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Isolation of Echovirus Type 13 from Patients of Aseptic Meningitis

Masaaki Keino*, Masahiko Kanno, Kyoko Hirasawa, Tomoko Watari, Masahide Mikawa, Kimio Saito, Kazuo Kato, Masahiko Katayose1 and Hiromu Yoshida2

Department of Microbiology, Fukushima Prefectural Institute of Public Health, Mitouchi 16-6, Hokida, Fukushima 960-8560,
1Department of Pediatrics, Public Soma General Hospital, Tsugobasako 142, Ninnuma, Soma 976-0011 and
2Department of Virology II, National Institute of Infectious Diseases, Gakuen 4-7-1, Musashimurayama, Tokyo 208-0011

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A district of Fukushima Prefecture experienced an outbreak of echovirus type 13 (E13) in September, October, and November 2001. The virus was isolated from 36 patients with clinical manifestations such as fever, headache, respiratory illness, vomiting, abdominal pain, and rash. The number of cases was 8 in September, 15 in October, and 13 in November. Such a focal E13 outbreak had not been reported before in Japan, though an isolated case was registered in 1980. The 36 cases were children of 6 months to 15 years of age (the precise ages of three cases were not recorded, however). The number of patients in each age group from 0 to 15 years was 2, 4, 1, 0, 5, 1, 4, 5, 3, 1, 2, 0, 0, 0, and 1, respectively. Children under 12 constituted the majority in accordance with the reports from the United States (U.S.) (1) and Germany (2) but in discordance with the situation in the United Kingdom (U.K.) (3) where two-thirds of the isolates were from patients over 15 years of age.

The male to female ratio among the patients was 7 to 5 in the present outbreak; a similar tendency was observed in the report from the U.S. (1). However, it has not yet been established that the males are more prone to E13 infection.

The patients consulted pediatricians at a sentinel hospital participating in the National Epidemiological Surveillance of Infectious Diseases in the district and were diagnosed as aseptic meningitis (24 cases), gastroenteritis (5 cases)*, tonsillitis (4 cases)*, pharyngitis (3 cases)*, pneumonia (1 case)*, bronchitis (1 case)*, tympanitis (1 case)*, fever convulsion (1 case)* and viral exanthema (1 case), where diagnosis with asterisks contained cases with more than one diagnostic name. In the cases of aseptic meningitis, a fever of 37.0-39.7°C persisted for 2-4 days and a headache for 4-6 days. All patients were admitted to the hospital but discharged without residual effects. Generally, the meningitis in the present outbreak was relatively mild.

A total of 65 E13 isolates were obtained from the 36 cases, 28 from throat swab specimens, 26 from rectal swab specimens, and 11 from cerebrospinal fluid specimens. Among 24 meningitis patients, E13 was positive in the cerebrospinal fluids of 11 cases, but not in those of other 13 patients. The throat and/or rectal swab specimens of the latter group were, however, positive for the virus. Similar isolation rates of E13 from meningitis patients among all the E13 positive cases had been observed previously (1,3).

For virus isolation, the specimens were inoculated onto RD-18s, HEP-2, Vero, and HME-II cells. Of these cells, only RD-18s cells developed a clear cytopathic effect, such as rounding of the cells at 2 to 5 days followed by cell lysis. The cytopathic effect in Vero cells was equivocal. The isolated virus could be propagated efficiently in RD-18s cells, and the virus titers attained 10⁶-10¹¹ TCID₅₀/ml after the three passages. The isolates were not neutralized by an anti-echovirus serum pool EP95 which contained antibodies against echovirus 3, 4, 5, 6, 7, 9, 11, 14, 16, 17, 18, 22 (human parechovirus 1), 24, 25, and 30. The isolates were neutralized by anti-coxsackie virus A6 and by anti-A9 sera. As a consequence, we used the modified RT-PCR with pan-EV primer pairs developed by Centers for Disease Control and Prevention (CDC) (4-6). By RT-PCR with two primer pair combinations, i.e., 187, 188, 249, 250, and 251, PCR products of the VP1 region of the enteroviruses were obtained. The sequence analysis of the PCR products showed about 80% cDNA sequence homology with the E13 prototype, Del Carmen strain, and 98 to 99% homology with the U.S. and the European E13 isolates obtained in the last two years. The neutralization test with an anti-E13 serum confirmed that the isolates were E13.

CDC reported that among 76 E13-positive cases reported from 13 states in the U.S. in March-June 2001, 50 cases were diagnosed as aseptic meningitis (1). The CDR Weekly (3) reported that among 67 E13-positive cases in the first 30 weeks of year 2000 in the U.K., 38 were diagnosed as viral meningitis. Germany, where E13 cases were rare, reported an increase of E13 meningitis (2). The source of infection of the present outbreak in Fukushima Prefecture is not known; a link to a visit to the above epidemic countries cannot be established. As of 11 January 2002, 26 additional cases including two cases suspected of aseptic meningitis appeared in the district. The clinical specimens collected during December 2001 are now under virological examination.

It should be mentioned that a voluntary fax from another hospital in the same district informed Fukushima Prefectural Institute of Public Health of a sporadic occurrence of meningitis already in the middle of August. The Institute took immediate public health measures by warning district physicians of the possibility of an increase in the number of meningitis patients through the weekly report on infectious diseases.
REFERENCES


