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Outbreak of aseptic meningitis caused by echovirus 30 in Kushiro, Japan in 2017

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ABSTRACT

Background: Echovirus 30 (E30) is one of the most common causative agents for aseptic meningitis.

Objectives: In the autumn of 2017, there was an outbreak caused by E30 in Kushiro, Hokkaido, Japan. The aim of this study was to characterize this outbreak.

Study design: Fifty-nine patients were admitted to the Department of Pediatrics, Kushiro Red Cross Hospital (KRCH) with clinical diagnosis of aseptic meningitis. Among those, 36 patients were finally diagnosed as E30-associated aseptic meningitis by the detection of viral RNA using reverse transcription-polymerase chain reaction (RT-PCR) and/or the evidence of more than four-fold rise in neutralizing antibody (NA) titers in the convalescent phase relative to those in the acute phase. We investigated these 36 confirmed cases.

Results: The median age was 6 years (range: 6 months–14 years). The positive signs and symptoms were as follows: fever (100%), headache (94%), vomiting (92%), jolt accentuation (77%), neck stiffness (74%), Kernig sign (29%), and abdominal pain (28%). The median cerebrospinal fluid (CSF) white cell count, neutrophil count, and lymphocyte count were 222/μL (range: 3–1434/μL), 144/μL (range: 1–1269/μL), and 85/μL (range: 2–354/μL), respectively. Although the detected viral genes demonstrated same cluster, they were different from E30 strains observed in Japan between 2010 and 2014.

Conclusion: We mainly showed clinical and virological features of the E30-associated aseptic meningitis outbreak that occurred in Kushiro. To prevent further spread of E30 infection, continuous surveillance of enterovirus (EV) circulation and standard precautions are considered essential.

1. Background

Echovirus 30 (E30) belongs to the genus Enterovirus (EV) and is one of the most common causative agents of aseptic meningitis [1–8]. Local outbreaks of aseptic meningitis caused by E30 have been reported in numerous countries [8–22].

2. Objectives

In the autumn of 2017, there was an outbreak of aseptic meningitis caused by E30 in Kushiro, Hokkaido, Japan. It began in early August and continued until early December.

We investigated clinical signs, symptoms, laboratory findings, and virological characteristics of the collected specimens for these patients who were hospitalized at the Department of Pediatrics, Kushiro Red Cross Hospital (KRCH) to characterize the outbreak and obtain a better understanding of this disease.

Abbreviations: E30, Echovirus 30; EV, enterovirus; KRCH, Kushiro Red Cross Hospital; RT-PCR, reverse transcription-polymerase chain reaction; NA, neutralizing antibody; CSF, cerebrospinal fluid; CODEHOP, consensus-degenerate hybrid oligonucleotide primer; VP, viral protein; NT, neutralization test; IgG, immunoglobulin G; CRP, C-reactive protein

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3. Study design

3.1. Case definitions

Clinical diagnosis of aseptic meningitis was made by the presence of the following: citation for signs of meningitis/encephalitis and symptoms with fever, headache, or vomiting. Confirmed cases were defined by the presence of E30 using reverse transcription-polymerase chain reaction (RT-PCR) and/or neutralizing antibody (NA) test.

3.2. Patients and data acquisition

A retrospective study was performed on confirmed cases at the Department of Pediatrics, KRCH. Medical records and the interview with the patients themselves or their parents were utilized. The following information was collected: age, gender, duration of fever, clinical signs and symptoms, laboratory values on admission, cerebrospinal fluid (CSF) parameters, and the presence of the cases with aseptic meningitis among their families or classmates.

3.3. RT-PCR

The consensus-degenerate hybrid oligonucleotide primer (CODEHOP) semi-nested RT-PCR method [23] for amplifying viral protein (VP) 1 region [24] and the nested RT-PCR method for amplifying VP4-VP2 region [25] were used to detect EV genes.

3.4. Phylogenetic analysis

Phylogenetic analysis was performed on the determined nucleotide sequence of VP1 region using the neighbor-joining method.

3.5. Virus isolation

CSF specimens were cultured for virus isolation using human rhabdomyosarcoma cell line, RD-18S, and human colorectal adenocarcinoma cell line, Caco-2. All the isolates positive for EV were subjected to a neutralization test (NT) with echovirus pool antisera “EP-95” (provided by the National Institute of Infectious Disease, Tokyo) to determine the type of the virus [26].

3.6. E30 serological determination

In accordance with the manufacturer’s instructions, E30 immunoglobulin G (IgG) antibodies were tested using NA test (SRL, Inc., Tokyo, Japan). The presence of acute infection of E30 was determined by the evidence of more than four-fold rise in NA titers in the convalescent phase relative to those in the acute phase.

3.7. Statistical analysis

Statistical analyses were performed with EZR (Saitama Medical Center, Jichi Medical University, Saitama, Japan), which is a graphical user interface for R 3.5.2 (The R Foundation for Statistical Computing, Vienna, Austria) [27]. More precisely, it is a modified version of R commander (version 2.5-1) designed to incorporate statistical functions frequently used in biostatistics. The Wilcoxon signed-rank test was used to compare two groups of dependent, non-parametric numerical data. The Kruskal Wallis H-test was used to compare three groups of independent, non-parametric numerical data. A two-sided p value of < 0.05 was considered statistically significant.

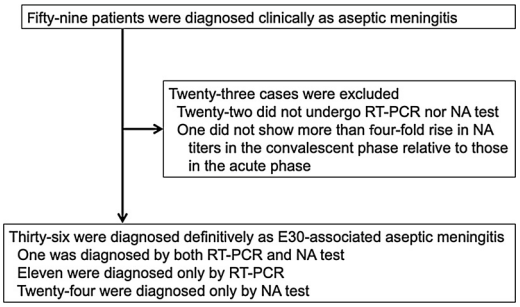

Fig. 1. Pediatric patients in the Kushiro Red Cross Hospital.

4. Results

4.1. Patients

Fifty-nine patients were admitted to the Department of Pediatrics of KRCH with clinical diagnosis of aseptic meningitis; out of these, thirty-six patients were finally diagnosed as confirmed cases (Fig. 1).

4.2. Clinical investigation

The clinical features and laboratory data are shown in Table 1.

The median age was 6 years (range: 6 months–14 years). Twenty-two patients (61%) were male, and 14 (39%) were female (male:female ratio 1.6:1). There were no pre-existing medical conditions, including immunodeficiency. Additionally, 29 patients (81%) had at least one case of aseptic meningitis in their families or classmates.

Median duration of fever was 3 days (range: 1–8 days). Median duration of hospitalization was 4 days (range: 2–7 days). The positive signs and symptoms were as follows: fever (100%), headache (94%),

Table 1
Clinical features and laboratory data of confirmed cases.

A. Clinical features	Number of patients (%)
Number of patients	36
Sex ratio (male : female)	1.6:1
Age	
< 1 yr	1/36 (2)
1—6 yr	20/36 (56)
> 6 yr	15/36 (42)
Medical history	
Immunosuppressed status	0/36 (0)
Contact with meningitis	29/36 (81)
Signs and symptoms	
Fever	36/36 (100)
Headache	34/36 (94)
Vomiting	33/36 (92)
Jolt accentuation	20/26 (77)
Neck stiffness	26/35 (74)
Kernig sign	9/31 (29)
Abdominal pain	10/36 (28)
Duration of fever (median and range in days)	3 (1–8)
Duration of hospitalization (median and range in days)	4 (2–7)

B. Laboratory data	Median (range)
CSF white cell count (/μL)	222 (3–1434)
CSF neutrophil count (/μL)	144 (1–1269)
CSF lymphocyte count (/μL)	85 (2–354)
CSF glucose (mg/dL)	70 (46–86)
CSF protein (mg/dL)	31.7 (11.9–77.7)
Peripheral white cell count (/μL)	10.7×10^3 (4.69×10^3–21.5×10^3)
CRP (mg/dL)	3.01 (0.17–11.7)
Procalcitonin (ng/mL)	0.15 (0.02–1.43)

Any signs or symptoms not described in the medical record were not counted.

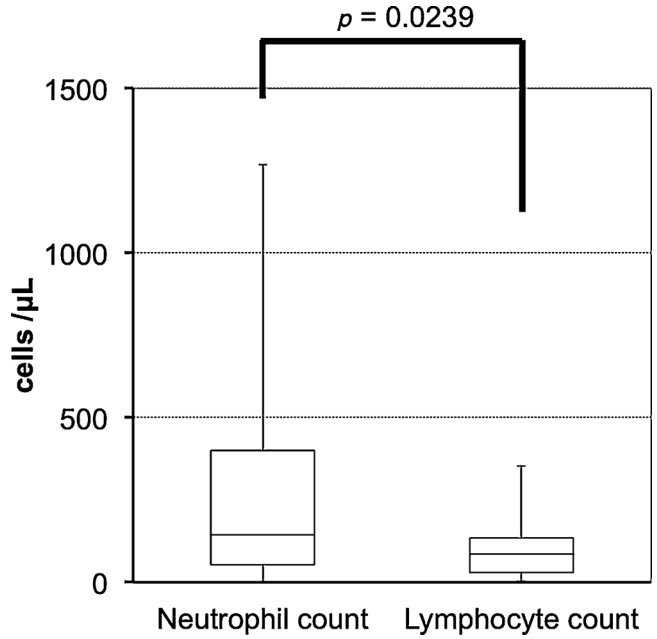

Fig. 2. CSF parameters. In box plots, horizontal lines represent the median, lower and upper edges represent interquartile ranges, and whiskers represent the range. *p* value for the Wilcoxon signed-rank test is shown.

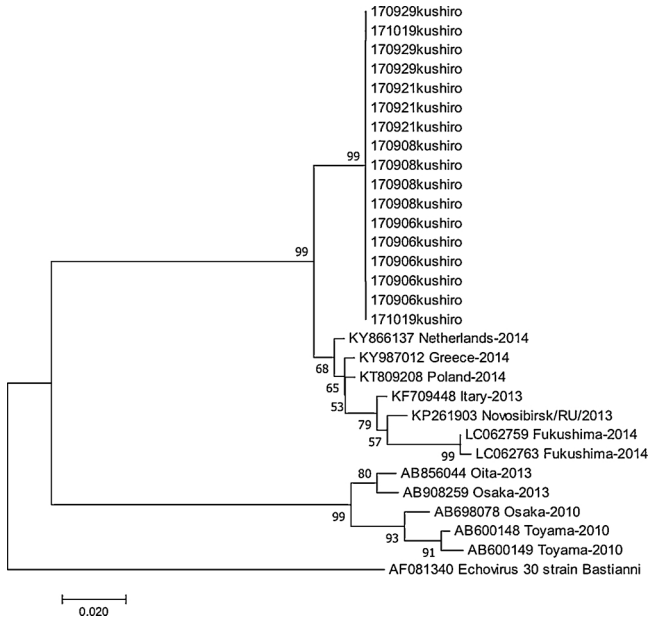

Fig. 3. Phylogenetic tree of the E30 sequences detected in this study. The neighbor-joining method with a maximum likelihood distance matrix was used. The sequences from cases in Kushiro are designed by sampling date (YYMMDD) and the word "kushiro". For example, 170929kushiro is the sequence obtained from a sample in Kushiro on September 29, 2017. Numbers at the nodes represent the percentage of 1000 bootstrap pseudoreplicates.

vomiting (92%), jolt accentuation (77%), neck stiffness (74%), Kernig sign (29%), and abdominal pain (28%). All the confirmed patients recovered and were discharged within 7 days.

CSF examination was performed in 21 out of the 36 patients. The median CSF white cell count, neutrophil count, and lymphocyte count were 222/μL (range: 3–1434/μL), 144/μL (range: 1–1269/μL), and 85/μL (range: 2–354/μL), respectively (Fig. 2). Neutrophil count was significantly higher than lymphocyte count ($p = 0.0239$). Fifteen out of 21 CSF samples (71%) had a neutrophil predominance. The median CSF glucose and protein levels were 70 mg/dL (range: 46–86 mg/dL) and 31.7 mg/dL (range: 11.9–77.7 mg/dL), respectively. We divided 21 cases who underwent lumbar puncture into three groups according to the period between the onset of the symptoms to lumbar puncture, which were < 24 h, 24–48 h, and > 48 h groups. The median of CSF white cell count was lower in < 24 h group, and the neutrophil percentage was higher in > 48 h group (Table 2). However, there was no statistical difference in CSF white cell count ($p = 0.426$) or neutrophil percentage ($p = 0.351$) in any group.

The median peripheral white cell count was 10.7×10^3/μL (range: 4.69×10^3–21.5×10^3/μL). All 36 peripheral samples had a neutrophil predominance (range: 48.3–92.6%). The median C-reactive protein (CRP) and procalcitonin levels were 3.01 mg/dL (range: 0.17–11.7 mg/dL) and 0.15 ng/mL (range: 0.02–1.43 ng/mL), respectively.

4.3. Virological investigation

Samples of CSF and serum from 12 patients were tested for VP1 and VP4-VP2 regions by RT-PCR. VP1 and VP4-VP2 regions were both detected in CSF samples of all 12 patients. In serum samples, both VP1 and VP4-VP2 regions were detected in 2 patients, only VP1 region was detected in 3 patients, and only VP4-VP2 region was detected in 2 patients. The 325 bp VP1 nucleotide sequence of the viruses in the samples was determined, whereby all the viruses were identified as E30. The nucleotide sequences derived from the samples of these 12 patients were completely identical.

Phylogenetic analysis showed they all belong to the same cluster, which was different from those observed in Japan between 2010 and 2014 (Fig. 3).

Furthermore, the virus was isolated from 7 of these 12 CSF samples, and all the isolates were identified as E30 by viral NT. The nucleotide sequence of VP1 region identified by RT-PCR with one of the cultured specimens was identical to those identified with the primary specimens.

5. Discussion

Clinical manifestations of the outbreak showed similar results when compared with those previously reported [10, 13–15, 17–20, 28]. Although there were reports of having neurological sequelae or fatal outcome in some cases of E30-associated aseptic meningitis [18], all of our pediatric patients recovered and were discharged.

A neutrophil predominance was shown in CSF samples in many patients in this outbreak. Although neutrophil predominance in CSF was atypical for aseptic meningitis, it has been shown previously for aseptic meningitis caused by E30 [17–19]. Although it has been

Table 2
Number of patients, CSF white cell count, and neutrophil percentage in three groups according to the period between the onset of the symptoms to lumbar puncture, which were < 24 h, 24—48 h, and > 48 h groups.

Time from onset to lumbar puncture (hour)	< 24	24—48	> 48	*p* value
Number of patients	3	9	9	
CSF white cell count (/μL)	12 (3–591)	230 (102–676)	222 (93–1434)	0.426
Neutrophil percentage (%)	69 (33–83)	68 (6–87)	85 (3–99)	0.351

Data presented as the median and range. *p* values for the Kruskal Wallis H-test are shown.

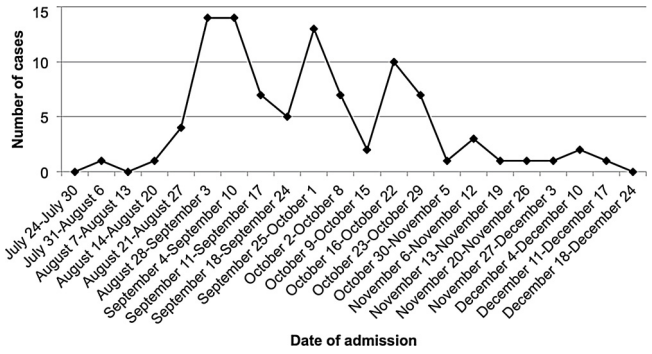

Fig. 4. A timeline of the outbreak reported by Kushiro Center of Public Health.

reported that the neutrophil percentage in CSF tends to decrease with the duration of the disease [20,29], we observed the opposite. CSF white cell count greatly differed among the cases in this study, which made it difficult to diagnose the causative agent as viruses according to CSF white cell count. E30 was detected in CSF by RT-PCR even though the white cell count was 3/μL in a four-year-old patient. The absence of CSF pleocytosis in E30-associated aseptic meningitis has been shown previously [14,16,18], and reported in EV meningitis in children, especially in neonates [30,31]. These studies indicated that CSF pleocytosis is not absolutely necessary for the diagnosis of aseptic meningitis. Two cases were diagnosed as E30-associated aseptic meningitis by RT-PCR or NA testing, although the CSF white cell count was more than 1000/μl. Such cases have also been reported [19,30]. RT-PCR was a very effective means to detect E30 and enabled early diagnosis in this study.

It was desirable to conduct RT-PCR in all the specimens in this study. However, as RT-PCR is an expensive method and not covered by health insurance in Japan, we did not use it for diagnosis once the causative agent was found to be E30 due to our limited funding. Instead, we performed neutralizing assay as an alternative.

All E30 strains identified were identical, indicating that all the cases shared an epidemiological link. Unexpectedly, they were more closely related to E30 strains prevalent in Europe than those circulating in Japan shown in Fig. 3.

During this outbreak, 95 patients were reported to the Kushiro Center of Public Health as having a diagnosis of aseptic meningitis. Eighteen were infants, 25 were primary school children, 5 were junior high school students or high school students, 5 were 19–29 years of age, 29 were 30–39 years of age, and 3 were 40 years of age or older, respectively [32]. The child to parent ratio was high, suggesting that infection spread within the family. A timeline of the outbreak reported by Kushiro Center of Public Health is shown in Fig. 4 [32]. This outbreak initially occurred at a childcare center, and then spread to an elementary school. At the school where aseptic meningitis was prevalent, students did not appear to have been instructed to wash hands correctly. Since EVs are transmitted via fecal-oral route [33], such insufficient hygiene practices may have contributed to the spread of E30 infection. Thus, it is important to inform the community that standard precautions such as frequent and adequate hand washing are recommended to prevent the spread of E30.

In conclusion, we demonstrated the clinical and virological features of E30-associated aseptic meningitis outbreak that occurred in Kushiro. Continuous surveillance of EV circulation and standard precautions are essential to prevent further spread of aseptic meningitis.

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Competing interests

None to declare.

Ethical approval

Ethical approval was obtained by the KRCH Ethics Committee.

CRediT authorship contribution statement

Yuji Maruo: Conceptualization, Methodology, Formal analysis, Investigation, Data curation, Writing - original draft. **Masanori Nakanishi:** Conceptualization, Investigation, Project administration, Writing - review & editing, Supervision. **Yasuto Suzuki:** Investigation. **Yosuke Kaneshi:** Investigation. **Yukayo Terashita:** Investigation. **Masashi Narugami:** Investigation. **Michi Takahashi:** Investigation. **Sho Kato:** . **Ryota Suzuki:** Investigation. **Akiko Goto:** Investigation. **Masahiro Miyoshi:** Investigation. **Hideki Nagano:** Investigation. **Takahisa Sugisawa:** Resources. **Motohiko Okano:** Writing - review & editing.

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Appendix A. Supplementary data

Supplementary material related to this article can be found, in the online version, at doi:https://doi.org/10.1016/j.jcv.2019.05.001.

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