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An Outbreak of Aseptic Meningitis in a Nursery School Caused by Echovirus Type 30 in Kobe, Japan

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Human enteroviruses infect a large number of people every year. The great majority of infections are asymptomatic or cause only mild respiratory illness (common cold). However, these viruses may cause various clinically distinct diseases including aseptic meningitis (1). In the summer of 2006, there was an outbreak of aseptic meningitis in a nursery school in Kobe City, Japan.

The school is situated in an urban district, where 21 staff care for 112 children (from 6 months old to 6 years old). On July 30, a 5-year-old boy (Patient A) demonstrated a high fever, headache and vomiting, and was hospitalized with a diagnosis of aseptic meningitis. Then, three children (5 to 6 years old) were hospitalized with similar symptoms on July 31 and August 1 (Patient B, C, D), followed by a 23-year-old nursery teacher on August 3 (Patient E). Three to 4 children were constantly absent from school with symptoms similar to those of a common cold between July 31 and August 9. From August 10 to 14, the school was partially closed for summer vacation, though a small number of children continued coming. There were no meningitis patients appearing for more than a week and the outbreak seemed to be terminated. However, on August 14, a 4-year-old boy was hospitalized with a diagnosis of aseptic meningitis (Patient F); he was the last patient in this outbreak. In all cases, the meningeal symptoms were self-limited and the patients recovered without any neurological abnormalities.

From the 6 patients described above, clinical specimens (2 cerebrospinal fluids, 4 stools and 5 throat swabs) were obtained (Table 1). RD-18S, HEp-2, FL and Vero-E6 cells were used for virus isolation. The virus was isolated from 7

Table 1. The result of the viral culture and neutralizing antibody titers

Patient	Viral culture			Neutralizing Ab titers	
	CSF	Throat swab	Stool	acute phase	recovery phase
A	Negative	ND	ND	80	ND
B	ND	Negative	KOBE/908/06	160	640
C	ND	KOBE/909/06	KOBE/910/06	20	640
D	ND	KOBE/911/06	KOBE/912/06	20	1,280
E	Negative	Negative	KOBE/913/06	20	160
F	ND	KOBE/0158/06	ND	ND	ND

Ab, antibody; CSF, cerebrospinal fluid; ND, not done.

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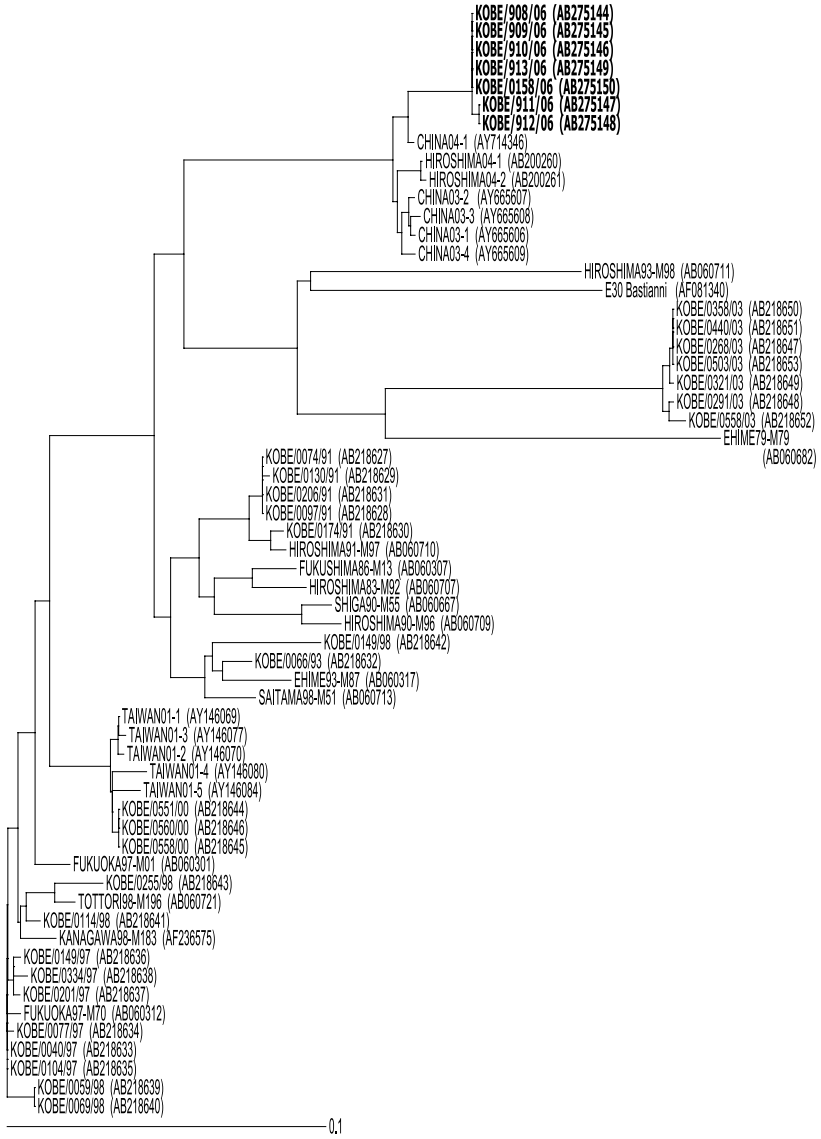

Fig. 1. Phylogenetic tree based on VP1 nucleotide sequences of E30. A total of 60 strains from Kobe, other Japanese regions, Taiwan and China were examined. Numbers in parentheses show DDBJ accession numbers of the nucleotide sequences.

specimens (4 stools and 3 throat swabs) of 5 patients (B, C, D, E, F) using RD-18S. All were identified as echovirus type 30 (E30) on neutralization tests with pooled antisera Echopool 95 (EP95) and an antiserum specifically against E30, provided by the National Institute of Infectious Diseases.

The neutralizing antibody titers against the E30 (KOBE/912/06) were examined in sera obtained during the acute and recovery phases of 4 patients (B, C, D, E) (Table 1). In all cases, the antibody titers were 4 to 64 times higher in the recovery phase, confirming that the infection was caused by E30. With Patient A, only acute-phase serum was provided, and the neutralizing titer was 80. In order to detect IgM, the serum was treated with 2-mercaptoethanol (2ME), and dialyzed against the buffer. As a control, the serum was treated with medium in the same manner. The antibody titer of the 2ME-treated serum was 10, while that of the control was 80. Consequently, the majority of the neutralizing antibody was IgM, which strongly suggested that active infection caused by E30 was occurring when the serum had been obtained.

Finally, genetic analysis of 7 isolated viral strains was performed as described previously (2). Briefly, from viral cDNA, an amplicon of approximately 750 bp encompassing the 3′ region of VP1 was produced using a primer pair, 187.188.189 (a mixed primer) and 011. Then, the products were sequenced with BigDye Terminator v1.1 Cycle Sequencing Kit, and were analyzed by the ABI Prism 310 automatic sequencer (Perkin Elmer, Foster City, Calif., USA). With 5 strains from 4 patients (B, C, E, F), 749 nucleotide bases were analyzed and all were the same. In 2 strains from Patient D, a nucleotide mutation was detected (A to G); however, it did not cause amino acid substitution. The fact that the virus isolated from Patient F had the same nucleotide sequences as the virus isolated from earlier patients suggested that the virus had been carried by children at the school, either asymptomatically or with common cold symptoms, for almost 10 days.

The resulting sequences were compared with the E30 VP1 sequences in the GenBank database of the National Center for Biotechnology Information (NCBI), and nucleotide identity scores were highest (96%) with 2004 isolates (CHINA04-1) from Zhejiang Province, which is situated on the coastal area of China. Then the phylogenic analysis of partial sequences on the VP1 region was constructed by the neighbor-joining method from ClustalW of the DNA Data Bank of Japan

(DDBJ) (Figure 1). The 2006 Kobe isolates were not part of the same cluster as previous Kobe isolates (3), but formed a cluster with the 2003 isolates from Jiangsu, China and the 2004 isolates from Zhejiang, China and those from Hiroshima, Japan. As Kobe is a large international harbor city, it is suggested that the virus recently arrived in Kobe from China.

Thus, it was clearly demonstrated that the outbreak of aseptic meningitis in the nursery school in Kobe City, Japan was caused by E30 derived from the 2004 isolates from China.

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REFERENCES

1. Khetsuriani, N., LaMonte-Fowlkes, A., Oberste, M.S., et al. (2005): Enterovirus surveillance – United States, 1970 - 2005. Morbid. Mortal. Wkly. Rep., 55 (SS08), 1-20.
2. Oberste, M.S., Maher, K., Flemister, M.R., et al. (2000): Comparison of classic and molecular approaches for the identification of untypeable enteroviruses. J. Clin. Microbiol., 38, 1170-1174.
3. Akiyoshi, K. and Haruta, T. (2006): Genetical analysis of echovirus type 30 isolated in Kobe City during 1991 - 2003. Clin. Virol., 34, 164-173 (in Japanese).