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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

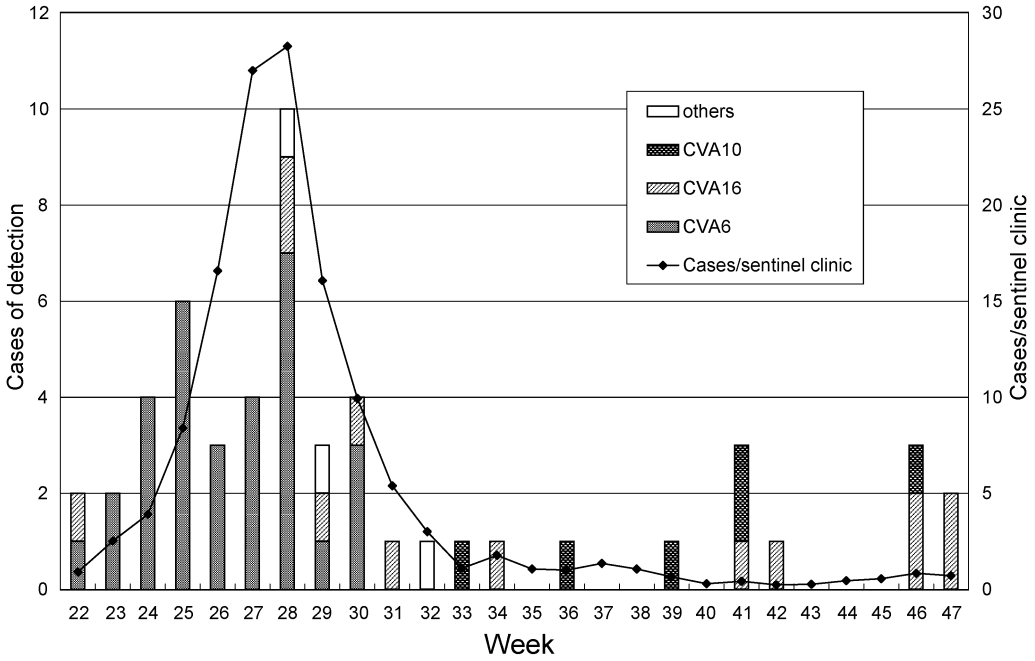

Fig. 1. Weekly cases of hand, foot, and mouth disease (HFMD) per pediatric sentinel clinic, and incidences of infection with viruses among HFMD, herpangina, and exanthema patients in Kobe during 2011.

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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-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.

Table 1. Viruses detected in the 53 cases

	Disease	Throat swab	Stool	Contents of blister	Saliva	No. of cases
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			1
CVB3	HFMD	1	1			1
CVB4	HFMD	1				1
Rhinovirus	HFMD		1			1
Total		44	6	8	1	53

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

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-

Table 2. Cases with multiple episodes of hand, foot, and mouth disease in Kobe, Japan, 2011

	Onset	Date of onset	Week	Virus detected	Temperature	Symptom	Remarks
Patient 1	1st	6/19	24	ND	39.2°C	Large blisters more than 1 cm, and skin of hand which peeled off	
	2nd	7/14	28	CVA16	No fever	Vesicular exanthema	
	3rd	11/12	45	CVA10	No fever	Many vesiculrar exanthema of the oral mucosa and peripheral extremities	
Patient 2	1st	6/9	23	CVA6[1)]	Unknown	Unknown	The younger brother developed HFMD on June 4
	2nd	7/16	28	CVA16	No fever	Conventional HFMD	The younger brother developed the second HFMD on July 16
Patient 3 (Brother of Patient 1)	1st	6/24	25	ND	No fever	Many large blisters on skin of buttocks	The younger brother developed HFMD on June 19
	2nd	7/20	29	CVA16	No fever	Conventional HFMD	The younger brother developed second HFMD on July 15
Patient 4	1st	7/11	28	ND	Fever	Large blisters more than 1 cm, and skin of hand which peeled off	
	2nd	7/31	30	CVA16	No fever	Conventional HFMD	
Patient 5	1st	6/28	26	ND	37.0°C	Severe symptoms of oral mucosa	
	2nd	8/22	34	CVA16	No fever	Many vesiculrar exanthema of palms and soles, and many aphthae of tongue	
Patient 6	1st	6/24	25	CVA6	Fever	Blisters which fused and peeled off became scabs	
	2nd	10/12	41	CVA10	39.0°C	A lot of small rash	
Patient 7	1st	7/14	28	ND	39.0°C	Small rash on palms and soles without oral mucosa, and nail shedding 1 month later	
	2nd	10/10	41	CVA16	39.0°C	Severe inflammation of oral mucosa	

[1)]: CVA6 was detected in a specimen of the younger brother obtained on June 6.
ND, not done. Other abbreviations are in Table 1.

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.

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Conflict of interest None to declare.

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