from 6.3 to 100.[30–33] The present study was cross-sectional with a hospital- and health center-based population; thus, these differences in study design could influence this conclusion. Epidemiologic factors that could explain this phenomenon include the fact that in our study a higher percentage (15%) of DHF/DSS cases was observed in primary infections than the $< 10\%$ usually reported,[34] combined with the high rate of secondary infections found in non-severe cases (66%) in our study population. An earlier study reported that in Nicaragua in 1994, five (71%) of seven presumed DHF cases were secondary infections; however, the numbers were small, and an OR was not calculated.[7] The occurrence of DHF in primary infections could be related to viral strain and serotype; dengue viral virulence has long been proposed as an alternative to the immune enhancement hypothesis.[35–37] Interestingly, other studies have found that DEN-3 causes DHF in primary infections to a greater extent than DEN-2 or DEN-4.[28,33] It has been hy-

pothesized that determinants for primary disease severity may be different from those associated with severe disease in secondary infections.[33] The high percentage of secondary infections in non-severe as well as severe dengue patients in this study also contributes to the lack of association between disease severity and immune status. These findings are consistent with results of a serosurvey in 1997 in Nicaragua that found an average of 77% of the population in cities in the Pacific region of the country to be seropositive as measured by hemagglutination inhibition, ranging from 66% in children 1–4 years old to 87% in individuals more than 50 years old (de los Reyes J, Balmaseda A, Huelva G, Gutierrez CM, Cerda S, and Amador JJ, unpublished data). Lastly, host factors could play a role in this phenomenon, and the Nicaraguan population has not been studied extensively with respect to the epidemiology of dengue. We have continued this study in Nicaragua to further investigate these issues.

Relatively high rates of hospitalization were observed for classic dengue (34%) and DF with hemorrhagic manifestations (63%). The mean duration of hospitalization for all disease categories was 5.8 days and for DHF/DSS was 6.1 days, as calculated from 186 (57%) of the hospitalized patients for which information was available; this figure is similar to that reported for hospitalization of DHF/DSS cases in Puerto Rico.[23] The cost of hospitalizing dengue patients in Nicaragua is very high (US$130 per day for a hospital bed),[38] especially considering the level of poverty in the country (per capita GNP of US$469 per year [Banco Central de Nicaragua, 1999]). Thus, it is clear that dengue exacts a large economic burden; in the 1994 dengue epidemic in Nicaragua, the cost of medical care accounted for 64% of the overall cost of the epidemic.[38] In Puerto Rico as well, dengue has been shown to have a large economic impact, in terms of direct costs associated with medical care, hospitalization, and epidemic control measures, as well as indirect costs (lost production of ill workers and parents of ill children).[39] A recent study found that disability-adjusted life year (DALY) losses due to dengue in Puerto Rico were on the order of those due to malaria, tuberculosis, or hepatitis in the Latin American/Caribbean region.[40]

The importance of laboratory confirmation of clinical diagnosis was reaffirmed, as a number of dengue cases were misdiagnosed based solely on clinical suspicion (ranging from 47% in DF cases to 17% in DSS cases and 10% in DSAS cases). This is to be expected, since the symptoms are consistent with other diseases such as rubella, malaria, influenza, typhoid, and leptospirosis, and is consistent with other reports.[7,23,28,41] Importantly, we found that 25% of dengue specimens with high IgG titers taken over five days since the onset of symptoms produced negative results in the IgM assay; therefore, the IgM ELISA alone is not sufficient for capturing all positive cases. DEN-3 was found to be the predominant serotype, accounting for 90% of the viruses identified, while the rest were attributed to DEN-2. DEN-2 was first identified in July and continued to spread through the end of the year;[9] as predicted, it became the predominant serotype in the 1999 epidemic (Balmaseda A, Sandoval E, and Harris E, unpublished data). Both serotypes were associated with severe disease (Table 4); this is consistent since the subtypes of both DEN-3 (''Sri Lanka'') and DEN-2 (''Jamaica'')[9] are of Southeast Asian origin and have been previously associated with DHF.[42,43]

In summary, this study demonstrates that the epidemiology of dengue varies from country to country and possibly according to the serotype and strain of the virus, underscoring the importance of defining the characteristics of dengue epidemics in different regions. It also emphasizes the importance of building laboratory capacity in endemic countries and strengthening the ties between the laboratory, epidemiologic, and clinical sectors. We have provided new information about dengue epidemiology specific to the Central American region, and now the resources are in place to continue and expand these studies.

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REFERENCES

1. Halstead SB, 1988. Pathogenesis of dengue: challenges to molecular biology. *Science 239:* 476–481.
2. Morens DM, 1994. Antibody-dependent enhancement of infection and the pathogenesis of viral disease. *Clin Infect Dis 19:* 500–512.
3. Monath TP, 1994. Dengue: the risk to developed and developing countries. *Proc Natl Acad Sci USA 91:* 2395–2400.
4. Gubler DJ, Trent DW, 1994. Emergence of epidemic dengue/dengue hemorrhagic fever as a public health problem in the Americas. *Infect Agents Dis 2:* 383–393.
5. Pan American Health Organization, 1994. *Dengue and Dengue Hemorrhagic Fever in the Americas: Guidelines for Prevention and Control.* Washington, DC: Pan American Health Scientific Organization. Publication #548.
6. Kouri G, Valdez M, Arguello L, Guzmán MG, Valdes L, Soler M, Bravo J, 1991. Dengue epidemic in Nicaragua, 1985. *Rev Inst Med Trop Sao Paulo 33:* 365–371.
7. Guzmán MG, Vásquez S, Martínez E, Alvarez M, Rodríguez R, Kourí G, de los Reyes J, Acevedo F, 1996. Dengue en Nicaragua, 1994: reintroducción del serotipo 3 en las Américas. *Bol Oficina Sanit Panam 121:* 102–110.
8. Harris E, Roberts TG, Smith L, Selle J, Kramer LD, Valle S, Sandoval E, Balmaseda A, 1998. Typing of dengue viruses in clinical specimens and mosquitoes by single-tube multiplex reverse transcriptase-PCR. *J Clin Microbiol 36:* 2634–2639.
9. Balmaseda A, Sandoval E, Pérez L, Gutiérrez CM, Harris E, 1999. Application of molecular typing techniques in the 1998

dengue epidemic in Nicaragua. *Am J Trop Med Hyg 61:* 893–897.
10. Miagostovich MP, dos Santos FB, Gutiérrez CM, Riley LW, Harris E, 2000. Rapid subtyping of dengue virus serotypes 1 and 4 by restriction site-specific (RSS)-PCR. *J Clin Microbiol 38:* 1286–1289.
11. Word Health Organization, 1997. *Dengue Haemorrhagic Fever: Diagnosis, Treatment, Prevention, and Control.* Second edition. Geneva: World Health Organization.
12. Soldin SJ, 1997. Pediatric reference ranges. Soldin S, Brugnara C, Gunter K, Hicks J, eds. *Pediatric Reference Ranges.* Washington DC: AAAC Press.
13. Nogueira RMR, Miagostovich MP, Lampe E, Souza RW, Zagne SMO, Schatzmayr HG, 1993. Dengue epidemic in the state of Rio de Janeiro, Brazil, 1990–1: co-circulation of dengue 1 and dengue 2 serotypes. *Epidemiol Infect 111:* 163–170.
14. Vives JL, Aguilar JL, 1997. *Manual de Técnicas de Laboratorio en Hematología.* Second edition. Barcelona: Masson SA.
15. Kuno G, Gomez I, Gubler DJ, 1991. An ELISA procedure for the diagnosis of dengue infections. *J Virol Methods 33:* 101–113.
16. Fernandez R, Vasquez S, 1990. Serological diagnosis of dengue by an ELISA Inhibition method. *Mem Inst Oswaldo Cruz 85:* 347–351.
17. Clark DH, Casals J, 1958. Techniques for hemaglutination and hemaglutination inhibition with arthropod-borne viruses. *Am J Trop Med Hyg 7:* 561–563.
18. Seah CLK, Chow VTK, Tan HC, Chan YC, 1995. Rapid, single-step RT-PCR typing of dengue viruses using five NS3 gene primers. *J Virol Methods 51:* 193–200.
19. Kliks SC, Nimmanitya S, Nisalak A, Burke DS, 1988. Evidence that maternal antibodies are important in the development of dengue hemorrhagic fever in infants. *Am J Trop Med Hyg 38:* 411–419.
20. Nimmannitya S, 1987. Dengue hemorrhagic fever in Thailand. *Southeast Asian J Trop Med Public Health 18:* 291–294.
21. Zagne SMO, Alves VGF, Nogueira RMR, Miagostovich MP, Lampe E, Tavares W, 1994. Dengue haemorrhagic fever in the state of Rio de Janeiro, Brazil: a study of 56 confirmed cases. *Trans R Soc Trop Med Hyg 88:* 677–679.
22. Guzmán MG, Alvarez M, Rodríguez R, Rosario D, Vásquez S, Valdés L, Cabrera MV, Kourí G, 1999. Fatal dengue hemorrhagic fever in Cuba, 1997. *Int J Infect Dis 3:* 130–135.
23. Rigau-Perez JG, Puerto Rican Association of Epidemiologists, 1997. Clinical manifestations of dengue hemorrhagic fever in Puerto Rico, 1990–1991. *Pan Am J Public Health 1:* 381–388.
24. Bravo JR, Guzman MG, Kouri GP, 1987. Why dengue haemorrhagic fever in Cuba? 1. Individual risk factors for dengue haemorrhagic fever/dengue shock syndrome (DHF/DSS). *Trans R Soc Trop Med Hyg 81:* 816–820.
25. Halstead SB, 1997. Epidemiology of dengue and dengue hemorrhagic fever. Gubler DJ, Kuno G, eds. *Dengue and Dengue Hemorrhagic Fever.* New York: CAB International.
26. Halstead SB, Nimmannitya S, Cohen SN, 1970. Observations related to pathogenesis of dengue hemorrhagic fever. IV. Relation of disease severity to antibody response and virus recovered. *Yale J Biol Med 42:* 311–328.
27. Kouri GP, Guzman MG, Bravo JR, Triana C, 1989. Dengue haemorrhagic fever/dengue shock syndrome: lessons from the Cuban epidemic, 1981. *Bull World Health Organ 67:* 375–380.
28. Murgue B, Deparis X, Chungue E, Cassar O, Roche C, 1999. Dengue: An evaluation of dengue severity in French Polynesia based on an analysis of 403 laboratory-confirmed cases. *Trop Med Int Health 4:* 765–773.
29. Sumarmo WH, Jahja E, Gubler DJ, Suharyono W, Sorensen K, 1983. Clinical observations on virologically confirmed fatal dengue infections in Jakarta, Indonesia. *Bull World Health Organ 61:* 693–701.
30. Burke DS, Nisilak A, Johnson DE, Scott RM, 1988. A prospective study of dengue infections in Bangkok. *Am J Trop Med Hyg 38:* 172–180.
31. Sangkawibha N, Rojanasuphot S, Ahandrik S, Viriyapongse S, Jatanasen S, Salitul V, Phanthumachinda B, Halstead SB, 1984. Risk factors in dengue shock syndrome: a prospective epidemiologic study in Rayong, Thailand. I. The 1980 outbreak. *Am J Epidemiol 120:* 653–669.
32. Thien S, Aung MM, Shwe TN, Aye M, Zaw A, Aye K, Aye KM, Aaskow J, 1997. Risk factors in dengue shock syndrome. *Am J Trop Med Hyg 56:* 566–572.
33. Vaughn DW, Green S, Kalayanarooj S, Innis BL, Nimmannitya S, Suntayakorn S, Endy T, Raengsakulrach B, Rothman AL, Ennis FA, Nisalak A, 2000. Dengue viremia titer, antibody response pattern, and virus serotype correlate with disease severity. *J Infect Dis 181:* 2–9.
34. Monath TP, Heinz FX, 1996. Flaviviruses. Fields BN, Knipe DM, Howley PM, eds. *Fields Virology.* Philadelphia: Lippincott-Raven Publishers.
35. Gubler DJ, Reed D, Rosen L, Hitchcock JCJ, 1978. Epidemiologic, clinical, and virologic observations on dengue in the kingdom of Tonga. *Am J Trop Med Hyg 27:* 581–589.
36. Rosen L, 1989. Disease exacerbation by sequential dengue infections: Myth or reality? *Rev Infect Dis 11:* S840–S842.
37. Rosen L, 1977. The Emperor's New Clothes revisited, or reflections on the pathogenesis of dengue hemorrhagic fever. *Am J Trop Med Hyg 26:* 337–343.
38. Ferrando JE, 1995. *Estimate of the Costs of the Dengue Epidemic in 1994 in Nicaragua.* Washington, DC: Pan American Health Organization. Consultancy Report. OPS/HCP/HCT/95.64.
39. Von Allmen SD, Lopez-Correa RH, Woodall JP, Morens DM, Chiribiga J, Casta-Velez A, 1979. Epidemic dengue fever in Puerto Rico, 1977: A cost analysis. *Am J Trop Med Hyg 28:* 1040–1044.
40. Meltzer MI, Rigau-Perez JG, Clark GG, Reiter P, Gubler DJ, 1998. Using disability-adjusted life years to assess the economic impact of dengue in Puerto Rico: 1984–1994. *Am J Trop Med Hyg 59:* 265–271.
41. Deparis X, Murgue B, Roche C, Cassar O, Chungue E, 1998. Changing clinical and biological manifestations of dengue during the dengue-2 epidemic in French Polynesia in 1996/97—description and analysis in a prospective study. *Trop Med Int Health 3:* 859–865.
42. Lanciotti RS, Lewis JG, Gubler DJ, Trent DW, 1994. Molecular evolution and epidemiology of dengue-3 viruses. *J Gen Virol 75:* 65–75.
43. Rico-Hesse R, Harrison LM, Alba Salas R, Tovar D, Nisalak A, Ramos C, Boshell J, De Mesa MTR, Nogueira RMR, Travassos Da Rosa A, 1997. Origins of dengue type 2 viruses associated with increased pathogenicity in the Americas. *Virology 230:* 244–251.