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Prevalence of Coxsackievirus A5, A6, and A10 in Patients with Herpangina in Aichi Prefecture, 2005

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Herpangina is caused primarily by coxsackievirus A (CV-A) 1, 2, 3, 4, 5 (CV-A5), 6 (CV-A6), 8, 10 (CV-A10), and 22 (1) in the genus *Enterovirus* of the family *Picornaviridae*. The illness is characterized by an acute onset of fever and sore throat (2). In Aichi Prefecture, located near the center of Japan's main island of Honshu, the number of case reports of herpangina increased from the 23rd week of 2005 and resulted in occurrence exceeding 12 cases per sentinel in the 27th week of 2005 (a diagram is available at http://www.pref.aichi.jp/eiseiken/herpan_graph.html). The number was twice as great as the average of the last 5 years. We detected CV-A5, -A6, and -A10 from these patients.

Feces and/or throat swabs were collected from 64 herpangina patients who visited 9 pediatric clinics in Aichi Prefecture between April and July 2005. Their ages ranged from 4 months to 7 years. Their samples were suspended in Veal infusion broth and centrifuged at 10,000 × g for 20 min. The supernatants were inoculated onto RD cells and used for RT-PCR, as previously described (3). Briefly, the supernatants were mixed with TRIzol and followed by isopropanol precipitation. The pellet was suspended in RNase-free water. RT-PCR was performed using Access Quick RT-PCR System (Promega, Madison, Wis., USA). Primers OL68-1(+) and MD91(-), designed by Olive et al. (4) and Rotbart et al. (5), were used for RT-PCR. Primers OL68-1(+) and EVP4(-), designed by Rotbart et al. (5), were used for the nested PCR. Following the PCR, amplified products were purified and introduced into a pGEM-T vector (Promega). The DNA

Table 1. Detection of coxsackievirus A from patients with herpangina between 2000 and 2005

Virus	Year/No. patients					
	2000	2001	2002	2003	2004	2005
	120	51	40	40	77	64
CV-A 2		11		2	4	
CV-A 4	11	5	25	1	34	
CV-A 5		3	2	1		1
CV-A 6	17		1	14		10
CV-A 8		12				
CV-A10	2	3	2	1	2	19
CV-A12				4	1	
CV-A16			1	2	1	

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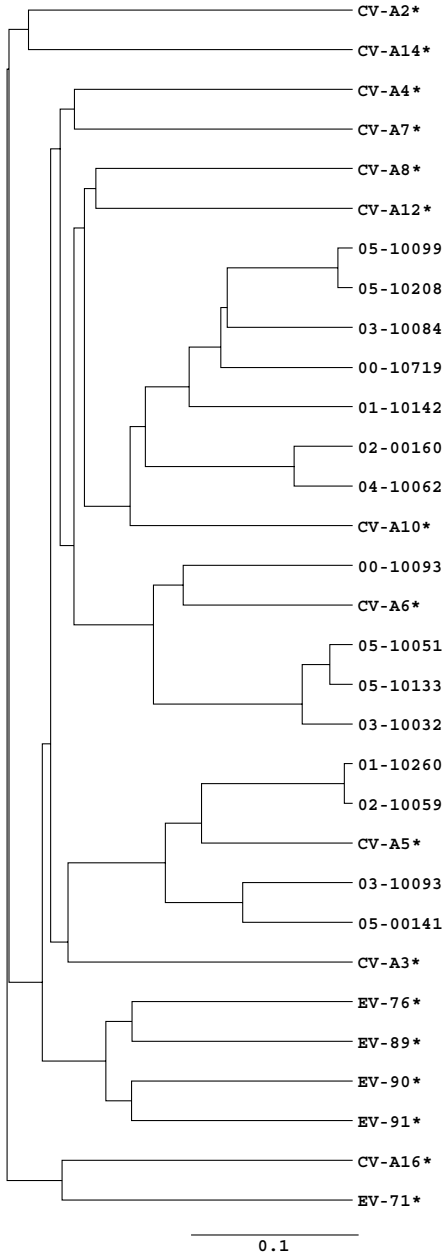

Fig. 1. Phylogenetic relationship among prototype strain of coxsackievirus type A and isolates from patients with herpangina based on nucleotide sequences of the VP4 region. The sequences of this year (05-10099, 05-10208, 05-10051, 05-10133, and 05-00141) clustered with CV-A10, CV-A6, or CV-A5, and were most closely related with the isolates in 2003 (03-10084, 03-10032, or 03-10093). *: prototype strain.

sequence was determined by a Model-4000 automated DNA sequencer (Li-cor, Lincoln, Nebr., USA). Serotype was determined from the sequence by comparison to a database of all enterovirus VP4 sequences using the Genetyxi program (Genetix, New Milton, Hampshire, UK).

No cytopathic effect (CPE) was observed in any of the inoculated RD cells, but the virus RNA could be detected in 30 of 64 (46.9%) patients using PCR. Of 30 positive samples, the sequences of 19 samples were most closely related to that of CV-A10, 10 to CV-A6, and one to CV-A5 (Table 1). The sequences in the same group of this year were at least 99% identical to one another. CV-A10 isolates were about 79% identical to the prototype strain and 87% to the isolated strain in 2003. CV-A6 isolates were about 80% identical to the prototype strain and 95% to the isolates in 2003. These pairwise comparisons were consistent with the results of phylogenetic reconstruction (Fig. 1). The sequences of CV-A10 isolates in these last 6 years cluster into 4 major phylogenetic groups. The sequences from this year were most closely related to the isolates from 2003. The sequences of CV-A6 from this year were most closely related to that from 2003 (Fig. 1). The patients with CV-A10 ranged in age from 4 months to 7 years, and those with CV-A6 were up to 3 years old.

In 1994, CV-A10 was the most prevalent serotype in patients with herpangina in Aichi Prefecture. Since then, the number of isolates has become fewer. It could be isolated every season between 2000 and 2004, but the number was not as high as another serotype that was the most prevalent in each year (Table 1). CV-A6 was the most prevalent serotype in 2000 and 2003. This year, CV-A10 and -A6 were more prevalent, and resulted in a larger number of patients.

Until 2003, we used suckling mice for isolation of viruses from patients with herpangina. However, this method from a practical standpoint is difficult, and we were able to confirm that our PCR was as sensitive as the use of suckling mice (6). Therefore, we have used PCR for identification of CV-A since 2004.

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