Abstract. Dengue fever infection was first documented in Jeddah, Saudi Arabia, by virus isolation of dengue type 2 virus in 1994 at the virology laboratory of Dr. Soliman Fakeeh Hospital. Dengue virus surveillance was established after that time. Blood samples were collected from 985 patients (710 male patients and 275 female patients) with suspected cases of dengue from February 1994 to December 1999. Dengue virus isolates were obtained in 207 patients (21%; 162 male patients and 45 female patients). Dengue type 2 was the predominant serotype (138 of 207 isolates, 66.7%), followed by dengue type 1 with (56 of 207 isolates, 27%) and dengue type 3 (13 of 207 isolates, 6.3%). The largest number of isolates (186 of 207 isolates, 90%) was in 1994, a year during which there was a dengue epidemic. In the next 5 years, 1995–1999, only 21 isolates (10%) were isolated. Immunoglobulin M capture enzyme-linked immunosorbent assay was positive in 160 acute samples; 52 of them were from virus culture-positive cases and 108 (11%) from culture-negative cases. The total number of cases diagnosed by both methods was 315 (32%).

The prevalence of dengue immunoglobulin G antibodies, as assessed on the basis of immunofluorescent assay, hemagglutination inhibition titers ≥ 1/20, or both, in the acute samples was 314 (32%) of 985, indicating past Flavivirus infection. Two patients died, one man with dengue hemorrhagic fever and one woman with dengue shock syndrome. Both fatal dengue cases were due to infection with type 2 virus. All other cases were simple dengue fever. To our knowledge, this is the first report confirming the circulation of 3 dengue serotypes in Jeddah.

INTRODUCTION

Dengue fever (DF), and its more severe forms, dengue hemorrhagic fever (DHF) and dengue shock syndrome (DSS), is the most important arthropod-transmitted viral disease affecting humans in the world today. Each year, 50–100 million cases of DF and several hundred thousand cases of DHF occur. The dengue pandemic has intensified in the past 20 years, with expanding geographical distribution of both the mosquito vector and the viruses and increased epidemic activity. Today, dengue is a global disease of the tropics and is one of the most important emerging tropical diseases, with over half of the world population at risk.1 Dengue fever and DHF are caused by the dengue viruses, which belongs to the genus Flavivirus, family Flaviviridae. There are 4 antigenically related but distinct dengue virus serotypes, dengue virus types 1–4 (DEN-1, DEN-2, DEN-3, and DEN-4), all of which can cause DF-DHF.2 The viruses are transmitted to humans by the bite of Aedes aegypti mosquitoes.

Epidemics of dengue-like disease appeared in the Arabian peninsula in the late 19th century (1870–1873). The disease appeared in Zanzibar, in Dar el Salam, on the East African coast, in Arabia (Aden, Mecca, and Madina), and in Jeddah, Saudi Arabia. However, this epidemic was preceded by earlier major epidemics affecting the 3 continents of Asia, Africa, and America in 1779 and 1780.1 In 1984, on the basis of serologic results, Jimenez-Lucho and others2 reported a case of DF with hemorrhagic manifestations in a patient from Yemen, a country south of Saudi Arabia on the Red Sea.

In 1994, dengue virus was isolated in Jeddah, Saudi Arabia, for the first time at the virology laboratory of Dr. Soliman Fakeeh Hospital (SFH) from a fatal case of dengue hemorrhagic fever and from another nonfatal case. The 2 isolates were confirmed to be DEN-2 at both Yale Arbovirus Research Unit, New Haven, Connecticut, and the Centers for Disease Control and Prevention (CDC), Fort Collins, Colorado (Zaki A, Shope RE, Gubler DJ, unpublished data). Since that time, doctors in Jeddah were alerted to the clinical syndromes associated with dengue infection. Blood samples from patients with clinically suspected dengue were referred to the virology laboratory of SFH for laboratory diagnosis by virus culture and serological methods.3 In this article, we report the results of virus culture and immunoglobulin (Ig) M enzyme-linked immunosorbent assay (ELISA) on suspected cases. We also report the clinical picture of the first fatal case.

MATERIALS AND METHODS

Patient selection. All hospitals and clinics in Jeddah were alerted and asked to report suspected cases of DF or DHF according to the following case definition: fever with thrombocytopenia and or leucopenia, eye pain, generalized body aches, headache, and skin rash with or without bleeding. Acute blood samples were collected from 985 patients during February 1994 to December 1999, but convalescent samples were collected from only 152 (15.4%) of these patients. Supernatant of clotted blood before centrifugation was used for virus isolation from acute samples. One portion of the supernatant was kept at −80°C; the rest of blood was centrifuged, and serum was separated and kept at −20°C for serologic testing. Convalescent or second blood samples were collected whenever possible after 2 weeks (range, 3–30 days); convalescent serum was separated and kept at −20°C.

Virus isolation. Supernatant from clotted blood was inoculated onto C6/362 and Vero cell lines grown in tissue culture tubes (Nunclon delta 1-56758, Nunc, Denmark). Inoculated Vero cells were incubated at 37°C for 2 weeks for detection of cytopathogenic effects. Cultures of C6/36 were incubated at 33°C for 1 week; then the cells were scraped from the tubes and centrifuged at 2,000 rpm for 10 min. The supernatant was passed and deposit was spotted on Teflon-coated slides and tested by indirect immunofluorescent assay
(IFA) by use of polyclonal anti-dengue antibody; viruses in positive cultures were identified to serotype by IFA by use of serotype-specific monoclonal antibodies.6–8

**Animal inoculation.** A total of 20 acute samples for which clinical data were highly indicative of viral infection were inoculated by the intracerebral route into in 1-day-old suckling mice.5

**Serologic tests.** The hemagglutination inhibition (HI) test was performed according to the method of Clarke and Casals,9 modified for use in microtiter plates. The test used 8 units of DEN-1 and DEN-2 antigens supplied by the Division of Vector-Borne Infectious Diseases (DVBDI), CDC, Fort Collins, Colorado. The IgM antibody reactive with dengue viruses was measured by isotype capture enzyme immunoassay by use of a modification of the IgM capture ELISA described by Kuno and others.6–9 Serum samples were diluted 1:100 for testing with a cocktail of DEN-1 and DEN-2 antigens.10,11 Specimens were considered positive if the optical density value was ≥ 0.20. Indirect IFA for IgG was performed with infected Vero cells.8

**Case definition.** Dengue infection was defined as a febrile illness associated with the isolation of dengue virus, the detection of anti-dengue IgM antibody, a 4-fold rise in HI titer, illness associated with the isolation of dengue virus, the detection of anti-dengue IgM antibody, a 4-fold rise in HI titer, or a sustained elevation (≥ 1:1,280) of HI antibody. Dengue infections were defined as primary or secondary according to World Health Organization criteria.13

**RESULTS**

**Isolation and typing of dengue viruses.** Dengue virus infection was confirmed in 207 (21%) of 985 suspected cases by virus isolation. Typing of the isolated dengue viruses by type-specific monoclonal antibodies showed that DEN-2 is the most commonly isolated type (138 isolates, 66.7%), followed by DEN-1 (56 isolates, 27.0%) and DEN-3 (13 isolates, 6.3%). Most of the dengue isolates (186 of 207 isolates, 90%) were obtained in 1994, which was an epidemic year, and only 21 isolates (10%) were isolated in the next 5 years. Table 1 shows the distribution of the laboratory-confirmed cases per year. Dengue virus-reactive IgM antibodies were detected by IgM capture ELISA in 160 patients, 52 of them from culture-positive cases. In total, 108 (11%) of 985 patients were diagnosed by single IgM capture ELISA.

A second blood sample was obtained in only 17 of these IgM-positive patients, and seroconversion was detected in all of them. Table 2 shows the age distribution of laboratory-diagnosed dengue cases. The number of isolates was significantly lower in the first and last groups than other groups (P = 0.002). One isolate of DEN-3 was isolated in 1996 and 12 in 1997, whereas DEN-2 and DEN-1 were isolated over the course of the entire study period. Testing acute sera for HI antibodies, IFA antibodies, or both of IgG type showed that 314 (32%) serum samples had titers of ≥ 1/20, indicating previous dengue infection or Flavivirus infection. Only 2 patients died: one man died of DHF complicated by fulminant hepatitis, and a woman died of DSS.

**Clinical findings of a fatal case of DHF.** A 29-year-old Saudi man was admitted to SFH on October 13, 1993, with a 3-day history of fever, vomiting, upper abdominal pain, diarrhea, and repeated attacks of fainting. At admission, he was semiconscious, with ecchymosis over the back, neck, and right shoulder. The rectal body temperature was 37.8°C; his pulse was 96/min, and blood pressure was 100/70. Chest and heart examinations revealed nothing abnormal. Abdominal examination showed only abdominal distension. Laboratory examination revealed the following: white blood cell count, 3.4 × 10^9/L; hemoglobin, 18.7 g/dL; erythrocyte volume fraction, hematocrit 0.565; platelets 7 × 10^9/L. Prolonged prothrombin time was 25 sec (control, 12 sec), partial thromboplastin time (PPT) 46 sec (control, 32 sec), and thromboplastin time (TT) 37 sec (control, 19 sec). Elevated liver enzymes were evident: the patient had an alanine aminotransferase (ALT) level of 315 U/L and an aspartate aminotransferase (AST) level of 1,088 U/L. Total bilirubin was 3.5 mg/dL, with direct bilirubin 2.1 mg/L.

The patient received urgent transfusion of fresh frozen plasma, platelet concentrates, and other supportive treatments. However, despite treatment, the patient’s level of consciousness deteriorated. He also experienced deterioration of liver function (ALT, 5,687 U/L; AST, 18,624 U/L), and creatinine levels were elevated (6.1). He became anuric and jaundiced, with widespread ecchymosis and petechiae. The patient died on October 22 as a result of hepatorenal failure.

This patient was diagnosed clinically with fulminant viral hepatitis with acute renal impairment. The blood of this patient was kept at −80°C until we received C6/36 cell lines and reagents from the Yale Arbovirus Research Unit in New Haven, Connecticut, which allowed us to diagnose DHF in January 1994. Diagnosis of dengue was confirmed by isolation of DEN-2 from the patient’s blood on C6/36 cell line. The isolated virus identity was confirmed at both the Yale Arbovirus Research Unit and the CDC, Fort Collins, Colo-
rado. This case of DHF was the first to be diagnosed at the SFH virology laboratory.

**DISCUSSION**

This study confirms the presence of dengue virus infection in Jeddah, Saudi Arabia, for the first time on the basis of laboratory data by virus isolation and typing and by serologic techniques. It is important to note that because we were unable to get a second blood sample in many patients, some of the negative cases in our study could actually have been cases of dengue infection. Also, because of the presence of cases of mild dengue, a number of patients with DF may not be included here. Dengue virus infection was confirmed by virus isolation from 207 patients (21%) by use of culture on C6/36 cell line and by IgM capture ELISA in 108 (11%) patients, giving a total of 315 patients (32%). All dengue virus strains were isolated in C6/36 cell line and typed by use of type-specific monoclonal antibodies. This method, although less sensitive than mosquito inoculation, it is more sensitive, rapid, and less costly than the direct plaque assay that used LLC-MK2 cell and plaque reduction neutralization test for identification of dengue virus.\(^5\) One of the DEN-1 isolates failed to grow in C6/36 at primary isolation from patient’s blood but was detected by intracerebral inoculation into suckling mice and passage into C6/36 cells; this raises the possibility that some of the patients classified as negative may actually have had similar strains.

Most of isolates were from blood samples collected from the first 5 days of symptoms, a common finding by many researchers.\(^7,12,14,15\) The number of isolates was significantly lower in the age group 1–10 years and the age group > 50 years, but was significantly higher in all other age groups (\(P = 0.002\)). The reason for the lower number of isolates in the younger age group could be due to improper clinical selection of cases; DF in younger age groups manifests as rather undifferentiated illness, such as upper respiratory-like infection accompanied by headache and mild gastrointestinal complaints; often, DF lacks an obvious rash and has an atypical clinical presentation.\(^16\) Age is the best-described modulator of the severity of dengue illness. Preadolescent children exhibit a DF-like illness but are not as severely incapacitated as adults. The disease in adults is severe enough that patients feel sick and demand medical attention. This seems to be the reason why adult patients are particularly apparent during dengue epidemics.\(^17\)

In this study, a good number of patients were adult construction workers who slept outdoors near water reservoirs, which harbored large numbers of mosquitoes. However, the lower number of isolates in the > 50 age group could be explained by higher immunity to dengue in this group. There was a statistically significant difference in the number of isolates between female and male subjects (\(P = 0.02\)), with a higher number isolated from male subjects. However, because of the significant difference in the mean ages between male and female subjects (\(P = 0.0002\)), this difference in number of isolates could be partly due to difference in age between male and female subjects.

Typing of the virus isolates by use of type-specific monoclonal antibodies\(^5\) showed that DEN-2 was the predominant serotype (138 of 207 isolates, 66.7%), followed by DEN-1 (56 of 207 isolates, 27%) and DEN-3 (13 of 207 isolates, 6.3%). Despite the circulation of 3 dengue serotypes, very few patients with DHF or DSS were observed in the group of patients we examined; we saw only 2 cases in adults. The lack of cases of DHF or DSS in the studied group could be attributed to many factors. The typing of the few DEN-2 strains at the CDC in Fort Collins by RNA fingerprinting showed that the strains were of African origin. Dengue hemorrhagic fever and DSS are common with secondary infection with dengue viruses of Asian origin.\(^18\) Age is also an important factor for development of severe DHF and DSS, with children being the most susceptible to these complications.\(^21\) However, none of the children examined showed any severe complications of DHF or DSS, which could be explained by Race\(^25\) or by a virus factor.\(^18\)

Most of the dengue isolates (186 of 207, 90%) were in 1994, the year of a dengue epidemic; the rest of isolates (21 of 207, 10%) were isolated in the next 5 years. The termination of the outbreak by the end of 1994 can be attributed to many factors, including accumulation of a large number of immune people, a lower temperature, which decreased mosquito activity, and the use of mosquito-control measures, which was started early after the discovery of the first 2 cases in February 1994. Of these factors, the most important, in our opinion, is the accumulation of large number of immune people from the infection. Attack rates during outbreaks are high and may reach 80–90%, but more commonly 40–50%, leaving a large number of the population with residual immunity.

All laboratory-confirmed cases were self-limiting DF, with the exception of the 2 fatal cases, one of DSS and the other of DHF with severe impairment of the liver function and renal failure, mimicking other hemorrhagic fevers, such as yellow fever, Rift Valley fever, or Crimean Congo hemorrhagic fever.\(^23\)

In 1979, Gubler and others,\(^24\) on the basis of earlier observations by Russell and others\(^25\) in 1967, classified as secondary infections those patients in whom acute serum specimens had HI titers \(\geq 10\). When we used this criteria in our study and raised the HI titer to \(\geq 20\), secondary dengue infection was detected in 121 patients (38.4%), whereas 194 (61.6%) patients were considered to represent cases of primary dengue infection. We preferred to use this criteria, rather than the World Health Organization criteria, because of our inability to obtain convalescent blood samples in most of the patients. There is a possibility of outbreaks of DHF and DSS because of the large number of people already being infected with dengue viruses, the presence of 3 dengue types, and the possibility of introduction of new strains or genetic changes in existing strains.\(^26\) Recent reports from Cuba indicate a reemergence of dengue there, with more cases of DHF in adults than in children.\(^27\)

This study confirms the circulation of 3 dengue virus serotypes in Jeddah, Saudi Arabia. Most clinically and laboratory-confirmed cases are mild DF; DHF infection is rare (with only 2 adults affected), and no hemorrhagic manifestations were seen in children. Immunoglobulin M ELISA can be used to screen patients who display dengue-like fever for infection with the dengue virus, and virus culture can be initiated once positive IgM results are obtained to minimize the cost of surveillance. Effective mosquito control programs
are important to prevent further transmission of dengue viruses.

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