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Enteroviruses in Patients Experiencing Multiple Episodes of Hand, Foot, and Mouth Disease in the Same Season in Kobe, Japan, 2011

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In 2011, the largest hand, foot, and mouth disease (HFMD) epidemic was experienced since the start of the National Epidemiological Surveillance of Infectious Diseases in Japan (July 1981). Although the most frequent virus detected was coxsackievirus A type 6 (CVA6), several enteroviruses (EVs) such as CVA16, CVA10, and CVA6 caused the HFMD epidemic in various parts of Japan (1). In Kobe, the epidemic (weekly cases per Kobe pediatric sentinel clinic exceeding 1.0) started on week 23 and ended on week 38 (a duration of 16 weeks). The main epidemic peaked at week 28 of 2011 (28.3 cases/sentinel) (Fig. 1).

Clinical specimens were collected from patients with HFMD and herpangina during routine infectious agent surveillance between June and November, 2011. All samples were transferred to the Kobe Institute of Health for laboratory diagnosis.

Specimens were analyzed using consensus-degenerate hybrid oligonucleotide primer (CODEHOP) VP1 RT-seminested PCR (2–4) to detect EVs. For EV typing, the partial VP1 sequences derived from the CODEHOP products (about 290 base pairs) were determined. The
resulting sequences were compared with the sequences in the GenBank database of the DNA Data Bank of Japan (DDBJ) using BLAST. Virus isolation was performed using RD-18S, HEp-2, FL, and Vero-E6 cells.

Viruses were detected in 59 samples from 53 patients (47 HFMD, 5 herpangina, and 1 exanthema patients) between June 2 (week 22) and November 21, 2011 (week 47). The number of patients infected with CVA6, CVA16, CVA10, CVB3, CVB4, and rhinovirus were 31, 13, 6, 1, 1, and 1, respectively (Table 1).

As CVA6 did not grow well in cultured cells, it was directly detected from the clinical specimens using CODEHOP. Though 2 CVA10 strains were isolated using RD-18S, and it was detected by CODEHOP in the other 5. Ten CVA16 strains were isolated using Vero-

\begin{table}
\centering
\caption{Viruses detected in the 53 cases}
\begin{tabular}{lccccc}
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Disease & Throat swab & Stool & Contents of blister & Saliva & No. of cases \\
\hline
CVA6 & HFMD & 21 & 3 & 7 & 1 & 29 \\
& Herpangina & 2 & & & & 2 \\
CVA16 & HFMD & 13 & 1 & & & 13 \\
CVA10 & HFMD & 2 & & & & 2 \\
& Herpangina & 3 & & & & 3 \\
& Exanthema & 1 & 1 & & & \\
CVB3 & HFMD & 1 & & & & 1 \\
CVB4 & HFMD & 1 & & & & 1 \\
Rhinovirus & & 1 & & & & 1 \\
Total & & 44 & 6 & 8 & 1 & 53 \\
\hline
\end{tabular}
\end{table}

CVA6, coxsackievirus A type 6; CVB3, coxsackievirus B type 3; HFMD, hand, foot, and mouse disease.

E6, RD-18S, or/and FL and the other 4 strains from throat swabs were detected using CODEHOP. CVA10 and CVA16 strains were identified using isolates, by neutralization tests with antisera provided by the National Institute of Infectious Diseases or by sequencing analysis. CVB3 and CVB4 strains were isolated using FL, HEp-2, and Vero-E6, and identified by neutralization tests with antisera (Denka Seiken, Tokyo, Japan). Rhinoviruses were detected by CODEHOP.

CVA6 accounted for 82% of viruses detected between weeks 22 and 30, but was not detected after week 31. CVA16 was detected from week 22 in the prevalent early stage, while CVA16 accounted for 13% of detected viruses between weeks 22 and 30 and was detected sporadically until week 47. CVA10 and CVA16 were detected in 6 and 7 samples, respectively, between weeks 33 and 47. This suggested that the spread of CVA10 and CVA16 occurred at the same time.

The median ages of patients infected with CVA6, CVA16, and CVA10 were 1.8, 2.3, and 3.5 years, respectively. Two patients infected with CAV6 were aged over 30 years, suggesting that the infection had spread to adults.

Febrile illness was observed in 84%, 46%, and 83% of patients infected with CVA6, CVA16, and CVA10, respectively. No aseptic meningitis case was found. CVA6-positive patients also showed the characteristic skin symptom of large blisters. Some CVA6-positive patients showed nail shedding, several weeks after infection. CVA16-positive patients showed the characteristic skin symptoms found in HFMD. Half of the CVA10-positive patients were diagnosed with herpangina.

Between June and November 2011, 7 patients were af-
fected by multiple occurrences of HFMD (Table 2). The first onset in these 7 cases was between weeks 23 and 28. The first specimen was analyzed in only 1 (Patient 6) of the 7 cases. CVA6 was detected in this patient. The first specimen from Patient 2 was not analyzed; however, CVA6 was detected in the younger brother of Patient 2 who developed HFMD at the same time. In the first HFMD episode in Patients 1, 3, 4, and 7, characteristic skin symptoms of large blisters measuring more than 1 cm in diameter and nail shedding several weeks later, strongly suggested infection with CVA6.

At the second HFMD episode in the 7 patients, CVA16 and CVA10 were detected in 6 and 1 patients, respectively. Furthermore, Patient 1 developed HFMD 3 times, suggesting that Patient 1 was infected with 3 different viruses (CVA6, CVA16, and CVA10) successively in the season.


Conflict of interest None to declare.

REFERENCES