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Prevalence of Enterovirus in Osaka, Japan, between April and September 2008

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The prevalence of enterovirus (EV) infection in infants and young children is high between spring and autumn, and the incidence of the disease generally peaks in the summer months. EV can cause a spectrum of clinically distinct syndromes. Here, we report on the detection of EV in Osaka, Japan, between April and September 2008. To enable the rapid reporting of test results, we detected EV using reverse transcription-polymerase chain reaction (RT-PCR) in parallel with viral isolation. Given the presence of several common nucleotide sequences in the EV genus, PCR amplifies commonality with EV, such as viral protein (VP) 4, the whole area (1-3), or part of the VP1 (4-6). In the present study, we performed PCR that specifically amplifies the VP4 area.

Viral RNA was extracted from patient samples (throat swab,

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spinal fluid, fecal matter) using a commercially available kit (High Pure Viral RNA kit; Roche Diagnostics, Mannheim, Germany). For the first PCR experiment, we used the primers OL68-1 (2) (antisense 5′-GGT AAY TTC CAC CAC CAN CC-3′) and EVP2 (7) (sense 5′-CCT CCG GCC CCT GAA TGC GGC TAA T-3′). The reverse transcription reaction was run at 42°C for 30 min, after which amplification involved 40 cycles of denaturation at 94°C for 30 s, annealing at 55°C for 30 s, and elongation at 72°C for 1 min. The primers OL68-1 and EVP4 (7) (sense 5′-CTA CTT TGG GTG TCC GTG TT-3′) were used for the semi-nested PCR experiment and were amplified under the same conditions as the first round of PCR. After the PCR products were gel-isolated, the base sequence was determined by direct sequencing, and then BLAST was used to search for homogeneous sequences (see http://blast.ncbi.nlm.nih.gov/Blast.cgi).

Our sequencing detected 64 strains of EV in 52 (35%) of the 147 patients (Table 1). Twenty-eight strains were detected in samples obtained from throat swabs, 24 in fecal samples, and 12 in samples from spinal fluids. In total, 10 EV serotypes were detected, of which coxsackievirus B (CB) 5 and echovirus (Echo) 30 accounted for half. Several serotypes were detected in herpangina patients. Both coxsackievirus A (CA) 16 and EV71 were detected in hand, foot, and mouth disease (HFMD) patients. Two of the three HFMD patients with EV71 also had coincidental aseptic meningitis. Further, the prevalence of both CB5 and Echo30 was markedly elevated in these aseptic meningitis patients, a finding that reflects nationwide incidence. In the past 5 years, CB5 and Echo30 have comprised a large portion of the EV viruses detected in aseptic meningitis patients, with a combined incidence nearly equivalent to that of Echo6 and Echo18. The prevalence of Echo5 has also increased in Japan since 2006. In the present study, 4 of 9 children aged less than 1 year, all attending the same preschool, had a fever and displayed exanthematous symptoms in June 2008, and Echo5 was detected in 2 subjects.

Table 1. Virus prevalence listed by disease

	Herpangina	HFMD	Aseptic meningitis	Anthema symptoms
Echo5	1	0	3	2
Echo9	1	0	0	0
Echo18	0	2	0	0
Echo30	2	0	13	0
CA2	1	0	1	0
CA4	1	1	0	0
CA16	2	6	0	0
CB4	6	1	0	0
CB5	3	0	14	0
EV71	1	3	2	0
Total	18	13	33	2

HFMD, hand, foot, and mouth disease; Echo, echovirus; CA, coxsackievirus A; CB, coxsackievirus B; EV, enterovirus.

Given its high speed and greater sensitivity than the tissue culture method (8), RT-PCR may be useful for enteroviral surveillance.

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