

Coxsackievirus B5 Aseptic Meningitis in Infants in Chiba Prefecture, Japan, in 2016

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Background: Infantile aseptic meningitis is a rare infection of the central nervous system. Coxsackievirus B5 (CVB5) is an enterovirus that is sometimes associated with aseptic meningitis and encephalitis.

Case presentation: We report on three isolated infants with aseptic meningitis caused by CVB5 in the spring and summer of 2016 with nearly identical 404-bp CVB5 Viral Capsid Protein 1 (VP1) sequences. In addition, viral analysis of sewage samples from Chiba Prefecture in 2016 showed similar 404-bp CVB5 VP1 sequences from May to September 2016.

Conclusion: These results indicate that viral screening of sewage water may help detect occult outbreaks of viral infection, particularly for enterovirus strains. (*J Nippon Med Sch* 2018; 85: 187–190)

Key words: infant, meningitis, coxsackie B5, sewage sample

Introduction

Infantile aseptic meningitis is a rare infection of the central nervous system. Coxsackievirus B5 (CVB5) is an enterovirus that is sometimes associated with aseptic meningitis and encephalitis. Outbreaks due to CVB5 were reported in the USA in 1972¹. Here, we report on three isolated infants with aseptic meningitis caused by CVB5 in the spring and summer of 2016.

Case Presentation

Case 1

A 2-month-old boy was admitted to our hospital in May 2016 due to high-grade fever for one day without significant lethargy or abnormal neurological findings, except for minor anterior fontanelle bulging. Laboratory examination showed C-reactive protein (CRP) 0.17 units, white blood cells (WBC) 20,820 units (Neutrophils 42%, Lymphocytes 49%) and Hematocrit 31.5%, and cerebrospinal fluid (CSF) examination showed a cell number of 184/μL with 41% mononuclear cells. Bacterial cultures from the blood and CSF were negative. After 3 days of treatment with intravenous fluid infusion and administration of antibiotics, his temperature returned to normal

and he was discharged on day 8 after admission without neurological sequelae.

Case 2

A 2-month-old girl was admitted to our hospital in August 2016 due to high-grade fever for 12 hours with dysthithia but no abnormal neurological findings, except for minor anterior fontanelle bulging. Laboratory examination showed CRP <0.05 units, WBC 15,370 units (Neutrophils 38%, Lymphocytes 53%) and Hematocrit 31.5%, and CSF examination showed a cell number of 268/μL with 99% mononuclear cells. Bacterial cultures from blood and CSF were negative. After 5 days of treatment with intravenous fluid infusion and administration of antibiotics, and intravenous gamma globulin, her temperature returned to normal and she was discharged on day 9 after admission without neurological sequelae.

Case 3

A 1-month-old boy was admitted to our hospital in September 2016 due to high-grade fever for 12 hours, with no abnormal neurological findings, including anterior fontanelle bulging. Laboratory examination showed CRP <0.05 units, WBC 13,360 units and Hematocrit 27.7%, and CSF examination showed a cell number of

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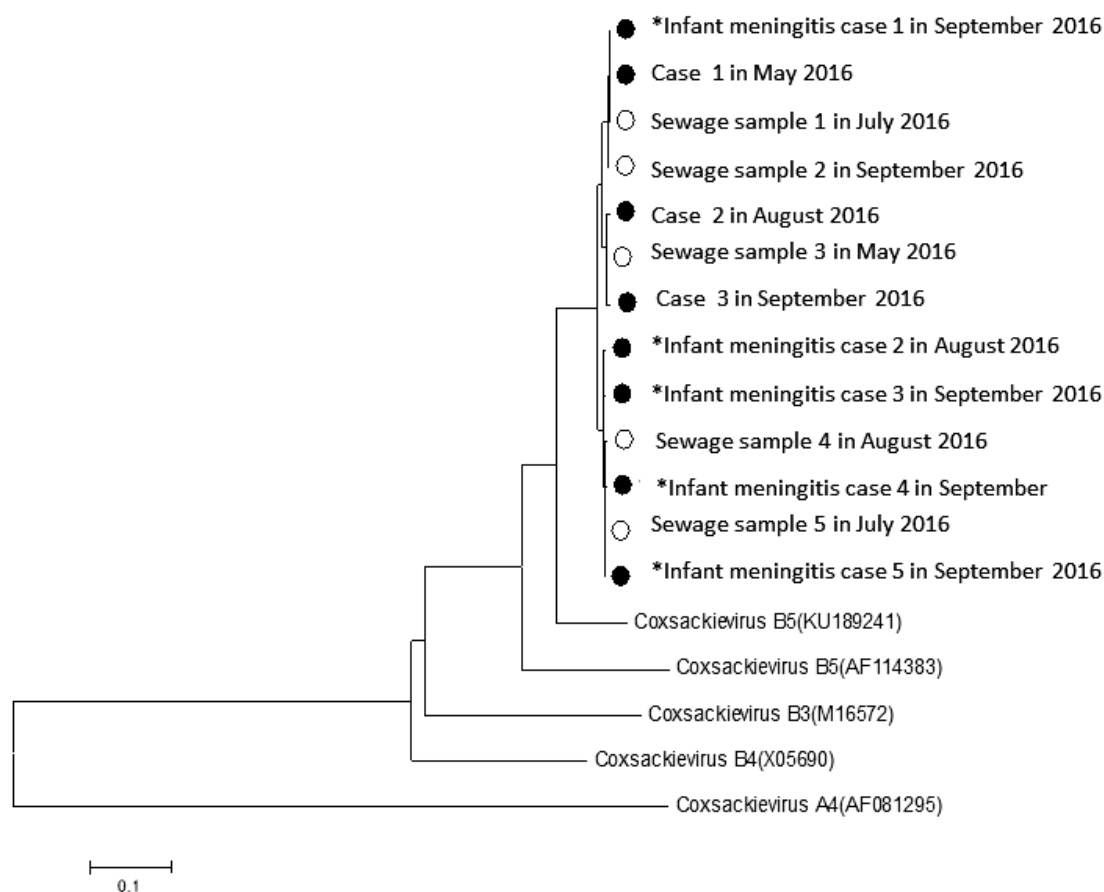


Fig. 1 Phylogenetic analysis of VP1 sequences (404 bp) in isolates from Chiba Prefecture. Viral sequence analysis in the present infant meningitis cases (cases 1, 2 and 3) and those from other regions of Chiba Prefecture (*infant meningitis case), and sewage samples, depicted as a phylogenetic tree of nucleotide sequences. Bar and 0.1: scale bar

469/ μ L with 22% mononuclear cells. Bacterial cultures from blood and CSF were negative. After 3 days of treatment with intravenous fluid infusion and administration of antibiotics, his temperature returned to normal and he was discharged on day 9 after admission without neurological sequelae.

These patients lived at least 2 km apart, and they had had no prior contact prior to admission.

Sample Collection, Viral Examination, and Sequence and Phylogenetic Analysis

All specimens were collected for diagnostic tests, and the remaining portion of the specimens was used for viral investigations. Following collection, samples were centrifuged at 1,500 rpm for 5 min in order to remove cells, divided into aliquots, and immediately frozen on dry-ice and stored at -80°C . The Institutional Review Board of Nippon Medical School Chiba Hokusoh Hospital approved the collection and investigation of samples and written informed consent was obtained from the guardi-

ans of all patients.

Viral RNA from CSF and serum were detected by a conventional RT-PCR assay^{2,3} using CODEHOP primers. Molecular methods based on the VP1 region have been established as the gold standard for enterovirus typing, and phylogenetic analysis of VP1 nucleotide sequences allows tracking of viral transmission⁴. PCR products were further analyzed using direct sequencing of the 404-bp VP1 region in the enterovirus. Phylogenetic trees were constructed by the Neighbor-joining method using Mega software version 6.0. We also performed a comparison with CVB5 data from sewage obtained from Chiba Prefecture as a part of the National Epidemiological Surveillance of Vaccine Preventable Diseases in Japan.

Viral Results

All three patients were positive for the CVB5 gene by conventional RT-PCR. Phylogenetic analysis showed that 404-bp of CVB5 VP1 sequences were quite similar in all 3 isolates (Fig. 1). In addition, a sewage sample collected in

May 2016 also showed a similar 404-bp CVB5 VP1 sequence as in Cases 2 and 3, while the sequence in a sewage sample collected in July 2016 showed an identical 404-bp CVB5 VP1 sequence as in Case 1.

Discussion

CVB5 is an enterovirus that causes aseptic meningitis, encephalitis and paralysis. Outbreaks due to CVB5 have been reported worldwide, including in the USA in 1972². Here, we reported on three infant cases (admitted to our hospital in May, August, and September) with aseptic meningitis caused by CVB5, with nearly identical 404-bp VB5 VP1 sequences. In addition, viral analysis of sewage samples from Chiba Prefecture in 2016 showed similar 404-bp CVB5 VP1 sequences from May to September 2016. A comparison of 404-bp CVB5 VP1 sequences in each sample, including our cases, cases in other regions of Chiba, and sewage water, showed over 94% homology¹.

We consider that the screening of enterovirus strains in sewage may not only predict epidemic strains, but may also help in predicting severe symptoms due to meningitis from such strains.

Conclusion

These results indicate that viral screening of sewage water may help detect occult outbreaks of viral infection, particularly for enterovirus strains.

List of Abbreviations

CVB5 Coxsackievirus B5
CSF cerebrospinal fluid
VP1 viral capsid protein 1

Declarations

Ethical Approval and Consent to Participate

This study was approved by the Ethics Committee of Nippon Medical School Chiba Hokusoh Hospital. Informed consent was obtained from the parents of all patients for publication of this case report.

Consent

The Institutional Review Board of Nippon Medical School, Chiba Hokusoh Hospital, approved the collection and investigation of samples and written informed consent was obtained from the guardians of all subjects.

Consent for Publication

All authors, participants (patients/legal guardians), and partners consent to the publication of the data presented in this manuscript.

Availability of Data and Materials

The data supporting the conclusions are included within the manuscript.

Competing Interests

The authors declare that they have no competing interests.

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Authors' Contributions

Study concept and design: TA
Collection of clinical data and patient care: KT, CM
Analysis and interpretation of data: TA, TO
Drafting the manuscript: KT, TA
Critical revision of the manuscript for important intellectual content: TA, TO
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Administrative, technical and material support: HN, CH
Study supervision: TA, TO
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Conflict of Interest: The authors declare no conflict of interest.

References

1. Marier R, Rodriguez Y, Chloupek RJ, Brandt CD, Kim HW, Baltimore RS, Parker CL, Artenstein MS: Coxsackievirus 85 Infection and Aseptic Meningitis in Neonates and Children. *Am J Dis Child* 1975; 129: 321-325.
2. <http://www.nih.go.jp/niid/images/lab-manual/AsepticMening.pdf>.
3. Nix WA, Oberste MS, Pallansch MA: Sensitive,

Seminested PCR Amplification of VP1 Sequences for Direct Identification of All Enterovirus Serotypes from Original Clinical Specimens. *J Clin Microbiol* 2006; 44: 2698–2704.

4. Oberste MS, Schnurr D, Maher K, al-Busaidy S, Pallansch M: Molecular identification of new picornaviruses and

characterization of a proposed enterovirus 73 serotype. *J Gen Virol* 2001; 82: 409–414.

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