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Viral Titeres in the Sera of Dengue Patients among Travelers at the Quarantine Station of Kansai International Airport

Yukimasa Ooi1,2*, Akihiro Hayashi1, Hiroaki Aoki1,2, Junji Eda1, Masaru Hamada1, Shunro Imura1,2, Takashi Nakano1, Kouichi Sano1 and Etsuro Kashiwagi1

1Kansai Airport Quarantine Station, Ministry of Health, Labour and Welfare, Osaka 549-0011, and 2Department of Microbiology and Infection Control, Osaka Medical College, Osaka 569-8686, Japan

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Dengue is an acute viral febrile exanthema transmitted by mosquitoes, Aedes aegypti, among and between humans and monkeys. In Japan, outbreaks of dengue attributed to the mosquito, Aedes albopictus occurred in the early 1940s by travel of infected humans (1-4). Dengue is categorized into dengue fever, which has mild symptoms, and dengue hemorrhagic fever combined with dengue shock syndrome (DHF-DSS), which has severe symptoms.

DHF-DSS tends to occur from infection in infants born to dengue-immune mothers and secondary infections in children. On the basis of this evidence, immunized status is not preferable even though the primary infections usually result in the mild disease. Since plural outbreaks from importation, even though they are transient outbreaks, increase the number of immunized individuals and opportunities for secondary infection, the importation of dengue should be surveyed and be controlled.

Concerning importation of the virus, it is important to clarify the level of viremia at the entrance of Japan. At the Kansai Airport Quarantine Station, we examined 158 blood samples of suspected carriers of dengue, namely, travelers who had a fever, and found 8 cases positive for dengue virus between 2005 and 2007.

The laboratory diagnosis consisted of reverse transcriptase polymerase chain reaction (RT-PCR), IgM-capture enzyme-linked immunosorbent assay (ELISA), rapid immunochromatographic test and virus culture for titration. All examinations were performed at Kansai Airport Quarantine Station, and repeated at the Department of Virology 1, National Institute of Infectious Diseases, Tokyo, for confirmation. In brief, for RT-PCR, RNA was extracted from the sera using QIAamp Viral RNA Mini kit (QIAGEN, Hilden, Germany) and 5 μl of RNA solution was applied to TaqMan RT-PCR as previously described (5). Virus typing was performed by the RT-PCR assay.

For IgM detection, an IgM-ELISA kit (FOCUS technologies, Cypress, Calif., USA) and/or rapid immunochromatographic test kit (Panbio Tech Co, Brisbane, Australia) was used according to the manufacturer’s instructions.

For virus titration, a plaque assay was performed. In brief, the patients’ serum samples were inoculated to monolayer Vero cells cultured for 2 days in 6-well plates. After adsorption at 37°C for 90 min, 1% methylene blue-supplemented MEM was layered on the monolayer, and the cells were cultured at 37°C in 5% CO2 for 7 days. The cells were then fixed with ice-cold methanol, washed with tap water, and stained with methylene blue. The number of plaques was counted and the titers of dengue virus were calculated as plaque-forming units (PFUs).

In this article, “onset” is defined as the appearance of fever. The results of the examinations are summarized in Table 1. Seven out of the 8 patients were infected with dengue virus type 3 and one patient was infected with dengue virus type 1. Seven of the patients had high fever. The period after the onset was 2.6 days on average, and the titer of dengue virus was 1.3 × 107 PFU/ml on average.

Yamada et al. (6) reported that the titers of dengue virus of 39 patients collected in various phases of disease progress were between 1.0 × 107 and 2.9 × 107 PFU/ml. They had 4 cases of very early-stage disease, and found that the levels of viremia varied. Six out of our 8 cases were examined within 2 days after onset, and had very high titers of the virus. These findings indicate that the virus may be imported through travelers with high level of viremia at the very early-stage disease, suggesting that a survey of imported dengue virus is important to prevent the spread of the disease in Japan.

Six of 8 patients were determined to be negative for anti-dengue IgM antibodies. Five cases negative for IgM antibodies were examined at the onset or first day of the disease. We detected a high level of IgM in Case no. 6, which revealed low-level viremia. The patient’s serum IgM antibody against dengue virus may neutralize the infectivity of the virus. Yamada al. (6), however, showed 2 cases positive for IgM antibodies and infective viruses, and other cases positive for IgM antibodies and dengue-specific gene. Those previous cases and ours suggest that the specific IgM antibody against dengue virus does not always neutralize the virus.

A. albopictus is a common mosquito in Japan. If dengue patients who have high levels of viremia, such as our cases, are bitten by A. albopictus, the mosquitoes may mediate the spread of dengue virus in Japan. Via the infected mosquitoes, susceptible individuals may be infected with dengue. Thus, it is possible that outbreaks like the recent outbreak of Chikungunya fever in Italy in September, 2007 (7) could occur in Japan. Further understanding of diseases mediated by mosquitoes including dengue, not only in relation to dengue-carrying foreign visitors but also to citizens visiting dengue-endemic areas, will be needed in the future. And, it is considered that further strengthening of the Ministry of Health,
Labour and Welfare’s quarantine system, especially for infectious diseases with high fevers, is required for all quarantine stations in Japan.

In conclusion, we discovered high levels of viremia in patients at very early stages of dengue virus infection, suggesting that dengue virus is routinely imported to Japan.

We thank staff members of the Department of Virology 1, National Institute of Infectious Diseases for their laboratory confirmation.

REFERENCES


<table>
<thead>
<tr>
<th>Case no.</th>
<th>Year</th>
<th>Fever (°C)</th>
<th>Treatment</th>
<th>Days after onset</th>
<th>Virus type</th>
<th>Virus load (PFU/ml)</th>
<th>IgM antibody (Reciprocal of dilution1)</th>
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</thead>
<tbody>
<tr>
<td>1</td>
<td>2005</td>
<td>39.1</td>
<td>–</td>
<td>1</td>
<td>3</td>
<td>4.5 × 10⁶</td>
<td>ND²</td>
</tr>
<tr>
<td>2</td>
<td>2005</td>
<td>38.1</td>
<td>febrifuge</td>
<td>8</td>
<td>3</td>
<td>4.0 × 10⁵</td>
<td>ND</td>
</tr>
<tr>
<td>3</td>
<td>2005</td>
<td>40.2</td>
<td>–</td>
<td>0</td>
<td>1</td>
<td>2.7 × 10⁵</td>
<td>ND</td>
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<tr>
<td>4</td>
<td>2006</td>
<td>39.2</td>
<td>febrifuge</td>
<td>1</td>
<td>3</td>
<td>1.9 × 10⁵</td>
<td>ND</td>
</tr>
<tr>
<td>5</td>
<td>2006</td>
<td>38.7</td>
<td>–</td>
<td>1</td>
<td>3</td>
<td>2.4 × 10⁵</td>
<td>ND</td>
</tr>
<tr>
<td>6</td>
<td>2007</td>
<td>35.9</td>
<td>febrifuge</td>
<td>8</td>
<td>3</td>
<td>3.0 × 10³</td>
<td>×6,400</td>
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<tr>
<td>7</td>
<td>2007</td>
<td>38.5</td>
<td>–</td>
<td>1</td>
<td>3</td>
<td>4.7 × 10⁵</td>
<td>ND</td>
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<tr>
<td>8</td>
<td>2007</td>
<td>37.6</td>
<td>febrifuge</td>
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<td>3</td>
<td>2.3 × 10⁵</td>
<td>×100</td>
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<td>Average</td>
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<td>50%</td>
<td>2.6</td>
<td></td>
<td>1.3 × 10⁷</td>
<td></td>
</tr>
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</table>

1): For titration of IgM antibody, serum samples were diluted 100-fold first, serially 2-fold diluted and titrated.

2): ND: not detected as less than detection limit.