

# Laboratory-confirmed Dengue in Children in Three Regional Hospitals in the Philippines in 2009–2010

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**Background:** The burden of dengue is high in the Philippines but the prevalence of confirmed cases is unknown, and the disease is subject to under-reporting because surveillance of suspected cases is passive. We conducted a prospective epidemiological study to estimate the proportion of laboratory-confirmed dengue among clinically suspected hospitalized cases in the pediatric wards of 3 regional hospitals in the Philippines and to describe the clinical and laboratory features, age distributions, case fatality rates and serotype distributions of these hospitalized cases.

**Methods:** Patients  $\leq 18$  years and hospitalized for suspected dengue were included if they had an axillary temperature  $\geq 38^{\circ}\text{C}$  for 2–7 days and 2 or more dengue-associated symptoms. Dengue infection was confirmed in acute blood samples by serotype-specific reverse transcription-polymerase chain reaction and IgM immunoassay.

**Results:** We confirmed dengue infection in 1809 (86.1%) cases of 2103 suspected cases between November 2009 and November 2010. The 6- to 10-year-old age group had the highest proportion of cases overall (36.7%). Fever, anorexia, myalgia, abdominal pain and headache were the most common symptoms at admission. Hemorrhagic manifestations, signs of plasma leakage, thrombocytopenia and leucopenia were all significantly more common in confirmed than in nonconfirmed cases. Most cases (76.5%) developed dengue hemorrhagic fever or dengue shock syndrome, and the overall case fatality rate was 0.94%. Distributions of all 4 virus serotypes varied at each hospital.

**Conclusions:** The clinical burden of pediatric dengue continues to be substantial in the Philippines. Most hospitalized cases of suspected pediatric dengue can be laboratory confirmed and most develop severe disease.

**Key Words:** epidemiology, dengue fever, dengue hemorrhagic fever, children, hospitalization

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Dengue fever (DF) is caused by infection with 1 of 4 dengue virus (DENV) serotypes (1–4).<sup>1,2</sup> Although many dengue infections are asymptomatic or cause only mild to moderate fever for 2–7 days, severe cases can progress to dengue hemorrhagic fever (DHF), which may lead to shock, organ failure or death. Currently, there are no dengue-specific treatments or vaccines available, although one vaccine candidate is in the late phases of clinical development.<sup>3–7</sup> Additional and improved vector control measures are also needed to prevent disease.

The global burden of dengue is already large and continues to grow.<sup>8–11</sup> Recent estimates put the annual number of dengue infections at 390 million, of which 96 million are symptomatic.<sup>8</sup> According to an evidence-based consensus study, DENV transmission occurs in 128 countries worldwide, with 3.97 billion people at risk of dengue infection, of which at least 70% live in the Asia-Pacific region.<sup>8,12</sup>

Dengue disease is hyperendemic in the Philippines.<sup>13</sup> All 4 serotypes circulate throughout the country, and children aged 5–14 years are primarily affected.<sup>13–15</sup> To monitor disease patterns and outbreaks, the Philippine Integrated Disease Surveillance and Response program implemented in 2008 includes virological and serological testing. However, because of limited resources and laboratory capacity, only 3%–5% of all reported cases are submitted for laboratory confirmation.<sup>16</sup> Consequently, little serotype distribution information is available for different regions of the Philippines. In addition, dengue has been historically subject to underreporting, and the epidemiology of laboratory-confirmed dengue disease in children has not been well described.

We conducted an epidemiological study of all suspected dengue cases in the pediatric wards of 3 sentinel hospitals in the Philippines to estimate the proportion of laboratory-confirmed disease among hospitalized cases, to describe the clinical features of confirmed dengue in children and the age groups most affected and to determine regional serotype distributions.

## MATERIALS AND METHODS

### Study Design

This was a descriptive, prospective, epidemiological study of dengue disease in the pediatric wards of 3 sentinel regional hospitals in the Philippines (see Fig., Supplemental Digital Content 1, <http://links.lww.com/INF/C197>) using data collected between November 2009 and November 2010. The 3 hospitals were the José B. Lingad Memorial Regional Hospital (JBL); Davao Medical Center (DMC; now the Southern Philippines Medical Center), both of which care for patients aged  $\leq 15$  years, and the Western Visayas Medical Center (WVMC), which cares for patients aged  $\leq 18$  years. These 3 hospitals are located on the 3 major islands of the country: Luzon, Mindanao and the Visayas. The primary objective of this study was to estimate the proportion of laboratory-confirmed dengue cases among the clinically suspected dengue cases hospitalized in these pediatric wards. Secondary objectives were to describe the clinical and laboratory features of suspected

and laboratory-confirmed dengue; to describe the age distributions, case fatality rates and serotype distributions of laboratory-confirmed dengue and to assess the feasibility of case detection and laboratory confirmation.

### Ethical Considerations

This study was conducted in accordance with the latest revision of the Declaration of Helsinki (Seoul, Republic of Korea, October 2008), the Guidelines for Good Epidemiology Practices and local regulatory requirements. Before the enrollment of the first patient, the protocol was approved by the Institutional and Ethical Review Board of the Research Institute for Tropical Medicine (RITM) and by the Hospital Research Committee of the 3 hospitals. All patients (or parents or legal representatives if aged <18 years) provided written and signed informed consent.

### Study Population

To be considered for inclusion, patients had to be  $\leq 18$  years and hospitalized in the pediatric ward of a study hospital, and to have currently or to report having within the previous several days, an axillary temperature  $\geq 38^\circ\text{C}$  for 2–7 days. Patients also had to have at least 2 of the following dengue-associated signs or symptoms: a positive tourniquet test, hemorrhagic manifestations (eg, epistaxis, petechiae, ecchymosis, gum bleeding, hematemesis, melena or other bleeding), flushing, abdominal pain, anorexia, headache, retro-orbital pain, myalgia, congested pharynx, injected conjunctivae, restlessness, hemoconcentration (hematocrit  $>20\%$  over baseline for age and sex), thrombocytopenia (platelet count  $<100,000/\text{mm}^3$ ), signs of circulatory collapse (eg, shock, rapid weak pulse, narrow pulse pressure, hypotension, cold clammy skin) or other signs or symptoms suggesting dengue, as specified by the physician. Patients were excluded from the study if they had a known diagnosis other than dengue (eg, bronchopneumonia, gastroenteritis, meningitis).

### Clinical Procedures

At inclusion, a member of the hospital's medical staff performed a physical examination and recorded patient characteristics, vital signs, features of dengue-associated illness and clinical signs of any concomitant disease. A blood sample (5 mL) was also collected to obtain a complete blood count and to confirm dengue disease by virological and serological analyses. After admission, patients received the hospital's standard medical care and treatment for dengue. During hospitalization, the clinical development of the case was recorded, including any signs of severity that appeared during hospitalization (hemorrhagic manifestations, signs of plasma leakage and signs of shock) and any further hematological findings (elevated hematocrit or thrombocytopenia).

### Clinical Diagnosis at Discharge

Clinical diagnosis of dengue disease was made according to 1997 World Health Organization guidelines.<sup>17</sup> Patients were diagnosed with DHF if, along with a history of fever, they had or developed 3 of the following signs: (1) hemorrhagic tendencies indicated by a positive tourniquet test; petechiae, ecchymoses or purpura; bleeding from the mucosa, gastrointestinal tract, injection site or other site; hematemesis or melena; (2) thrombocytopenia ( $\leq 100,000/\text{mm}^3$ ); and (3) plasma leakage resulting in a hematocrit  $>20\%$  above average for the age and population or  $>20\%$  lower after fluid infusion treatment, or resulting in other signs (ascites, hypoproteinemia or pleural effusion). Patients with DHF were considered to have dengue shock syndrome (DSS) if they had or developed circulatory failure resulting in

all 3 of the following: (1) rapid and weak pulse; (2) narrow pulse pressure ( $\leq 20$  mm Hg) or hypotension and (3) cold, clammy skin and restlessness. Patients with DHF or DSS were considered to have severe dengue disease.

### Laboratory Assessments

Complete blood counts were performed on the same day it was collected. Serological and virological testing for dengue was performed by the Research Institute for Tropical Medicine virology laboratory (Muntinlupa City, Philippines). Serological confirmation was determined by an absorbance value  $>1.1$ -fold over the cutoff value in an anti-dengue IgM capture enzyme-linked immunosorbent assay (PanBio; Alere, Brisbane, Australia). Absorbance values were considered equivocal if they were 0.9–1.1 times the cutoff value and negative if  $<0.9$  times the cutoff value. For virological analysis, RNA was extracted from serum using a QIAamp Viral RNA Mini Kit (Qiagen, San Diego, CA). Viral RNA was then amplified by reverse transcription-polymerase chain reaction (RT-PCR) using dengue- and serotype-specific primers<sup>18</sup> and visualized on an agarose gel. Virological confirmation was determined by the presence of bands corresponding to viral RNA and to 1 or more DENV serotypes. Nonconfirmed cases were negative for RT-PCR and for serology, negative for RT-PCR with an equivocal serology result or no serology test performed or had no RT-PCR or serology test performed.

### Statistical Analyses

Statistical analyses were performed using Stata 10 software (StataCorp LP, College Station, TX). Normally distributed data were compared using Student's *t* test for continuous variables and the  $\chi^2$  test for categorical variables. Fisher exact test was used for small sample sizes. *P* values  $\leq 0.05$  were considered to indicate significant differences.

## RESULTS

### Study Population

Overall, 2103 suspected dengue cases were enrolled between November 2009 and November 2010 in the pediatric wards of the study hospitals, with 182 cases at JBL, 798 at WVMC and 1123 at DMC (Table 1). Patient ages ranged from infants  $<1$  years to 18-year-old adolescents, with 85% of all suspected cases between 3 and 15 years of age. Mean age was  $9.0 \pm 3.5$  years at JBL,  $7.5 \pm 3.6$  years at DMC and  $10.3 \pm 4.8$  years at WVMC (the only hospital where patients aged 16–18 years were admitted). Sex ratios were generally equivalent at each hospital.

### Laboratory-Confirmed Cases

Of the 2101 cases with blood samples available for analysis, 1809 (86.1%) were confirmed by the presence of anti-dengue IgM, viral RNA or both (Table 2). Of the remainder, 277 (13.2%) lacked detectable levels of either IgM or viral RNA and were considered nonconfirmed, and 15 (0.7%) had an ambiguous or missing serology result and undetectable levels of viral RNA. Overall, 81.1% of the confirmed cases were positive for IgM, 73.1% were positive for viral RNA and 54.2% were positive for both. Confirmation rates for the individual hospitals were between 75.3% and 90.5% although the corresponding proportions of IgM-positive and viral RNA-positive cases varied at each hospital. Between 68.0% and 90.0% of confirmed cases were IgM-positive and between 66.0% and 89.8% were viral RNA-positive.

The distributions of the suspected and confirmed cases among the pediatric age groups were similar for the 3 hospitals

**TABLE 1.** Study Population by Center

Characteristic	JBL	WVMC	DMC	Total
	N = 182	N = 798	N = 1123	N = 2103
Age group, n (%)				
<1 yr	1 (0.6)	8 (1.0)	28 (2.5)	37 (1.8)
1–2 yr	9 (5.0)	32 (4.0)	109 (9.7)	150 (7.1)
3–5 yr	29 (15.9)	149 (18.7)	305 (27.2)	483 (23.0)
6–10 yr	75 (41.2)	256 (32.1)	441 (39.3)	772 (36.7)
11–15 yr	67 (36.8)	223 (27.9)	240 (21.4)	530 (25.2)
16–18 yr	1 (0.6)	130 (16.3)	0 (0.0)	131 (6.2)
Mean age ± SD	9.0 ± 3.5	10.3 ± 4.8	7.5 ± 3.6	8.7 ± 4.3
Range, yr	0.9–16.1	0.0–19.0	0.0–15.1	0.01–19.0
Sex, n (%)				
Male	86 (47.3)	415 (52.0)	565 (50.3)	1066 (50.7)
Female	96 (52.7)	383 (48.0)	558 (49.7)	1037 (49.3)

N indicates total number of patients; n, number of patients with the characteristic.

**TABLE 2.** Results of Laboratory Confirmation Analyses

Laboratory Results	JBL, N = 182	WVMC, N = 798	DMC, N = 1123	Total, N = 2103
Cases tested (% of total)	182 (100.0)	798 (100.0)	1121 (99.8)	2101 (99.9)
Total confirmed cases, n (% of all cases tested)	137 (75.3)	657 (82.3)	1015 (90.5)	1809 (86.1)
Confirmation, n (% of confirmed cases)				
IgM <sup>+</sup> and RT-PCR <sup>+</sup>	91 (66.4)	320 (48.7)	569 (56.0)	980 (54.2)
IgM <sup>+</sup> and RT-PCR <sup>-</sup>	14 (10.2)	127 (19.3)	345 (34.0)	486 (26.9)
IgM <sup>-</sup> and RT-PCR <sup>+</sup>	28 (20.4)	192 (29.2)	81 (8.0)	301 (16.6)
IgM <sup>±</sup> and RT-PCR <sup>+</sup>	4 (2.9)	18 (2.7)	20 (2.0)	42 (2.3)
Nonconfirmed cases*	45 (24.7)	134 (16.8)	98 (8.7)	277 (13.2)
IgM <sup>±</sup> and RT-PCR <sup>-</sup>	0 (0.0)	7 (0.9)	8 (0.7)	15 (0.7)
Total RT-PCR <sup>+</sup> , n (% of confirmed cases)	123 (89.7)	530 (80.6)	670 (66.0)	1323 (73.1)
DENV-1 only, n (% of RT-PCR <sup>+</sup> )	36 (29.3)	20 (3.8)	220 (32.8)	276 (20.9)
DENV-2 only	28 (22.8)	345 (65.1)	57 (8.5)	430 (32.5)
DENV-3 only	54 (43.9)	122 (23.0)	311 (46.4)	487 (36.8)
DENV-4 only	4 (3.3)	24 (4.5)	70 (10.5)	98 (7.4)
All mixed infections	1 (0.8)	19 (3.6)	12 (1.8)	32 (2.4)
DENV-1 and 2	1 (0.8)	3 (0.6)	1 (0.2)	5 (0.4)
DENV-1 and 3	0 (0.0)	0 (0.0)	4 (0.6)	4 (0.3)
DENV-1 and 4	0 (0.0)	0 (0.0)	1 (0.2)	1 (0.1)
DENV-2 and 3	0 (0.0)	5 (0.9)	3 (0.5)	8 (0.6)
DENV-2 and 4	0 (0.0)	8 (1.5)	0 (0.0)	8 (0.6)
DENV-3 and 4	0 (0.0)	3 (0.6)	3 (0.5)	6 (0.5)

N indicates total number of cases; n, number of patients with the characteristic; IgM<sup>±</sup>, ambiguous IgM serology result or serology not done.

\*Tested but with an IgM<sup>-</sup> result and a RT-PCR<sup>-</sup> result.

(Fig. 1). The age groups with the highest number of confirmed cases were the 11 to 15-year group at JBL and the 6 to 10-year group at DMC and WVMC. Confirmation rates were high for nearly all age groups at each hospital, were over 70% for each age group overall, and did not correlate with age.

### Dengue Serotype Distributions

All 4 DENV serotypes were found at each hospital (Table 2). Overall, in patients infected with a single serotype, DENV-3 was the most common followed by DENV-2 and DENV-1. Serotype distributions also differed at each hospital, with DENV-3 the most common at JBL and DMC, and DENV-2 the most common at WVMC. Thirty-two patients overall had mixed infections with 2 DENV different serotypes.

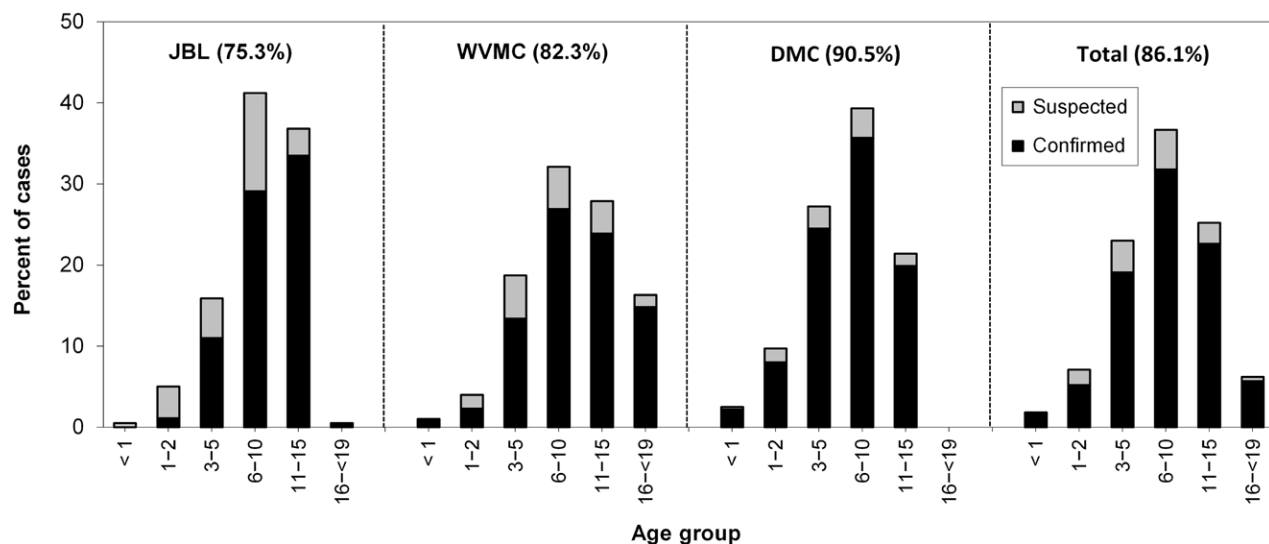
### Fever Patterns in Confirmed Dengue

Only 26.1% of the confirmed cases overall and 24.9–32.3% at each hospital had fever at admission, with the majority of cases reporting a prior episode of fever (see Table, Supplemental Digital Content 2, <http://links.lww.com/INF/C198>). More cases overall (44.0%) and at each hospital

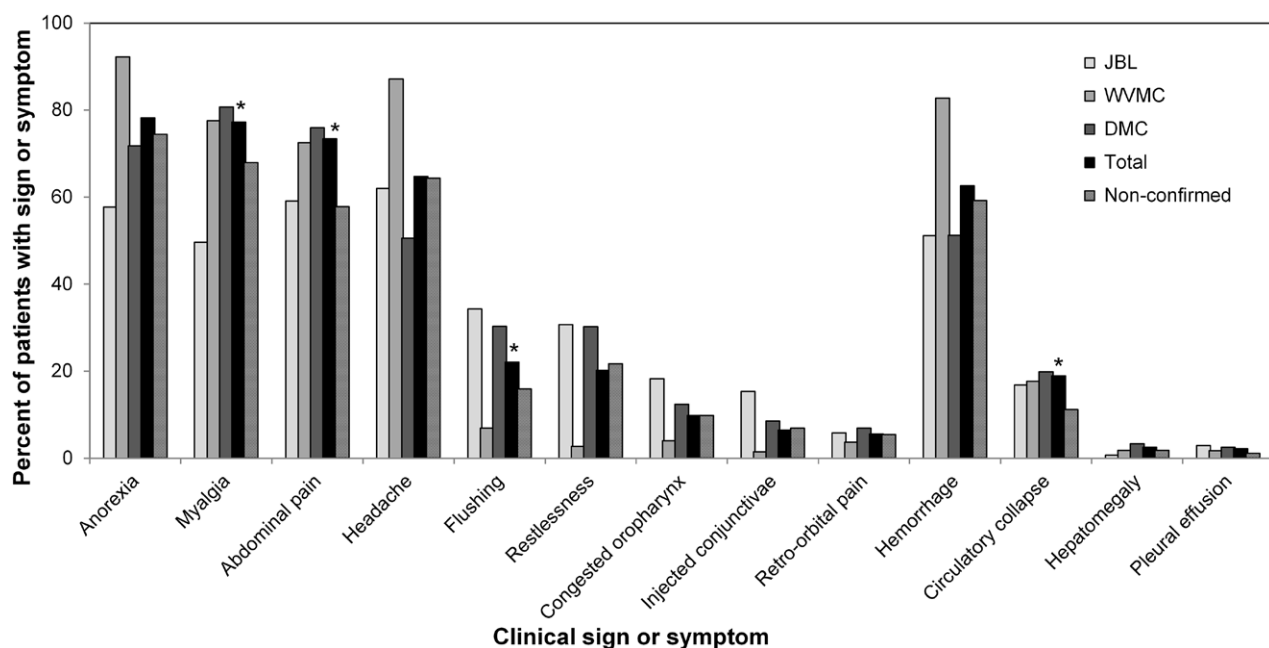
(38.7%–52.7%) had fever during hospitalization, indicating that fever returned or spiked during hospitalization for at least 324 cases (17.9%). Fever began 3–5 days before admission for most confirmed cases overall (71.0%) and at each hospital (61.0%–78.7%; see Fig., Supplemental Digital Content 3, <http://links.lww.com/INF/C199>).

### Clinical Features of Confirmed Dengue at Admission and Signs of Deterioration During Hospitalization

At admission, anorexia, myalgia, abdominal pain, headache and hemorrhage were the most common signs and symptoms and were present in >55% of the confirmed and nonconfirmed cases overall and in >50% of the confirmed cases at each hospital (Fig. 2). Myalgia ( $P = 0.001$ ), abdominal pain ( $P < 0.001$ ), flushing ( $P = 0.019$ ) and signs of circulatory collapse ( $P = 0.002$ ) were all significantly more common in confirmed than in nonconfirmed cases. Some signs of hemorrhage, such as a positive tourniquet test (45.5% vs 34.7%;  $P = 0.001$ ) and petechiae (20.8% vs 13.4%;  $P = 0.004$ ), were significantly more common in confirmed than in nonconfirmed cases, but signs of hemorrhage overall were not. At



**FIGURE 1.** Age-group distributions of suspected and confirmed dengue at the three sentinel hospitals. Cases of suspected (gray bars) and confirmed (black bars) dengue in each age group for each hospital and overall are shown as a percentage of the total number of cases. The values in parentheses are the case-confirmation rates at each hospital and overall. JBL, José B. Lingad Memorial Regional Hospital; WVMC, Western Visayas Medical Center; DMC, Davao Medical Center.



**FIGURE 2.** Clinical signs and symptoms at admission for confirmed and non-confirmed dengue. The percentage of cases with each clinical sign or symptom at admission are shown for the confirmed cases at each hospital, for the total confirmed cases, and for the total non-confirmed cases. Asterisks indicate signs or symptoms with significantly different percentages ( $P < 0.05$ ) for the total confirmed cases vs the total non-confirmed cases.

admission, shock was reported for only 8 confirmed cases. Retro-orbital pain, hepatomegaly and pleural effusion were infrequent among all cases overall.

Elevated hematocrits (>42% and >45%), thrombocytopenia (<100,000/mm<sup>3</sup>) and leucopenia (<5000/mm<sup>3</sup>) were all significantly more common in confirmed cases than in nonconfirmed cases (all  $P < 0.001$ ).

During hospitalization, between 17.7% and 45.4% of the confirmed cases at each hospital presented with dengue-related

signs of deterioration, which were significantly more common overall in confirmed (28.4%) than in nonconfirmed cases (18.1%;  $P < 0.001$ ; see Table, Supplemental Digital Content 4, <http://links.lww.com/INF/C200>). Hemorrhagic manifestations (especially petechiae and epistaxis) and signs of plasma leakage (especially hemoconcentration) were significantly more common in confirmed than in nonconfirmed cases. Signs of shock developed in 3.6%–13.1% of the confirmed cases at each hospital. Among the mixed serotype infections, 11 cases had signs of deterioration, including



**TABLE 3.** Clinical Diagnoses, Outcomes and Case Fatality Rates of Confirmed and Nonconfirmed Dengue

	Confirmed, N = 1809	Nonconfirmed*, N = 277	All Suspected, N = 2086
Diagnosis, n (%)			
Dengue	1788 (98.8)	252 (91.0)	2040 (97.8)
Dengue fever	292 (16.1)	117 (42.2)	409 (19.6)
Dengue hemorrhagic fever	1462 (80.8)	133 (48.0)	1595 (76.5)
Dengue shock syndrome	34 (1.9)	2 (0.7)	36 (1.7)
Not dengue, n (%)	21 (1.2)	25 (9.0)	46 (2.2)
Outcome, n (%)			
Recovered	1778 (98.3)	268 (96.8)	2046 (98.1)
Discharged against medical advice	9 (0.5)	6 (2.2)	15 (0.7)
Transferred to another hospital	5 (0.3)	0 (0.0)	5 (0.2)
Died	17 (0.9)	3 (1.1)	20 (1.0)
Case fatality rate, % (n)			
JBL	1.46 (2)		
WVMC	0.61 (4)		
DMC	1.08 (11)		
Overall	0.94 (17)	1.08 (3)	0.96 (20)
<1 yr	0 (0)		
1–2 yr	0 (0)		
3–5 yr	1.24 (5)		
6–10 yr	1.35 (9)		
11–15 yr	0.42 (2)		
16–18 yr	0.84 (1)		

N indicates total number of cases; n, number of patients with the characteristic.

\*IgM/RT-PCR.

hemorrhagic manifestations (7 cases), signs of plasma leakage (6 cases) and shock (2 cases).

### Clinical Diagnosis at Discharge, Outcome and Case Fatality Rate

Overall, 98.8% of the confirmed cases were clinically diagnosed with dengue disease at discharge (Table 3). Most (82.7%) were severe dengue (DHF or DSS), and 16.1% were DF. Of the mixed infections, 26 (81.3%) were severe, and 6 (18.7%) were DF. Despite laboratory confirmation (not known at time of discharge), 21 confirmed cases were clinically diagnosed with an illness other than dengue. Many of these cases were diagnosed with acute viral infections (7 cases) or pneumonia (6 cases) or other infections that involve fever. Nearly all of the nonconfirmed cases were also diagnosed with dengue. Overall, 98.3% of the confirmed cases recovered and 20 patients died, 17 of which had laboratory-confirmed dengue. None of the mixed serotype infections were fatal. Case fatality rates (CFR) for confirmed dengue were between 0.61% and 1.46% at each hospital and 0.94% overall. Most fatal confirmed cases were in patients aged 4–16 years, with a mean age of  $8.0 \pm 3.4$  years. Six (35.3%) were boys, and 11 (64.7%) were girls.

### Seasonal Infection Patterns

During the study, infection numbers at all 3 hospitals peaked during the Philippine rainy season (June–November) for all 4 DENV serotypes (Fig. 3).

## DISCUSSION

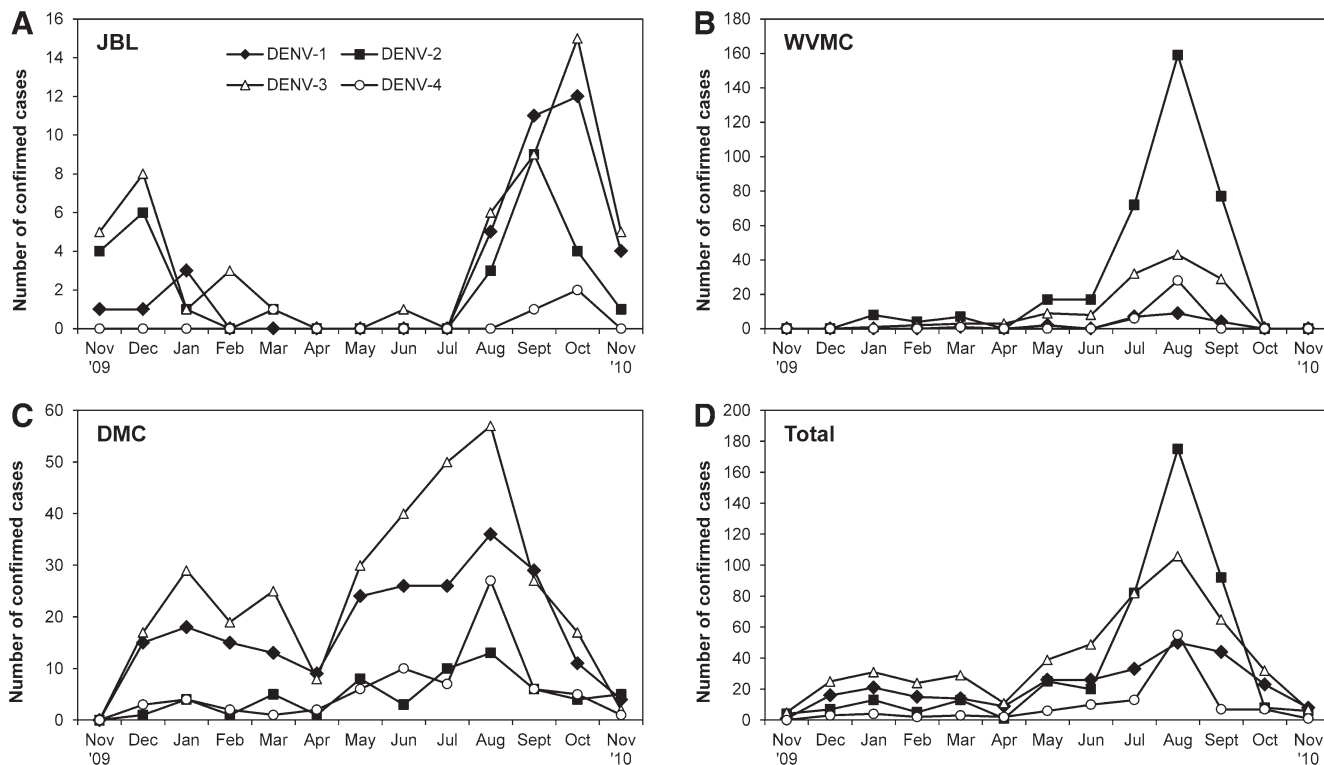
In the Philippines, children and adolescents are at the highest risk of dengue infection. In this study of 2103 hospitalized pediatric cases of suspected dengue, 86.1% were laboratory confirmed, and over 80% were considered severe dengue. The clinical signs and symptoms at admission were those typically associated with dengue. Almost one-third of the confirmed cases worsened during hospitalization and 17 were fatal, although the overall case fatality rate was <1%. The distributions of all 4 DENV serotypes were seasonal and varied at the three hospitals.

This study yielded valuable regional age distribution information for pediatric dengue in the Philippines. The proportions of confirmed infections tended to be highest in the 6- to 10-year-old age group and to decrease in the older age groups. These results are consistent with data from the Philippine Department of Health showing that the highest proportions of DF and DHF cases were in the 5- to 14-year-old age group in 2000–2009.<sup>13</sup> Although we are unable to compute the age group incidence rates for these areas and did not investigate adult transmission rates, these results suggest that dengue susceptibility in the Philippines peaks between 6 and 10 years of age and then decreases. These findings are consistent with the hyperendemicity and the high burden of dengue disease in the Philippines, where dengue immunity increases with age.<sup>19</sup>

A high proportion of the suspected cases in the study were laboratory confirmed, indicating that the clinical diagnosis at admission was generally accurate and that the combination of laboratory analyses was an effective means of confirming dengue disease. Indeed, both assays were important for case confirmation, and we calculate that 343 (18.9%) case confirmations would have been missed using only serological analysis and 486 (26.9%) would have been missed using only virological analysis. That the study included only cases severe enough to warrant hospitalization likely also contributed to the high confirmation rate. Severe cases tend to have higher viral loads and are more likely to be confirmed than mild cases.<sup>20–22</sup>

Many of the confirmed cases had no fever at admission but instead had reported previous fever. Fever in dengue may be short-lived (2–7 days), so fever for some patients may have already subsided during the natural course of the infection. However, at least 10.2%–26.0% of the patients with confirmed dengue at each hospital experienced a second bout of fever while hospitalized. This may have also occurred as a natural course of the infection as fever waned and spiked.

Nearly all of the confirmed cases received a clinical diagnosis of dengue. For those that did not, some may have had a false positive test, whereas a dengue diagnosis may have been justified for others but was missed because of the unavailability of test results or



**FIGURE 3.** Seasonal prevalence of dengue serotypes. Numbers of cases in which the infecting serotype was identified by RT-PCR are shown for each hospital and for the overall (total) for each month of the study (November 2009 to November 2010).

to an unrelated coinfection (eg, typhoid or ascariasis). With many of the same nonspecific clinical features as the confirmed cases, 91% of the nonconfirmed cases were also diagnosed with dengue despite the absence of laboratory confirmation. It is possible that some of these cases were authentic dengue infections that produced false negative test results because of cleared or low viremia or a weak or excessively late IgM response. Another possibility is that some cases were misdiagnosed because of bias or overreporting, which is common in endemic areas and during outbreaks.<sup>23</sup>

The overall CFR (0.94%) was comparable with the national values reported by the Philippine Department of Health for 2008 (0.94%) and 2009 (0.95%), however, it was higher than that reported for 2010 (0.59%).<sup>24</sup> The higher CFR found in this study during the same period may be related to the pediatric age groups studied and to the hospitalization requirement, which would include the most severe cases. Indeed, the highest CFRs were in the 3- to 5-year and 6- to 10-year age groups. By comparison, CFRs rarely exceed 1% in endemic countries in the Western Pacific.<sup>1,2</sup>

Our study may be the first to describe concurrent serotype distributions at 3 different locations in the Philippines. All 4 DENV serotypes were found at all 3 hospitals, but the distributions and seasonal patterns were different at each one. As disease severity may vary with serotype,<sup>20,25</sup> these results may not reflect the true proportions of the serotypes circulating in the local populations but only the proportions associated with hospitalization.

The 32 mixed infections with multiple serotypes we detected was another indication of the high burden of disease and hyperendemicity in the Philippines. Although a mixed infection was not detected until 1982,<sup>26</sup> they are not uncommon in hyperendemic regions if appropriate virological techniques, including serotype-specific RT-PCR, are used for surveillance.<sup>27</sup> Indeed, mixed infections have been reported in many countries.<sup>27-32</sup> It is unlikely that

mixed infections in this study resulted from sample contamination or different technological standards because all samples from the 3 hospitals were tested at the same laboratory. Determining the outcome and impact of these types of infections will require detailed virological testing and follow-up of suspected cases in hyperendemic areas, although most mixed infections appear to be no more severe than single infections.<sup>27,32,33</sup>

This study is strengthened by the accurate clinical diagnosis and comprehensive laboratory testing, which resulted in confirmation of >85% of suspected cases. We also investigated dengue in 3 distinct geographical areas in the Philippines, allowing the clinical features, age distributions and serotype distributions of dengue to be compared across regions. We present some of the first serotype distribution information for the islands of Visayas and Mindanao. We also recorded signs of deterioration during hospitalization. One limitation was the lack of catchment information for the 3 hospitals, which prevented us from estimating the incidence and burden of dengue hospitalization in these areas, and from determining why enrolment levels were different at each hospital. Finally, the study included only hospitalized cases, which are more severe than outpatient cases, so we are unable to estimate laboratory confirmation rates for outpatient symptomatic cases or burdens of disease in these areas.

In conclusion, most hospitalized cases of pediatric dengue in the Philippines can be laboratory confirmed. Over 85% of the cases we studied were in children 3–15 years of age, and over 80% of these cases were severe dengue with classic signs and symptoms. Regional and seasonal variations in the distributions of all 4 DENV serotypes were detected at each hospital, but transmission was highest during the rainy season. Finally, the very high seasonal caseloads of dengue at each hospital, the age-group distributions

and the abundance of mixed infections all point to an extremely high clinical disease burden in the Philippines and emphasize the need for additional control measures.

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