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The Features of Imported Dengue Fever Cases from 1996 to 1999

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Dengue viruses are transmitted by infected mosquitoes, mainly by *Aedes aegypti* and *Aedes albopictus* (1). There are four serotypes, namely, dengue virus types 1, 2, 3, and 4 (2). The clinical manifestations of dengue virus infections range from asymptomatic infection to two forms of illness (3). Dengue fever (DF) is a self-limited febrile illness. Some patients with dengue virus infection develop a severe, life-threatening syndrome called dengue hemorrhagic fever (DHF). The World Health Organization categorizes DHF cases into four grades, from less severe (grade 1) to severe (grade 4). Grades 3 and 4, in which plasma leakage is so profound that shock occurs, are also referred to as dengue shock syndrome (DSS) (3).

Dengue virus infections are a serious cause of morbidity and mortality in many areas of the world: Southeast and South Asia, Central and South America, the Caribbean, and Africa. It is estimated that 100 million cases of dengue fever and 250,000 cases of DHF occur annually (4). Thus, DF/DHF is one of the most important infectious diseases in the world. The regions in which dengue virus infections are shown to be serious health problems have been expanding, and the number of DHF cases is increasing. The World Health Organization reports that domestic dengue virus infections occur in nearly 100 countries and regions.

A dengue outbreak occurred in Osaka, Kobe, Hiroshima, and Nagasaki from 1942 to 1945, and dengue virus type 1 was responsible for the outbreak (5). Dengue virus infections have never reached epidemic proportions in Japan since then, and there are currently no domestic dengue virus infections in contemporary Japan. However, imported dengue cases have been reported (6). We have been performing serodiagnosis of dengue virus infection upon request from hospitals and clinics. The features of imported dengue cases that we serodiagnosed from 1985 to 1995 at the Department of Virology 1, National Institute of Infectious Diseases, Japan (NIID) were previously reported (6).

Serum specimens were obtained from dengue-suspected cases in clinics and hospitals in Japan from 1996 to 1999, and were sent to the Department of Virology 1, NIID for the laboratory diagnosis of dengue. In the present manuscript, we report features of these dengue cases. Dengue virus infections were diagnosed by IgM-capture enzyme-linked immunosorbent assay (ELISA), reverse transcriptase-polymerase chain reaction (RT-PCR), and hemmagglutination inhibition (HI) test, as previously reported (7,8).

IgM-capture ELISA was performed as previously reported (7,8). Briefly, 96 well microplates were sensitized with goat anti-human IgM (μ chain specific) antibody (Zymed Laboratories, Inc., South San Francisco, Calif.). One hundred microliters of serum samples diluted at 1:101 in phosphate buffered saline (PBS) containing 10% calf serum (Wako Pure Chemical Industries, Osaka) was added to the wells and incubated for 1 h at room temperature. After washing with PBS, each of four monovalent and uninfected control antigens were added to wells and incubated for 1 h at room temperature. Plates were washed and peroxidase-conjugated human anti-flavivirus IgG was added and incubated at 1 h at room temperature. After washing, enzyme substrate, *o*-phenylenediamine 2HCl, and H_2O_2 in 0.1 M citrare buffer, pH 5.0, was added and incubated for 30 min at room temperature. Index value was calculated by the following formula: Index value = A_{492} absorbance with viral antigen/A_{492} absorbance with uninfected control antigen. We defined an Index value equal to or greater than 2.0 as positive.

RT-PCR was performed as previously reported (7,8). Briefly, RNA was isolated from 0.05 m*l* of serum specimen using 0.2 m*l* of Isogen-LS (Nippon gene, Tokyo) and 0.04 m*l* of chloroform. RT and PCR were done in a single tube. The tubes were set in an oil bath-type thermal programmer (Iwaki, Co., Tokyo) and subjected to programmed incubation at 53°C for 10 min for reverse transcription. This was followed by 30-40 PCR cycles of amplification. PCR products were then subjected to agarose gel electrophoresis. Amplified DNA fragments were visualized by ethidium bromide staining. Target size and the primer sequences used to amplify each serotype of dengue virus were previously reported (7,8). HI tests adapted for microtiter plates were performed using 4 hemagglutinin units of dengue-2 viral antigen, as previously described (6). The highest dilution of serum samples which provided positive HI was considered as HI titer.

Table 1 shows the number of dengue cases confirmed by laboratory tests from 1996 to 1999. The numbers of dengue cases were 15, 6, 42, and 11 in 1996, 1997, 1998, and 1999, respectively. All of the cases were DF cases; there were no DHF cases during this period of time. Of 74 total patients with DF, 53 were male and 21 were female (Table 2). The patients' age distribution is also shown in Table 2. The youngest patient was 16 years old and the oldest was 62 years old. Approximately half of the cases were between the ages of 21 and 30 (inclusive). Each of the three age groups, 11-20, 31-40, and 41-50 accounted for approximately 15 % of the total

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Table 1. Number of imported dengue cases confirmed at the National Institute of Infectious Diseases from 1996 to1999

Year	Total number of tested cases	Dengue fever (DF)	Dengue hemorrhagic fever (DHF)
1996	35	15 (M 14, F 1)*	0
1997	26	6 (M 3 , F 3)	0
1998	90	42 (M 28 , F14)	0
1999	53	11 (M 8, F 3)	0

*M and F indicate male and female, respectively.

Table 2. Age distribution of imported dengue cases confirmed at the National Institute of Infectious Diseases from 1996 to1999

Age	Number of DF cases*
11-20	11 (15%)
21-30	35 (49%)
31-40	11 (15%)
41-50	10 (14%)
51-60	3 (4%)
61-70	1 (1%)

*Ages of 3 cases were not known.

Table 3. Countries visited by dengue patients before they developed symptoms

Countries*	1996	1997	1998	1999	Total
Asia					
Thailand	2	1[d]	16[e]	2[k]	21
India	4[a]	0	5[f]	3	12
Philippines	1	1	8	1	11
Indonesia	3	1	5	0	9
Singapore	1	1	2[g]	0	4
Myanmar	0	0	2	2	4
Malaysia	2[b]	0	0	1	3
Bangladesh	1[c]	0	1[h]	0	2
Maldives	0	0	1[i]	1	2
Nepal	0	1	0	0	1
Cambodia	0	0	0	1	1
Oceania					
Tahiti	0	1	0	0	1
Central America					
Guatemala	0	0	1	0	1
Africa					
Nigeria	1	0	0	0	1
Cote d'Ivoire	0	0	1[j]	0	1
Total	15	6	42	11	74

*When patients visited more than one country, the countries which patients visited last were counted.
[a] One patient also visited Thailand.
[b] One patient also visited Thailand.
[c] One patient also visited India.
[d] One patient also visited Laos and Vietnam.
[e] Two patients also visited Cambodia. One patient also visited China, Vietnam and Laos. One patient also visited Laos and Myanmar.
[f] Two patients also visited Thailand.
[g] One patient also visited China,Vietnam, Cambodia, Thailand and Malaysia.
[h] One patient also visited Nepal and India.
[i] One patient also visited Srilanka.
[j] One patient also visited Liberia.
[k] Two patients also visited Myanmar.

number of cases.

Table 3 shows the countries visited by these dengue patients before they developed illness. Most of the Japanese dengue patients were infected with dengue viruses in Southeast and South Asia, especially in Thailand, India, the Philippines, Indonesia, Singapore, and Myanmar. There were patients who had visited Tahiti, Guatemala, Nigeria, and Cote d'Ivoire in recent years; these patients developed dengue illness after returning to Japan.

Although the age distribution of dengue patients ranges from 16 to 62 years old, approximately 50% of the cases were in the age group of 21-30. This probably reflects the number of travellers who visited dengue epidemic areas. Furthermore, it is important to note that there were patients who visited Oceania, Central America, and Africa before developing dengue illness. Thus, dengue is not to be considered as a differential diagnosis only in the case of patients who return from Asian countries.

We believe that these dengue cases account for only a small part of the total imported cases; the exact number of imported cases in Japan remains unknown. The new infectious disease control law is effective as of April 1, 1999 in Japan. DF/DHF is one of the diseases which physicians are requested to report by this law. Thus, it is expected in the near future that the exact annual number of dengue cases will be known in Japan. Nearly 5 million Japanese annually visit these countries in tropical and subtropical areas; nearly 2 million people visit Japan from these areas. Therefore, DF/DHF is a significant infectious disease worthy of more attention from the medical community in Japan. DF/DHF should be included in the differential diagnosis when febrile patients have just returned from tropical and subtropical regions, including those in Asia, Oceania, Central and South America, and Africa. Such a diagnosis should be considered irrespective of sex or age.

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