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BRIEF REPORT

Detection of diarrheal viruses circulating in adult patients in Thailand

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Abstract A total of 332 fecal specimens collected during January-December 2008 from adult patients with diarrhea were screened for group A and C rotaviruses, noroviruses GI and GII, sapovirus, Aichi virus, human parechovirus, enterovirus, adenovirus and astrovirus by RT-multiplex PCR. The detection rate for diarrheal viruses was 4.2 %. Adenovirus and enterovirus were equally detected as the most predominant viruses, with prevalence of 1.2 %, followed by Aichi virus (0.9 %) and norovirus GII (0.6 %). Mixed infection with norovirus GII and human parechovirus was also detected (0.3 %). This study provides epidemiological data for a wide variety of diarrheal viruses circulating in adult patients with diarrhea in Chiang Mai, Thailand.

Keywords Diarrheal viruses · Adult · Epidemiology · Thailand

Acute gastroenteritis (AGE) is one of the most common diseases in children and adults and continues to be a significant cause of morbidity and mortality worldwide. The most common etiology is diarrheal viruses. Among various types of diarrheal viruses, norovirus (NoV) and rotavirus (RV) are considered to be the major cause of diarrhea [3]. Moreover, associations with other viruses such as adenovirus (AdV), sapovirus (SaV) and astrovirus (AstV) have also been reported in sporadic and outbreak cases of diarrhea [7, 19, 26, 27]. NoV is now recognized as the main cause of epidemic gastroenteritis in all age groups [20]. Among adults and elderly patients, NoV is responsible for 4.4 to 8.7 % of AGE cases [10]. RV is more common in children less than 5 years of age. Studies conducted in adults with gastroenteritis in several countries from Europe, America, Asia, and Australia have demonstrated that the prevalence of RV ranges from 2 to 40 % [2, 6, 9, 24]. Enteric AdV can also cause AGE in adults, but at a lower rate than those by RV or NoV infections, ranging from 1.5 to 5.4 % [11, 17]. AstV has also been shown to associate with AGE, with a frequency ranging between 2 and 26 % [19]. For SaV, although it is known to cause diseases primarily in children, it has also recently been reported to affect young adults to the elderly [12]. In addition, there are several reports of newly discovered enteric viruses that are associated with AGE in humans, including Aichi virus (AiV), human parechovirus (HPeV), enterovirus (EV), and human cosavirus (HCoSV) [1, 5, 15, 23, 25].

In Thailand, there have been far fewer epidemiological studies of diarrheal viruses in adults than in children. Therefore, it is of interest to investigate the molecular epidemiology of diarrheal viruses in adults with diarrhea in Chiang Mai, Thailand.

A total of 332 fecal specimens were collected from adult patients with diarrhea, with the ages ranging from 15 to 90 years, who attended Chiang Mai University Hospital, Chiang Mai province, Thailand, during the period of January to December 2008. The specimens were stored at −20 °C until used. 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).

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Table 1 Oligonucleotide primers for detection of diarrheal viruses

Virus	Primer	Nucleotide sequence (5′-3′)	Position	Length (bp)	Reference
SaV	SLV5317	CTC GCC ACC TAC RAW GCB TGG TT	5124-5146	100	[30]
	SMP-R	CMW WCC CCT CCA TYT CAA ACA C	5202-5223		[14]
AiV	C94b	GAC TTC CCC GGA GTC GTCGTC T	6398-6419	158	[29]
	AiMP-R	GCR GAG AAT CCR CTC GTR CC	6536-6555		[14]
RVC	GCMP-F	CAA ATG ATT CAG AAT CTA TTG	500-520	205	[14]
	G8NA2	GTT TCT GTA CTA GCT GGT GAA	684-704		[31]
HPeV	Ev22(+)	CYC ACA CAG CCA TCC TC	312-328	270	[13]
	Ev22(−)	TRC GGG TAC CTT CTG GG	565-581		[13]
NoV GI	G1SKF	CTG CCC GAA TTY GTA AAT GA	5342-5361	330	[30]
	G1SKR	CCA ACC CAR CCA TTR TAC A	5653-5671		[30]
NoV GII	COG2F	CAR GAR BCN ATG TTY AGR TGG ATG AG	5003-5028	387	[30]
	G2SKR	CCR CCN GCA TRH CCR TTR TAC AT	5367-5389		[30]
EV	F1	CAA GCA CTT CTG TTT CCC CGG	160-180	440	[32]
	R1	ATT GTC ACC ATA AGC AGC CA	580-599		[32]
AdV	Ad1	TTC CCC ATG GCT CAY AAC AC	1834-1853	482	[31]
	Ad2	CCC TGG TAK CCR ATR TTG TA	2296-2315		[31]
RVA	VP7′(F)	AAA GGA TGG CCA ACA GGA TCA GT	373-395	569	[31]
	End 9 (s)	GTA TAR AAH ACT TGC CAC CAT	921-941		[14]
AstV	PreCAP1	GGA CTG CAA AGC AGC TTC GTG	4235-4255	719	[30]
	82b	GTG AGC CAC CAG CCA TCC CT	4934-4953		[30]

The viral genome was extracted from a 10 % fecal suspension using a Geneaid Viral Nucleic Acid Extraction Kit II (Geneaid, Taipei, Taiwan). Then, the specimens were tested for the presence of SaV, AiV, group A rotavirus (RVA), group C rotavirus (RVC), HPeV, NoV GI and GII, EV, AdV, and AstV by RT-multiplex PCR using the protocol described previously by Khamrin et al. [14]. Positive and negative controls were also concurrently included along with the test samples. The oligonucleotide primers for the detection of each virus are shown in Table 1.

The PCR products obtained from the specimens that were positive for diarrheal viruses were subjected to direct sequencing using a BigDye Terminator Cycle Sequencing Kit (Applied Biosystems, Foster City, CA, USA). The sequences obtained were compared with reference sequences by searching for closely related reference sequences in the NCBI GenBank database using the BLAST server (http://www.ncbi.nlm.nih.gov/blast). The nucleotide sequences of diarrheal viruses described in the present study have been deposited in the GenBank database. The accession numbers are as follows: KJ643239-KJ643242 for AdVs, KJ643243-KJ643246 for EVs, KJ643247 for HPeV, and KJ643248-KJ643250 for NoVs GII.

Screening by RT-multiplex PCR showed that 14 out of 332 (4.2 %) samples were positive for five types of diarrheal viruses. Among these, AdV, EV, AiV, NoV GII, and HPeV were detected, while RVA, RVC, SaV, NoV GI, and AstV were not found in this study. AdV and EV were detected as the most predominant viruses (1.2 %, 4 out of 332 for each virus), followed by AiV (0.9 %, 3 out of 332) and NoV GII (0.6 %, 2 out of 332). In addition, a mixed infection with NoV GII and HPeV was also detected in one fecal specimen (0.3 %), as shown in Table 2.

Based on nucleotide sequence analysis, all three NoV GII specimens detected in the present study belonged to the GII.4 genotype. For AdV, three different genotypes were identified, including AdV24, AdV25, and AdV40. In addition, four strains of EV found in this study belonged to two different species, *Enterovirus B* and *Enterovirus C*. Interestingly, AiV of both genotypes A and B were also detected in this surveillance. Furthermore, one HPeV strain detected in this study belonged to genotype 1 (Table 2).

In this study, 4.2 % of fecal specimens collected from adults with diarrhea were positive for diarrheal viruses. Five types of viruses out of a total of 10 were detected in this study. Monoinfections with one type of virus were found for AdV, EV, AiV, and NoV GII. In addition, a mixed infection with NoV GII and HPeV was found in one case, but the impact of the mixed infection on clinical severity was not determined for this patient. It had been reported previously that no significant difference was found in the clinical symptoms of patients with multiple

Table 2 Genotypes of diarrheal viruses in adult patients with diarrhea

Virus	No. of cases (%)	Genotype	Sample code	Date of collection	Accession no.
Adenovirus	4 (1.2)	AdV24	CMHA158/08/THA	April 18, 2008	KJ643240
		AdV25	CMHA009/08/THA	January 21, 2008	KJ643239
		AdV40	CMHA263/08/THA	May 12, 2008	KJ643241
		AdV40	CMHA599/08/THA	August 22, 2008	KJ643242
Enterovirus	4 (1.2)	EVB	CMHA059/08/THA	February 22, 2008	KJ643244
		EVC	CMHA042/08/THA	January 31, 2008	KJ643243
		EVC	CMHA136/08/THA	April 2, 2008	KJ643245
		EVC	CMHA414/08/THA	June 7, 2008	KJ643246
Aichi virus	3 (0.9)	A	CMHA135/08/THA	April 2, 2008	KF414962
		B	CMHA032/08/THA	January 28, 20008	KF414960
		B	CMHA317/08/THA	May 20, 2008	KF414961
Norovirus GII	2 (0.6)	GII.4	CMHA010/08/THA	January 21, 2008	KJ643248
		GII.4	CMHA049/08/THA	February 20, 2008	KJ643249
		GII.4	CMHA552/08/THA	August 7, 2008	KJ643250
Human parechovirus (mixed infection with NoV GII.4)	1 (0.3)	HPeV1	CMHA049/08/THA	February 20, 2008	KJ643247

viral infections as compared to monoinfection [8]. The prevalence of diarrheal viruses detected in the present study is consistent with the findings reported previously in all age groups in Thailand, where the prevalence was reported at 5 % [17].

AdV infection occurs worldwide and may involve several systems and organs, including the upper and lower respiratory tract, the gastrointestinal (GI) tract, the urinary tract, and the eyes [28]. The AdV types that commonly infect the GI tract, the so-called enteric adenoviruses, are AdV40 and AdV41 in subgroup F. Sequence analysis showed that adenovirus detected in this study belonged to two distinct species (D and F) with three genotypes (Ad24, Ad25, and Ad40). When the AdV sequences detected in this study were compared to those from previous studies, the data clearly demonstrated that the AdV genotypes identified in children and adults are different. The AdV strains found in adults were AdV24 and AdV25 of subgroup D and AdV40 of subgroup F, while the strains identified previously in children were AdV1 of subgroup D, AdV3 of subgroup B, and AdV41 of subgroup F [7]. It is interesting to note that in the present study we detected AdV24 and AdV25, in addition to AdV40, in adult patients with diarrhea. The AdV types 24 and 25 commonly infect the eyes, causing conjunctivitis and epidemic keratoconjunctivitis [28]. To our knowledge, this is the first report of AdV24 and AdV25 in adult patients with diarrhea in this area. In this study, the EV detection rate in adult patients was 1.2 %, which is somewhat lower than that reported previously for children in Thailand in 2007 (2.5 %) [4]. Nucleotide sequence analysis of four EV strains detected in this study revealed that they belonged to species B and C. For AiV, the prevalence in adults is similar to that in children, where the detection rate is as low as 0.9 %. Molecular genetic analysis of the only AiV strain detected previously in a child with diarrhea in Chiang Mai, Thailand, revealed that it was genotype A [21]. It is interesting, however, to note that both genotype A and B of AiVs were detected in the present study. These data clearly demonstrate that the AiV strains circulating in this area are genetically diverse. Several epidemiological reports of NoV infections have shown that NoV GII, particularly GII.4, is the most predominant genotype in all parts of the world. Most recently, surveillance of NoV in Thailand revealed that the prevalence of NoV infection in all age groups is as high as 44.7 %. NoV GII was shown to be the most predominant genotype and accounted for 64.8 % of cases [18]. However, the NoV GII detection rate in adults with diarrhea in the present study was as low as 0.9 %. It is possible that NoV GII is not the major pathogen causing diarrhea in adults in this area. In addition, this study clearly demonstrates that HPeV is an unusual cause of acute gastroenteritis in adults compared to other viruses. The prevalence of HPeV infection in Thailand has only been reported in children with acute gastroenteritis at an infection rate of 14.6 % in 2005 [22] and 6.1 % in 2009-2011 [5]. The HPeV genotype found in adults in this study is genotype 1, which was the predominant genotype detected in diarrheal children. However, HPeV genotypes identified in children with diarrhea were highly diverse, including HPeV 1-6, 10, and 14 [5, 22]. It should be pointed out that rotavirus, which is the most important cause of diarrhea in

pediatric patients, was not detected in adult diarrheic patients in the present study. The low prevalence and lack of detection of rotavirus infection in adult patients observed in this study might be due to immunity to rotavirus resulting from natural infection.

The relatively low rate of diarrheal virus detection in adults with diarrhea in this study suggests that acute gastroenteritis in adults in this area may be caused by other pathogens. Further investigation for bacterial or parasitic infections may help to clarify this point. Nevertheless, several other diarrheal viruses that may cause diarrhea, including Saffold virus, pestivirus, coronavirus, picobirnavirus, and torovirus [16, 27], were not included in the screening protocol in this study. In addition, the sensitivity limit of the multiplex PCR method or low amount of target viruses may also affect the detection rate.

In conclusion, this study demonstrates that a wide variety of viral pathogens that are associated with diarrhea are circulating in adult patients in Chiang Mai, Thailand. Since epidemiological information about gastroenteritis viruses in adults is limited, it is important to continue further surveillance, which may provide a better understanding of the whole picture of gastroenteritis virus epidemiology in the adult population.

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Conflict of interest The authors declare that they have no conflict of interest.

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