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Endemic Transmission of Echovirus 30 in Toyama, Japan in 2010 Is Verified by Environmental Surveillance

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Although human enteroviruses are the most commonly identified causes of aseptic meningitis in infants and children and are known to circulate worldwide, infection with these viruses is mostly asymptomatic.

Echovirus 30 (E30) was a major causative agent of aseptic meningitis in Japan in 2007–2008 (1), although in 2010 relatively few E30 isolates were reported in Japan (1). In contrast, outbreaks of aseptic meningitis caused by E30 were reported in European countries such as Latvia and Serbia (2,3). In Toyama, E30 was isolated from 2 children aged 1 and 4 years with gastroenteritis and upper respiratory illness diagnosed in pediatric clinics in April and July 2010, respectively. E30 was isolated from fecal specimens and a throat swab, respectively, using RD-18S cells and identified by a neutralization test with rabbit antisera against enteroviruses (25U; Denka-Seiken, Tokyo, Japan and National Institute of Infectious Diseases of Japan). We reported previously that environmental surveillance is a sensitive method for detecting silently circulating viruses in the community as environmental water, such as raw sewage, could contain enteric viruses shed by infected individuals (4–7). During the same survey period from September 2007 to August 2010, 89 E30 isolates were obtained from raw sewage samples in Toyama.

To identify the origin and lineage of the E30 isolates obtained from the above-mentioned patients, nucleotide sequences in a partial VP1 region (746 bases) of the virus were determined and phylogenetically compared with those of randomly selected E30 isolates (21 of 89 strains) obtained from raw sewage (environmental isolates) in Toyama between 2007 and 2010, and with E30 strains available in GenBank (8–15). Viral RNA was extracted from 140 μL of the culture fluid of cells that appeared to be cytopathic using a QIAamp Viral RNA Mini Kit (Qiagen, Hilden, Germany). cDNA was synthesized for 15 min at 37°C using an RT reagent kit (Takara, Otsu, Japan) with a random hexamer, according to the manufacturer's procedures, then submitted to polymerase chain reaction (PCR). PCR was performed using the primers 187 (sense; 5′-ACI GCI GYI GAR ACI GGN CA-3′) and 011 (antisense; 5′-GCI CCI GAY TGI TGI CCR AA-3′), which amplify an 809-bp DNA fragment corresponding to nucleotides 2553–3361 of E30, Bastianni (GenBank accession no. AF311938), as described by Oberste et al. (16). The PCR products were used directly for sequence analysis using an ABI Prism BigDye Terminator v3.1 cycle sequencing kit and an ABI Prism 3100 DNA sequencer (Applied Biosystems, Foster City, Calif., USA). The genetic relationship between the isolates and reference strains was analyzed using the MEGA 3.1 software (17) for the partial VP1 regions (746 bases of nucleotides 2573–3318).

Figure 1 shows the phylogenetic relationships of the partial VP1 region for the E30 isolates. The clinical isolates obtained in Toyama in 2010 were found to be closely related to the environmental isolates obtained in the period January 2008 to August 2010. The identities in the nucleotide sequences of the clinical isolates compared with the environmental isolates in 2008 and 2009–2010 were 96.4–97.2% and 98.0–99.6%, respectively. The nucleotide sequences of these isolates were also similar to those of the E30 strains isolated in Kobe, Japan, Korea, and Malaysia (93–96%) in 2003–2004. On the other hand, the similarity with the nucleotide sequences for the strains isolated in the United Kingdom in 2007–2008, Korea in 2008, and Russia in 2008–2009 was low (approximately 80%). The E30 isolates obtained in Toyama in 2010 were therefore not related to those from outbreaks in Europe. The clinical isolates obtained in Toyama in 2010 were genetically similar to environmental isolates obtained in the period 2008–2010 and the strains isolated in Kobe in 2003, thus suggesting that they were derived from viruses that had been circulating among individuals in Japan for several years. As enteric viruses from raw sewage are thought to reflect mostly asymptomatic infection in communities (4–6), E30 may have been transmitted asymptomatically in Toyama since 2008.

The nucleotide sequences determined in this study were deposited in GenBank under accession no. AB600148 to AB600170.

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Conflict of interest None to declare.

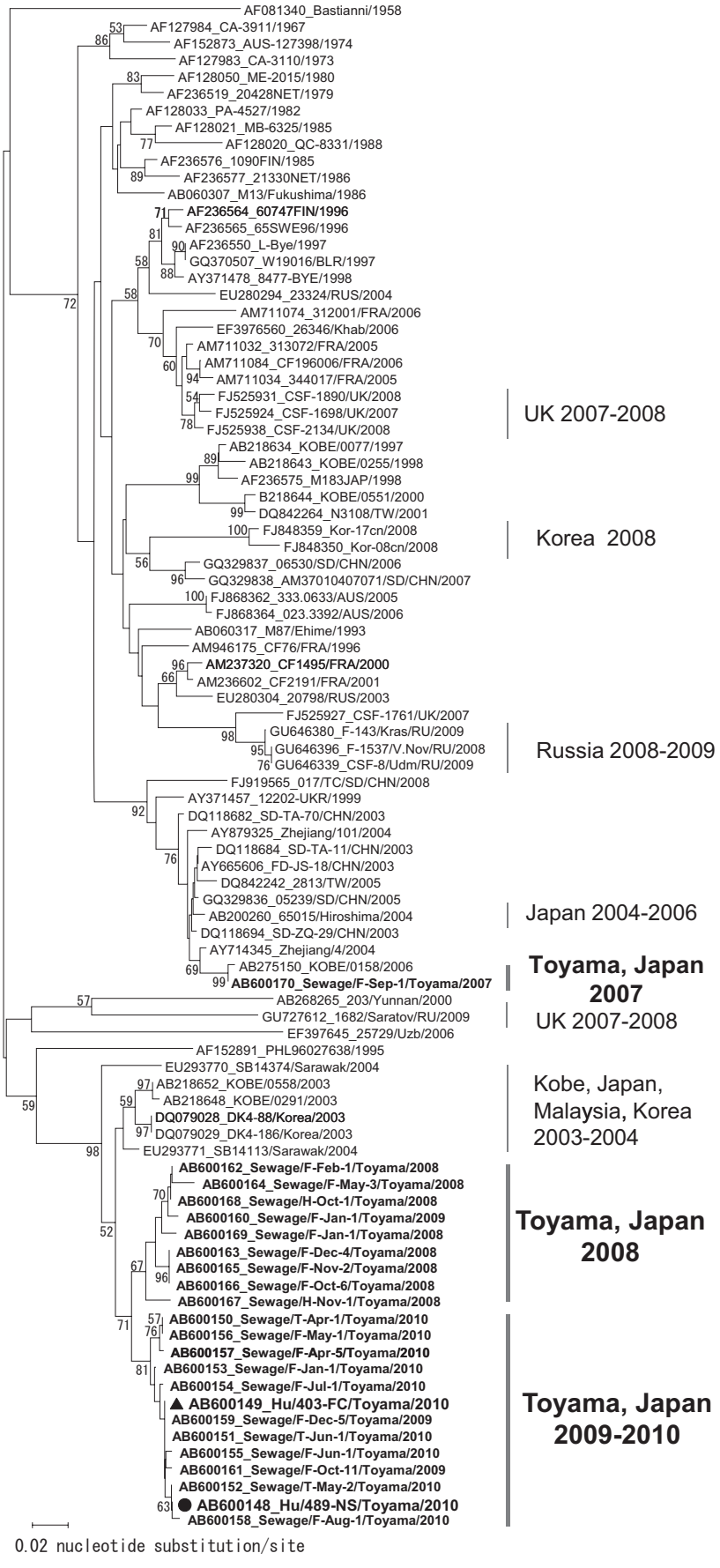

Fig. 1. Phylogenetic relationships among E30 isolates. Phylogenetic tree of E30 using the partial VP1 region (746 bases) was generated by the neighbor-joining method. Bold, triangle (▲), and circle (●) specify environmental and clinical isolates with gastroenteritis and upper respiratory illness, respectively. The strains are presented as accession number_strain name/year. Bootstrap values (in percentages) for 1,000 replicated trees are indicated.

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