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High Incidence of Human Echovirus Type 3 among Children in Osaka, Japan during the Summer of 2010

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The human echovirus, which comprises of 28 serotypes, causes a wide spectrum of clinical manifestations (1). Individual serotypes have often emerged at regular intervals (2). Human echovirus type 3 (E3) is a rare serotype of human echovirus (2), and few studies on this serotype have been reported. Only a small number of E3 strains have been isolated in Osaka City in the last 11 years. Three E3 strains were isolated from less than 4 patients in both 1999 and 2000. Since then, E3 strains have not been detected in this city.

In 2010, E3 was suddenly detected in a virus surveillance system conducted in Osaka (Fig. 1). Between July and November of that year, 635 specimens were submitted to be tested by this system. The E3 surveillance protocol comprised of virus isolation using RD-18S cell lines, serotyping using polyvalent antiserum (Denka Seiken Co., Tokyo, Japan), and molecular classification using VP4 sequences (3). E3 strains were isolated from 13 (2.0%) of the 635 specimens. Most of the E3 strains were isolated from nasal discharge samples (6 strains), followed by stool samples (5 strains), cerebrospinal fluid (1 strain), and sputum samples (1 strain). The E3 strains were isolated from 11 (1.1%) of 542 patients aged between 0 months and 38 years. All E3-positive patients, except 1, were children under 5 years of age. The E3-positive patients had respiratory inflammation (3 patients), status epilepticus (2 patients), hepatic failure (1 patient), aseptic meningitis (1 patient), gastroenteritis (1 patient), systemic symptoms such as fever (1 patient), and other symptoms (2 patients). The monthly peak for E3 isolation was in August, during which 7 of the 13 strains were isolated (Fig. 1). Thus, compared to the number of E3 isolates obtained in the last 11 years, the number obtained in 2010 was unusually high, and the number of E3 isolates obtained in 2010 was significantly higher than that detected in all previous years (Poisson distribution, P < 0.0001).

We determined the complete genome sequences of all isolates to investigate the evolutionary relationship of the E3 isolates obtained in Osaka in 2010. Viral RNA was extracted with a QIAamp Viral RNA Mini Kit (Qiagen K.K., Tokyo, Japan). We used the rapid amplification of 5'-cDNA ends system (Life Technologies Japan Ltd., Tokyo, Japan) or the methodology described elsewhere to amplify the 5'- and 3'-ends of the viral RNA. cDNA was synthesized using the reverse transcription reagents (Takara Bio, Shiga, Japan) and then amplified by performing polymerase chain reaction (PCR). PCR products were directly sequenced with an ABI Prism 3130 DNA Sequencer (Life Technologies Japan). A phylogenetic tree was constructed on the basis of the analysis of a 306-nucleotide region of VP1 (nucleotides 118–423 in the VP1 region of the prototype strain Morrissey, accession no. AY302553), as this region is present in most of the available sequences in GenBank.

The percent identities between the complete nucleotide sequence of the strains isolated from Osaka in 2010 (hereafter referred as 2010 Osaka strains) were 98.1%–99.8%. The nucleotide and amino acid sequences of capsid region of 2010 Osaka strains showed identity of 81.0%–81.3% and 96.0%–96.6%, respectively, with...
Fig. 2. Phylogenetic relationship among human echovirus type 3 (E3) isolates. A phylogenetic tree of E3 was generated by the neighbor-joining method using a partial VP1 region (306 nucleotides). The reference strains are presented as accession number_strain name. The E3 strains isolated in Osaka are presented in boldface. The bootstrap values for the major genotype clusters are displayed on the branches.

Those of the Morrisey strain. The difference in the complete genome sequences of the 2010 Osaka strains and that of the Morrisey strain was less than 20%. Thus, the complete genome sequence of the 2010 Osaka strains was similar to that of the Morrisey strain.

Phylogenetic comparison of a part of VP1 of the 2010 Osaka strain with that of other strains revealed that the 2010 Osaka strains were genetically close to the environmental strains isolated in the same year in Fukuoka City but not close to the strains isolated in Osaka in 1999 and 2000. This region of 2010 Osaka strains was 99.6%–97.7% identical to that of the strains isolated in Fukuoka in 2010 but only 78.1%–75.4% identical to that of the strains isolated in Osaka in 1999 and 2000. As shown in Fig. 2, according to the phylogenetic relationship among the E3 strains, these strains could be grouped into 2 major phylogenetic clusters. The strains isolated from Osaka in 1999 and 2000 and most of the E3 strains isolated worldwide before 2005 were grouped in 1 cluster (Cluster I). This cluster has been reported in several studies (4,5). Therefore, the E3 strains grouped in Cluster I might have been the dominant lineages among E3 strains worldwide prior to 2005. In contrast, the second cluster (Cluster II) comprised of the strains isolated from Osaka and Fukuoka in 2010, Pakistan in 2009, Bangladesh in 1999-2002, and Atlanta in 2002-2005. Thus, the E3 strains grouped in Cluster II might have co-circulated with those grouped in Cluster I before 2005 and might have become the dominant lineages after 2009. Nevertheless, it is difficult to derive a conclusion regarding the epidemiology of Cluster II strains, as there are few reports of this cluster.

In this study, we report a high incidence of E3 infections in Osaka City, Japan, during the summer of 2010. Like other echoviruses, E3 leads to a broad spectrum of clinical manifestations in children. Since few studies on
E3 infection have been reported and few E3 sequences are available in GenBank, our results will provide new insights into the epidemiology of E3 infections in Japan.

The nucleotide sequence of E3 reported here has been deposited in the EMBL/GenBank/DDBJ databases and assigned accession numbers AB647316–AB647326.

Conflict of interest None to declare.

REFERENCES