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Detection and characterization of a novel human parechovirus genotype in Thailand

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ABSTRACT

Human parechoviruses (HPeV), member in the family *Picornaviridae*, cause respiratory symptoms primarily in infants and young children. Currently, 16 genotypes have been described based on phylogenetic analysis of VP1 sequences, all of which have a global distribution. The purpose of this study was to investigate the prevalence and genotype distribution of HPeV in Thailand. A total of 171 fecal specimens collected during October 2012 to May 2013 from children with diarrhea in Chiang Mai, Thailand were investigated for HPeV by RT-PCR and sequence analysis. HPeVs were found in 3 out of 171 (1.8%) fecal specimens tested. Of these, one was HPeV1 which is commonly detected in children with gastroenteritis and another one was uncommon HPeV14 genotype. Most interestingly, the sequence of the third HPeV positive sample (CMH-N185-12) did not cluster with any of the known 16 genotypes and therefore is proposed as a candidate HPeV genotype 17.

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1. Introduction

Human parechoviruses (HPeVs) belong to the family *Picornaviridae*, a highly diverse group of small, non-enveloped, single-stranded RNA genome of positive polarity, and many of which cause diseases in humans (Harvala and Simmonds, 2009). The genera within the family are important human pathogens such as rhinoviruses, enteroviruses, polioviruses and hepatoviruses. HPeVs have been recognized as an important pediatric viral pathogen causing mild to severe infections including gastroenteritis, respiratory infections, encephalitis and flaccid paralysis in children under 5 years of age (Stanway et al., 2000). HPeV1 and HPeV2 genotypes were originally isolated from children with acute gastroenteritis (AGE) and described previously as echovirus 22 (EV22) and EV23, respectively, but later have been classified into a new genus *Parechovirus* based on distinct molecular and biological characteristics from other echoviruses (Stanway et al., 1994). Since the reclassification, the number of HPeV genotypes has increased continuously and currently a total of 16 genotypes have been described worldwide (www.picornaviridae.com/parechovirus/hpev/hpev.htm), while published data are available for only 12 genotypes, HPeV1–8, 10, 11, 12 and 14 (Joki-Korpela and Hyypia, 2001; Baumgarte et al., 2008; Drexler et al., 2009; Harvala and Simmonds, 2009; Ito et al., 2010; Pham et al., 2010b,c, 2011; Alam et al., 2012). Although 16 genotypes have been described on the basis of the phylogenetic analyses, the majority of published genotypes of HPeV are genotypes 1–8. As the HPeVs are comprised of highly diverse genotypes, high rates of recombinations have been reported (Ito et al., 2004). Even though HPeV1 and HPeV2 cause mild gastrointestinal or respiratory illness, however, the more serious diseases have also been reported occasionally, including myocarditis, encephalitis, pneumonia, meningitis, flaccid paralysis, Reye syndrome, and fatal neonatal infection (Stanway et al., 2000; Khetsuriani et al., 2006). HPeV3 causes not only mild gastrointestinal and respiratory tract illness, but also severe illness such as sepsis and central nervous system (CNS) diseases (Boivin et al., 2005; Harvala and Simmonds, 2009), whereas HPeV4-8 seem to cause the diseases similar to those associated with HPeV1 and HPeV2 infections (Levorson and Jantausch, 2009).

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Table 1
Characteristics of patients and HPeVs genotypes.

Strains	Date of specimen collection	Age (year/month/day)	HPeV genotypes	Co-infected viruses	GenBank accession no.
CMH-N166-12	24/11/2012	1 Y 4 M	1	–	KM407606
CMH-N185-12	26/11/2012	3 M	Novel	Norovirus GII.4	KM407607
CMH-N002-13	14/01/2013	1 Y 3 D	14	Enterovirus	KM407608

HPeVs replicate mainly in the gut and are transmitted by the fecal-oral route, although infections may also occur through respiratory route and virus shedding is readily detectable in respiratory secretions (Harvala and Simmonds, 2009). This suggests that HPeVs can also replicate in the cells of respiratory tract. The most predominant genotypes in pediatric patients are HPeV1 and HPeV3, followed by HPeV2, but HPeV4-6 are less common (Benschop et al., 2008; Tapia et al., 2008; Ito et al., 2010; Pham et al., 2010b). In addition, HPeV7-16 are new genotypes that have been discovered recently. Thus, the information of the epidemiology and prevalence of these genotypes have not been fully established.

In Thailand, few epidemiological surveillance studies of HPeV infection in children with diarrhea have been conducted (Pham et al., 2010b; Chieochansin et al., 2011). The prevalence of HPeV infections in children with acute gastroenteritis in Thailand was 14.6% in 2005 (Pham et al., 2010b) and 6.1% in 2009–2011 (Chieochansin et al., 2011). In addition, HPeV infection in adults with diarrhea has been reported at 0.3% (1 out of 332) (Saikruang et al., 2014).

The present study describes the surveillance study of HPeV and reports a candidate of novel HPeV17 genotype detected in children suffering from acute gastroenteritis in Chiang Mai, Thailand.

2. Materials and methods

2.1. Specimen collection

A total of 171 fecal specimens were collected from children admitted to the hospitals with acute gastroenteritis in Chiang Mai, Thailand during October 2012 to May 2013. Only the pediatric patients who had a clinical diagnosis of acute gastroenteritis with watery diarrhea were included in this study. The age of the patients ranged from neonate up to 13 years old. The study was conducted with the approval of the ethical committee for human rights related to human experimentation, Faculty of Medicine, Chiang Mai University (No. 181/2554). All stool samples were stored at −20 °C until used.

The stool specimens have been screened for other diarrheal viruses including group A, B, C rotaviruses, adenovirus, norovirus GI and GII, sapovirus, astrovirus, Aichi virus, enterovirus, and bocavirus based on the protocols described previously (Pham et al., 2010a; Yan et al., 2003, 2004).

2.2. HPeV detection

The fecal specimens were diluted with PBS to 10% suspensions and were clarified by centrifugation at 10,000×g for 10 min. The RNA genome of HPeV was extracted from 10% fecal suspension, using Geneaid Viral Nucleic Acid Extraction Kit II (Geneaid, Taipei, Taiwan), according to the manufacturer's protocol. The viral RNA was reverse transcribed by random primer and enzyme reverse transcriptase (Fermentas, Glen Burnie, MD, USA), and the cDNA was stored at −20 °C for further used as a template for virus detection.

The presence of HPeVs in fecal specimens was detected by PCR using forward primers ev22(+), 5′-CYCACACAGCCATCCTC-3′ and reverse primer ev22(−), 5′-TRCGGGTACCTTCTGGG-3′ to amplify a 270 bp PCR product of the 5′ untranslated region (UTR) of the viral genome (Joki-Korpela and Hyypia,1998). Positive and negative controls were also concurrently included along with the test samples in order to monitor any possible contamination that might be occurred in the PCR process.

2.3. HPeV genotype characterization and nucleotide sequence analysis

The identification of HPeV genotypes was performed based on the sequence analysis of VP1 region in comparison with the reference sequences. Amplification of VP1 was done by RT nested-PCR. The first round PCR reaction was conducted by using forward primer Cap-parEcho-F, 5′-TCHACWTGGATGMGRAARAC-3′ and reverse primer Cap-parEcho-R5′-TCYARYTCACAYTCYTCYTC-3′ (Pham et al., 2010b). Then, the second round PCR reaction was performed with forward primer VP1-parEchoF1, 5′-CCAAAATTCRT GGGGTTC-3′ and reverse primer VP1-parEchoR1, 5′-AAACCYCTRTC TAAATAWGC-3′. The expected PCR product size was 760 bp (Benschop et al., 2006).

The amplified VP1 PCR products of HPeVs were subjected to direct sequencing using BigDye Terminator Cycle Sequencing Kit (Applied Biosystems, Foster City, CA). The sequences of VP1 were compared with those of reference strains available in the NCBI GenBank database using BLAST server (http://www.ncbi.nlm.nih.gov/blast). Multiple sequence alignment was conducted using ClustalW, and a phylogenetic tree was constructed using MEGA (v5.05) software (Tamura et al., 2011). The sequences were annotated, aligned, and managed by using softwares of sequence difference including BioEdit (v7.2.0) (Hall, 1999), and SSE (v1.1) (Simmonds, 2012). The partial nucleotide sequences of HPeV VP1 described in the present study have been deposited in the GenBank database under the accession numbers KM407606-KM407608.

3. Results

Out of 171 fecal specimens tested, 3 (1.8%) were positive for HPeV (CMH-N166-12, CMH-N185-12, CMH-N002-13) by RT-PCR screening method. The HPeV-positive samples were from a 3 months old girl and two of 1 year old boys (Table 1). Of these, two specimens (CMH-N166-12 and CMH-N185-12) were collected in November 2012 and one specimen (CMH-N002-13) in January 2013. Among 3 cases, one child (CMH-N166-12) was infected only with HPeVs, while the other two children were co-infected with other gastroenteritis viruses, of which one (CMH-N185-12) was co-infected with norovirus GII.4 whereas another one (CMH-N002-13) was co-infected with enterovirus (Table 1).

The detected HPeV strains were characterized further by sequencing of the partial VP1 region and compared with reference strains of all 16 established HPeV genotypes available in the GenBank database (HPeV1–HPeV16). Analysis of partial VP1 nucleotide sequences (760 nucleotides long) of all 3 HPeV strains detected in the present study indicated that CMH-N166-12 was closely related to the HPeV1 reference strain detected previous in Sri Lanka (AB300965) with the nucleotide sequence identity of 91%, while CMH-N002-13 was closely related to the uncommon type of HPeV14 reference strain which was reported previously in

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