Dengue is a mosquito-borne viral disease caused by one of four antigenically distinct dengue flaviviruses: DEN-1, DEN-2, DEN-3, and DEN-4. Primary infection with any serotype may lead to acute illness defined as fever with two or more of the following symptoms: headache, retroorbital pain, myalgia, arthralgia, rash, and hemorrhagic manifestations (1,2). Fever and other symptoms may subside after 3 or 4 days, and the patient may recover completely, or the fever may return with a rash within 1 to 3 days (3). Secondary exposure to the same serotype generally does not produce illness because of pre-existing antibodies. However, secondary exposure to a different serotype may lead to another dengue fever episode, and the patient may be at risk for more serious forms of infection, dengue hemorrhagic fever (DHF) or dengue shock syndrome (DSS) (3). Dengue virus infection may also cause a nonspecific febrile illness that can be easily confused with measles or influenza. Therefore, laboratory testing is essential to clinical diagnosis and public health reporting.

Dengue-viremic persons are usually infectious to the mosquito vector 1 day before the onset of the febrile period and remain so for 6-7 days. When a mosquito ingests virus in a blood meal, the virus replicates during an extrinsic incubation period of 8 to 12 days, after which the mosquito remains infective for life (4,5). The life span of Aedes aegypti, the primary vector of dengue in the Americas, is usually 21 days, although life span and incubation periods depend on temperature and rainfall (6). Both A. aegypti and A. albopictus, a recently introduced vector species (7), have been found throughout Florida, and A. aegypti breeds year-round in south Florida (8).

The recent introduction of DEN-3 in Mexico and Central America is of public health importance because most of the population in the tropical Americas is susceptible to infection with this serotype (17,18). The presence of the vector, the rapid spread of the virus, and increased air travel and immigration contribute to the possibility of future dengue transmission in the

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**Synopses**

**Dengue Surveillance in Florida, 1997–98**

Julia Gill,* Lillian M. Stark,* and Gary G. Clark†

Florida Department of Health, Bureau of Laboratories and University of South Florida, College of Public Health, Tampa, Florida, USA; and †Centers for Disease Control and Prevention, San Juan, Puerto Rico, USA

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continental United States (19-21). A serosurvey conducted after the first confirmed dengue outbreak in Peru in 1990 clearly demonstrated earlier undetected dengue transmission (22). Silent transmission of dengue was also demonstrated in 1992 in an area of Taiwan believed free of the disease (23). In both cases, an early warning system based on immunoglobulin (Ig)M antibody-capture enzyme-linked immunosorbent assay (MAC-ELISA) laboratory tests was recommended for disease monitoring. Active surveillance, an essential component of an early warning system for detection of dengue, provides information vital to defining epidemiologic aspects of cases and enabling educational and mosquito control efforts (24-27).

Recent outbreaks of dengue in nearby Caribbean and Central and South American countries may increase the likelihood of future autochthonous transmission in Florida (15). Mosquito vectors are widely distributed in the state, and travelers returning from dengue-endemic areas place at risk the resident population, which has minimal (if any) immunity to dengue viruses. Because physicians’ awareness of dengue is low and specialized laboratory diagnostic methods are not available locally, low-level dengue transmission may go undetected. Imported dengue may thus be underreported in Florida, which has relied on passive surveillance. We used an educational campaign for county epidemiologists and health-care providers and an active laboratory-based surveillance program that facilitated prompt, accurate diagnosis of dengue to assess the risk for local dengue fever transmission in Florida.

The Study

The first phase of the surveillance program was the design of a dengue information packet for all 67 county health department epidemiologists in Florida, to be distributed to hospital emergency rooms, clinics, health departments, and infectious disease physicians in the county. The letter included information on case reporting, the dengue case definition, specimen requirements and transport instructions, and a dengue case investigation form.

Under cooperative agreements with two Florida commercial clinical laboratories (national reference clinical laboratories), specimens from patients with suspected dengue were forwarded to the state laboratory for free testing. In cases where specimens were tested at commercial laboratories only, dengue antibody-positive results were forwarded to county health departments and to the state laboratory for inclusion in this study. In Florida, dengue testing is offered only by the state laboratory and some commercial clinical laboratories.

Before this study, the hemagglutination inhibition (HAI) assay was the only serologic test for dengue offered at the state laboratory. Laboratory capabilities were enhanced to include testing for IgM antibodies to dengue. Acute- and convalescent-phase serum specimens were tested for dengue antibodies by both HAI assay and MAC-ELISA, using a DEN1-4 serotype cocktail (28-30). Available specimens positive for IgM antibodies to dengue, tested at the Florida state laboratory, were forwarded to CDC’s Dengue Branch laboratory for virus isolation, serotyping, and confirmation of serologic results.

Cases were classified as DHF if all the following were present: fever, hemorrhagic tendencies, thrombocytopenia (100,000/mm³ or less), and evidence of plasma leakage (hematocrit level increased by ≥ 20%) or other objective evidence of increased capillary permeability (31). If all the above were present, plus hypotension or pulse pressure ≤ 20 mm Hg, the case was classified as DSS.

In this study, a case was classified as presumptive dengue on the basis of serologic evidence of an HAI titer ≥ 1:1280, an equivalent IgG titer, or a positive dengue IgM antibody test on a single serum sample. A confirmed dengue case required a fourfold rise in HAI, IgG, or IgM antibody titers between acute- and convalescent-phase serum specimens; isolation of virus; or detection of viral antigen by immunohistochemistry, immunofluorescence, or viral nucleic acid detection. Confirmed or presumptive dengue cases are referred to as laboratory-diagnosed cases.

A case was classified as undetermined if sufficient information was not available on the timing of specimen collection in relation to onset of symptoms or a convalescent-phase serum was not available to demonstrate a fourfold rise in antibody titers. A case was also considered undetermined if the acute-phase serum was negative for antibodies and a convalescent-phase serum was not available.

Epidemiologic data were obtained from dengue case investigation forms that accompanied the patients’ specimens. Suspected as well
as confirmed cases of dengue are reportable in Florida (32). County health departments were notified of suspected cases, and a convalescent-phase serum was requested.

We used the Epi Info Software package for data analysis (33). Comparisons were made with historical data on reported cases of dengue (9,16,34-36).

From April 1, 1997, to March 31, 1998, 83 suspected cases of dengue were studied. Commercial clinical laboratories referred specimens for analysis for 36 (43%) of these cases. The rest were referred through county health departments, hospital laboratories, infection control practitioners, or physicians. Recent dengue infection was laboratory diagnosed in 18 (22%) of these cases. Twelve (67%) of the 18 confirmed dengue specimens were referred by commercial clinical laboratories. Virus isolation or polymerase chain reaction of five cases yielded all four dengue serotypes. Dengue was ruled out as the etiologic agent in 24 (29%) cases. The remaining 41 (49%) cases were undetermined because convalescent-phase serum samples were not available (Table 1).

Table 1. Characteristics of 83 suspected cases of dengue investigated in Florida, April 1997–March 1998

| Characteristic                        | Yes  | No   | Undetermined |
|--------------------------------------|------|------|--------------|
| History of recent travel to dengue-endemic area | 41   | 14   | 28           |
| (49%) (17%) (34%)                    |      |      |              |
| Fits dengue fever case definition    | 30   | 8    | 45           |
| (36%) (10%) (54%)                    |      |      |              |
| Flavivirus antibody detected         | 41   | 42   |              |
| (49%) (51%)                         |      |      |              |
| Convalescent-phase specimen provided | 25   | 58   |              |
| (30%) (70%)                         |      |      |              |
| Laboratory confirmation of recent dengue infection | 18   | 24   | 41           |
| (22%) (29%) (49%)                   |      |      |              |

Most (65%) of suspected-dengue patients were male (chi-square goodness of fit test p value = 0.006). Among suspected cases, the mean age was 41 years (1 day to 79 years). Forty-one (49%) initially tested positive for anti-flavivirus antibodies. Convalescent-phase serum was obtained in 25 (30%) of the cases. The average age of patients with confirmed dengue cases was 37 years (8 to 69); 14 (78%) of the 18 patients were male.

Laboratory-diagnosed cases were identified from five counties in central and extreme southeastern Florida (Figure 1). Cases were confirmed in persons residing in the following counties: Dade (8), Hillsborough (4), Orange (3), Palm Beach (2), and Broward (1). Table 2 lists Florida counties with laboratory-diagnosed dengue cases, case travel history, and dengue virus serotypes detected. All 18 laboratory-diagnosed dengue cases were in persons who had

Figure 1. County of residence for 18 laboratory-diagnosed dengue cases detected between April 1997–March 1998.

Table 2. Laboratory-diagnosed dengue cases in Florida by county, area of travel, and serotype, April 1997 and March 1998

| Area of travel – number of cases (dengue serotype) |
|-----------------------------------------------|
| County                                       | (n = 18)     |
| Broward                                      | Barbados – 1 |
| Dade                                         | Colombia – 1 |
|                                              | Haiti – 3    |
|                                              | Puerto Rico – 1 (DEN-2) |
|                                              | Venezuela – 2 (DEN-1) |
|                                              | Unknown – 1  |
| Hillsborough                                 | Colombia – 1 (DEN-2) |
|                                              | Nicaragua – 1 (DEN-3) |
|                                              | Thailand – 1  |
|                                              | Unknown – 1  |
| Orange                                       | Haiti – 2 (DEN-4) |
| Palm Beach                                   | Puerto Rico – 1 |
|                                              | Puerto Rico – 1 |
recently traveled to dengue-endemic areas and were therefore classified as imported. We included out-of-state cases in our analysis because the acute phase of their illness occurred while they were in Florida. Current county health department policy dictates that only cases in Florida residents are reported to the state epidemiologist for recording in the weekly and yearly morbidity statistics. Other case reports are forwarded to the county and state of primary residence of the patient.

Hemorrhagic manifestations were reported in 7 (39%) of the 18 confirmed cases; one met the DHF case definition; however, it was not possible to classify the remaining six cases with hemorrhage because information on hemoconcentration and plasma leakage was incomplete. Encephalopathy was present in one case. Antibody titers suggested secondary dengue infections in 10 (56%) of the 18 cases. Only 2 (11%) of the 18 cases appeared to involve primary infections. Laboratory tests necessary to determine infection status (primary vs. secondary) were not available in the other six cases. A woman with acute secondary dengue infection with hemorrhagic manifestations gave birth to a healthy uninfected baby.

Conclusions

During the year of active surveillance, 18 laboratory-diagnosed cases of dengue were detected. On the basis of the previous 10-year mean of 1.3 cases per year (Figure 2), the probability of detecting 18 cases was virtually 0% (Poisson distribution rare event vs. standard test). These cases were identified in Florida counties with high rates of international travel and large immigrant populations, as well as year-round breeding of A. albopictus and A. aegypti mosquitoes. According to Florida Department of Commerce statistics, of the 6 million international visitors to Florida in 1995, 38.4% traveled from South and Central America, the Caribbean, Mexico, Asia, and other tropical areas (37) in which dengue is endemic.

All four dengue serotypes were detected in five specimens during this study. Improved specimen handling should increase the rate of virus isolation. The serotype of the infecting dengue virus was identified in only five cases for the entire United States in 1995, when 79 laboratory-diagnosed dengue cases were documented (12). In the same year, 22 imported and seven indigenous cases were detected in Texas (15). In 1996, the infecting dengue serotype was identified in 5 of the 43 laboratory-diagnosed cases of imported dengue in the United States (three cases of DEN-1 and two of DEN-2) (38).

This study found multiple problems with routine clinical laboratory confirmation and follow-up of dengue infections: Tests requested by physicians and performed at clinical laboratories were not always optimal for identifying a current dengue infection. Even though the dengue IgM test is the most appropriate assay for determining current infection, it is not routinely performed at commercial laboratories and may not be readily available if requested. Test results are frequently misinterpreted, e.g., a single positive indirect fluorescent antibody test performed at a commercial laboratory may be interpreted as positive for current dengue infection when it only indicates infection with a flavivirus (e.g., dengue, St. Louis encephalitis, Japanese encephalitis) or vaccination (e.g., yellow fever) at an undetermined time in the past. In addition, cases are rarely investigated, and the convalescent-phase serum samples needed for confirmation are rarely requested. When an investigation indicates need for further testing, specimens may have already been discarded. Finally, positive test results are often not forwarded to the county and state epidemiologists in a timely manner. In cases tested only at commercial laboratories, delays of 2 to 4 months before positive cases were reported to the state Bureau of Epidemiology preclude prompt follow-up.

Three of the confirmed dengue cases in this study tested at commercial laboratories had not

Figure 2. Reported dengue cases in Florida, 1987–1997.
(*1997 = study year April 1, 1997–March 31, 1998)
been reported to the state epidemiologist by the county health departments because the patients were primary residents of other states, although they became ill while in Florida.

This study indicates that surveillance efforts should be concentrated in densely populated counties with large numbers of international travelers (Dade, Palm Beach, Orange, and Hillsborough), especially during dengue season in the Caribbean (July to November). As a part of the epidemiologic investigation of imported dengue cases, an attempt should be made to identify secondary cases.

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