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**Enterovirus Detection Status from Patients with Herpangina and Hand, Foot and Mouth Disease in Kanagawa Prefecture, Japan**

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The number of weekly reported patients of herpangina in the Kanagawa Prefecture area (excluding Yokohama city and Kawasaki city) in 2006 exceeded 1.0 case per sentinel clinic at week 22 (May 29 - June 4), and this appearance began approximately 2 weeks earlier than the past 5 years. A major prevalence like the one in 2001 was predicted, but this year it peaked at 7.07 cases per sentinel at week 25 (June 19 - 25), fell below 1.0 case per sentinel at week 31 (July 31 - August 6), and at present, is moving towards termination (Fig. 1). Meanwhile, the number of weekly reported patients of hand, foot and mouth disease (HFMD) remained at about 1.0 case per sentinel at week 27 (July 3 - 9) with no major prevalence predicted (Fig. 2). However, there was a regional prevalence in the western area (at Odawara and Ashigara-kami Public Health Centers) at week 23 (June 5 - 11); in particular, it reached 8.0 cases per sentinel at week 30 (July 24 - 30) in the Odawara district, and the prevalence can still be seen there.

For patients with either herpangina or HFMD, throat swab specimens were brought in from 5 pediatric clinics in the Kanagawa Prefecture area (excluding Yokohama city, Kawasaki city, Yokosuka city, Sagami-hara city, and Fujisawa city), and viral isolation was conducted using 6 types of cell lines, RD-18S, HeLa, Vero, HEP-2, LLC-MK2, VeroE6, and suckling mice. The cell isolate was identified by the neutralization test using antisera distributed by the National Institute of Infectious Diseases (NIID) and commercial antiserum. The suckling mice isolate was identified by the CF test using NIID's antisera and in-house ascitic fluid. For the isolates that were difficult to identify, gene analyses of the enterovirus VP1 and VP4 regions were conducted using the RT-PCR method and analyzed by homology search using

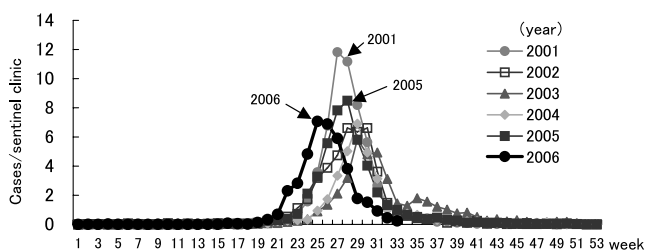


Fig. 1. Weekly cases of herpangina in Kanagawa Prefecture area (2001 - 2006).

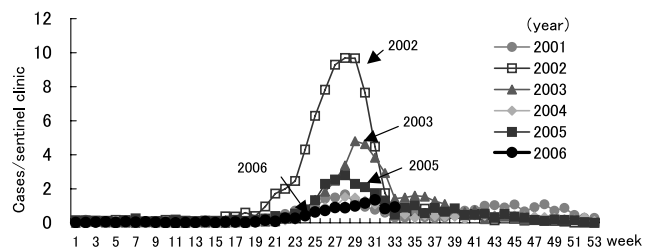


Fig. 2. Weekly cases of hand, foot and mouth disease in Kanagawa Prefecture area (2001-2006).

NCBI's BLAST analysis.

During the period from January to August, 2006, 37 throat swab from herpangina patients were brought in and 20 strains of coxsackievirus A4 (CA4), 1 strain of human herpes simplex virus type 1 (HSV-1), and 2 strains of adenovirus (Ad) type 3 were isolated (Table 1). From these results, the major virus for herpangina prevalence this summer was thought to be CA4.

Furthermore, from 39 throat swabs of HFMD patients, 6 strains of CA16, 3 strains of CA4, and 1 strain of HSV-1 have been isolated (Table 2). Of the 6 CA16 isolations, 4 were from the Odawara district; hence, the prevalent strain in the western area was presumed to be CA16. Enterovirus

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Table 1. Number of virus isolation from herpangina cases in Kanagawa Prefecture area (2001 - 2006)

Virus	2001	2002	2003	2004	2005	2006 8/31 present
CA2	6			3	1	
CA4	7	4 <sup>1)</sup>	4	5	1	20 <sup>2)</sup>
CA5	7				1	
CA6		3	1	3	15	
CA8		1				
CA10			8		3	
CA12			9	1	1	
CA16		2		1		
CB2		1				
CB5	4					
E13		1				
Ad1		1 <sup>1)</sup>				
Ad2				1		
Ad3				2	1	2 <sup>2)</sup>
HSV-1	2	1			3	1
Polio1+2	1					
Para Inf 3				1		
No. of isolates	27	13 (1) <sup>1)</sup>	22	17	26	22 (1) <sup>2)</sup>
Negative	1	15	10	4	9	4
Under examination	-	-	-	-	-	11
No. of specimens	28	28	32	21	35	37
Isolation rate (%)	96	46	69	81	74	59

<sup>1)</sup>: superinfection: CA4 · Ad1 1 case (2002).

<sup>2)</sup>: superinfection: CA4 · Ad3 1 case (2006).

CA, coxsackievirus A; CB, coxsackievirus B; E, echovirus; Ad, adenovirus; HSV, herpes simplex virus; Polio, poliovirus; Para Inf, Para influenza.

71 (EV71), which was prevalent in the Kanagawa Prefecture area in 2003 and 2005, was not seen in 2006. For the HFMD, although it is of a small scale, it can continually be seen in the whole of the prefecture and so caution is required.

Cultured cells and suckling mice are used simultaneously at our institute to isolate higher percentages of CA from all specimens taken from herpangina and HFMD patients.

Table 2. Number of virus isolation from hand, foot and mouth disease cases in Kanagawa Prefecture area (2001 - 2006)

Virus	2001	2002	2003	2004	2005	2006 8/31 present
EV71		3	5		13 <sup>1)</sup>	
CA2	1					
CA4			1			3
CA6		3			8 <sup>1)</sup>	
CA14					2	
CA16	14	31		8	17 <sup>1)</sup>	6
CB3					1 <sup>1)</sup>	
E13		1				
Ad5					1 <sup>1)</sup>	
HSV-1						1
Not identified				1		
No. of isolates	15	38	6	9	37 (5) <sup>1)</sup>	10
Negative	9	14	10	5	10	4
Under examination	-	-	-	-	-	25
No. of specimens	24	52	16	14	47	39
Isolation rate (%)	63	73	38	64	79	26

<sup>1)</sup>: superinfection: EV71·CA6 2 cases, EV71·CB3 1 case, CA16·CA6 1 case, CA6·Ad5 1 case (2005).

EV, enterovirus. Other abbreviations are in Table 1.

Of the 23 CA4 strains isolated this season, 23 were from suckling mice and 2 strains were from RD-18S cells. The detection rate of CA4 was higher using the suckling mice method than the cultured cell method. Further, we experienced superinfectious cases in which the cultured-cell method and suckling-mouse method detected EV71 and CA6, respectively, from specimens of HFMD patients for the previous season, and CA4 and Ad3, respectively, from specimens of herpangina patients this season.

In order to understand the current prevalence status, we will continue to survey these pathogens using these isolation and identification methods.

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