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Research paper

Prevalence of human cosavirus and saffold virus with an emergence of saffold virus genotype 6 in patients hospitalized with acute gastroenteritis in Chiang Mai, Thailand, 2014–2016

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ABSTRACT

Human cosavirus and saffold virus are both newly discovered members of the *Picornaviridae* family. It has been suggested that these viruses may be the causative agents of acute gastroenteritis. In this study, 1093 stool samples collected from patients with acute gastroenteritis between January 2014 and December 2016, were screened for cosavirus and saffold virus using reverse transcription-polymerase chain reaction. The viral genotypes were then established via nucleotide sequencing. Here, cosavirus was detected in 16 of 1093 stool samples (1.5%) and saffold virus was detected in 18 of 1093 stool samples (1.6%). The saffold virus genotypes 1 (16.7%), 2 (50%) and 6 (33.3%), and the cosavirus genetic groups A (87.5%), C (6.25%) and D (6.25%), were all identified across the three-year study period. Interestingly, saffold virus genotype 6 has now been detected for the first time in Thailand. The present study provides the prevalence of cosavirus and saffold virus with the emergence of saffold virus genotype 6 in Thailand.

1. Introduction

Acute gastroenteritis is a common cause of morbidity and mortality worldwide and is responsible for an estimated 2.2 million deaths globally, which are most commonly occurring in infants aged 6–24 months (Dennehy, 2011). The cause of gastroenteritis can be from bacteria, but viruses are often the most common causative agents (Thongprachum et al., 2016). The viruses in the *Picornaviridae* family are comprised of 12 genera of both human and animal viruses (Adams et al., 2016). *Cardiovirus* is one genus of the *Picornaviridae* family that was previously believed to infect just rodents and pigs, but not humans (Blinkova et al., 2009). However, in 2007 a new member of the *Cardiovirus* genus, named saffold virus (SAFV), was isolated from an 8-month-old infant presenting with fever of unknown origin (FUO) in America (Jones et al., 2007). This suggests the existence of a human-specific *Cardiovirus* (Jones et al., 2007). Furthermore, in 2008 another member of the *Picornaviridae* family, human cosavirus, was identified (Kapoor et al., 2008). Human cosavirus (HCoSV), of the *Cosavirus* genus, was initially isolated from the stools of non-polio acute flaccid paralysis (AFP) cases and healthy children in Pakistan (Kapoor et al., 2008). Like all other members of the *Picornaviridae* family, HCoSV and SAFV both consist of a positive sense, single stranded ribonucleic acid (RNA) genome (Jones et al., 2007; Kapoor et al., 2008). The HCoSV genome is 7632 base pairs (bp) long and codes for seven non-structural proteins (2A, 2B, 2C,3A, 3B, 3C, 3D) and four structural proteins (VP1, VP2, VP3, VP4) (Kapoor et al., 2008). The SAFV genome is 7846 bp long with a single open reading frame (ORF) that also codes for four structural proteins (VP4, VP2, VP3, VP1) and seven non-structural proteins (2A, 2B, 2C, 3A, 3B, 3C, 3D) (Jones et al., 2007). However, HCoSV has 6 genetic groups (A-F) and consists of more than 30 genotypes (Kapusinszky et al., 2012) whereas SAFV has 11 established genotypes (SAFV 1–11) (Naeem et al., 2014).

HCoSV and SAFV both have a worldwide distribution. Both viruses have been detected in the stools of patients with acute gastroenteritis in several countries such as: Japan, China, Brazil, and Thailand; each reporting different prevalence rates and incidences of co-infection with other diarrhea-causing viruses (Chiu et al., 2008; Holtz et al., 2008; Ren et al., 2009; Dai et al., 2011; Khamrin et al., 2011; Stocker et al., 2012; Nielsen et al., 2013; Okitsu et al., 2014; Yodmeeklin et al., 2015). Previous studies have also found HCoSV in healthy controls (Dai et al., 2010). However, there have been instances of sole HCoSV and SAFV infection in acute gastroenteritis samples (Nielsen et al., 2013; Okitsu et al., 2014; Yodmeeklin et al., 2015). Therefore, it can be difficult to determine the pathogenicity of SAFV and HCoSV in acute gastroenteritis alone (Khamrin and Maneekarn, 2014; Khamrin et al., 2011). It is also important that the pathogenicity of HCoSV and SAFV

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is established due to possible neurovirulence (Kotani et al., 2016; Zhang et al., 2015; Rezig et al., 2015).

It is still crucial to screen for both viruses in order to gain a full understanding of their genetic diversity, the association with gastroenteritis and how the viruses are distributed globally. Therefore, the aim of this study is to screen for SAFV and HCoSV from pediatric patients who were admitted to hospitals with acute gastroenteritis in Chiang Mai, Thailand over the period of 2014–2016. Positive samples were further analyzed via genetic characterization to gain a deeper understanding of the different genotypes and prevalence of these two potentially harmful viruses.

2. Materials & methods

2.1. Specimen collection

During the period of January 2014 to December 2016, a total of 1093 samples were collected from patients with acute gastroenteritis admitted to Nakhon Ping hospital, Maharaj Nakorn Chiang Mai hospital, and San Pa Tong hospital. Overall, 268 samples were collected in 2014, 335 samples were collected in 2015, and 490 samples were collected in 2016. The age of patients enrolled in this study ranged from neonate to 14 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 (MIC-2557-02710). The same set of fecal specimens were also screened for several other diarrhea-causing viruses, including rotavirus, adenovirus, norovirus, sapovirus, astrovirus, enterovirus, bocavirus, parechovirus, salivirus, and Aichivirus, using reverse transcription polymerase chain reaction (PCR), multiplex PCR, nested-PCR and nucleotide sequencing.

2.2. RNA extraction and reverse transcription

The viral genome was extracted from the supernatant of 10% fecal suspension in phosphate buffered saline solution according to the manufacturer's instructions using the viral nucleic acid extraction kit II (Geneaid, Taipei, Taiwan). The RNA genome was then reverse transcribed into cDNA using the Thermo Scientific RevertAid Firststrand cDNA synthesis kit (Thermo Scientific, USA) and stored at −80 °C.

2.3. Multiplex PCR and nested PCR

The cDNA was used as a template for PCR to detect both SAFV and HCoSV using a thermocycler machine (peqSTAR 96 universal, Isogen Life Science, Netherlands). The first round of PCR was multiplex and was for the screening of the SAFV and HCoSV 5′untranslated region (UTR) using a combination of previously published primers (Kapoor et al., 2008; Drexler et al., 2008). The primers DKV-N5U-F1 and DKVN5 U-R2 were used to detect HCoSV by amplifying a fragment size of 441 bp, whereas the primers CF188 and CR990 were used to detect SAFV by amplifying a fragment size of 800 bp. The thermal cycling conditions for multiplex PCR were as follows: 94 °C for 3 min followed by 35 cycles of 94 °C for 1 min, 55 °C for 1 min, 72 °C for 1 min and followed by a final extension at 72 °C for 10 min.

The second round of nested-PCR was then conducted separately for each virus. To detect SAFV, the primers CF204 and CR718 were used to amplify a 500 bp fragment. To detect HCoSV, the primers DKV-N5U-F2 and DKV-N5 U-R3 were used to amplify a 316 bp fragment. The thermal cycling conditions were the same as mentioned previously except for the annealing temperature for SAFV was 54 °C and the annealing temperature for HCoSV was 65 °C. All of the primer sequences are shown in Table 1.

2.4. Sequence and phylogenetic analysis

Amplification of HCoSV 5′UTR was achieved using the same primers and thermal cycling conditions as those for the HCoSV multiplex screening method mentioned above. The SAFV positive samples in this study were further analyzed via amplification of the partial viral protein 1 (VP1) via two rounds of nested-PCR using a combination of previously published primers (Itagaki et al., 2010). First round of amplification used the primers 315F and 738R and the second round of amplification used the primers 316F and 621R. The thermal cycling conditions for both rounds were as follows: 94 °C for 3 min followed by 40 cycles of 94 °C for 40 s, 50 °C for 40 s, 72 °C for 1 min, followed by a final extension at 72 °C for 10 min. All information for the primers used in amplification is shown in Table 1.

All positive PCR products were purified using Geneaid Gel/PCR kit protocol (Geneaid, Tapei, Taiwan) and subsequently sequenced (First Base Laboratories SDNBHD Selangor Darul Ehsan, Malaysia). The sequences of HCoSV and SAFV were each separately compared with reference strains available in the NCBI GenBank database, using the BLAST server (https://blast.ncbi.nlm.nih.gov/Blast.cgi). The selection of the reference sequences from the NCBI GenBank database was based on several criteria. Firstly, a few strains that were most closely related to our strain from BLAST results were selected as the reference strains. Secondly, the strains that were commonly used by other studies as the prototype strains for particular genotypes were also selected. Thirdly, the strains isolated from the same geographical area or from the countries in the same continent were included for our analysis. Multiple sequence alignment and sequence editing was performed by Clustal X (1.81) and Bioedit (v7.0.5.3). Phylogenetic trees were then constructed using the neighbour joining method via MEGA (v6.0) software (Tamura et al., 2013). The HCoSV phylogenetic tree was constructed using the maximum likelihood composite model with a bootstrap value of 1000. The SAFV phylogenetic tree was constructed using the Kimura 2-parameter model with a bootstrap value of 1000.

2.5. Accession numbers

The nucleotide sequences of HCoSV and SAFV described in this study have been deposited in the GenBank database under the accession

Table 1
Description of primers used in this study for the screening and genotyping of SAFV and HCoSV.

Virus	Primer Name	Primer nucleotide sequence	Orientation	Usage	Reference
HCoSV	DKV-N5 U-F1	CGTGCTTTACACGGTTTTTGA	+	Multiplex PCR 1st round	Kapoor et al., 2008
HCoSV	DKVN5 U-R2	GTACCTTCAGGACATCTTTGG	–	Multiplex PCR 1st round	Kapoor et al., 2008
SAFV	CF188	CTAATCAGAGGAAAGTCAGCAT	+	Multiplex PCR 1st round	Drexler et al., 2008
SAFV	CR990	GACCACTTGGTTTGGAGAAGCT	–	Multiplex PCR 1st round	Drexler et al., 2008
SAFV	CF204	CAGCATTTTCCGGCCCAGGCTAA	+	Nested PCR 2nd round	Drexler et al., 2008
SAFV	CR718	GCTATTGTGAGGTCGCTACAGCTGT	–	Nested PCR 2nd round	Drexler et al., 2008
HCoSV	DKV-N5 U-F2	ACGGTTTTTGAACCCCACAC	+	Nested PCR 2nd round	Kapoor et al., 2008
HCoSV	DKV-N5 U-R3	GTCCTTTCGGACAGGGCTTT	–	Nested PCR 2nd round	Kapoor et al., 2008
SAFV	315F	HAARCARGRRYTGGARYTTYNTNATGTT	+	Nested PCR 1st round	Itagaki et al., 2010
SAFV	738R	DDGBCKDGGRCARUAVACYCTCAT	–	Nested PCR 1st round	Itagaki et al., 2010
SAFV	316F	AARCARGRYTGGARYTTYDTHATGTTYTC	+	Nested PCR 2nd round	Itagaki et al., 2010
SAFV	621R	RRTRKKRAARTYNGMRDANCYRTTRAACCA	–	Nested PCR 2nd round	Itagaki et al., 2010