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Epidemic myalgia associated with human parechovirus type 3 infection among adults occurs during an outbreak among children: Findings from Yamagata, Japan, in 2011

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ABSTRACT

Background: Based on our findings in Yamagata, Japan, in 2008, we reported that human parechovirus type 3 (HPeV3) could be associated with epidemic myalgia among adults, although HPeV3 is generally associated with infectious diseases in children.
Objectives: To clarify the relationship between community outbreaks among children and myalgia through the continued surveillance of HPeV3 infections.
Study design: In the summer season (June–August) of 2011, we collected 586 specimens from children with infectious diseases, and throat swabs, and stool and serum specimens from 5 patients with myalgia. We detected HPeV3 using virus isolation and reverse-transcription PCR, and carried out phylogenetic analysis. We also performed screening for HPeV3 using 309 stocked frozen specimens collected in 2008 for a comparison between 2008 and 2011 strains.
Results: We detected HPeV3 in 59 children and isolated HPeV3 from all myalgia patients. Phylogenetic analysis indicated that the HPeV3 strains circulating in 2008 and 2011 could be clearly distinguished, apart from two strains. Further, we detected HPeV3 strains with identical nucleotide sequences from children and adults in 2008 and 2011, respectively. Two children belonging to one myalgia patient had upper respiratory infections prior to the onset of their father's illness, and the HPeV3 isolates from these three patients had identical nucleotide sequences.
Conclusions: These findings suggest that HPeV3, circulating among children in the community, infects their household, including parents, a portion of whom may subsequently show symptoms of myalgia. Our observations in 2008 and 2011 strongly suggest that clinical consideration should be given to HPeV3 in children as well as in adults during summer seasons in which an HPeV3 outbreak occurs among the children in the community.

1. Background

Human parechovirus (HPeV) is a positive-sense, single-stranded RNA virus belonging to the genus *Parechovirus* of the family *Picornaviridae*.[1,2] HPeV type 1 (HPeV1) and HPeV2 were initially classified as echovirus types 22 and 23 of genus *Enterovirus*, but were recently reclassified.[1,2] HPeV3 was first isolated from a stool specimen of a 1-year-old Japanese child with transient paralysis

Abbreviations: HPeV1, human parechovirus type 1; VP, viral protein; RT, reverse-transcription; NESID, National Epidemiological Surveillance of Infectious Diseases.

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in 1999.[3] There are 8 types of HPeV (HPeV1-8) and one newly reported strain that remains untyped for which the complete genome sequences are available, and the viral protein (VP) 1 coding region sequences have been recently reported for HPeV9-16.[4,5]

HPeV infections are mainly asymptomatic or associated with mild respiratory and gastrointestinal illness, although more serious diseases, such as myocarditis, encephalitis, pneumonia, meningitis, flaccid paralysis, and sepsis-like syndrome have been occasionally reported.[1,2] HPeV3, in particular, has peaks in summer about every 2–3 years and often causes severe illness involving the central nervous system and sepsis-like syndrome in very young children.[1,3,6–15] Serological and virological studies have suggested that HPeV1-3 infections usually occur in early infancy.[1–3,6–15]

We previously reported an outbreak of epidemic myalgia associated with HPeV3 infection among adults in the summer season between June and August 2008 in Yamagata, Japan, and postulated that an outbreak of HPeV3 among children might be a necessary background condition for outbreaks of epidemic myalgia.[16] To prove this hypothesis, we decided to continue surveillance of HPeV3 outbreaks in children as well as epidemic myalgia in adults and to accumulate data to confirm their relationship. Furthermore, it was necessary to disseminate our observations regarding epidemic myalgia among adults in the summer season in Yamagata and wait for similar reporting from areas other than Yamagata in order to establish HPeV3-associated epidemic myalgia as a disease. We identified another HPeV3 outbreak among children in combination with cases of adult epidemic myalgia in the summer of 2011 in Yamagata, which we describe herein.

2. Objectives

To clarify the relationship between community outbreaks of HPeV3 among children and epidemic myalgia among adults through continued surveillance of both diseases.

3. Study design

3.1. Surveillance of viral infectious diseases among children

We have been taking part in the national surveillance of viral infectious diseases in Japan based on the Infectious Diseases Control Law. Between June and August, 2011, 559 nasopharyngeal swabs and 27 stool specimens ($n = 586$) were collected from patients at pediatric clinics. Among these patients, 418 (71.3%) were from patients <5 years old, 110 (18.8%) were from patients between 6 and 9 years old, 41 (7.0%) were from patients between 10 and 14 years old, 7 (1.2%) were from patients >14 years old and 10 (1.7%) were from patients of unknown age. The clinical diagnoses for these patients were as follows: upper respiratory infections (273, 46.6%), lower respiratory infections (63, 10.8%), herpangina (59, 10.1%), hand-foot-mouth disease (53, 9%), viral exanthema (52, 8.9%), gastroenteritis (26, 4.4%), and others (60, 10.2%).

Nasopharyngeal swab specimens were placed immediately into tubes containing 3 ml of transport medium, and stool specimens were put in a stool container and transported to the Department of Microbiology, Yamagata Prefectural Institute of Public Health for virus isolation and reverse-transcription (RT)-PCR screening. A 10% water suspension of the fecal specimen was centrifuged at 9100 × *g* for 10 min using a microcentrifuge, and the supernatant was filtered using a 0.45 μm filter (Steradisc 25, S-2504, KURABO, Osaka, Japan). Virus isolation was carried out using a previously described microplate method.[17] Briefly, HEF, HEp-2, Vero E6, MDCK, RD-18S, and GMK cell lines were prepared in the wells of a 96-well microplate (Greiner Bio-One, Frickenhausen, Germany). As the LLC-MK2 cell line is sensitive to HPeV3,[16] we also prepared a separate 96-well microplate of this cell line. After a change of medium, nasopharyngeal swabs and 10% stool suspension specimens were centrifuged at 450 × *g* for 20 min, and 75 μl of the supernatant was inoculated onto two wells of each of the cell lines. The inoculated plates were centrifuged for 20 min at 1500 × *g*, incubated at 33 °C in a 5% CO_2 incubator, and assessed for cytopathic effect. We also screened for HPeV3 using RT-PCR. RNA was extracted from 200 μl of each nasopharyngeal swab specimen or 10% stool suspension using a High Pure Viral RNA kit (Roche Diagnostics, Manheim, Germany) according to the manufacturer's instructions, transcribed into cDNA, and the PCR assay was then performed as previously described.[16] The remainder of each specimen and the cDNA specimens were stored at −80 °C.

3.2. Detection of HPeV3 from adult patients diagnosed with epidemic myalgia

A total of 5 patients visited the Department of Neurology, Yamagata Prefectural Central Hospital, and were clinically diagnosed with epidemic myalgia between July and August 2011 (Table 1). All patients were male and aged between 31 and 41 years. We tried to detect HPeV3 from these patients using the virus isolation and RT-PCR methods described above.

3.3. Detection of HPeV3 from stocked frozen specimens in 2008

Since we knew from data in the National Epidemiological Surveillance of Infectious Diseases (NESID) system[18] that there was an outbreak in the summer–autumn season in Japan in 2008, we also carried out screening for HPeV3 strains retrospectively using the frozen stocked specimens collected from children with acute respiratory infections at the Yamanobe Pediatric Clinic between June and September (307 specimens) and one stool and one throat swab specimen, which were collected from two myalgia-suspected adult patients at the Yamagata Prefectural Central Hospital, in order to compare strains between 2008 and 2011.

3.4. Sequence analysis of the HPeV3 strains

For specimens found to be positive for HPeV3 by virus isolation and/or RT-PCR method, sequence analysis was carried out as described previously using the reported primers[16] as well as those shown in the Appendix Table. Sequence data were registered under GenBank accession numbers AB759146–AB759207. Sequence data for the VP1 region were used and analyzed with CLUSTAL W version 1.83, and a phylogenetic tree was constructed via the neighbor-joining method using the same software.[19]

Supplementary data related to this article found, in the online version, at http://dx.doi.org/10.1016/j.jcv.2013.05.019.

4. Results

4.1. Detection of HPeV3 in the surveillance of viral infectious diseases among children

Our surveillance system based on virus isolation using a microplate method can isolate a wide range of viruses such as influenza viruses, parainfluenza viruses, and a variety of enteroviruses.[17] However, herein we limit our results to those for HPeV3. During the study period, we isolated HPeV3 strains from 29 specimens using the LLC-MK2 cell line and detected the HPeV3 genome in 59 specimens using the RT-PCR method. We succeeded in detecting the HPeV3 genome in all specimens from which the virus was isolated. Among the 59 HPeV3-positive specimens, we detected not only HPeV3 but also other viruses in 22 specimens