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DIFFERENCES IN DENGUE SEVERITY IN INFANTS, CHILDREN, AND ADULTS IN A 3-YEAR HOSPITAL-BASED STUDY IN NICARAGUA

SAMANTHA NADIA HAMMOND,† ANGEL BALMASEDA,† LEONEL PÉREZ, YOLANDA TELLEZ, SAIRA INDIRA SABORÍO, JUAN CARLOS MERCADO, ELSA VIDEA, YORYELIN RODRIGUEZ, MARIA ANGELES PÉREZ, RICARDO CUADRA, SORAYA SOLANO, JULIO ROCHA, WENDY IDIAQUEZ, ALCIDES GONZALEZ, AND EVA HARRIS*

Division of Infectious Diseases, School of Public Health, University of California, Berkeley, California; Departamento de Virología, Centro Nacional de Diagnóstico y Referencia, Ministerio de Salud, Managua, Nicaragua; Infectious Diseases Unit, Hospital Infantil Manuel de Jesús Rivera, Managua, Nicaragua; Hospital Escuela Oscar Danilo Rosales Arguello, León, Nicaragua

Abstract. To investigate age-related differences in dengue severity, 114 infants, 1,211 children, and 346 adults with laboratory-confirmed dengue virus (DEN) infections presenting to three hospitals in major urban centers in Nicaragua were recruited from 1999 to 2001. The age distribution of dengue cases and the circulating serotype (predominantly DEN2) were representative of national data. Similar results were obtained when either dengue hemorrhagic fever/dengue shock syndrome or its principal manifestations (vascular permeability, internal hemorrhage, marked thrombocytopenia, and/or shock) were analyzed in relation to age and immune status. The burden of disease and of severe dengue was found predominantly in infants 4–9 months of age and in children 5–9 years old, and secondary DEN infection was a risk factor for severity in children. Age-related differences were identified in the prevalence of specific clinical manifestations as well as in their association with a confirmed DEN diagnosis. This represents one of the few comprehensive studies to analyze characteristics of dengue in infants, children, and adults in the same population and highlights age-related differences in dengue severity.

INTRODUCTION

Caused by the four serotypes of the dengue flavivirus and transmitted by mosquitoes, dengue affects an estimated 50–100 million people annually around the world, principally in tropical and subtropical regions.[1] Dengue virus (DEN) causes a spectrum of clinical disease ranging from the self-limited dengue fever, usually accompanied by arthrolgia, myalgia, and headache, to dengue hemorrhagic fever (DHF) marked by thrombocytopenia, hemorrhagic manifestations, and increased vascular permeability (plasma leakage), to dengue shock syndrome (DSS), which when untreated may lead to death. The infecting serotype and an individual's previous exposure to other DEN serotypes are known to influence disease severity.[2]

Although unique disease characteristics in infants[3–5] as well as clinical differences between pediatric and adult cases[6,7] have been noted, few publications have compared all three age groups in a single population. Furthermore, dengue in Asia has been much more extensively characterized than dengue in the Americas.[8] Here, we provide a comprehensive description of the clinical manifestations of dengue in infants, children, and adults in a hospitalized population in Nicaragua over a 3-year period and enumerate the age-specific differences in the occurrence of DHF/DSS as well as in the presence of severe and classic manifestations of dengue.

MATERIALS AND METHODS

Study population. This study was conducted in three major hospitals in the two largest cities in Nicaragua: Managua (population 1,336,000) and León (population 388,000). The 336-bed Hospital Escuela Oscar Danilo Rosales Arguello (HEODRA) is the only public tertiary care facility that serves León's pediatric and adult populations. The Hospital Infantil Manuel de Jesús Rivera (HIMJR) in the capital city of Managua contains 221 beds; it is the National Pediatric Reference Hospital and treats the great majority of children seeking tertiary care in Managua. The Hospital "Roberto Calderon," also located in Managua, is one of the city's public hospitals serving adults, with 205 beds. The "Roberto Calderon" Hospital was included in the study only in 2001. This study was approved by the Committee for the Protection of Human Subjects at the University of California, Berkeley, and the Ethical Review Committee of the Centro Nacional de Diagnóstico y Referencia (CNDR) of the Nicaraguan Ministry of Health (MOH).

Study design. From January 1999 to December 2001, all patients presenting to the hospitals who were suspected of dengue underwent the informed consent process and were invited to participate in the study. After consenting, a blood sample was collected for serologic and virologic testing. A second blood sample, taken upon hospital discharge, served as the convalescent phase sample. Each sample was accompanied by a standardized questionnaire that recorded the subject's demographic and epidemiologic characteristics as well as information regarding their illness. Clinical evolution of hospitalized patients was monitored daily using study forms that were supplemented by chart review. As per routine hospital procedure, blood samples were collected daily from patients hospitalized for dengue for monitoring of hematocrit and platelet levels until normal levels were obtained post-defervescence.

Definitions. Study participants 0–11 months of age were considered infants, those 1–14 years old were considered children, and adults were defined as those participants 15 years old and above. A suspected dengue case consisted of an acute febrile illness with two or more of the following symptoms and signs: headache, retro-orbital pain, myalgia, arthralgia, rash, and hemorrhagic manifestations. The World Health Organization (WHO) and Pan American Health Organization (PAHO) definitions and criteria were used to classify dengue disease.[9,10] Classic dengue fever (DF) and dengue fever with

† These two authors contributed equally to this study.
* Address correspondence to Eva Harris, Division of Infectious Diseases, School of Public Health, 140 Warren Hall, University of California, Berkeley, CA 94720-7360. E-mail: eharris@berkeley.edu

hemorrhagic manifestations (DFHem) were considered mild diseases. Dengue hemorrhagic fever (DHF) was defined as fever with hemorrhagic manifestations, thrombocytopenia (platelet count $\leq$ 100,000/mm^3), and hemoconcentration or other signs of plasma leakage (equivalent to WHO classification DHF grades I and II), and dengue shock syndrome (DSS) (equivalent to DHF grades III and IV) was classified using the DHF criteria plus either hypotension for age (systolic pressure < 80 mm of Hg for those < 5 years of age and < 90 mm of Hg for those $\geq$ 5 years of age) or narrow pulse pressure ($\leq$ 20 mm of Hg)[10] in the presence of clinical signs of shock (e.g., slow capillary filling, cold clammy skin). DHF and DSS were considered severe disease syndromes.

In parallel to the DHF/DSS classification, severe clinical manifestations of dengue were defined as internal hemorrhage, plasma leakage, shock, and/or platelet count $\leq$ 50,000/mm^3. Internal hemorrhage consisted of melena, hematemesis, hematuria, and/or menorrhagia. Plasma leakage was indicated by the presence of pleural effusion, ascites, and/or hemoconcentration ($\geq$ 20% increase in hematocrit over the value at discharge or hematocrit values $\geq$ 20% of the normal value for age and sex).[11] Shock was characterized by narrow pulse pressure or hypotension with or without documented clinical signs of shock. Marked thrombocytopenia was determined by analyzing different cutoff values for platelet counts with respect to their association with the other critical manifestations of DHF/DSS. Platelet counts $\leq$ 50,000/mm^3 were significantly associated (OR 4.14, 95% CI 3.22–5.31) with the presence of shock, plasma leakage, and/or internal hemorrhage, whereas platelet counts of 50,000–100,000/mm^3 or $\geq$ 100,000/mm^3 were not (see accompanying paper; Balmaseda and others).

Dengue cases were considered to have a positive laboratory result if any of the following occurred: 1) DEN was isolated, 2) DEN RNA was demonstrated by reverse transcriptase-polymerase chain reaction (RT-PCR), 3) an IgM capture enzyme-linked immunosorbent assay (ELISA) was positive, 4) antibody titer by inhibition ELISA was $\geq$ 2,560 (equivalent to $\geq$ 1,280 by hemagglutination inhibition (HI)) or demonstrated a fourfold or greater increase in antibody titer in paired acute and convalescents sera.[11] Subjects 1 year and older were considered to have experienced a primary DEN infection when the antibody titer was < 20 in acute samples and/or < 2,560 in convalescent samples as determined by inhibition ELISA (equivalent to < 10 or < 1,280 by HI, respectively). Secondary DEN infection was defined by an antibody titer by inhibition ELISA of $\geq$ 20 in acute samples and/or $\geq$ 2,560 in convalescent samples.[11] Immune status was not ascertained in infants.

Laboratory methods. Platelet count was determined by the Neubauer method,[12] and hematocrit was obtained by manual centrifugation or by using the Sysmex automated counter (Sysmex Corp., Kurashiki City, Japan) at the associated clinical laboratories. Periodic training in clinical hematologic methods was conducted by the Clinical Chemistry Division of the CNDR of the Nicaraguan MOH. In hospitalized patients, hematologic analysis was conducted at least once per day, and the values were recorded on 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 MAC-ELISA[13] was modified to decrease the time required for the assay.[14] Total antibody levels were measured using an inhibition ELISA[15] that had been previously validated against the HI assay,[11] resulting in values that were approximately one dilution higher than HI titers (Balmaseda A and others, unpublished data). Viral isolation and RT-PCR detection of viral RNA were perfomed with sera collected within 5 days since onset of symptoms. Viral isolation in C6/36 cells and subsequent immunofluorescent detection of viral antigens were performed as described previously.[16,17] RNA was extracted, reverse transcribed, and amplified using serotype-specific primers directed to the capsid region[17,18] or NS3 gene[19] with minor modifications. Restriction site specific PCR (RSS-PCR) for determination of DEN genotype was performed as previously described.[20]

Statistical analysis. Data were entered and analyzed using Epi-Info (Centers for Disease Control and Prevention, Atlanta, GA) and STATA (StataCorp LP, College Station, TX). Crude odds ratios and their Cornfield 95% confidence intervals were calculated. Chi-square analysis for significance was performed in Epi-Info.

RESULTS

Study population. A total of 3,173 suspected dengue cases presenting to the HIMJR and Roberto Calderon hospitals in Managua and to the HEODRA hospital in León were enrolled, including 205 (6%) infants, 2,185 (69%) children, and 783 (25%) adults. Of these, 114 infants, 1,211 children, and 346 adults were confirmed as positive for DEN infection, consitituting 56%, 55%, and 44% of each age group, respectively. Of laboratory-confirmed cases, 5 (4%) infants, 296 (24%) children, and 285 (82%) adults were seen at the hospital in León, and 109 (96%) infants, 915 (76%) children, and 61 (18%) adults were attended at the hospitals in Managua (Table 1). The great majority of the 3,173 patients were presenting as their first hospital visit: 35 (3.0%), 16 (2.1%), and 44 (3.5%) patients in 1999, 2000, and 2001, respectively, were referred from other district hospitals.

The most affected age group was children 5–9 years of age, which accounted for 58% of all confirmed dengue cases in the study population (Figure 1A). Among children enrolled in the study, the burden of disease was also observed predominantly in infants (Figure 1B). The burden of disease stratified by age group paralleled the trends observed in the MOH dengue surveillance system (data not shown). The ratio of females to males in the study population was 3:2 in adults and 1:1 in infants and children (Table 1). The average hospital stay was 6.4 days, 6.0 days, and 5.2 days for infants, children, and adults, respectively.

Differences between calendar years. To monitor year-to-year variation, data from the three calendar years of the study were compared. Little to no difference in circulating DEN serotype was observed between the three calendar years. In 1999, DEN2, DEN3, and DEN4 were isolated in a ratio of 21:2:3, respectively, from hospitalized patients in both cities. In 2000 and 2001, only DEN2 isolates were obtained from the study population. This trend is consistent with data obtained by the national dengue surveillance system (data not shown). The Asian-American ("Jamaica") DEN2 genotype was main-

TABLE 1
Demographic data on study participants and distribution of participants by calendar year

	Infants		Children		Adults	
	Enrolled *N* (%)	Lab-confirmed positive *N* (%)	Enrolled *N* (%)	Lab-confirmed positive *N* (%)	Enrolled *N* (%)	Lab-confirmed positive *N* (%)
Year						
Sum	205 (100)	114 (64)	2,185 (100)	1,211 (63)	783 (100)	346 (50)
1999	74 (36)	42 (37)	799 (37)	504 (42)	279 (36)	111 (32)
2000	34 (17)	19 (17)	464 (21)	215 (18)	256 (33)	117 (34)
2001	97 (47)	53 (46)	922 (42)	492 (40)	248 (32)	118 (34)
Age						
Mean (SD)	9.2 mo (2.8)	9.5 mo (2.6)	6.8 yr (3.9)	6.9 yr (3.8)	28.9 yr (13.3)	29.1 yr (12.8)
Sex						
F	105 (51)	62 (54)	1,097 (50)	620 (51)	469 (60)	205 (59)
M	100 (49)	52 (46)	1,087 (50)	591 (49)	314 (40)	141 (41)
City						
Managua	184 (90)	109 (96)	1,445 (66)	915 (76)	114 (15)	61 (18)
Leon	21 (10)	5 (4)	740 (34)	296 (24)	669 (85)	285 (82)

tained in circulation throughout the years of study, as determined by RSS-PCR.[16] Over the 3-year period, the number of people who presented and who were diagnosed with dengue at the study hospitals varied, with 1,152, 754, and 1,267 individuals presenting during 1999, 2000, and 2001, respectively. The drop in 2000 is again consistent with MOH surveillance data, which registers suspected and confirmed dengue cases from hospitals and health centers throughout the country (data not shown).

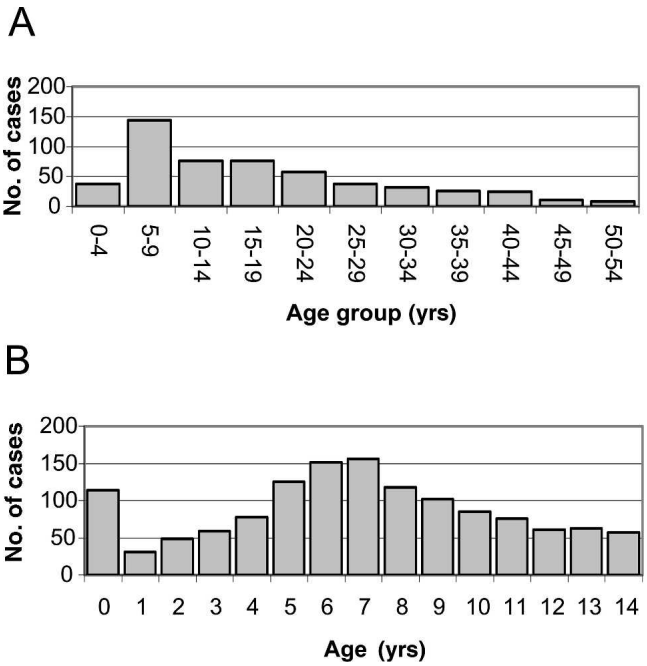

FIGURE 1. Age distribution of study cohort. **A**, Age distribution of study participants in León by age group, with all three years combined. Because the HEODRA hospital in León was the only public facility serving both children and adults in León and surrounding territories, study participants should represent the age distribution of individuals of all ages requiring tertiary attention for dengue. Due to the participation of the National Pediatric Reference Hospital in our study, which introduces a bias toward young age, the data from Managua was not appropriate for this comparison of age distribution, which included both children and adults. **B**, Age distribution of study participants 0–14 years of age, including both León and Managua.

Age-related differences in dengue severity. To characterize age-related differences in dengue disease in our study population, the presence of DHF/DSS as defined by the WHO[9] as well as four key severe clinical manifestations associated with dengue (shock, plasma leakage, marked thrombocytopenia, and internal hemorrhage) were investigated in infants, children, and adults. Overall, 64%, 55%, and 36% of infants, children, and adults, respectively, displayed one or more of the four severe clinical manifestations studied. When analyzed separately, shock, plasma leakage, and marked thrombocytopenia were more prevalent as age decreased, whereas the frequency of internal hemorrhage augmented as age increased (Figure 2). Signs of plasma leakage and marked thrombocytopenia varied most substantially between age groups, with 40–46% in infants, 30–31% in children, and 15–17% in adults. Internal hemorrhage increased from 6.3% of infants, to 9.4% in children, to 15.2% in adults.

Analysis of severity by age and immune status. The association of age and immune status with DHF/DSS or its severe manifestations was analyzed in the study population. Infants and children 4–6 years of age were significantly more likely than adults to develop DHF/DSS or manifestations of severe clinical illness (Figure 3). A graph of the odds ratios of age associated with severe illness demonstrates the risk associated

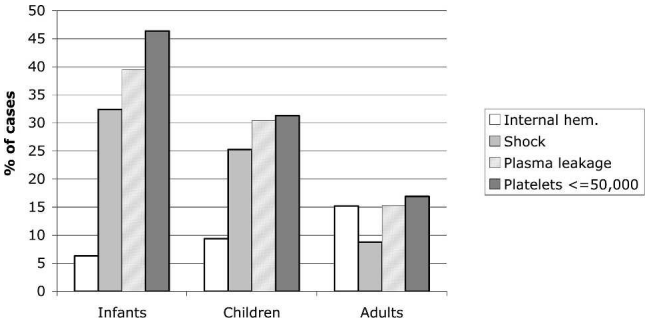

FIGURE 2. Prevalence of severe clinical manifestations of dengue in infants, children, and adults. The percentage of cases in each age group presenting with internal hemorrhage, shock, signs of plasma leakage (hemoconcentration, pleural effusion, and/or ascites), and/or marked thrombocytopenia (platelet count $\leq$ 50,000/mm^3) was plotted, as indicated. Infants, < 1 year old; children, 1–14 years old; adults, > 14 years old.

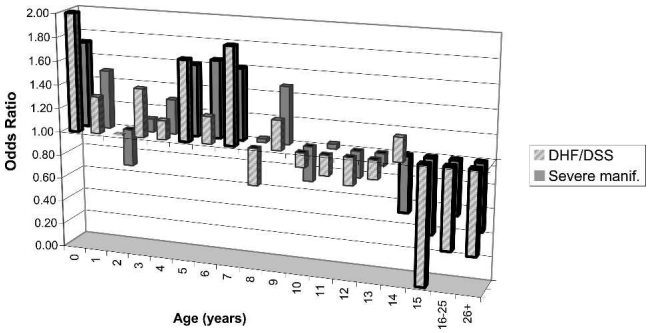

FIGURE 3. Age as a risk factor for DHF/DSS or for the presence of severe clinical manifestations of dengue. The risk of presenting with DHF/DSS or shock, signs of plasma leakage, internal hemorrhage, and/or platelet count $\leq 50{,}000/mm^3$ by year of age was calculated. Bar height indicates the value of the odds ratio, and vertical bars with heavy black borders indicate $P < 0.01$. Infants and children ages 5–7 years old were at significant risk for severe dengue, whereas adults and adolescents 14 years and older were significantly less at risk for severe disease (OR < 0.5). The risk of presenting with any of the four severe clinical manifestations closely reflected the risk of developing DHF/DSS.

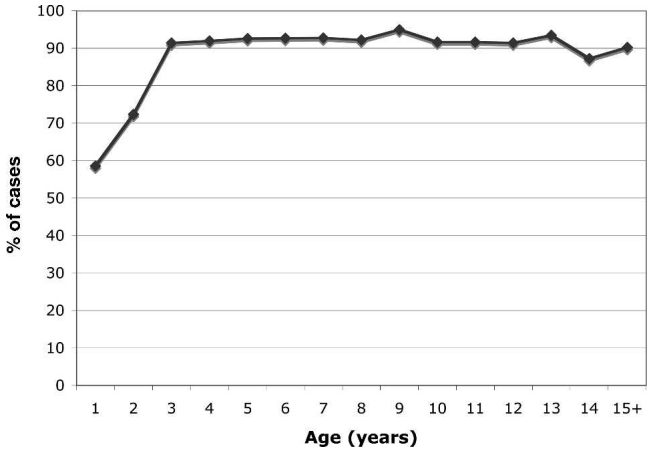

FIGURE 5. Immune response by age in children with confirmed dengue. Cases were classified as primary or secondary DEN infections, and secondary infections were plotted according to year of age.

with infants and children 4–6 and protection in older adolescents and adults. The presence of severe clinical manifestations or DHF/DSS stratified by year of age mirrored the number of dengue cases by year of age (compare Figure 1B to Figure 3). Dengue among infants was analyzed by month of age. Cases ranged from 1 to 11 months of age, peaking at 6 months of age. Similar age distribution curves, which also peaked at 6 months, were observed in infants with DHF/DSS and in infants presenting with any of the severe clinical manifestations (Figure 4).

The study population consisted almost entirely of secondary DEN infections. By 1 year of age, more than 50% of confirmed dengue cases were due to secondary DEN infections, and by 3 years of age this percentage had risen to 90% of confirmed dengue cases (Figure 5). Secondary immune status proved to be a risk factor for severe disease in children but not in adults. In children, a secondary DEN infection was significantly associated with internal hemorrhage, marked thrombocytopenia, presence of one or more of the four severe manifestations, and DHF/DSS, whereas it was not associated with plasma leakage or shock (Table 2). In adults, secondary immune status was not significantly associated with any of the severe clinical manifestations or DHF/DSS (data not shown). Infants were excluded from this analysis due to the presence of maternal antibodies, which complicates analysis of the immune response.

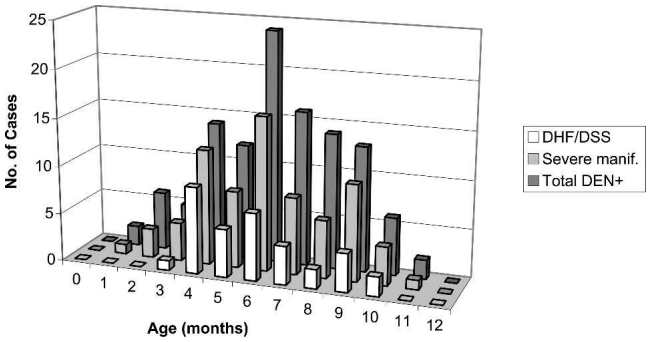

FIGURE 4. Age distribution of infants with confirmed dengue, DHF/DSS, or severe clinical manifestations. The number of infants laboratory-confimed as DEN-positive ("Total DEN+"), classified as DHF/DSS cases ("DHF/DSS"), or presenting with shock, signs of plasma leakage, internal hemorrhage, and/or platelet count $\leq 50{,}000/mm^3$ ("Severe manif.") were graphed by month of age.

Signs and symptoms. A comparison of the prevalence of various signs and symptoms between age groups was performed, although symptoms were not analyzed in infants. The classic symptoms of dengue, such as headache, arthralgia, myalgia, and retro-orbital pain, were present in more than 60% of children and adults with confirmed dengue (Table 3). External bleeding (petequiae, positive torniquet test, epistaxis, or gingival bleeding) and chills were also present in more than 50% of children and adults with confirmed dengue. Fever, external hemorrhagic manifestations, and rash were present in more than 50% of infants. When pairwise comparisons were made between children and either infants or adults, the percentage of specific signs and symptoms (with the exception of the torniquet test, melena, and pleural effusion) were significantly different between children and infants or between children and adults, and in some occasions between infants and children and adults. Epistaxis, hypotension, and anorexia were present to a significantly greater degree in children than

TABLE 2

Association of secondary immune response with severe clinical manifestations or DHF/DSS in laboratory-confirmed dengue cases in children 1 to 14 years of age

	1° DEN infection *N* (%)	2° DEN infection *N* (%)	OR (95% CI)*
Internal hemorrhage	4 (4)	106 (10)	**2.99** (1.0–9.8)
Plasma leakage	28 (26)	334 (31)	1.33 (0.8–2.2)
Shock	26 (27)	254 (26)	0.95 (0.6–1.6)
Platelet count $\leq 50{,}000/mm^3$	15 (16)	330 (33)	**2.68** (1.5–5.0)
Severe manifestations†	50 (46)	609 (57)	**1.59** (1.1–2.4)
DHF/DSS	13 (12)	231 (22)	**2.06** (1.1–3.9)

DHF, dengue hemorrhagic fever; DSS, dengue shoch syndrome.
* **Bold** indicates statistical significance.
† Presence of one or more of the following severe clinical manifestations: internal hemorrhage, signs of plasma leakage, shock, platelet count $\leq 50{,}000/mm^3$.

TABLE 3

Prevalence of signs and symptoms in infants, children, and adults, with significant differences in prevalence noted between children and infants and between children and adults*

	Infants *N* (%)	OR (95% CI)	Children *N* (%)	OR (95% CI)	Adults *N* (%)
Other					
Fever	106 (100)	N/A	1,134 (95)	0.23 (0.07–0.65)	340 (99)
Headache	N/A	N/A	873 (78)	0.32 (0.20–0.50)	268 (92)
Myalgia	N/A	N/A	743 (66)	0.37 (0.26–0.53)	244 (84)
Arthralgia	N/A	N/A	726 (62)	0.39 (0.28–0.52)	274 (81)
Abdominal pain	N/A	N/A	740 (62)	2.42 (1.88–3.12)	138 (41)
Anorexia	42 (38)	0.43 (0.28–0.65)	697 (59)	2.62 (2.03–3.40)	120 (35)
Retro-orbital pain	N/A	N/A	693 (59)	0.38 (0.28–0.50)	271 (79)
Chills	31 (29)	0.41 (0.26–0.64)	584 (50)	–**	164 (51)
Rash	69 (63)	1.97 (1.29–3.01)	545 (46)	–	136 (40)
Platelets ≤ 50,000	51 (46)	1.94 (1.28–2.93)	346 (31)	2.19 (1.51–3.18)	41 (17)
Cough	42 (39)	–	387 (33)	1.79 (1.33–2.42)	71 (21)
Hepatomegaly	29 (26)	–	260 (22)	5.63 (3.27–9.84)	16 (5)
Diarrhea	31 (28)	2.00 (1.25–3.18)	196 (17)	–	53 (16)
Hemorrhage					
Any hemorrhagic signs	67 (60)	0.64 (0.42–0.97)†	842 (70)	1.88 (1.45–2.42)	190 (56)
External bleeding††	64 (59)	–	763 (65)	1.75 (1.36–2.26)	170 (51)
Positive torniquet test	44 (40)	–	494 (42)		124 (38)
Petequiae	53 (48)	–	454 (38)	1.31 (1.01–1.71)†	108 (32)
Epistaxis	9 (8)	0.33 (0.16–0.69)	251 (21)	2.07 (1.42–3.02)	39 (12)
Gingival bleeding	3 (3)	–	60 (5)	0.55 (0.34–0.89)	30 (9)
Hematemesis	3 (3)	–	72 (6)	3.06 (1.34–7.33)	7 (2)
Hematuria	2 (2)	–	22 (2)	0.30 (0.15–0.58)	20 (6)
Melena	4 (3)	–	46 (4)	–	8 (2)
Menorrhagic (females only)	0 (0)	N/A	18 (3)	0.18 (0.09–0.34)	30 (15)
Internal bleeding	7 (6)	–	112 (10)	0.57 (0.40–0.83)	52 (16)
Shock					
Clinical shock§	46 (40)	–	419 (35)	4.05 (2.82–5.84)	40 (12)
Arterial shock¶	33 (32)	1.59 (1.00–2.51)†	280 (23)	3.13 (2.01–4.89)	26 (9)
Cold skin	32 (29)	–	250 (21)	4.04 (2.49–6.61)	21 (6)
Narrow pulse press	31 (30)	1.78 (1.11–2.84)†	221 (20)	2.79 (1.76–4.45)	24 (8)
Slow capillary fill	20 (19)	–	168 (15)	2.89 (1.66–4.93)	17 (6)
Hypotension	6 (6)	0.35 (0.14–0.85)†	169 (15)	5.68 (2.78–12.02)	9 (3)
Plasma leakage					
Plasma leakage	45 (40)	–	368 (30)	2.41 (1.74–3.36)	53 (15)
Hemoconcentration	30 (40)	2.63 (1.56–4.43)	156 (20)	–	13 (13)
Pleural effusion	12 (11)	–	73 (6)	–	11 (3)
Ascites	12 (11)	2.06 (1.02–4.08)†	40 (3)	–	11 (3)

* All P values < 0.01 unless otherwise indicated.
** –, not significant.
† $P < 0.05$.
†† Petequiae, positive torniquet test, gingival bleeding, epistaxis.
§ Slow capillary filling, cold clammy skin, cold extremities.
¶ Narrow pulse pressure or hypotension for age.

in either infants and adults. All signs and symptoms associated with shock, including narrow pulse pressure, all signs and symptoms associated with plasma leakage, and low platelet count were more prevalent in infants followed by children and then adults, with the exception of hypotension, which was more prevalent in children than infants.

Signs and symptoms were also analyzed for their association with a laboratory-confirmed dengue diagnosis. Signs indicating plasma leakage, thrombocytopenia, and hepatomegaly were significantly associated with confirmed dengue in all age groups (Table 4). The presence of petechiae and/or a positive tourniquet test was statistically associated with a confirmed laboratory diagnosis in all age groups, whereas the nature of the other hemorrhagic symptoms varied between age groups. Assessment of the torniquet test as a diagnostic test for confirmed dengue yielded the following: for infants, a sensitivity of 33%, specificity of 76%, positive predictive value (PPV) of 71%, and negative predictive value (NPV) of 38%; for children, a sensitivity of 36%, specificity of 73%, PPV of 69%, and NPV of 40%; for adults, a sensitivity of 37%, specificity of 86%, PPV of 73%, and NPV of 57%. Rash, abdominal pain, and clinical signs of shock (e.g., slow capillary filling, cold clammy skin) were significantly associated with confirmed disease in children; leukopenia was associated with confirmed disease in adults; and chills were correlated with dengue in infants. The only sign or symptom statistically associated with a negative dengue diagnosis was cough in children (OR = 0.75, 0.6–0.9), which was present in 393 (33%) of positive cases and 274 (40%) of negative cases.

Mortality. In the years 1999 to 2001, 13 patient deaths were reported among the study participants, 10 (77%) of which were in children. Seven patient deaths were classified as DHF/DSS, and all 13 fatal cases demonstrated evidence of plasma leakage, internal hemorrhage, and/or shock.

DISCUSSION

Since 1985, Nicaragua has reported endemic dengue, including both classic disease and DHF/DSS. This study examines the clinical features of hospitalized dengue cases during

TABLE 4
Signs and symptoms significantly associated with a laboratory-confirmed positive dengue among cases with a laboratory-confirmed result*

Signs and symptoms	Infants			Children			Adults		
	Positive cases *N* (%)	Negative cases *N* (%)	OR (95% CI)	Positive cases *N* (%)	Negative cases *N* (%)	OR (95% CI)	Positive cases *N* (%)	Negative cases *N* (%)	OR (95% CI)
Plasma leakage									
Ascitis	–**	–	–	66 (6)	6 (1)	6.8 (2.8–17.6)	11 (3)	2 (1)	5.7 (1.2–37.3)
Hemoconcentration	37 (34)	7 (13)	3.5 (1.3–9.5)	311 (28)	90 (16)	2.0 (1.5–2.6)	27 (16)	6 (5)	4.0 (1.5–11.1)
Plasma leakage	45 (40)	8 (13)	4.5 (1.8–11.3)	368 (30)	100 (14)	2.7 (2.1–3.5)	53 (15)	14 (4)	4.3 (2.3–8.4)
Pleural effusion	12 (11)	1 (2)	7.4 (0.94–158)	73 (6)	7 (1)	6.4 (2.8–15.4)	11 (3)	1 (0)	10.9 (1.4–230)
Shock									
Clinical signs	–	–	–	419 (35)	151 (21)	2.0 (1.6–2.5)	–	–	–
Hemorrhage									
Any hemorraghic. sign	67 (60)	26 (40)	2.2 (1.1–4.4)†	–	–	–	188 (56)	114 (33)	2.5 (1.8–3.4)
Gingival bleeding	3 (3)	0	N/A	60 (5)	18 (3)	2.0 (1.1–3.6)†	30 (9)	14 (4)	2.3 (1.1–4.6)†
Hematemesis	–	–	–	72 (6)	20 (3)	2.2 (1.3–3.8)	–	–	–
Melena	–	–	–	11 (2)	46 (4)	2.5 (1.2–5.2)	–	–	–
Petechiae	53 (48)	13 (21)	3.5 (1.6–7.7)	–	–	–	106 (32)	54 (16)	2.4 (1.6–3.5)
Positive torniquet test	–	–	–	–	–	–	122 (37)	48 (15)	3.5 (2.4–5.2)
Positive torniquet test and/or petechaie	63 (58)	21 (35)	2.5 (1.3–5.2)	665 (57)	273 (40)	2.0 (1.6–2.4)	154 (47)	70 (22)	3.2 (2.2–4.6)
Vaginal bleeding	–	–	–	–	–	–	30 (9)	11 (3)	3.0 (1.4–6.3)
Platelet count									
$\leq$ 150,000/mm^3	94 (86)	31 (55)	4.7 (2.1–10.8)	895 (80)	297 (53)	3.5 (2.8–4.4)	136 (56)	67 (35)	2.4 (1.6–3.6)
$\leq$ 100,000/mm^3	79 (72)	18 (32)	5.4 (2.5–11.6)	695 (62)	173 (31)	3.6 (2.9–4.5)	94 (39)	38 (20)	2.6 (1.6–4.1)
$\leq$ 50,000/mm^3	51 (46)	10 (18)	4.0 (1.7–9.5)	351 (31)	59 (11)	3.9 (2.9–5.3)	41 (17)	15 (8)	2.4 (1.2–4.8)
Other									
Abdominal pain	–	–	–	747 (63)	309 (45)	2.1 (1.7–2.6)	–	–	–
Hepatomegaly	29 (26)	3 (5)	7.0 (1.9–30.9)	262 (22)	69 (10)	2.6 (1.9–3.5)	15 (4)	5 (1)	3.1 (1.0–9.8)†
Leukopenia	–	–	–	–	–	–	110 (48)	51 (29)	2.2 (1.4–3.4)
Rash	–	–	–	551 (47)	197 (29)	2.19 (1.8–2.7)	–	–	–
Chills	31 (29)	6 (10)	3.72 (1.3–10.8)	–	–	–	–	–	–

* Chi-squared analysis performed. All P values are < 0.01 unless otherwise specified. Cases with indeterminate laboratory diagnosis were excluded from analysis.
** Not significant.
† $P < 0.05$.

the years 1999 to 2001 in infants, children, and adults. As the DEN2 serotype predominated over the period studied, data from all 3 years were combined. Overall, 3,173 suspected dengue cases were enrolled, and 1,671 (53%) were laboratory-confirmed as DEN infections. The burden of disease severity was concentrated in infants and children 5–9 years of age. Profiles of disease in infants consistent with the effects of maternal antibody enhancement of dengue illness were observed. Numerous age-related differences in the clinical profile of classic and severe dengue were documented. This study is one of the first comparisons of these three age groups simultaneously.

The major burden of disease in Nicaragua lies in infants and children 5 to 9 years of age. A peak at this age in children can be expected in a country that has been endemic for dengue for more than 15 years.[5,21] Countries with a shorter or nonendemic history of DEN circulation report cases principally in the adolescent and adult population.[22,23] In this study, the distribution of the burden of disease was similar to that of DHF/DSS and/or severe clinical manifestations in both infants and children. In other years, however, the age of greatest disease burden has differed from the age most highly associated with severity (Balmaseda A and others, unpublished data), as has been described elsewhere.[7,21,24] This difference may be due to variations in circulating DEN serotype and strain or to changes in susceptibility to infection or enhanced disease due to immune status. In our study population, DHF/DSS and DSS alone were more prevalent in infants than children or adults. This finding is consistent with data from other countries where the burden of severe illness lies predominantly in infants.[3,5,21] Similarly, the presence of severe clinical manifestations (shock, plasma leakage, and marked thrombocytopenia) was also more prevalent in infants, followed by children and then adults. Only the frequency of internal hemorrhage increased with age, affecting one in seven adults.

Infants suspected of dengue underwent mandatory hospitalization at one of the study hospitals; therefore, the burden of illness in this age group may be overrepresented. However, as a greater percentage of infants present with severe clinical manifestations and/or DHF/DSS, this policy may well be warranted. The peak of dengue cases in infants 4–9 months of age is consistent with the theory of maternal antibody enhancement of disease, as maternal antibodies wane from protective to enhancing levels.[3–5,25,26] Maternal antibodies to dengue would be present at birth in virtually all infants due to the high seroprevalence in the adult population (Balmaseda A and others, unpublished data).[27] This is the first reported data supporting the maternal antibody effect by month of age in the Americas. Unlike other studies that have categorized most or all infants as DHF/DSS cases,[3–5,26] only 30% of the infants in this study fulfilled strict DHF/DSS criteria, while 64% presented at least one of the severe clinical manifestations of dengue. Therefore, maternal antibodies may enhance symptomatic dengue, not just DHF/DSS.

The great majority of the dengue cases in this study were due to secondary DEN infection: 58% of children 1 year old had secondary DEN infections, and by age 3, more than 90% of confirmed DEN-positive cases were secondary infections. In children only, secondary infection was a risk factor for

DHF/DSS and for the presence of severe clinical manifestations. In adults, no statistically significant differences in disease severity were found according to immune status; however, this analysis may have been affected by the low number of primary DEN infections in adults. In an earlier publication, we reported that during the 1998 dengue epidemic in Nicaragua, secondary infection was not significantly correlated with DHF/DSS.[11] In 1998, 91% of cases were associated with DEN3 infection, whereas in this study, the great majority were DEN2 infections. This finding is consistent with studies that show that disease due to DEN2 is principally associated with secondary infection, whereas DEN1 and DEN3 can cause symptomatic disease and DHF/DSS in primary infections (Balmaseda A and others, unpublished data).[28,29]

An extensive analysis of signs and symptoms revealed both similarities and significant differences between the three age groups examined. In children and adults, headache, myalgia, arthralgia, and retro-orbital pain were present in more than 60% of DEN-positive cases; the age-related rise in their prevalence may be due to difficulties for young children to communicate the presence of such symptoms. Signs of internal bleeding varied between age groups. Hematuria and menorrhagia were significantly more prevalent in adults than in children or infants and comprised the bulk of internal bleeding in adults. Hematemesis and melena, on the other hand, were more prevalent in children, followed by infants and then adults. Internal hemorrhage contributed substantially to the number of severe dengue cases that the DHF/DSS definition did not identify in adults; in fact, bleeding, especially but not exclusively in adults, has been described as a disease distinct from DHF/DSS.[30]

The principal complication of dengue illness is vascular permeability,[9] which decreases with increasing age; greater capillary fragility at younger ages likely accounts for this age-related trend.[31,32] In our study population, signs of plasma leakage and reduced platelet count were more prevalent in infants, followed by children and then adults, and were strongly associated with a positive laboratory dengue diagnosis. Of note, platelet counts of less than 150,000/mm^3, 100,000/mm^3, and 50,000/mm^3 were equally associated with a positive laboratory dengue diagnosis across all ages. The frequency of shock also decreased with increasing age. With the exception of hypotension, all signs and symptoms associated with shock were most significantly associated with infants, then children and adults. In lieu of hypotension, narrow pulse pressure, which typically precedes hypotension in children and is therefore one of the earliest manifestations of shock,[33] predominated as the primary sign of shock in infants. We found that slow capillary filling was documented in two-thirds of patients with shock. As dengue exists in regions of the world, including Nicaragua, where pediatric blood pressure cuffs are not readily available, an investigation into the usefulness of slow capillary filling as a surrogate marker for low blood pressure as measured with a sphygomanometer may be warranted.

A positive tourniquet test and petequiae, the milder hemorrhagic manifestations that are primarily associated with increased capillary fragility,[32] displayed similar age-related trends as plasma leakage. The presence of petequiae was associated with a confirmed laboratory diagnosis in infants. Interestingly, a positive tourniquet test appeared to be significantly associated with confirmed DEN infection in adults. However, when the use of the torniquet test was evaluated as a diagnostic test for dengue, it was found to have low sensitivity, consistent with previous reports.[34] Hepatomegaly was less prevalent in our study population than in other studies.[5,35–37] Nonetheless, its presence was significantly associated with a confirmed dengue diagnosis in all age groups. Other studies have indicated elevated levels of hepatic enzymes as an early indicator of disease severity,[35,38,39] and a study in French Polynesia suggested that hepatic failure should be considered as a distinct severe form of dengue.[38]

Dengue will continue to spread worldwide until a safe and effective vaccine is available alongside sustainable mosquito control practices. Furthermore, as the eventual implementation of a vaccine will shift the burden of disease, the age-related differences in clinical manifestations described in this report indicate the importance of comparing a wide range of ages in future clinical studies of dengue.

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Authors' addresses: Angel Balmaseda, Leonel Pérez, Yolanda Tellez, Saira Indira Saborío, and Juan Carlos Mercado, Departamento de Virología, Centro Nacional de Diagnóstico y Referencia, Ministerio de Salud, Complejo de Salud Dra. Concepcion Palacios, Primero de Mayo, Managua, Nicaragua, Telephone/Fax: 011-505-289-7723. Samantha Nadia Hammond and Eva Harris, Division of Infectious Diseases, School of Public Health, 140 Warren Hall, University of California, Berkeley, CA 94720-7360, Telehone: 510-642-4845, Fax: 510-642-6350. Maria Angeles Pérez, Sheyla Silva, and Crisanta Rocha, Infectious Diseases Unit, Hospital Infantil Manuel de Jesus Rivera, Barrio Ariel Darce, Distrito no. 5, Managua, Nicaragua, Telephone: 011-505-289-7702, Fax: 011-505-289-7408. Ricardo Cuadro and Julio Rocha, Hospital Escuela Dr. Oscar Danilo Rosales Arguello (HEODRA), Iglesia Catedral 1 cuadra al sur, León, Nicaragua., Telephone/Fax: 011-505-311-5939.

Reprint requests: Eva Harris, Division of Infectious Diseases, School of Public Health, University of California, Berkeley, 140 Warren Hall, Berkeley, CA 94720-7360. E-mail: eharris@berkeley.edu

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