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Original Report

Expansion of Epidemic Dengue Viral Infections to Pakistan

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ABSTRACT

Objectives: Antibodies to dengue viruses have occasionally been reported in individuals in Pakistan, but the frequency of occurrence of dengue infection in Pakistan is unclear. The first confirmed dengue hemorrhagic fever outbreak in Pakistan occurred in 1994. In October 1995, the authors investigated an outbreak of a febrile illness among employees of a construction contractor at a power generation plant in Baluchistan, Pakistan, to determine the cause of illness and recommend appropriate preventive measures.

Methods: The work site and living arrangements were inspected, a questionnaire was administered, and serum samples were collected from all consenting contractor employees and their families if they lived at the camp. Sera were analyzed for IgM against dengue virus, using an enzyme-linked immunosorbent assay.

Results: Interviews were conducted with 76 persons (mean age, 42 y); 95% were men. Forty-two persons (55%) reported having experienced fever, headache, or myalgia in the preceding 6 weeks. Fifty-seven subjects (75%) had IgM antibodies against at least one dengue serotype; 45 subjects (59%) had IgM antibodies against dengue serotype 2.

Conclusion: This was an outbreak of dengue fever due to multiple serotypes of dengue virus. This confirms that epidemic dengue infection was present in southern Pakistan for 2 consecutive years.

Key Words: *dengue, enzyme immunoassay, epidemiology, fever, Pakistan, travel*

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Dengue is the most widely distributed of the mosquito-borne viruses that affect man.[1] Hundreds of thousands of cases of dengue and dengue hemorrhagic fever are reported each year in tropical regions of the Americas, Africa, Asia, and Oceania.[1-3] The prevalence of dengue infection in Pakistan prior to 1994 is unclear. Serosurveys conducted in the 1960s and 1980s among apparently healthy Pakistanis in Rawalpindi and Peshawar areas,[4,5] parts of Punjab province,[6,7] and Karachi[8] found that substantial proportions of the populations tested positive for neutralization and hemagglutination inhibition antibodies to dengue virus. However, these persons also tested positive for antibodies to other flaviviruses, and so the positive dengue tests may only reflect cross-reacting antigens. In 1994, the first serologically and virologically confirmed outbreak of dengue hemorrhagic fever in Pakistan was reported.[9]

On October 19, 1995, company officials of Contractor A requested assistance from the Aga Khan University Department of Community Health Sciences after 16 of their 100 employees became so ill that they missed work during a 1-week period. Contractor A is an Italian firm assisting in constructing an oil-fueled electrical generation plant in the Baluchistan province of Pakistan, approximately 60 kilometers west of Karachi, Pakistan's largest city. An investigation was conducted to determine the cause of the illness, and to determine appropriate preventive measures that could be recommended. This investigation documented an outbreak of dengue fever a year after the 1994 Karachi outbreak.

METHODS

The initial visit involved inspection of the construction site and interviews of ill persons, company officials, and the doctors who treated ill employees who worked at the power plant. A second visit was scheduled to verify the cause of the outbreak. A questionnaire was prepared to gather data about demography, travel history (to assess previous exposure in expatriates, or travel to areas highly endemic for dengue), illness (including onset, duration, symptomatology, severity, and whether subjects had experienced any bleeding episode), and exposures (duration of work at the open powerhouse or outdoor sites, sleeping quarters, and use of repellents). Subjects were classi-

fied as being clinically ill if they had fever, headache, or muscle ache during the preceding 6 weeks.

The questionnaire was completed by all employees of Contractor A who were present on the day of the field evaluation and consented to be interviewed. Family members of the employees who lived at the powerplant site also were interviewed. In addition to the questionnaire, for those persons who were acutely ill at the time, blood pressure, postural drop (i.e., change in systolic blood pressure with change of posture from sitting to the standing position), temperature, presence of petechiae, and capillary fragility by the tourniquet test were recorded.

Investigators collected 10 mL of blood in a neutral tube from all employees and family members living on the site who agreed to have their blood tested. Serum was separated at the site, saved in two aliquots of 500 μL each and kept frozen in a dry-ice canister for transportation to the Aga Khan University Hospital Laboratory where it was later stored at -80°C until it was analyzed. An additional 5 mL of blood was collected in anticoagulant-containing bottles from those ill at the time of the investigation, for platelet counts, hematocrit, and thick and thin smears for malarial parasites. These samples were transported in a wet-ice box and analyzed at the Aga Khan University Hospital Laboratory. Available diagnostic tests for those subjects who had been ill during the preceding 6 weeks were reviewed, specifically, the complete blood count, malarial parasite smear, and cultures for *Salmonella typhi.*

Serum IgM and IgG antibodies to dengue viruses (serotype 1, 2, 3, and 4) and IgM antibodies to West Nile and Japanese encephalitis were determined by enzyme-linked immunosorbent assay (ELISA). Lyophilized inactivated dengue antigen (DEN 1, 2, 3, and 4) in a dilution of 1:320 in 0.05 M carbonate and bicarbonate buffer pH 9.6 was used to coat flat-bottomed microtiter plates. The plates were washed three times with washing buffer that contained 5% bovine serum albumin and 1% Tween 20. Test serum samples (negative and positive controls) were added in a dilution of 1:100 in serum dilution buffer (2.5% bovine serum albumin, 0.01% Tween in 0.01 M phosphate buffered saline pH 7.4). Plates were covered and incubated at 37°C for 1 hour. Wells were again washed five times, using phosphate buffered saline in a total volume of 200 μL per well. Antihuman IgM and IgG alkaline phosphatase conjugate was added in a dilution of 1:25,000, and plates were incubated again for 1 hour at 37°C. Presence of dengue-specific IgM and IgG was detected by using chromogenic substrate para-nitrophenyl phosphate. The cutoff value for each assay was calculated by taking the mean absorbance of negative controls for that assay, and multiplying that by three standard deviations.

During a third visit, 3 weeks later, sera were collected from convalescent individuals who had been acutely ill and those who had been ill in the previous 2 weeks and also had tested negative for IgM antibody to the four dengue serotypes. Any persons who had fallen ill since the previous visit were sought and included in the study.

The proportion of categoric differences between groups was compared by relative risks, confidence intervals were estimated by Cornfield's approximation, and Yates corrected chi-square test or Fisher's exact test was used where appropriate. Statistical analysis was performed using Epi Info 6.0 (Centers for Disease Control and Prevention, Atlanta, GA).

RESULTS

Contractor A employed 60 expatriate and 40 Pakistani workers. On the initial visit investigaters learned that the main symptoms among affected persons were fever, body aches, and weakness. Although both expatriate and local employees of the company were affected by the illness, they ate at separate cafeterias. Close contacts of ill employees were not affected, but workers from other subcontracted companies were. The local clinics reported a 50% increase in the number of patients presenting with fever. The workers at the plant reported a large number of mosquitoes, especially in the powerhouse, an open area where the main turbines were being installed. There was a narrow pool of stagnant water in a shallow trench dug around three sides of the plant, under which was submerged a utility line. Interim recommendations for mosquito control were made and a second visit was planned.

Table 1. Frequency of Symptoms Reported among Contractor A Employees in the 6 Weeks Prior to October 24, 1995, Baluchistan, Pakistan

Symptom	*Occurrence among Interviewed Contractor A Employees (n = 76) (%)*	*Occurrence among 42 Individuals Who Met Case Definition* (n = 42) (%)*
Fever	35 (46)	35 (83)
Headache	32 (42)	32 (76)
Chills	26 (34)	26 (62)
Weakness	25 (33)	25 (60)
Muscle ache	24 (32)	24 (57)
Joint pain	20 (26)	19 (45)
High fever	19 (25)	19 (45)
Diarrhea	18 (24)	14 (33)
Dizziness	15 (20)	15 (36)
Nausea	15 (20)	14 (33)
Vomiting	14 (19)	14 (33)
Abdominal pain	13 (17)	13 (31)
Sore throat	13 (17)	1 (2)
Rash	12 (16)	11 (26)
Cough	11 (15)	9 (21)
Rhinorrhea	10 (13)	9 (21)
Rigors	9 (12)	8 (19)
Eye pain	8 (11)	7 (17)
Chest pain	6 (8)	5 (12)
Abnormal bleeding	3 (4)	3 (7)

*Case definition of illness: fever or headache or myalgia in the 6 weeks prior to being interviewed.

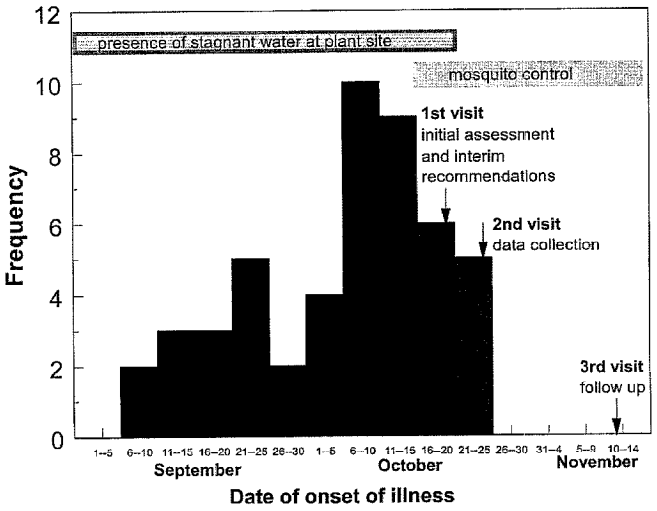

Figure 1. Date of onset of illness among those who met the case definition of illness (i.e., fever, headache, or muscle ache during 6 weeks preceding interview), among Contractor A employees, September to November 1996, Baluchistan, Pakistan.

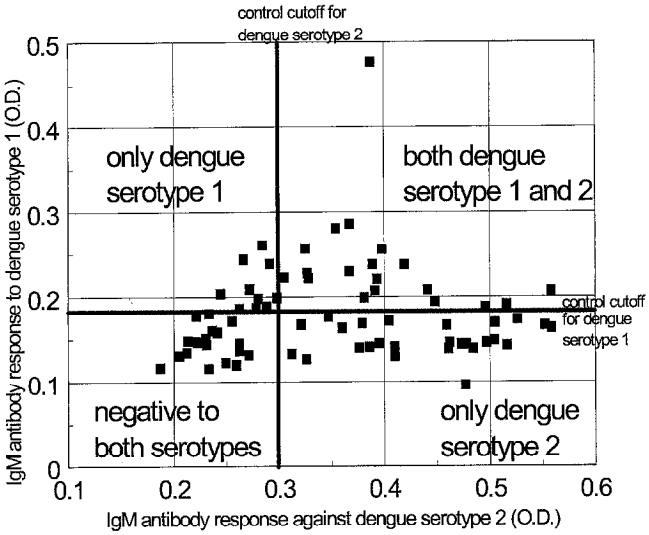

Figure 3. Relationship of IgM response to dengue serotype 2 and dengue serotype 1 among Contractor A employees, October to November 1995, Baluchistan, Pakistan. O.D. = optical density.

During the second visit 72 employees and 4 family members agreed to be interviewed; 51 (67%) were expatriates and 25 (33%) were Pakistanis (mean age, 42 y). There were 72 (95%) males and 4 (5%) females (who were the spouses and children of three of the expatriates). Forty-two (55%) met the definition of illness; that is, they had fever, headache, or muscle ache, during the 6 weeks preceding the interview (Table 1). Thirty-eight (50%) individuals reported being sufficiently ill to miss work. Only three persons complained of abnormal bleeding. Most of the patients fell ill between October 5 and 25, 1995 (Figure 1).

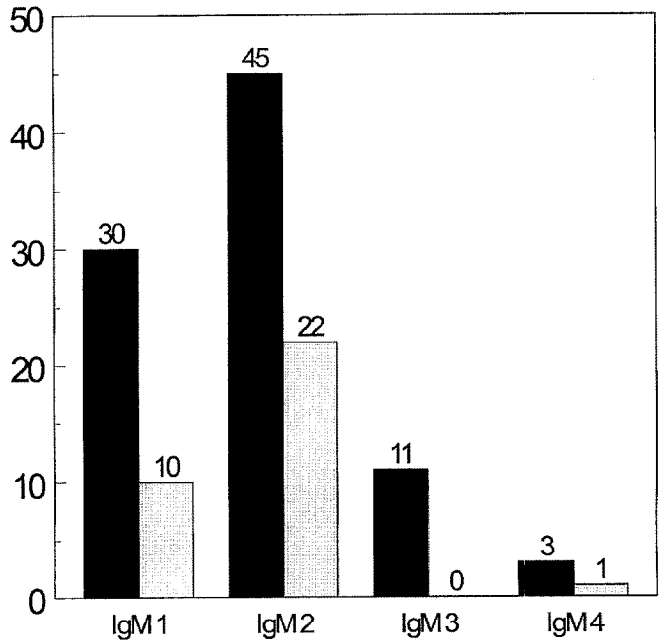

Figure 2. IgM antibody reaction to dengue virus in employees of Contractor A, October to November 1995, Baluchistan, Pakistan. Black columns indicate number of subjects positive to one or more serotypes; grey columns indicate number of subjects positive to only one serotype.

Seven persons (9%) were ill at the time of the first visit, and three other persons had laboratory tests performed when they were ill. Three (42%) of the seven persons reviewed at the time of the visit had fever and a postural drop of 15 mmHg or more. None of the seven ill patients had petechiae; each had a negative tourniquet test. Only one of the 10 individuals whose platelet count was obtained had an abnormal result, 42,000 cells/mm^3; hematocrits of nine individuals were normal. Blood cultures collected on two ill persons yielded no pathogens. None of the 10 specimens examined had malarial parasites.

In 2 days 76 convalescent sera and 7 acute sera samples were collected; 57 (75%) convalescent sera samples were positive for IgM antibodies to one or more dengue serotypes (Figure 2); 45 (59%) had antibodies against dengue serotype 2. The optical densities obtained against dengue serotype 2 antibodies were higher than those against dengue serotype 1 antibodies (Figure 3). Twenty-six persons (34%) reacted only to serotype 2; 19 (25%) reacted to both dengue serotype 1 and 2. Ten (13%) persons reacted to dengue 1 alone (see Figure 3). None of the 76 sera were positive to IgG antibody for dengue serotype 2 or IgM antibody to West Nile or Japanese encephalitis virus.

In the 6 weeks prior to the initiation of mosquito control measures, the median range of mosquito bites

reported was between one and five bites per day. Seventeen (22%) individuals reported having more than 10 bites per day; only 10 (13%) reported the use of repellents. Forty-five (60%) of the respondents reported wearing short-sleeved shirts. There was no significant association between those who tested positive to dengue virus antibody and measures of exposure, such as number of mosquito bites, use of repellents, time spent at powerhouse or wearing short-sleeved shirts.

DISCUSSION

This investigation confirmed that dengue virus was responsible for an outbreak of febrile illness at the powerplant in Baluchistan in October 1995. The clinical presentation was typical. There was substantial exposure to mosquitoes, and the serological tests demonstrated IgM antibody against dengue virus, which is a sensitive and specific marker of acute dengue infection.[10]

The epidemiology of dengue virus-induced illness in Pakistan is not well characterized. In the 1960s, in Rawalpindi and Peshawar (two cities in the north of Pakistan), 4 of 30 randomly collected sera tested positive to neutralization antibodies to dengue serotype 1.[4,5] Studies to test for antibodies to flaviviruses were done among residents of Chiniot and Changa Manga National Forest areas of Punjab Province during 1968 and 1978. Eleven of 50 sera collected in 1968 and 1 of 124 sera collected in 1978 to 1979 tested positive for hemagglutination inhibition (HI) antibodies against dengue serotype 3, but none of the positive sera had a higher titer against dengue serotype 3 than against West Nile virus.[6] Between 1983 and 1985, HI antibodies against dengue virus were reported from the sera of outpatients and healthy volunteer controls in a study at a government hospital in Karachi, the largest city of Pakistan, located in the south.[8] In all except one of the patients, the titer for dengue viral antibodies was equal or lower than that of Japanese encephalitis viral antibodies. Both West Nile and Japanese encephalitis viruses have been isolated within Pakistan.[5,11] Since hemagglutination inhibition and neutralizing antibodies cross-react to a number of flaviviruses,[10] whether or not the serosurveys actually reflect dengue infection is uncertain. In 1968, a virus that appeared to be a member of the DEN complex (probably DEN-3) was isolated from a sick child during an epidemic of encephalitis in Lahore.[7]

In 1994, there were many reports of hemorrhagic fever attributed to dengue virus following unusually heavy rainfall.[9] During this outbreak, investigation of acute phase sera from 16 patients in one hospital showed that 15 patients had IgM antibodies to dengue virus by IgM capture, ELISA using dengue serotype 2 and monoclonal antibodies, and dengue type 1 viral RNA and dengue type 2 RNA were recovered using semi-nested polymerase chain reaction (PCR) in the acute sera of three hospitalized patients.[9] Many senior physicians described this outbreak as something they had never seen before (Ahmed AJ. Personal communication). The outbreak in 1994 and this investigation in 1995 clearly document the presence of dengue viral infections in Pakistan, most probably due to the presence of susceptible individuals (both Pakistanis and expatriates) and increased rainfall in Karachi in 1994 (Karachi Regional Meteorological Center. Personal communication) that resulted in an increase in mosquito populations. No information on the mosquito species involved at the time of these studies was available, though the most common vector of dengue virus, *Aedes aegypti* has been collected previously in various areas of Pakistan, including areas surrounding Karachi.[7,12]

The elevation of monovalent IgM ELISA titers to dengue 1 and dengue 2 suggests that viruses in this outbreak may represent two different types of circulating dengue virus. In the 1994 epidemic in Karachi, both dengue type 1 and type 2 were identified through PCR amplification of RNA, but only dengue type 1 virus was cultured.[9] It also is possible that the differences in IgM-type specific titers reflect the nonspecificity of the ELISA test in the setting of dengue outbreaks in 2 consecutive years.

Dengue is endemic in neighboring India and Sri Lanka, and due to the presence of more than one dengue serotype, epidemic dengue hemorrhagic fever is also increasingly reported.[3,13-15] In 1996, an epidemic of dengue hemorrhagic fever in India resulted in at least 227 deaths and more than 4700 persons being admitted in Delhi government hospitals.[16,17] Most previous episodes of dengue in India were marked by only isolated cases of hemorrhagic fever. In these and other parts of tropical and subtropical Asia where dengue is reported, changing lifestyles, urbanization, explosive population growth, destruction of city water supplies, migration, and increased air travel are some of the reasons cited for increases in the prevalence of dengue infections and areas occupied by *A. aegypti* in the post-World War II era.[18-20]

In Pakistan, with similar social and economical changes, little information is available regarding viral and vector surveillance. This study confirms the presence of epidemic dengue in Pakistan for a second consecutive year and suggests that there may be more than one serotype present. Visitors to Pakistan should be warned about the possibility of dengue infection and encouraged to use mosquito repellents. Clinicians in Pakistan should consider dengue in their differential diagnosis of patients presenting with fever or hemorrhage of unknown cause. This could lead to improved clinical and virologic surveillance for dengue. Ultimately, in areas where dengue is identified, efforts to decrease *A. aegypti* breeding sources, by community participation in capping, cleaning, or emptying water containers; identifying and eliminating hidden or inaccessible mosquito breeding sites; and addition

of larvicidal agents to stored water can decrease the morbidity and mortality associated with dengue.

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