INCREASE OF LEPTOSPIROSIS IN DENGUE-NEGATIVE PATIENTS AFTER A HURRICANE IN PUERTO RICO IN 1966

EDUARD J. SANDERS, JOSÉ G. RIGAU-PÉREZ, HENK L. SMITS, CARMEN C. DESEDA, VANCE A. VORDAM, TIN AYE, RICHARD A. SPIEGEL, ROBBIN S. WEYANT, AND SANDRA L. BRAGG

Dengue Branch, Division of Vector-Borne Infectious Diseases, Centers for Disease Control and Prevention, San Juan, Puerto Rico; Epidemiology Program Office, and Meningitis and Special Pathogens Branch, Division of Bacterial and Mycotic Diseases, Centers for Disease Control and Prevention, Atlanta, Georgia; Royal Tropical Institute, Amsterdam, The Netherlands; Division of Epidemiology, Puerto Rico Department of Health, San Juan, Puerto Rico

Abstract. Leptospirosis has rarely been reported in Puerto Rico, although in the period from 1948 to 1952, 208 cases of leptospirosis and an island-wide seroprevalence of antibody to Leptospira of 14% were documented. In Puerto Rico in October 1996, following rainfall and a period of flooding generated by Hurricane Hortense, serum specimens of 4 patients with suspected dengue fever that were negative for dengue tested positive for Leptospira-specific IgM antibodies in a dipstick assay. Subsequently, we used an island-wide dengue laboratory-based surveillance system to determine the increase in leptospirosis after hurricane-generated floods. All anti-dengue IgM-negative patients (n = 142) with disease onset from August 8 to October 6, 1996 from prehurricane and posthurricane groups were investigated for leptospirosis. Laboratory-confirmed leptospirosis cases were defined as microscopic agglutination test titers ≥ 1:400 to 1 or more serovars, or positive immunohistochemistry in autopsy tissues. Four (6%) of 72 prehurricane and 17 (24%) of 70 posthurricane patients had laboratory-confirmed cases of leptospirosis (relative risk [RR] = 4.4, 95% confidence interval [CI] = 1.6–12.4). The mean age of case-patients was 34 years (range = 13–64). Eighteen (86%) of 21 confirmed case-patients were males, including one patient who died (31 years old). Patients were located in 18 (38%) of 48 municipalities that submitted serum samples. Clinical features significantly associated with leptospirosis were eye pain (RR = 1.5, 95% CI = 1.3–1.9), joint pain (RR = 1.4, 95% CI = 1.1–1.6), diarrhea (RR = 1.7, 95% CI = 1.2–2.5), and jaundice (RR = 3.3, 95% CI = 1.5–7.2). This study demonstrates the utility of a dengue laboratory-based surveillance system for the detection of an increase of leptospirosis, which most likely would have gone unrecognized. Leptospirosis is treatable with antibacterial agents; knowledge of this diagnosis may significantly reduce morbidity and mortality.

Leptospirosis, a commonly occurring spirochetal zoonosis of worldwide distribution, causes a wide spectrum of clinical manifestations, including subclinical infection, self-limited anicteric febrile illness with or without meningitis, and a severe and potentially fatal illness known as Weil disease that presents as hemorrhage, renal failure, and jaundice. The initial leptosomal syndrome of acute fever, chills, and myalgia with muscle tenderness is indistinguishable from dengue. Typical symptoms for dengue are frontal headache, retro-orbital pain, muscle and joint pain, and rash, while painful eyes, conjunctival suffusion, and severe muscle pain (often beginning in the calf) are symptoms more commonly ascribed to leptospirosis. From 1955 to 1994, the total annual number of reported cases of leptospirosis in the United States, including Puerto Rico, was less than 150. In 1994, the last year national surveillance was required for leptospirosis, 22 of the 38 reported cases in the United States occurred in Hawaii, and 2 cases were reported from Puerto Rico. Leptospirosis is endemic in most Caribbean countries and may be mistaken for dengue or dengue hemorrhagic fever. In Barbados, the average annual incidence (1979–1991) of severe leptospirosis was 13 cases per 100,000 population, with a case fatality rate of 14%. Leptospirosis has rarely been reported in Puerto Rico, except for the period from 1948 to 1952, when 208 cases of leptospirosis and an island-wide seroprevalence of antibody to Leptospira of 14% (range by region = 6–22%) were documented, and in 1974 when, following a case of suspected fatal leptospirosis, 8 (19%) of 43 persons investigated had evidence of recent leptospirosis. A temporal association between heavy rainfall and human leptospirosis has been reported in many settings. In Puerto Rico in October 1996, following rainfall and a period of flooding generated by Hurricane Hortense, serum specimens of 4 patients with suspected dengue fever that were negative for dengue tested positive for Leptospira-specific IgM antibodies in a dipstick assay developed by the Royal Tropical Institute (Amsterdam, The Netherlands) and used for research purposes at the Dengue Branch of the Centers for Disease Control and Prevention (CDC) (San Juan, PR). We hypothesized that leptospirosis was under-reported in Puerto Rico and that the 20-year-old laboratory-based surveillance system used for dengue fever might detect another acute public health problem, namely, the increase of leptospirosis following heavy rainfall.

METHODS

Patient selection. The CDC Dengue Branch receives serum specimens of suspected dengue patients from public health clinics, public and private hospitals, laboratories, and private physicians’ offices throughout Puerto Rico. Sera are accompanied by a dengue case investigation form. Rainfall data from the National Oceanic and Atmospheric Administration showed unusually high rainfall in Puerto Rico in September 1996: all six climate divisions had an increase averaging 6.6 inches (range = 2.5–14.4) higher than the historic mean (September 1960 to 1990). Most (70%) of the total rain fell on September 10 and September 11, 1996. This investigation was conducted in accordance with the human research subject guidelines of the Puerto Rico Department of Health and the guidelines of CDC for studies conducted in rapid response to public health emergencies. All patients who had negative results in an anti-dengue
IgM ELISA test were assigned to a prehurricane period or to a posthurricane period based on the dates of onset of illness. Review of signs and symptoms recorded on the dengue case investigation form was conducted for all of these patients. The extension of the posthurricane period was established by using the approximate duration of floods in Puerto Rico (September 11–17, 1996) and the range of the incubation period of leptospirosis (2–19 days). The posthurricane period extended from September 13 to October 6, 1996 (24 days). The prehurricane period extended from August 7 to September 12, 1996 (34 days), which allowed for the inclusion of an approximately equal number of dengue-negative serum samples as in the posthurricane period.

**Laboratory testing. Dengue.** Laboratory analysis of serum from suspected dengue patients was conducted according to procedures described in detail elsewhere. Briefly, acute-phase samples taken less than 6 days after onset of symptoms were tested for the presence of virus by methods described elsewhere. Acute-phase samples that failed to demonstrate the presence of virus, or which were not tested, were termed indeterminate. All serum specimens collected in the convalescent phase, between 6 and 90 days after onset of symptoms, were tested for IgM antibody to dengue by the IgM antibody-capture ELISA. The presence of IgM antibody to dengue is indicative of a dengue infection within the previous three months. If there was no significantly elevated level of IgM in these samples, they were considered negative for dengue and were used in this study.

**Leptospirosis.** The LEPTO-Dipstick (LEPTO-DST) is a *Leptospira*-specific IgM antibody assay developed at the Royal Tropical Institute (Amsterdam, The Netherlands). Serum dilutions (1:50) were made in the reconstituted detection reagent, a human IgM-specific monoclonal antibody conjugated to a colloidal suspension of palanyl red. Dipsticks were wetted in distilled water to prevent direct adsorption of the detection reagent to the solid support and then incubated for 3 hr in the mixture of diluted serum and detection reagent. After incubation, the dipsticks were rinsed with tap water and air-dried. Staining intensity was observed at the two bands of the dipstick: the internal control (the upper band) and the antigen band (the lower band). A red-stained antigen band indicated a positive reaction and was visually scored (according to the kit instructions) from 1+ to 4+ by one of the investigators (EJS). Serum samples that had a dipstick staining intensity ≥ 2+ were considered positive for leptospirosis. Convalescent-phase serum samples were requested from LEPTO-DST-positive patients. The microscopic agglutination test (MAT) was performed on convalescent-phase serum samples when available or on acute-phase serum samples from LEPTO-DST-positive patients, and on a systematic selection of the serum samples from LEPTO-DST-negative patients. LEPTO-DST-negative samples were listed and every third sample was selected for MAT testing.

The presence of antibodies to various serovars of *Leptospira interrogans* was determined by the MAT. The panel of antigens included the following 21 serovars: *ballum, canicola, copenhageni, icterohaemorrhagiae, bataviae, grippotyphosa, pyrogenes, autumnalis, pomona, wolfi, australis, tarassovi, georgia, alexi, cynopteri, mankarso, celledoni, djasiman, borincana, javanica*, and *bratislava*. The antigens were 4–7-day-old live cells cultured in PLM-5 broth (Technogen Company, Purchase, NY) and adjusted to the turbidity of a 0.5 McFarland standard. Serial 2-fold dilutions of serum in phosphate-buffered saline (50 µl/well) starting at 1:50 (1:100 after the addition of antigen) were mixed with an equal volume of antigen in 96-well, flat-bottom tissue culture plates (#3596; Costar Corp., Cambridge, MA). After the plates were incubated at room temperature (25°C) for 1.5–4 hr, the reactions were read by darkfield microscopy at 100× magnification. The reported titer is the reciprocal of the highest final dilution that agglutinated at least 50% of the leptospires relative to the buffer control. To provide a positive control for each antigen, homologous rabbit antiserum specimens were run in parallel with the patient serum specimens. In addition, *Leptospira* antigens in organ tissues obtained at autopsy from one LEPTO-DST-positive patient were demonstrated by immunofluorescence. Laboratory-confirmed leptospirosis was defined as an MAT-positive *Leptospira* agglutination titer ≥ 1:400 to one or more serovars, or demonstration of *Leptospira* by immunofluorescence in organ tissues.

**RESULTS**

A total of 142 dengue-negative patients were included in the study. Five (7%) of 72 prehurricane patients and 19 (27%) of 70 posthurricane patients had a positive LEPTO-DST result. A convalescent-phase serum sample was obtained from 20 (87%) of 23 surviving LEPTO-DST-positive patients (interval between onset and convalescent-phase serum sample: mean = 25 days, range = 6–64). Confirmatory testing was performed on 65 serum specimens (24 LEPTO-DST-positive, of which 20 were convalescent-phase, and 41 LEPTO-DST-negative serum samples, all acute-phase). Nineteen (79%) of 24 LEPTO-DST-positive serum samples and 1 (2%) of 41 LEPTO-DST-negative serum samples were MAT-positive. *Leptospira* antigen was demonstrated in liver and kidney tissues from one patient with a LEPTO-DST-positive sample (negative by MAT). Thus, leptospirosis was laboratory-confirmed in 21 patients.

There were 44 MAT negative specimens: 40 of 41 LEPTO-DST-negative specimens (i.e., true negatives), and 4 of 24 LEPTO-DST positive specimens (i.e., false positives). The LEPTO-DST showed a sensitivity of 95% (20 of 21) and a specificity of 91% (40 of 44), and concordance was 92% (60 of 65). Because of the good concordance between the dipstick and confirmatory testing, we accepted as leptospirosis negative all LEPTO-DST-negative samples that were not tested by the MAT. Therefore, 17 of 70 patients (24%) were laboratory-confirmed for leptospirosis in the posthurricane group, compared with 4 of 72, or 6% in the prehurricane group (relative risk [RR] = 4.4, 95% confidence interval [CI] = 1.6–12.4, Figure 1). The four prehurricane patients were 2 men (ages = 24 and 38 years) and 2 women (ages = 48 and 52 years). The 17 posthurricane patients were 16 males (median age = 28 years, range = 13–64), and 1 women (55 years old). One patient had a fatal case, a 31-year-old man who had onset of illness on September 20, 1996. Time period and sex-specific positivity for leptospirosis was 5% (2 of 40) and 35% (16 of 46) for males.
and 6% (2 of 32) and 4% (1 of 24) for females in the pre-hurricane and posthurricane periods, respectively (Table 1).

Dengue surveillance results did not reveal an unusual pattern during the study period (Figure 2). In 1996, laboratory results for 4,651 suspected dengue cases in Puerto Rico consisted of 1,903 (41%) positive, 2,183 (47%) indeterminate, and 565 (12%) negative determinations. The number of suspected dengue cases increased from July to October, and the proportion of dengue-positive specimens increased from July to November, corresponding with the seasonal pattern of dengue transmission in Puerto Rico (Figure 2). The proportion of specimens that were dengue-negative decreased in August, September, and October: 13% (65 of 483), 12% (72 of 609), and 10% (68 of 714), respectively.

Clinical findings noted in the dengue case investigation forms are shown in Table 2. The most common complaints for both groups were fever, chills, headache, body pain, joint pain, nausea or vomiting, and eye pain. Clinical features significantly associated with leptospirosis were eye pain (RR = 1.5, 95% CI 1.3–1.9), joint pain (RR = 1.4, 95% CI 1.1–1.6), diarrhea (RR = 1.7, 95% CI 1.2–2.5), and jaundice (RR = 3.3, 95% CI 1.5–7.2). In six (29%) of 21 laboratory-confirmed cases, leptospirosis was mentioned as a possible diagnosis in the remarks section of the dengue case investigation form.

Specimens of 20 patients that met the criteria of MAT seropositivity (≥ 1:400) were positive to 2–8 different serovars. From most to least common serovar, patient specimens were positive to copenhageni (80%), georgia (75%),icterohaemorrhagiae (70%), mankarso (70%), canicola (65%), bratislava (50%), autumnalis (40%), javanica (35%), ballum (25%), cynopteri (20%), bataviae (5%), pyrogenes (5%), tarassovi (5%), and alexi (5%).

Forty-eight (62%) of the 78 island municipalities submitted dengue-negative serum samples during the period of study, and 18 (38%) of these 48 municipalities had at least one patient with confirmed leptospirosis. One municipality had two confirmed cases of leptospirosis in the posthurricane period.

**DISCUSSION**

This study demonstrates the potential of the dengue surveillance system to detect an otherwise unsuspected increase in leptospirosis incidence after hurricane-generated flooding. Flooding after heavy rains is particularly favorable to leptospirosis in Puerto Rico during a pre-hurricane period (August 6 to September 12, 1996) and a posthurricane period (September 13 to October 6, 1996).

### Table 1

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Confirmed leptospirosis (%)</th>
<th>Leptospirosis-negative (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Prehurricane (n = 72)</td>
<td>Posthurricane (n = 70)</td>
</tr>
<tr>
<td>LEPTO-DST-positive*</td>
<td>19 (27)</td>
<td>24 (17)</td>
</tr>
<tr>
<td>Confirmed leptospirosis†</td>
<td>27 (38)</td>
<td>21 (15)</td>
</tr>
<tr>
<td>Total males</td>
<td>66 (61)</td>
<td>86 (61)</td>
</tr>
<tr>
<td>Total females</td>
<td>56 (39)</td>
<td>56 (39)</td>
</tr>
<tr>
<td>Cases in males</td>
<td>25 (35)</td>
<td>18 (21)</td>
</tr>
<tr>
<td>Cases in females</td>
<td>17 (24)</td>
<td>3 (5)</td>
</tr>
</tbody>
</table>

* Leptospirosis IgM dipstick.
† Microscopic agglutination test (titer ≥ 1:400 to one or more serovars) or positive immunohistochemistry in autopsy tissues.

* The denominator excludes nonresponses.
† P < 0.05, by two-tailed Fisher’s exact test.
‡ P < 0.01, by two-tailed Fisher’s exact test.

**Figure 2.** Laboratory diagnosis of suspected cases of dengue by month of onset of illness in Puerto Rico in 1996.
tospires; it prevents animal urine from being absorbed into the soil or evaporating so leptospires may pass directly into the surface waters or persist in mud.13 The more than 4-fold increase in laboratory-confirmed leptospirosis in the posthurricane period was attributable to male patients. It is conceivable that mostly males engaged in posthurricane relief work or played in flood waters and were thus at greater risk for infection.

This study represents a systematic analysis of a dengue surveillance system for detecting the occurrence of leptospirosis. Testing of relatively few dengue-negative serum samples revealed an island-wide occurrence of leptospirosis: 38% of the municipalities that submitted serum samples had at least one patient with confirmed leptospirosis. As in other countries, leptospirosis may be underreported in Puerto Rico for the following reasons: lack of physician awareness of Leptospirosis, absence of rapid diagnostic tests or laboratory facilities for the diagnosis of leptospirosis, and clinical similarity with dengue.

Leptospirosis is considered by physicians in Puerto Rico in its severe form: in 6 (29%) of 21 patients with laboratory-confirmed leptospirosis, leptospirosis was noted in the differential diagnosis on the dengue surveillance form. All six were severely ill, five were jaundiced, and one died. Seven (41%) of 17 leptospirosis patients were jaundiced in our study. Since less than 10% of symptomatic leptospirosis infections result in severe, icteric illness,17 milder leptospirosis cases may have been underestimated through the dengue surveillance system. In Puerto Rico in 1996, specimens were available from an additional 16 patients who died of suspected dengue; for 5 (31%), laboratory results diagnosed dengue, and for 5 (31%) laboratory-results diagnosed leptospirosis (CDC, unpublished data). All patients with fatal leptospirosis cases had onset of illness during the period July–December, which corresponded with the months of intense dengue transmission in Puerto Rico. Interestingly, the majority of leptospirosis cases studied in the period from 1948 to 1952 occurred during the 7-month period from July through January, and correlated with rainfall.8

Disease surveillance programs usually do not have a direct role in patient management since specimen collection, transportation to a laboratory, and laboratory investigations require too much time to provide feedback in the course of the disease. However, one of the functions of a surveillance system is to provide feedback to physicians on the suspected diagnosis, thus contributing to knowledge of correct patient management and care. The results of our study indicate that leptospirosis, and therefore laboratory testing for its diagnosis, should be considered part of the routine work-up of febrile patients with jaundice, and in febrile patients with less specific symptoms but with a history of exposure to flood waters or animal contact.

Combining testing for dengue and leptospirosis may help improve surveillance for leptospirosis. This outbreak could not have been suspected by looking at the proportion of dengue-negative samples for the period August through September. That a more than 4-fold increase in leptospirosis cases among dengue-negative patients was detected shows the potential of the dengue surveillance system for identification of other diseases. Additional leptospirosis cases could have been detected through the dengue surveillance system if convalescent-phase serum samples from patients with indeterminate samples had been sent for testing.

The potential of rapid diagnostic assays (dipsticks), which are not yet approved by the U.S. Food and Drug Administration, is substantial. However, since diagnostic results will not be available in the first five to six days of the illness, we recommend studies that evaluate clinical and laboratory algorithms for early differentiation of dengue from other febrile illnesses, including leptospirosis. A recent study of dengue in children in Thailand revealed that low white blood cell counts, absolute neutrophil counts, and absolute monocyte counts were significantly associated with dengue and not with other febrile illnesses,27,28 and leukocytosis was more common in nonsurvivors than in survivors in an evaluation of prognostic factors associated with mortality of leptospirosis.29

Our study did not obtain information from patients with confirmed leptospirosis about specific activities prior to onset of illness. The likelihood that cases were associated with flooding is supported by reports of exposure in 5 patients and studies conducted in Barbados and Nicaragua that identified walking through ponds of stagnant water as the most important risk factor.11,30 Hurricane predictions and predicting periods of extreme rainfall may be helpful in sensitizing the population to the potential for exposure to leptospires following sustained floodings. Predicting flooding is now possible months in advance, particularly where El Niño has been determined to have a strong effect on regional rainfall patterns.31

This study did not attempt to evaluate the sensitivity and specificity of the LEPTO-DST, which have been reported as 87% and 93%, respectively.23 Although we did not test all LEPTO-DST results against the MAT, among preselected serum samples of potential leptospirosis patients that were tested by the MAT, we documented an almost similar performance (sensitivity = 95%, specificity = 91%).

Given the wide range of serovar positivity, it is likely that many leptospiral strains are circulating in Puerto Rico and are responsible for causing disease. Between 1948 and 1952, 6 different serovars were isolated from humans in Puerto Rico and leptospirosis was found to be endemic. In addition, a high proportion of infected and serologically positive animals were found in a limited survey of wild and domestic animals.8 In our study, many patients were positive for serovars most commonly found in rodents (such as covoehagni andicterohaemorrhagiae), but others were also positive for dog-associated (canicola) and pig-associated and horse-associated (bratislava) strains. In 1980, serologic investigations of 116 stray dogs from several municipalities of Puerto Rico detected 73 (63%) with significant titers to predominant serogroupicterohaemorrhagiae, suggesting that the rat-to-dog and dog-to-dog cycles are the primary modes of transmission infecting canines in Puerto Rico.32 The elimination of stray dogs, control of reservoir hosts (i.e., rats and mice), and the vaccination of pets are preventive measures that should be practiced to help reduce the problem of leptospirosis.

Leptospirosis is treatable with antimicrobial agents, including penicillin and doxycycline, and knowledge of this diagnosis may significantly reduce morbidity and mortality. Physicians in Puerto Rico should consider leptospirosis in
suspected dengue patients, especially in those with exposure to flood waters. Avoidance of exposure to flooding, use of protective boots, and chemoprophylaxis (doxycycline, 200 mg/week) in the event of exposure in rescue work should be recommended. In our study, jaundice, eye pain, and diarrhea in dengue-negative patients were significantly associated with leptospirosis. In Barbados, patients who exhibit jaundice and accompanying thrombocytopenia are considered to have leptospirosis until proven otherwise. Since jaundice is relatively uncommon in cases of dengue, patients with jaundice and a dengue-like illness should be considered for early diagnostic testing for leptospirosis and for antibiotic treatment for leptospirosis until a laboratory-based diagnosis can be established.

Acknowledgments: We gratefully acknowledge the assistance of the staff of the Epidemiology Division, Puerto Rico Department of Health (San Juan, PR) for obtaining some of the patient information and some of the convalescent-phase samples; Ivette Gómez (CDC Dengue Branch, San Juan, PR) for performing a portion of the testing; and Sherif Zaki (National Center for Infectious Diseases, CDC, Atlanta, GA) for *Leptospira* immunofluorescence testing in autopsy tissues.

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Authors’ addresses: Eduard J. Sanders, Ethio-Netherlands AIDS Research Project (ENARP), Ethiopian Health and Nutrition Institute, PO Box 1242, Addis Ababa, Ethiopia, e-mail: enarp@telecom.net.et. José G. Rigau-Pérez and Vance A. Vornodam, Dengue Branch, Division of Vector-Borne Infectious Diseases, Centers for Disease Control and Prevention, San Juan, PR 00921-3200. Henk L. Smits, Royal Tropical Institute, Amsterdam, The Netherlands. Carmen C. Deseda, Division of Epidemiology, Puerto Rico Department of Health, San Juan, PR 00921-3200. Tin Aye, Richard A. Spiegel, Robbin S. Weyant, and Sandra L. Bragg, Meningitis and Special Pathogens Branch, Division of Bacterial and Mycotic Diseases, Centers for Disease Control and Prevention, Atlanta, GA 30333.

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