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An Imported Dengue Fever Case by Dengue Virus 3 (DENV-3) Infection in Gunma, Japan

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Dengue virus (DENV) of the genus Flavivirus, family Flaviviridae is an arthropod-borne virus that causes dengue fever (DF) and dengue hemorrhage fever (DHF). DENV is classified into four serotypes designated as DENV-1 to DENV-4, and is endemic in more than 100 countries in tropical and subtropical areas, South-East Asia, and the Western Pacific areas being most seriously affected (1). According to the World Health Organization, the global prevalence of DF has dramatically increased in recent decades (http://www.who.int/mediacentre/factsheets/fs117/en/). Thus, DF/DHF is a growing concern as one of the most important mosquito-borne human infectious diseases (2). In Japan, relatively large epidemics of DF occurred between 1942 and 1944 in Nagasaki, Kobe, and Osaka, originating from persons repatriating from the tropics during World War II; these epidemics were eliminated in 1946 (1). About 10 to 50 cases of DF/DHF have been reported annually in Japan in recent years, and all the cases were imported as a traveler’s disease (3-6). The number of reported DF/DHF cases has shown an increasing trend from the late 1990s (http://idsc.nih.go.jp/idwr/kansen/k04/k04_50/k04_50.html [in Japanese]).
In September 2007, we experienced a patient with DENV-3 infection who developed DF after returning to Gunma Prefecture, Japan, from Ho Chi Minh City, Vietnam. The case was a 26-year-old Japanese female who resided in Gunma Prefecture. She had no previous history of chronic or other diseases. She visited Ho Chi Minh City from August 22 to 24, 2007. She remembered clearly that she had been bitten by some mosquitoes on her legs on the night of August 22. She then visited Siem Reap, Cambodia, on August 24. On the morning of August 25, she developed a very high fever (40°C). She left Cambodia by plane and arrived in Japan in the early morning of August 26. She had no apparent memory of receiving mosquito bites while in Cambodia. After returning to Gunma Prefecture on August 27, she developed various symptoms such as continuous high fever (39 to 40°C), itching of the skin, profuse sweating, arthralgia, ague, diarrhea, vomiting, and subcutaneous hemorrhage. She consulted a physician in General Ota Hospital on August 27. On the first day of the hospital visit (hospital day 1), the patient’s clinical data were as follows: leukocyte count, 2.9 × 10³/μL (normal range, 3.5 to 9.0 × 10³/μL); platelet count, 1.3 × 10³/μL (normal range, 1.5 to 3.0 × 10³/μL); and C-reactive protein level, 1.5 mg/dL (normal range, <0.3 mg/dL). Ectopic enzymes (i.e., aspartate aminotransferase [AST], alanine aminotransferase [ALT], and lactate dehydrogenase [LDH]) and total bilirubin were within the normal range. She was treated with antibiotics (levofloxacin [300 mg/day, days 1 to 5], azithromycin [500 mg/day, days 3 to 5], cefepime [2,000 mg/day, days 3 to 6], and ciprofloxacin [600 mg/day, days 7 to 9]) and intravenous fluids for 9 days. On hospital days 3 and 4, 50 µg of granulocyte colony-stimulating factor (G-CSF) was intravenously administered to correct leukocytopenia. On hospital day 4, concentrated platelets (20 U/day) were administered to treat thrombocytopenia. High fever (<39°C) persisted through hospital day 5 and subsided on hospital day 7. On hospital days 1 to 8, a non-steroidal anti-inflammatory drug (acetaminophen, 400 mg/day, days 1 to 7), γ-globulin (5 g/day, days 2 to 4), gabexate mesilate (FOY, 2,000 mg/day, days 3 to 5), and ulinastatin (5 g/day, days 2 to 4), gabexate mesilate (FOY, 2,000 mg/day, days 3 to 6), and ciprofloxacin [600 mg/day, days 7 to 9]) and intravenous fluids for 9 days. On hospital days 3 and 4, 50 µg of granulocyte colony-stimulating factor (G-CSF) was intravenously administered to correct leukocytopenia. On hospital day 4, concentrated platelets (20 U/day) were administered to treat thrombocytopenia. High fever (<39°C) persisted through hospital day 5 and subsided on hospital day 7. On hospital days 1 to 8, a non-steroidal anti-inflammatory drug (acetaminophen, 400 mg/day, days 1 to 7), γ-globulin (5 g/day, days 2 to 4), gabexate mesilate (FOY, 2,000 mg/day, days 3 to 5), and ulinastatin (300,000 U/day, days 3 to 5) were intravenously administered to reduce inflammation and disseminated intravascular coagulation (DIC). On day 8, ectopic enzyme levels (AST, 267 IU/L; ALT, 211 IU/L; and LDH, 1,048 IU/L) were significantly elevated. The total bilirubin was within the normal range. Based on the symptoms and travel history, the examining physician suspected DENV infection.

A blood sample was collected on hospital day 3 and examined by reverse transcription-polymerase chain reaction (RT-PCR), using cross-reactive primers of DENV covering C-PrM (GenBank accession no. AB362209) and the DENV-3-specific primers covering E-NS1 (AB362210) (7). The DNA sequences of E-NS1 (320 nucleotides) regions were aligned. Homology analysis of the amplified E-NS1 product was performed by the ClustalW program. A phylogenetic tree was constructed by the “neighbor-joining” technique, specifically Kimura’s two-parameter method (neighbor-joining [N-J] method), using the Tree View (Win32) (ver. 1.6.6) software. The reliability of the tree was estimated using 1,000 bootstrap replications (8). Blood specimens were cultured for viruses or bacteria isolation using various cell lines (Vero, RD, HeP-2, and HEL cells) and agars, respectively; however, only the E-NS1 amplicon of DENV-3 was detected by RT-PCR from the blood samples.

The phylogenetic tree was constructed based on the E-NS1 sequences of DENV-3 (Fig. 1). Approximately 5% genetic diversity was detected among 16 strains that were isolated in Vietnam (10 strains), Thailand (4 strains), East Timor (1 strain), and Taiwan (1 strain). The sequence [E-NS1 (AB362210)] determined in the present study was located in a cluster with those that were isolated in Vietnam, and this sequence was most closely related to that of DENV detected in Aedes albopictus in Vietnam in 1996 (AF400029).

DF is an important infectious disease even in the countries in the temperate zone including Japan, as well as in tropical and subtropical countries. There is no vaccine available for human use. In addition, as shown in the present case, relatively severe clinical manifestations such as continuous high fever, hepatic disorder, leukocytopenia, and thrombocytopenia can develop (2). There is no evidence of domestic DENV transmission in Japan. However, Ae. albopictus, one of the main vectors, is widely distributed except for Hokkaido in Japan (9). Thus, it is possible that an outbreak of DF/DHF may occur in Japan, once the virus enters the country (1). Moreover, a large number of overseas travelers are at risk for DENV infection. The present case reconfirms the importance of laboratory diagnosis of DF in Japan.

REFERENCES