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Short communication

Diversity of human parechovirus in infants and children with acute gastroenteritis in Japan during 2014–2016

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ABSTRACT

A total of 972 stool samples were collected from infants and children with acute gastroenteritis (AGE) in pediatric clinics encompassing six localities (Hokkaido, Tokyo, Shizuoka, Kyoto, Osaka, and Saga) in Japan during the 2-year period from July 2014 to June 2016. Sixty six of the samples (6.8%) were found to be positive for human parechovirus (HPeV) by multiplex reverse transcriptase-polymerase chain reaction (RT-PCR) and subjected to genotyping based on viral protein 1 (VP1) sequences. Four different HPeV genotypes consisting of HPeV1, -3, -4 and -6 were detected, with HPeV1 clade B being predominant and followed by HPeV3 and -6. The first-time presence of HPeV1 clade A in Japan and rare HPeV4 were noted. This study provides up-to-date information on the genetic diversity of HPeV circulating in Japanese infants and children with AGE.

1. Introduction

Human parechovirus (HPeV) belongs to the *Parechovirus* genus of the *Picornaviridae* family and consists of 19 genotypes (HPeV1 to HPeV19) (http://www.picornastudygroup.com/types/parechovirus/parechovirus_a.htm). The HPeV genome contains a large open reading frame coding for a single polyprotein, which is post-translationally cleaved into three structural (VP0, VP3 and VP1) and seven non-structural proteins (2A–C and 3A–D (Hyypia et al., 1992)).

HPeV infections are mainly asymptomatic or associated with mild respiratory and gastrointestinal diseases (Baumgarte et al., 2008; Benschop et al., 2006; Ito et al., 2010; Stanway et al., 2000). However, more severe diseases such as meningitis, encephalitis, neonatal sepsis and sepsis-like, Reye's and haemolytic syndrome also have been reported (Boivin et al., 2005; Harvala et al., 2009; Koskiniemi et al., 1989; O'Regan et al., 1980; Wolthers et al., 2008). Different HPeV genotypes can cause different symptoms and diseases. Clinical studies have indicated that HPeV3 in particular is a predominant type associated with severe symptoms in neonates, and the main type infecting the central nervous system (Benschop et al., 2008; Verboon-Maciolek et al., 2008; Wolthers et al., 2008).

HPeV infection in children with acute gastroenteritis (AGE) has a prevalence rate from 1.8 to 29.4% worldwide (Baumgarte et al., 2008; Biscaro et al., 2018; Chuchaona et al., 2015; Mladenova et al., 2015; Patil et al., 2018; Pham et al., 2011b). HPeV1 is the most predominant genotype followed by HPeV3 and other genotypes such as HPeV2, -4, -6, -8, -10, and -11, which are less common in children with gastrointestinal diseases. In addition, recent studies from other countries have shown the diversity of HPeV genotypes circulating in children with AGE, in which many new genotypes such as HPeV13, -14, and -16 have been detected (Chen et al., 2018; Malasao et al., 2019; Patil et al., 2018). The purpose of this study was to investigate the epidemiological and genetic characterization of HPeV circulating in children with AGE in Japan during 2014–2016.

Abbreviations: Bp, Base pair; HPeV, Human parechovirus; RT-PCR, Reverse transcription- Polymerase chain reaction; AGE, Acute gastroenteritis; VP, Viral protein

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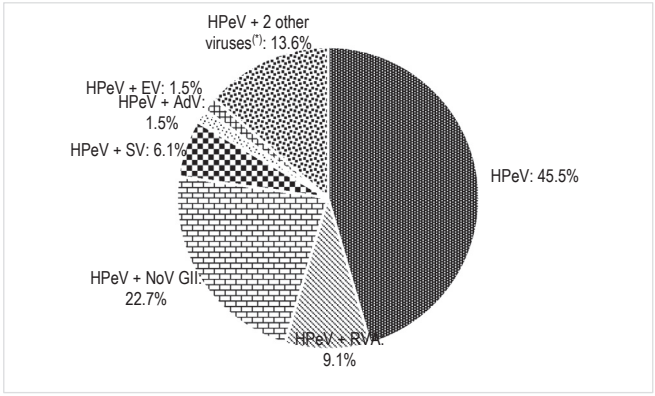


Fig. 1. HPeV mono-infection and co-infection with other enteric viruses.
(*) HPeV + NoV + Ade: (2 of 66) 3.03%; HPeV + RVA + SV: (1 of 66) 1.5%; HPeV + RVA + NoV: (4 of 66) 6.06%; and HPeV + NoV + SV: (2 of 66) 3.03%.

2. Materials and methods

2.1. Clinical specimens and HPeV genotyping

A total of 972 stool samples were collected from Japanese pediatric outpatients aged 1 month-15 years, who were diagnosed clinically with AGE in six prefectures encompassing Hokkaido, Tokyo, Shizuoka, Kyoto, Osaka, and Saga during the 2-year period from July 2014 to June 2016. Sixty six of the samples (6.8%) were found to be positive for HPeV by multiplex reverse transcriptase-polymerase chain reaction (RT-PCR) and subjected to genotyping based on viral protein 1 (VP1) sequences (Benschop et al., 2006; Pham et al., 2010). In addition, the samples were tested for other enteric viruses including group A rotavirus (RAV), norovirus genogroup I (NoVGI), norovirus genogroup II (NoVGII), adenovirus (AdV), astrovirus (AstV), sapovirus (SV), Aichi virus (AiV), enterovirus (EV), cosavirus (Cosa), bocavirus (BoV), and Saffold virus (Saffold) by multiplex RT-PCR (Thongprachum et al., 2017).

When samples failed to obtain polymerase chain reaction (PCR) products from the full VP1 region, a nested PCR was conducted using the known forward primer, VP1-parEchoF1 (Benschop et al., 2006), and a newly designed reverse primer, VP1-HPeV-R, 5′-GTCATYTGYTCHC-CWGCNGG-3′ (2808–2789), in order to amplify the 477-bp partial VP1 region. In addition, when samples still failed to amplify partial VP1, another PCR was performed to amplify a 300-bp fragment of the VP3-VP1 region, as described by Harvala et al. (2008) for identifying the HPeV genotype by the Basic Local Alignment Search Tool (BLAST).

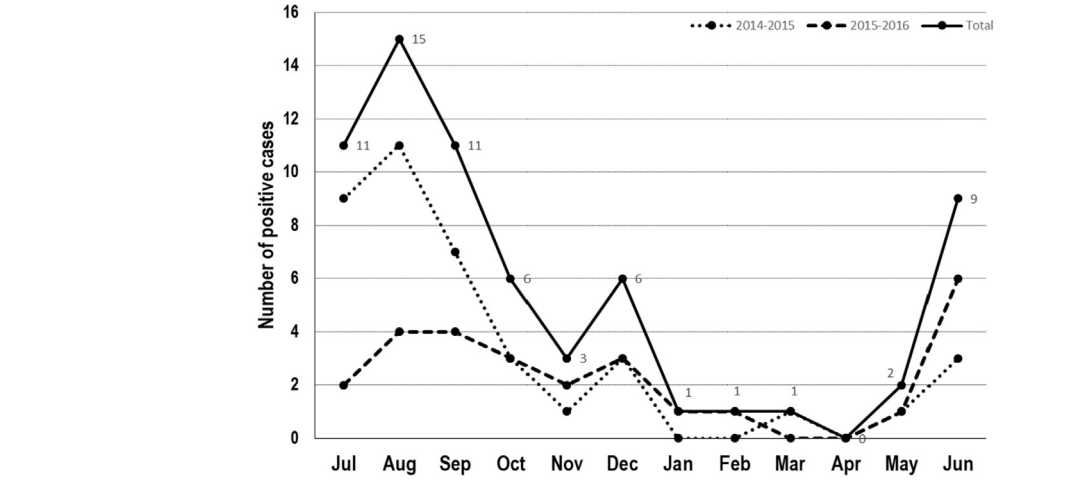
This study was approved by the Ethics Committee of the Nihon University School of Medicine, Tokyo, Japan (No. 29-9-0).

2.2. Sequencing and phylogenetic analysis

The PCR products were purified and sequenced using the BigDye Terminator Cycle Sequencing Kit (Perkin Elmer-Applied Biosystems, Inc., Foster City, CA, USA) on an automated DNA sequencer (ABI 3100; Perkin Elmer-Applied Biosystems, Inc., Foster City, CA, USA). The primers for amplifying the VP1 gene were used as sequencing primers. The nucleotide sequences were compared with those of the reference strains deposited in the GenBank database. The phylogenetic tree was constructed according to the neighbor-joining method based on the Kimura 2-parameter model, using MEGA version 7 software (Kumar et al., 2016). The nucleotide sequences of the HPeV strains described in this study were deposited in GenBank under accession numbers: MK558730-MK558774, MK558777-MK558778, MK558785-MK558792, and MN047159-MN047169.

3. Results and discussion

Of 972 stool samples collected during the study period, 66 (6.8%) were positive for HPeV. From these, 30 (45.5%) were HPeV mono-infection, whereas the remaining 36 (54.5%) were HPeV co-infection with other enteric viruses including RVA, NoVGII, AdV, SV, and EV. The double and triple infections were seen in 27 of the 66 samples (40.9%) and 9 of 66 (13.6%), respectively. Among these, NoVGII was the most predominant virus co-infected with HPeV (Fig. 1). This result is in agreement with previous study showing that co-infection with another enteric virus is common for HPeV, and dual infection is a



HPeV genotype		Jul	Aug	Sep	Oct	Nov	Dec	Jan	Feb	Mar	Apr	May	Jun
2014 – 2015	HPeV1	6	6	6	2	1	3					1	1
	HPeV3	1	3										
	HPeV4	1	2	1	1								
	HPeV6	1								1			2
2015 – 2016	HPeV1	2	4	4	2	2	2	1	1				2
	HPeV3											1	3
	HPeV6				1		1						1

Fig. 2. Seasonal pattern of HPeV infection in Japanese children with AGE during the 2-year period 2014–2016; and monthly distribution of HPeV genotypes identified in this study.

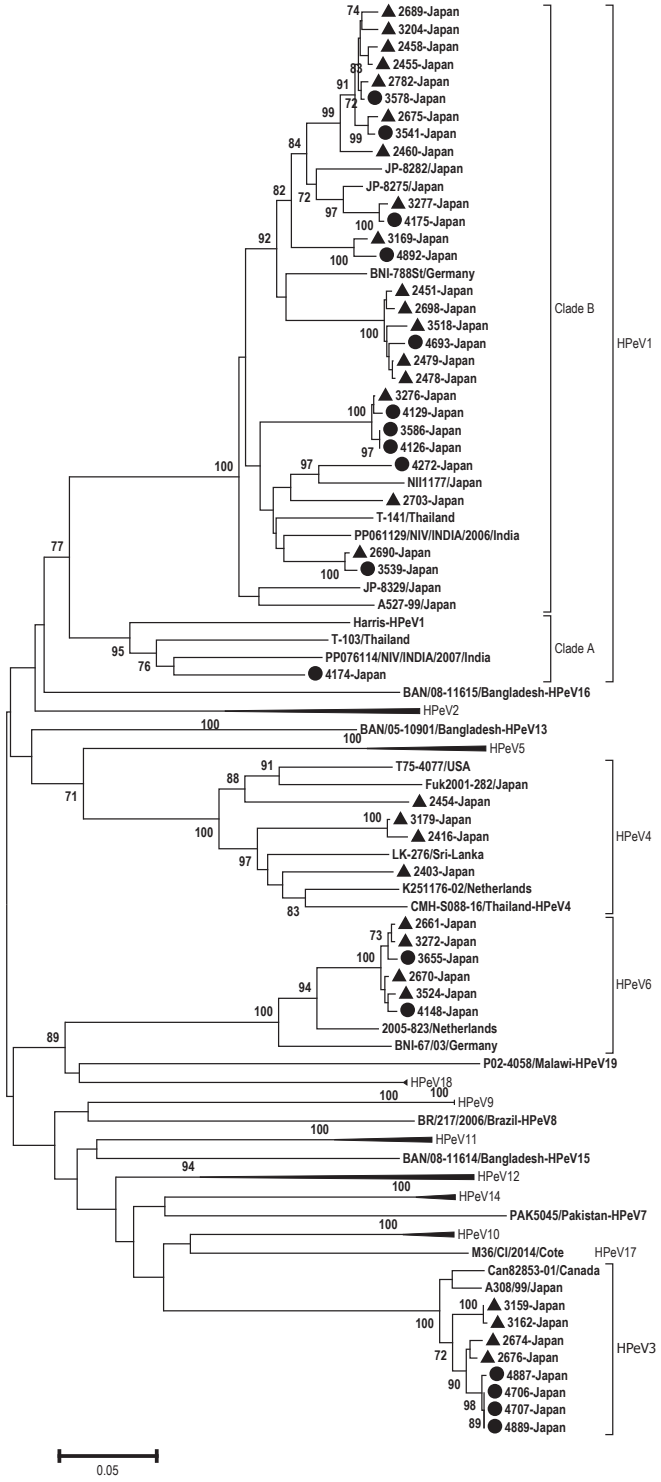


Fig. 3. Phylogenetic tree constructed from the partial VP1 region of the studied and reference HPeV (HPeV1 to 19) strains, with 500 bootstrap repetitions. Percentage bootstrap values above 70% are shown at the branch nodes. The strains studied are marked with a solid triangle (2014 - 2015) and circle (2015 - 2016). The nucleotide sequences of the reference HPeV strains were obtained from GenBank, with accession numbers in parentheses. HPeV1: Harris (L02971), T-103/Thailand (FJ648759), PP076114/NIV/INDIA/2007/India (KJ743635), JP-8282/Japan (FJ648741), JP-8275/Japan (HQ163882), BNI-788St/Germany (EF051629), NII1177-2014 (LC062506), T-141/Thailand (FJ648762), PP061129/NIV/INDIA/2006 (KJ743621), 8329/Japan (FJ648746), JP- A527-99/Japan (AB300928),; HPeV2: Williamson (AJ005695), T-144/Thailand (FJ648760); HPeV3: A308/99/Japan (AB084913), Can82853-01/Canada (AJ889918); HPeV4: T75-4077/USA (AM235750), K251176-02/Netherlands (DQ315670), Fuk2001-282/Japan (AB433630), LK-276/Sri-Lanka (HQ163879), CMH-S088-16 (MH094173); HPeV5: T92-15/USA (AM235749), CT86-6760/USA (AF055846); HPeV6: BNI-67/03/Germany (EU024629), 2005-823/Netherlands (EU077518); HPeV7: PAK5045/Pakistan (EU556224); HPeV8: BR/217/2006/Brazil (EU716175); HPeV9: BOL/02-10648-Bolivia (JX219577), BOL/02-10649B (JX219578); HPeV10: LK-103/Sri-Lanka (GQ402516), LK-106/Sri-Lanka (GQ402515); HPeV11: BAN/04-10905 (JX219574), LK-223 (HQ163877); HPeV12: BAN/04-10904 (JX219567), BOL/02-10667B-Bolivia (JX219566); HPeV13: BAN/05-10901 (JX219579); HPeV14: CMH-S034-14 (MH094184), CMH-N043-15 (MH094186); HPeV15: BAN/08-11614 (JX219573); HPeV16: BAN/08-11615 (JX219580); HPeV17: M36/CI/2014 (KT319121); HPeV18: GhanaA06 (KY931658), GhanaA36 (KY931660); and HPeV19: P02-4058 (MH339678).

possible mode of infection (Patil et al., 2018; Pham et al., 2011b; Zhang et al., 2011).

All of the patients, whose stool specimens showed positive for HPeV, were infants and children aged from 1 to 60 months, with a mean age of 14.4 months. The majority of patients (56 of 66; 84.8%) were from 6 to 24 months of age with the highest prevalence (31 of 66, 47%) in those aged from 12 to 24 months. Moreover, 5 of the 66 patients (7.6%) were less than 6 months old and the youngest was one month of age. The majority of HPeV-positive cases (46 of 66, 69.7%) were seen from July to September, with the peak occurring in August (Fig. 2). These findings are slightly different from previous reports that showed the majority of HPeV infection occurring in patients who were 7 to 12 months of age. This might be due to differences in the participants studied, because outpatient cohorts were targeted in this study, and previous studies focused on hospitalized patients (Guo et al., 2013; Patil et al., 2018; Zhang et al., 2011).

Regarding clinical characterization, symptoms other than diarrhea, such as abdominal pain, nausea, vomiting or fever were present in most of the patients. However, no dehydration or neurological symptoms were noted in this study.

This study determined HPeV genotypes by sequencing the full or partial VP1 region. Of the 66 HPeV strains, 46 were amplified successfully, with the full VP1 (760-bp) being sequenced, and they were analyzed phylogenetically with those of 43 HPeV1 to HPeV19 reference strains. The remaining 20 strains were successful in amplifying and sequencing the partial VP1 gene (477-bp) (19 strains) or VP3-VP1 (300-bp) (1 strain), and they were determined as HPeV1 (18 strains), HPeV4 (1 strain) and HPeV6 (1 strain) by BLAST.

Fig. 3 shows a phylogenetic tree construction based on the full VP1 gene sequences of 43 HPeV reference strains (HPeV1-19) and 46 studied strains. Four different HPeV genotypes consisting of HPeV1, -3, -4 and -6 were detected. The majority of strains studied belonged to HPeV1 (n = 28), followed by HPeV3 (n = 8), HPeV6 (n = 6) and HPeV4 (n = 4). This finding was in agreement with previous studies, which reported that HPeV1 was the predominant genotype in patients with AGE (Baumgarte et al., 2008; Guo et al., 2013; Han et al., 2011; Pham et al., 2011b; Pietsch and Liebert, 2019).

The majority of HPeV1 strains studied were grouped into clade B, whereas one (4147-Japan) that was clustered with the prototype Harris strain (clade A) had not been noted previously in Japan (Fig. 3) (Pham et al., 2011a). The clade A strain (4147-Japan) studied was related closely to the India strain (PP076114/NIV/INDIA/2007), with 87% nucleotide sequence identity (at 98.3% amino acid level). Among the 27 HPeV1 clade B strains studied, the nucleotide sequence identity ranged from 87% (at 96.7% amino acid level) to 99.5% (at 99.5% amino acid level).

The HPeV3 strains studied, which appeared as the second most prevalent genotype, clustered separately from the reference strains and shared 95.8–99.8% nucleotide (97.9–99.5% amino acid) identity with each other, while nucleotide and amino acid identities between the

studied and reference strains were 93.3–96.3% and 95.3–98.7%, respectively. The HPeV6 strains studied also formed a separate cluster and showed 98.3–99.6% nucleotide and 98.2–100% amino acid identities with each other.

HPeV4 in patients with AGE has been reported rarely in Japan. The first isolation was in 2005 from a 3-year-old boy with AGE in Fukuoka city, Japan, as described by Wakatsuki et al. (2008). All of the 4 HPeV4 strains in this study were detected in July, August and October 2014 (Fig. 2), from children aged 8 (Osaka), 15 (Shizuoka) 5 (Saga) and 13 (Kyoto) months. Their VP1 region shared 82.6% (2454-Japan and 2416-Japan) to 98.4% (2416-Japan and 3179-Japan) nucleotide and 94.9% to 100% amino acid identity with each other. This was the first HPeV4 isolated from children with AGE in Osaka, Shizuoka, Saga and Kyoto, Japan.

The alignment of deduced amino acid sequences of the 46 strains studied, which succeeded in fully sequencing the VP1, revealed that arginine-glycine-aspartic acid (RGD) was present in all of the HPeV1, -4 and -6 strains studied, but not in the HPeV3 strains studied (data not shown). This was consistent with previous studies, which showed that the RGD motif was identified in HPeV1, -4 and -6, but not present in HPeV3 (Al-Sunaidi et al., 2007; Ito et al., 2010; Watanabe et al., 2007). In addition, an amino acid insertion of cysteine was observed in HPeV4, but not in HPeV1, -3 or -6. This finding was in agreement with an earlier report by Malasao et al. (2019).

The detection and genotype distribution of HPeV in infants and children with AGE in Japan in 2007–2008 were reported previously, with only HPeV1 clade B and HPeV3 detected (Pham et al., 2011a). Diversity of the HPeV genotype, including HPeV1 clade A, HPeV1 clade B, HPeV3, -4 and HPeV6 was noted in this study.

In conclusion, this study provides up-to-date information on the genetic diversity of HPeV circulation in Japanese infants and children with AGE during 2014–2016, with the predominance of HPeV1 clade B followed by HPeV3, -6 and -4. In addition, the first-time presence of HPeV1 clade A and rare HPeV4 were noted.

Declaration of Competing Interest

No conflict of interest is declared.

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