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Abstract. From June 2005 to May 2006, a clinic-based enhanced surveillance system for dengue was implemented in a Puerto Rican municipality to provide a population-based measure of disease incidence and clinical outcomes. We obtained demographic and clinical information from suspected cases and performed serologic and virologic testing. We used World Health Organization (WHO) criteria to classify cases and applied a simplified case definition for severe dengue illness. There were 7.7 laboratory-positive cases of dengue per 1,000 population. The highest incidence, 13.4 per 1,000, was among 10 to 19 year olds. Of the 156 laboratory-positive cases, three patients (1.9%) met WHO criteria for dengue hemorrhagic fever, and 30 patients (19.2%) had at least one severe clinical manifestation of dengue infection. Our data suggest that in a community with endemic dengue, enhanced surveillance is useful for detecting symptomatic infections. Furthermore, the simplified case definition for severe dengue may be useful in clinic-based surveillance.

INTRODUCTION

Dengue is a mosquito-transmitted infection caused by the four dengue virus serotypes (DENV-1, DENV-2, DENV-3, and DENV-4). Many DENV infections, particularly in children, cause no symptoms or non-specific febrile illness, but each serotype can cause dengue fever or dengue hemorrhagic fever/dengue shock syndrome (DHF/DSS). Viral strain, host characteristics, and history of previous infection with a different dengue serotype are thought to affect disease severity.1,2 The World Health Organization (WHO) criteria for DHF have been challenged, and alternative case definitions for severe dengue illness have been proposed.3,4

In the Caribbean island of Puerto Rico, dengue fever and DHF are endemic conditions reportable by law. An islandwide, laboratory-based passive dengue surveillance system (PDSS) is maintained by the Puerto Rico Department of Health (PRDH) and the Centers for Disease Control and Prevention (CDC). Health care providers (HCPs) report suspected dengue cases by submitting serum samples with a dengue case investigation form. Results from the free diagnostic testing are given to the HCPs, and the summary data are used to plan and coordinate prevention and control activities. In Puerto Rico, dengue is seasonal, with a large peak of cases reported from August through November. Over the past 15 years, the average annual incidence of suspected dengue was 1.4 cases per 1,000 population in non-epidemic years, and 6.2 and 4.5 cases per 1,000 in the two most recent epidemic years, 1994 and 1998, respectively. Between 3 and 59 cases of laboratory-positive DHF were reported annually (CDC, unpublished data).5

As a passive reporting system, the PDSS is subject to under-reporting of cases. It is estimated that for every reported case of suspected dengue in Puerto Rico, 10 to 27 symptomatic cases are not reported, and for every reported DHF case, about 15 cases are not reported.6–8 Reporting of dengue to the PDSS can vary by HCPs’ awareness of dengue and diagnostic acumen and therefore might not produce representa-
infection caused by another pathogen (e.g., varicella or streptococcal pharyngitis).

Laboratory-positive dengue case. Suspected dengue case with either serologic (anti-dengue IgM seroconversion or single anti-dengue IgM positivity) or virologic (virus identification by reverse-transcriptase polymerase chain reaction [RT-PCR]) confirmation.9,10

Laboratory-negative dengue case. Suspected dengue case with no anti-dengue IgM antibodies identified in the convalescent sample, and neither dengue virus nor anti-dengue IgM detected in the acute sample.

Laboratory-indeterminate dengue case. Suspected dengue case with no convalescent sample and neither dengue virus nor anti-dengue IgM detected in the acute sample.

Primary dengue case. A laboratory-positive case in which the IgG-ELISA titer was < 1:160 in the acute sample.11

Secondary dengue case. A laboratory-positive case in which the IgG-ELISA titer was ≥ 1:160 in the acute sample.11

Dengue fever case. A laboratory-positive case identified through the EDSS.

Severe dengue illness. Three case definitions were used to characterize the severity of laboratory-positive cases identified by EDSS. They include:

**Dengue hemorrhagic fever (DHF) as defined by WHO criteria.** A patient with dengue fever who develops thrombocytopenia (platelets ≤ 100,000/mm³), hemorrhage (positive tourniquet test, petechiae, ecchymosis, epistaxis, bleeding gums, hematuria, hematemesis, melena, or menorrhagia), and evidence of capillary leak.2 Capillary leak was defined as pleural effusion, ascites, hypoalbuminemia (albumin ≤ 3.5 g/dL), or hemococoncentration (hematocrit value ≥ 20% above mean values for sex and age).12

**Provisional diagnosis of DHF as defined by WHO criteria.** A patient with dengue fever who develops hemorrhage and either thrombocytopenia or hemococoncentration or other evidence of capillary leak.2

**Dengue with severe clinical manifestations.** A patient with dengue fever who has any of the following: 1) marked thrombocytopenia (platelets ≤ 50,000/mm³), 2) internal hemorrhage (hematuria, hematemesis, melena, or menorrhagia), 3) hypotension (systolic pressure < 80 mm of Hg for those < 5 years of age and < 90 mm Hg for those > 5 years of age; or pulse pressure ≤ 20 mm of Hg), or 4) plasma leakage (pleural effusion, ascites, or hemococoncentration).4

**Enhanced dengue surveillance procedures.** Whereas PDSS involves the collection of data from unsolicited reports of suspected cases, and active surveillance involves active case finding, enhanced surveillance encourages and supports HCP reporting of dengue.13 The EDSS in Patillas was designed and implemented to optimize HCP identification and reporting of symptomatic dengue cases among residents of Patillas. The health center in Patillas provides primary care services for nearly 90% of the residents of the municipality (unpublished data, Centro de Servicios Primarios de Salud de Patillas).

Two full-time CDC staff members work at the health center in Patillas to implement EDSS and encourage HCPs to complete a dengue case investigation form for patients meeting the clinical case definition and submit serum samples for dengue diagnostic testing. CDC on-site staff verifies the accuracy and completeness of reporting by comparing medical chart data with data on the dengue case investigation form for all suspected dengue patients. Lastly, CDC staff provide systematic feedback to HCPs about the level and completeness of case reporting.

**Laboratory testing.** All blood samples were centrifuged and the serum separated in Patillas. Each sample consisted of at least 0.5 mL of serum. Samples were transported by CDC staff once a week to the CDC Dengue Branch in San Juan, Puerto Rico for dengue diagnostic testing. Serum samples collected within 5 days of the onset of symptoms (acute samples) were tested by serotype-specific RT-PCR to identify dengue virus and by an IgM antibody-capture enzyme-linked immunosorbent assay (MAC-ELISA) to identify IgM antibodies to dengue. Serum samples collected 6 to 30 days after symptom onset (convalescent samples) were tested by MAC-ELISA. All cases with positive PCR results or with IgM seroconversion from negative in the acute sample to positive in the convalescent sample were tested by quantitative IgG ELISA in the acute sample to determine primary or secondary status of current infections.

**Statistical analysis.** A descriptive analysis was performed by calculating frequencies of clinical, demographic, and laboratory features of suspected and laboratory-positive dengue cases. Overall dengue incidence was estimated as well as den-
gue incidence stratified by age groups and sex using 2000 U.S. census data for Patillas, Puerto Rico. Statistical differences in proportions were tested by applying Fisher’s exact test and $X^2$ ($X^2$) tests. Comparisons of continuous variables between groups were done by using $t$ tests and Mann-Whitney $U$ tests. All data analyses were conducted using SPSS software (version 12.0 for Windows).

Human subjects review approval. The protocol for this analysis was reviewed and approved by the CDC Human Subjects Review Committee.

RESULTS

Laboratory features of suspected and laboratory-positive dengue cases. A total of 1,393 cases of suspected dengue among Patillas residents were reported to the EDSS from June 2005 through May 2006. Of those, we found that 156 (11.2%) cases were laboratory-positive, 422 (30.3%) were laboratory-negative, and 815 (58.5%) were laboratory-indeterminate. DENV-2 was detected in 77 (49.4%) of the laboratory-positive cases and was the only serotype identified. Among the laboratory-positive cases, 28 (17.9%) were primary infections, 77 (49.4%) were secondary infections, and 51 were not classified as primary or secondary because the first serum sample was collected $>$ 5 days after the onset of symptoms.

Case reporting by month. Reporting of suspected cases and detection of laboratory-positive cases varied greatly over time (Figure 2). The period of highest dengue incidence was from July through September. The lowest number of suspected cases was reported in January.

Incidence by sex and age. The overall incidence of dengue in Patillas from June 2005 through May 2006 was 69.1 suspected dengue cases and 7.7 laboratory-positive dengue cases per 1,000 population (Table 1). The incidence of suspected and laboratory-positive dengue did not differ by sex but varied by age group. The incidence of laboratory-positive dengue was highest among 10 to 19 year olds (13.4 per 1,000), followed by 20 to 29 year-olds (9.9 per 1,000). The lowest incidence of laboratory-positive dengue was among those $\geq$ 40 years old (3.8 per 1,000).

Demographic and clinical features of primary and secondary infections. Patients with primary infections were younger ($P < 0.05$) than patients with secondary infection (median age of 14 years versus 27 years, respectively) (Table 2). Case patients with primary infections presented for medical care a median of 2 days after onset of illness compared with a median of 1 day for those with secondary infections ($P = 0.15$). Overall, seven (4.5%) laboratory-positive patients were hospitalized for 2 to 6 days (median of 4 days). No patients with primary infections were hospitalized whereas three (3.9%) patients with secondary infections required hospitalization.

Severe dengue illness. Among the 156 patients with laboratory-positive dengue, three (1.9%) met the WHO criteria for DHF: three men between 19 and 23 years of age. One DHF case was a secondary infection, and the other two DHF cases were unclassified because no acute sample was collected. All three DHF patients were hospitalized for 3 to 5 days and recovered.

Thirty laboratory-positive cases (19.2%) met the case definition for dengue with severe clinical manifestations. The most common severe feature among adults was plasma leakage whereas hypotension was the most common severe mani-

| TABLE 1 |

<table>
<thead>
<tr>
<th>Incidence (per 1,000)</th>
<th>Suspected*</th>
<th>Laboratory-positive†</th>
</tr>
</thead>
<tbody>
<tr>
<td>Overall</td>
<td>69.1</td>
<td>7.7</td>
</tr>
<tr>
<td>Age, yrs</td>
<td></td>
<td></td>
</tr>
<tr>
<td>0–4</td>
<td>307.8</td>
<td>7.5</td>
</tr>
<tr>
<td>5–9</td>
<td>102.4</td>
<td>8.1</td>
</tr>
<tr>
<td>10–19</td>
<td>74.8</td>
<td>13.4</td>
</tr>
<tr>
<td>20–29</td>
<td>55.1</td>
<td>9.9</td>
</tr>
<tr>
<td>30–39</td>
<td>46.7</td>
<td>9.3</td>
</tr>
<tr>
<td>40+</td>
<td>27.0</td>
<td>3.8</td>
</tr>
<tr>
<td>Sex</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Male</td>
<td>72.7</td>
<td>7.9</td>
</tr>
<tr>
<td>Female</td>
<td>65.6</td>
<td>7.7</td>
</tr>
</tbody>
</table>

* See Materials and Methods section for case definition.
† A laboratory-positive case was defined as a suspected dengue case with either serological (anti-dengue IgM seroconversion or single anti-dengue IgM positivity) or virological (virus identification through PCR) confirmation.
The proportions of patients with at least one reported severe clinical manifestation of dengue were similar between those with primary (25.0%) and secondary (16.9%) infections (Table 2). Six of the seven patients who were hospitalized with laboratory-positive dengue had at least one reported severe clinical manifestation. The hospitalized patient who did not meet the criteria for dengue with severe clinical manifestations was a 43-year-old man who was hospitalized for 4 days with suspected dengue and thrombocytopenia (platelet nadir of 56,000/mm³).

**DISCUSSION**

The implementation of a clinic-based enhanced dengue surveillance system in Patillas coincided with the onset of a DENV-2 outbreak. This provided a unique opportunity to accurately measure dengue disease burden in terms of incidence and severity of clinical disease. Incidence of laboratory-positive dengue was highest among 10 to 19 year olds and most patients with laboratory-positive dengue had mild disease. Few laboratory-positive infections met the WHO criteria for DHF whereas 1 in 5 cases met newly described criteria for serious dengue illness.¹

Compared with routine passive surveillance, the EDSS appears to detect more dengue infections. For example, the incidence of laboratory-positive dengue in Patillas under EDSS (7.7 laboratory-positive dengue cases per 1,000 population) was nearly three times higher than rates reported under the passive surveillance system for Patillas during the two most recent epidemics in 1994 and 1998 (1.3 cases per 1,000 in 1994 and 2.8 in 1998) (CDC, unpublished data). Furthermore, as enhanced surveillance is conducted at the principal health center that serves most of the municipality’s residents, a population-based incidence of symptomatic dengue can be measured with more accuracy than is possible with the island-wide PDSS.

Adolescents and young adults were at highest risk for dengue: 1.3% of 10 to 19 year olds and 1% of 20 to 29 year olds had laboratory-positive dengue. The burden of disease among adolescents and young adults is also reflected in the median age of primary infections (14 years) and the median age for

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**Table 2**

Demographic and clinical characteristics of laboratory-positive dengue cases by immune status (primary vs. secondary infections), Patillas, Puerto Rico—June 2005–May 2006

<table>
<thead>
<tr>
<th>Characteristics</th>
<th>Overall (N = 156)</th>
<th>Primary infection (N = 28)</th>
<th>Secondary infection (N = 77)</th>
<th>Unclassified* (N = 51)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Demographic</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Male, no. (%)</td>
<td>79 (50.6%)</td>
<td>12 (42.9%)</td>
<td>46 (59.7%)</td>
<td>21 (41.2%)</td>
</tr>
<tr>
<td>Median age, yrs (range)</td>
<td>22 (0.6–89.1)</td>
<td>14 (0.8–65.0)</td>
<td>27 (0.6–89.1)</td>
<td>19 (1.2–71.4)</td>
</tr>
<tr>
<td>Hospitalized, no. (%)</td>
<td>7 (4.5%)</td>
<td>0</td>
<td>3 (3.9%)</td>
<td>4 (7.8%)</td>
</tr>
<tr>
<td>Median length of stay, days (range)</td>
<td>4 (2–6)</td>
<td>–</td>
<td>5 (4–6)</td>
<td>3 (2–4)</td>
</tr>
<tr>
<td>Severe dengue illness, no. (%)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>DHF‡</td>
<td>3 (1.9%)</td>
<td>0</td>
<td>1 (1.3%)</td>
<td>2 (3.9%)</td>
</tr>
<tr>
<td>Provisional DHF¶</td>
<td>4 (2.6%)</td>
<td>0</td>
<td>2 (2.6%)</td>
<td>2 (3.9%)</td>
</tr>
<tr>
<td>Dengue with severe clinical manifestations**</td>
<td>30 (19.2%)</td>
<td>7 (25.0%)</td>
<td>13 (16.9%)</td>
<td>10 (19.6%)</td>
</tr>
</tbody>
</table>

* Unable to classify as primary or secondary due to lack of acute specimen.
† Days post onset of symptoms, at presentation.
‡ A dengue fever case with hemorrhage, platelets ≤ 100,000/mm³ and evidence of capillary leak (hemoconcentration, pleural effusion or ascites, or hypoalbuminemia).
¶ A dengue fever case with hemorrhage and either platelets ≤ 100,000/mm³ or evidence of capillary leak.
** A dengue fever case with any of the following: platelets ≤ 50,000/mm³, internal hemorrhage, plasma leakage (hemoconcentration, pleural effusion or ascites), or hypotension.

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**Figure 3**. Prevalence of severe clinical manifestations of dengue among children (1–15 years) and adults (≥ 16 years), Patillas, Puerto Rico, June 2005–May 2006. The percentage of laboratory-positive cases in each age group with reported internal hemorrhage, hypotension, signs of plasma leakage, and marked thrombocytopenia (platelets ≤ 50,000/mm³). No infants had severe manifestations reported.
secondary infections (27 years). Furthermore, all three DHF cases were young adults between 19 and 23 years of age.

Our data indicate that this simplified case definition for severe illness might be useful in clinic-based surveillance in dengue-endemic areas to detect a broader range of severe clinical manifestations than the WHO criteria for DHF. When the WHO case definition was applied, most laboratory-positive cases were dengue fever cases and few (1.9%) met the criteria for DHF. However, 19.2% of the laboratory-positive dengue cases had at least one severe clinical manifestation (platelets ≤ 50,000, plasma leakage, internal hemorrhage, or hypotension). Our findings are consistent with those of a recently published report that applied this new case definition to a patient population of European travelers presenting to outpatient clinics. The authors concluded that severe dengue infections could be missed if the WHO classification is strictly applied.

Limitations. We suspect that although EDSS improves detection of dengue infections, dengue was still under-reported. This surveillance does not detect asymptomatic infections or symptomatic infections among those who did not seek medical care. The EDSS likely under-reported DHF as well as other severe manifestations that are dependent on laboratory testing. Detection of thrombocytopenia and hemococoncentration (or other evidence of capillary leak such as hypoproteinemia) are needed to diagnose DHF. Only half (47%) of the 156 laboratory-positive dengue patients had platelet values recorded on either their health center medical chart or dengue case investigation form. Similarly, only 84 laboratory-positive patients (54%) had hematocrit values recorded. Thus, approximately half of the laboratory-positive patients could not be assessed for DHF and specific measures of severity such as marked thrombocytopenia.

CONCLUSION

Enhanced surveillance in a well-defined community in Puerto Rico allowed for a more accurate, population-based estimate of incidence and measure of clinical severity of dengue infection. Incidence of laboratory-positive dengue infection was high, particularly among adolescents and young adults. Although few of the laboratory-positive dengue cases met the WHO criteria for DHF, 10 times as many had at least one reported severe clinical manifestation of dengue infection. Our data suggest that this simplified case definition for severe illness could be useful in clinic-based surveillance and in testing the impact of future prevention efforts.

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REFERENCES


