Molecular Characterization of Rotaviruses, Noroviruses, Sapovirus, and Adenoviruses in Patients With Acute Gastroenteritis in Thailand

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Outbreaks of viral gastroenteritis occur worldwide including Thailand. Unfortunately, there is limited information since etiologic agents have not been identified in several outbreaks of non-bacterial gastroenteritis. The genotype of enteric viruses causing acute gastroenteritis in Thailand was determined using reverse transcription-multiplex polymerase chain reaction and DNA sequencing. From January 2006 to February 2007, stool samples were collected from patients with acute gastroenteritis of all age groups attending a hospital in Thailand, and patients with non-bacterial acute gastroenteritis (262 patients) were tested for enteric viruses. The overall positive detection rate of enteric viruses was 14.9%; group A rotaviruses (6.1%), noroviruses (6.5%): GI (0.8%) and GII (5.7%), adenoviruses (1.5%), and sapoviruses (0.8%) were found. Group B and C rotaviruses, and astroviruses were not detected in the enrolled patients. Viral acute gastroenteritis occurred in children less than 15 years of age (25.2%, 33/131) with higher frequency than in adults (4.6%, 6/131), P-value <0.001. Rotavirus G1 was the most predominant genotype, followed by G3, and G9. Among noroviruses, GI-2 was identified; whereas, GI was predominant with a high frequency of GI-4 observed, followed by GI-16, GI-2, GI-3, and GI-12. Sapovirus GI-3 and human adenoviruses were identified. This study suggests that enteric viruses play an essential role in patients with acute gastroenteritis attending hospital and mainly in children who have a higher prevalence of group A rotaviruses and noroviruses. The genetic analyses provide molecular epidemiological data for viruses important to public health. J. Med. Virol. 81:345–353, 2009. © 2008 Wiley-Liss, Inc.

KEY WORDS: rotaviruses; noroviruses; sapoviruses; adenoviruses; acute gastroenteritis; Thailand

INTRODUCTION

Acute gastroenteritis or acute diarrhea is one of the most significant diseases causing morbidity and mortality worldwide [Clark and McKendrick, 2004]. In Thailand, acute diarrhea is the primary cause of morbidity among diseases documented in the annual report of epidemiological surveillance [http://epid.moph.go.th]. Based on routine bacterial cultures of stool samples, only a few patients have been identified in the past with an underlying viral cause. However, a large proportion of patients with gastroenteritis were not identified with enteric viruses because virus identification has not been undertaken routinely in the country. Using improved molecular techniques, the etiologic role of viruses causing acute gastroenteritis has been established in various outbreaks [Koopmans, 2005; Siebenga et al., 2007; Svraka et al., 2007] and in studies on hospitalized children [Chen et al., 2007; Fabiana et al., 2007]. Enteric viruses that have been reported as a cause of nonbacterial acute gastroenteritis include group A rotaviruses, noroviruses, sapoviruses, astroviruses and enteric adenoviruses.

Rotaviruses (RVs) are members of the Reoviridae family, which consists of seven groups (A through G). Group A rotavirus causes acute diarrhea and is most commonly found among infants and young children [Glass et al., 2006]. Group B and C rotaviruses, however, infect children to a lesser extent [Phan et al., 2004; Barman et al., 2006]. RV is a nonenveloped virus containing 11 segments of double-stranded RNA [Estes...
and Kapikian, 2007]. There are 19G genotypes and 27P genotypes in RVs recovered from humans and animals [Matthijnssens et al., 2008]. The most prevalent human RVs are G1P[8], G2P[4], G3P[8], G4P[8], and G9P[8] [Santos and Hoshino, 2005]. Noroviruses (NoVs), which belong to the genus Norovirus in the family Caliciviridae, are the most common viral cause of acute gastroenteritis in all ages but are known to have more severity in young children and the elderly [Estes et al., 2006]. NoVs are nonenveloped, positive-sense, single-stranded RNA viruses and are classified into five distinct genogroups. Human NoV strains have been found in genogroups I (GI), II (GII), and IV (GIV) and are subdivided further into at least 8 genotypes for GI, and 19 genotypes for GII [Zheng et al., 2006]. Sapoviruses (SaVs) are members in the genus Sapovirus within the same Caliciviridae family as NoVs. Among those admitted to hospital, acute diarrhea caused by SaVs is more widespread in infants and young children. SaVs have a lower detection rate than NoVs [Hansman et al., 2004b; Khamrin et al., 2007a]. Human astroviruses (AsVs) belong to the Astroviridae family. The virion is nonenveloped and contains a positive single-stranded RNA genome [Mendez and Arias, 2007]. Besides rotaviruses and caliciviruses, AsVs are one of the major causes of acute gastroenteritis within young children and the elderly [Guix et al., 2005]. Human adenoviruses (AdVs) are only one DNA virus among other enteric RNA viruses and members of the family Adenoviridae, which consists of six subgenera (A through F). AdVs subgenus F are divided into two serotypes (AdV40 and AdV41) both of which are associated with diarrhea in children [Fabiana et al., 2007].

Rotaviruses and noroviruses are important enteric viruses in Thailand because they are often causes of acute gastroenteritis in infants and children admitted to hospital. In two studies, RVs were reported to be associated with approximately 37–43% of children with acute gastroenteritis admitted to hospital [Jiraphongsa et al., 2005; Khamrin et al., 2007c]. In other studies, NoVs were detected in hospitalized infants and children at a rate of 8.6–14.1% [Guntapong et al., 2004; Hansman et al., 2004b; Khamrin et al., 2007a]. Nevertheless, genetic analysis of the enteric viruses causing acute gastroenteritis has not been studied extensively. This particular study was carried out to determine the presence of gastroenteritis viruses in patients of all age groups attending hospital with acute gastroenteritis. The study used reverse transcription-multiplex polymerase chain reaction (RT-multiplex PCR) for screening and DNA sequencing for confirmation. Molecular characterization of the enteric viruses found in the stool samples of patients with acute gastroenteritis is included.

**MATERIALS AND METHODS**

**Stool Samples**

A total of 273 stool samples were collected from patients attending Lopburi Hospital, Lopburi Province, Thailand, and who had a clinical diagnosis of acute gastroenteritis or acute diarrhea. The study period was from January 2006 to February 2007, a 14-month period. The patients enrolled in this study had watery diarrhea for less than 7 days and one or more of the following symptoms; nausea, vomiting, abdominal cramps, headache, muscle pain and/or fever (≥38.0°C). Written informed consent was obtained from each patient or, if the patient was a child, their parent. The study was conducted with the approval of the Ethical Committee for Human Rights Related to Human Experimentation, Mahidol University, and of Lopburi Hospital. Stool samples were tested routinely for pathogenic bacteria, that is, Salmonella, Shigella, and Vibrio species. All stool samples were diluted in a ratio of 1:3 in 0.05 M phosphate-buffered saline, at pH 7.2. The suspensions were centrifuged at 500g for 15 min and the supernatant was stored at −70°C. Samples were sent to the laboratory and diluted 1:10 prior to RNA extraction.

**RNA Extraction and Reverse Transcription-Multiplex Polymerase Chain Reaction (RT-Multiplex PCR)**

Viral RNA was extracted from 140 μl of diluted supernatant (1:10) using a QIAamp Viral RNA Mini kit (QIAGEN GmbH, Hilden, Germany) following the manufacturer’s instructions. Screening for the presence of RV groups A, B, and C, NoV GI and GII, SaV, AsV, and AdV in the stool specimens was conducted using RT-multiplex PCR. Two sets of primers were used: set A, including the primers for the detection of RV groups A, B, C, and AdV; and set B, primers for the detection of NoV GI, GII, SaV, and AsV. The RT-multiplex PCR was performed according to the methods described previously by Yan et al. [2003, 2004].

Briefly, complementary DNA (cDNA) was prepared by adding 5 μl of RNA to 5 μl of random hexamers (50 ng/μl), 10 mM dNTP mix and DEPC-treated water (Invitrogen, Carlsbad, CA) and this was followed by incubation at 65°C for 5 min. The mixture was put on ice for 5 min. RT mixture (10 μl) containing 1 × RT buffer (20 mM Tris–HCl, pH 8.4, 50 mM KCl), 5 mM MgCl2, 1 mM of each dNTP, 0.1 M DTT, Rnase OUT™ (40 Units/μl), and Superscript® III RT (200 Units/μl), was then added to the sample. RT was carried out at 25°C for 10 min, which was followed by 50°C for 5 min. The reaction was terminated by incubation at 85°C for 5 min, and then chilling the mixture on ice. cDNA was centrifuged briefly, 1 μl of RNaseH was added and the resultant mixture was incubated at 37°C for 20 min.

In the multiplex PCR assay, 5 μl of cDNA was added to 20 μl of PCR mixture containing 1 × Taq DNA polymerase buffer (20 mM Tris–HCl, pH 8.4, 50 mM KCl), 200 μM dNTP, and 33 μM each of specific primers, 5 U/μl of Taq DNA polymerase, and nuclelease free water. Two specific primer sets were added in separate tubes. Set A contained Beg 9, VP7-1 [group A RV]; ADG9-1F, ADG9-1R [group B RV]; G8NS1, G8NS2 [group C RV];...
and Ad1, Ad2 [AdV]. While set B contained G1-SKF, G1-SKR [NoV GI]; COG2F, G2-SKR [NoV GII]; SLV 5317, SLV 5749 [SaV]; and PreCAP1, 82b [human AsV]. PCR was performed at 94°C for 3 min, followed by 35 cycles of 94°C for 1 min, 50°C for 1 min, 72°C for 1 min; and a final extension at 72°C for 3 min.

Monoplex PCR was also carried out for confirmation of virus-positive stool samples detected by RT-multiplex PCR using the procedure for RT-multiplex PCR but with only one pair of primers specific to each virus.

PCR products were electrophoresed in a 1.5% agarose gel, followed by staining with ethidium bromide (0.5 μg/ml) for 30 min, then visualized under ultraviolet light of a transilluminator. The results were recorded by photography. Specific DNA bands that corresponded to the product size for enteric viruses included group A RV (396 bp), group B RV (814 bp), group C RV (351 bp), AdV (462 bp), NoV GI (330 bp), NoV GII (387 bp), SaV (395 bp), and AsV (719 bp).

**DNA Sequencing and Phylogenetic Analysis**

DNA products were purified using the QIAquick PCR purification kit (QIAGEN GmbH, Hilden, Germany) and sequenced at the Bioservice Unit of the National Science and Technology Development Agency, Bangkok. The nucleotide sequences were compared with those of RV, NoV, SaV, and AdV strains deposited in the NCBI database using the BLAST (Basic Local Alignment Search Tool) program [Altschul et al., 1990]. Phylogenetic relationships of RV, NoV, and SaV were examined by aligning sequences with the ClustalX program. A phylogenetic tree was constructed according to the neighbor-joining method using MEGA version 3.1 [Kumar et al., 2004].

**Nucleotide Sequence Accession Numbers**

The nucleotide sequences of the study strains have been deposited at GenBank (accession nos., EU603407-EU603438 for RVs, EU603421 for NoV GI, EU603422-EU603436 for NoVs GII, EU603437 for SaV, and EU603438-EU603441 for AdVs).

**RESULTS**

**Characteristics of Patients**

From January 2006 to February 2007, a total of 273 patients with acute gastroenteritis or acute diarrhea were enrolled in this study. One hundred and ninety eight (72.5%) and 75 (27.5%) cases attended the Inpatient Department and Outpatient Department in Lopburi Hospital, respectively. The ratio of patients attending the Inpatient Department to Outpatient Department was 2.6:1. The ratio of males to females was 1.1:1. The median age was 12.5 years old (range 2 months–86 years old).

**Virological Testing**

In this study, enteropathogenic bacteria were identified in 11 patients and the samples from these patients were therefore excluded from the analysis. Among the 262 patients with nonbacterial acute gastroenteritis, 39 (14.9%) cases of enteric viruses were detected by RT-multiplex PCR and confirmed by RT-monoplex PCR. These enteric viruses included group A RVs (6.1%), NoVs (6.5%); GI (0.8%) and GII (5.7%), SaVs (0.8%) and AdVs (1.5%), as shown in Table I. After DNA amplification and gel electrophoresis with ethidium bromide staining, positive DNA bands were shown at 395 bp for group A RV, 330 bp for NoV GI, 387 bp for NoV GII, 434 bp for SaV, and 462 bp for AdV (Fig. 1). Group B RV, group C RV and AsV were not detected. The ratio of patients with viral gastroenteritis attending the Inpatient Department to Outpatient Department was 12:1. The patients with acute gastroenteritis in the age group 0–4 years had the highest frequency of viral infection. The median ages of patients infected with RVs and NoVs GII were 18 months (range 2 months–48 years) and 22 months (range 4 months–59 years), respectively.

### Table I. Enteric Viruses in Patients With Acute Gastroenteritis Detected Using RT-Multiplex PCR

<table>
<thead>
<tr>
<th>Patients</th>
<th>Nonbacterial gastroenteritis (%)</th>
<th>Rotavirus (%)</th>
<th>Norovirus</th>
<th>Sapovirus (%)</th>
<th>Adenovirus (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age group (years)</td>
<td>No.</td>
<td>GI (%)</td>
<td>GII (%)</td>
<td>No.</td>
<td>GI (%)</td>
</tr>
<tr>
<td>0–4</td>
<td>106</td>
<td>30 (28.3)</td>
<td>12 (11.3)</td>
<td>2</td>
<td>0.8</td>
</tr>
<tr>
<td>5–9</td>
<td>16</td>
<td>3 (18.8)</td>
<td>2 (12.5)</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>10–14</td>
<td>9</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>15–30</td>
<td>10</td>
<td>6 (50)</td>
<td>2 (17.5)</td>
<td>1</td>
<td>0.8</td>
</tr>
<tr>
<td>&gt;30</td>
<td>121</td>
<td>1.3</td>
<td>3.1</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>Male:female</td>
<td>1:1</td>
<td>1.3:1</td>
<td>3:1</td>
<td>All female</td>
<td>0.7:1</td>
</tr>
<tr>
<td>Days after onset</td>
<td>1–3</td>
<td>206</td>
<td>21 (10.2)</td>
<td>5 (2.4)</td>
<td>1</td>
</tr>
<tr>
<td>4–6</td>
<td>51</td>
<td>16 (31.4)</td>
<td>9 (17.6)</td>
<td>1</td>
<td>2.0</td>
</tr>
<tr>
<td>7–9</td>
<td>4</td>
<td>2 (50)</td>
<td>2 (50)</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>&gt;9</td>
<td>1</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>—</td>
</tr>
</tbody>
</table>

*Percentage of virus-infected patients in all nonbacterial gastroenteritis patients in each row.*
Infections with NoVs GI were found in one child aged 4 years and one adult aged 47 years. SaV infections occurred in children at the age of 6 and 8; whereas, all patients with AdV infections were less than 5 years old. Analysis by the Chi-square test was used to compare the two variables, the results showed that viral acute gastroenteritis occurred in children less than 15 years of age (33/131, 25.2%) with higher frequency than in adults (6/131, 4.6%) at P-value < 0.001. Viral gastro-enteritis occurred in males at a greater rate than in females. RV infection was observed in males at a rate three times greater than in females but for NoV GII, females were infected at a greater rate than males. The stool samples collected from the patients with acute gastroenteritis on days 1–3 after onset of illness were positive for RVs, NoVs, SaVs, and AdVs. Group A RVs were still detected in the stool samples collected on days 7–9 (Table I).

Season

Patients with rotaviral diarrhea occurred in the winter season, from January to March. A monthly analysis of cases with acute gastroenteritis, in order to determine the proportion of virus-infected patients, revealed a peak of RV infection in January. The patients infected with NoVs GI were found in October, while the patients with NoVs GII appeared between February and March and between August and November; the peak NoV GII infections was August and September. All patients with acute gastroenteritis caused by SaVs or AdVs were diagnosed in the early summer months (March and April), data not shown.

Genetic Analysis

Based on screening using RT-multiplex PCR, 16 group A RV (6.1%) cases were detected in 262 stool samples. Fourteen DNA products (87.5%) of the RVs detected were subjected to DNA sequencing of the partial VP7 capsid region. The genotypes of RVs were classified according to the phylogenetic analysis proposed by Khamrin et al. [2007b]. DNA amplification products were associated with RV reference strains and belonged to RVs G1 (11 samples), G3 (2 samples), and G9 (1 sample), as shown in Figure 2 and Table II. It was found that in this study G1 was the most predominant genotype, followed by G3 and G9. RVs G1 were only found in adults with acute gastroenteritis. Using the BLAST program and phylogenetic analysis, one sample positive for RV G1 showed 98% nucleotide sequence identity with human RV G1 isolate Chi-87 (DQ512998) within the same cluster as the reference KU strain; whereas, nearly all other RVs G1 showed 98–99% identity with rotavirus A strain 7265/JP (EP079066) within the same cluster as reference AU007 strain.

Fig. 1. Detection of enteric viruses in stool samples. Lanes: M, DNA marker (100-bp DNA Ladder); 1, adenovirus-positive sample (No. 057); 2, rotavirus-positive sample (No. 048); 3–9, virus-negative stool samples; 10, norovirus GI-positive sample (No. 105); 11, norovirus GII-positive sample (No. 085); 12, sapovirus-positive sample (No. 039). Gel electrophoresis of the RT-multiplex PCR products of adenovirus showed 462 bp; group A rotavirus, 395 bp; norovirus GI, 330 bp; norovirus GII, 387 bp; and sapovirus, 434 bp.

Fig. 2. Phylogenetic analysis of partial VP7 nucleotide sequences of group A rotavirus detected in patients with acute gastroenteritis (Lopburi002-069/2006/THA). The GenBank accession numbers for 15 known G genotypes include: G1; KU (D16343), AU007 (AB081799), G2; S2 (M11164), G3; P (AB118024), RV (AF293503), G4; Hochi (AB012078), G5; OSU (X04613), G6; NCDV (M12394), G7; CH2 (X56784), G8; B37 (J04334), G9; CH3574 (AY211068), 116E (L14072), G10; 61A (X53403), G11; YM (M23194), G12; L26 (M58290), G13; L338 (D13549), G14; CH3 (D25229), and G15; Hg18 (AF237666). The tree was generated based on the neighbor-joining method and the numbers on each branch indicate the bootstrap values.

Seventeen NoVs (6.5%) were detected in 262 stool samples; GI appeared in 0.8% of the samples (two NoVs GI) and GII appeared in 5.7% of the samples (15 NoVs GII). DNA products were sequenced and identified by phylogenetic analysis of the partial capsid region according to the phylogenetic clustering method described by Kageyama et al. [2004]. Only one sample of a DNA product from NoV GI was available for nucleic acid sequencing. Using the BLAST program and phylogenetic analysis, the DNA sequence showed 97% nucleotide sequence identity with human calicivirus SRSV/MI1/94/JP (AB005259), within the same cluster as the reference Southampton strain. This sequence belonged to GI-2 (Fig. 3 and Table II). A total of 15 NoVs GII were classified further into five genotypes: GII-2 (one sample), GII-3 (one sample), GII-4 (nine samples), GII-12 (one sample), and GII-16 (three samples), as shown in Figure 4 and Table II. The most common genotype was NoV GII-4. Two of these NoV GII-4 strains exhibited 99–100% nucleotide identity with NoV Hu/GII.4/Terneuzen70/2006/NL (EF126964); seven of the samples had 99–100% nucleotide identity with NoV Hu/GII.4/Nijmegen115/2006/NL (EF126966). NoV GII-16 was the second most frequent genotype found in this study. The distribution and genetic diversity of NoV GII genotype was observed in all ages.

Of 262 stool samples, two (0.8%) were positive for SaVs, as determined by RT-multiplex PCR and one DNA product was sequenced on the partial capsid region. The genotype was classified according to the clustering method mentioned by Phan et al. [2007b]. Using the BLAST program and phylogenetic analysis, this DNA product showed 94% nucleotide sequence identity with the cruise ship reference strain and classified into GII-3 (Fig. 5 and Table II). Four stool samples (1.5%) were positive for AdVs and all amplified DNA products specific to the hexon gene were analyzed by DNA sequencing. Using the BLAST program, two samples showed 96% and 97% nucleotide sequence identity with human adenovirus type 41 Tak prototype strain (X51783.1). The other two samples showed 96% nucleotide sequence identity with human adenovirus type 2 (EU128938.1) and 97% identity with human adenovirus type 38 (DQ149633.1).

**DISCUSSION**

Outbreaks of gastroenteritis caused by enteric viruses have been recognized and reported from all over the world. The disease burden and severity of viral acute gastroenteritis in Thailand has not been studied extensively since methods for detection of these viruses...
have not been used routinely in a hospital setting. In this study, the RT-multiplex PCR method has been used to investigate the role of enteric viruses in acute gastroenteritis in all age groups attending a hospital in Thailand. Identification and genetic analysis were undertaken by molecular technique.

Among 273 stool samples, enteropathogenic bacteria were identified only in 11 cases since most of the patients had been treated with antibiotics prior to attending the hospital. During the 14-month period study, 14.9% (39/262 patients) were detected with enteric viruses, using the RT-multiplex PCR. NoVs are the etiologic agents of acute gastroenteritis in people of all age groups but group A RVs, SaVs, AsVs, and AdVs are the cause of the infection mainly in children or the elderly [Koopmans, 2005]. The most common viruses found in the patients were NoVs as well as RVs, followed by AdVs and SaVs. Among NoV-infected patients, NoV GII was predominant. This finding is consistent with that reported previously [Hansman et al., 2004a,b; Fabiana et al., 2007; Nguyen et al., 2007; Papaventsis et al., 2007; Siebenga et al., 2007] including Thailand [Guntapong et al., 2004; Khamrin et al., 2007a]. However, the difference in NoV and SaV detection rates between this study and previous studies can be explained by the selection criteria for patients enrolled in this study. In this study, the patients enrolled were from all age groups, as opposed to the previous studies which enrolled children. Statistical significance indicates the enteric viruses caused acute gastroenteritis mainly in children less than 15 years old, as opposed to in adults.

The seasonal distribution of RV infection demonstrated a peak in January and occurred continually through March; this finding is consistent with the study.

Fig. 3. Phylogenetic analysis of partial capsid sequence of norovirus GI detected in a patient with acute gastroenteritis (Lopburi105/2006/THA). The GenBank accession numbers for 15 known GI strains include: GI-1; Norwalk (M87661), GI-2; Southampton (L07418), GI-3; DesertShield (U04469), GI-4; Chiba047 (AB042808), GI-5; Musgrove (AJ277614), GI-6; BS-5 (AF093797), GI-7; Winchester (AJ277609), GI-8; WUG1 (AB081723), GI-9; Saitama SuU5G1 (AB039774), GI-10; Boxer (AF538679), GI-11; Saitama KU8G1 (AB058547), GI-12; Saitama KU9G1 (AB058525), GI-13; Saitama T35aGI (AB112132), GI-14; Saitama T25aGI (AB112100), GI-15; Chiba030100/2003 (AJ844469), and for GI-1; Hawaii (U07611) as outgroup. The tree was generated based on the neighbor-joining method and the numbers on each branch indicate the bootstrap values.

Fig. 4. Phylogenetic analysis of partial capsid sequences of norovirus GII detected in patients with acute gastroenteritis (Lopburi020-159/2006/THA). The GenBank accession numbers for 18 known GII strains include: GII-1; Hawaii (U07611), GII-2; Merksham (X81879), GII-3; Saitama U201 (AB067342), GII-4; Bristol (X76716), GII-5; Hillington (AJ277607), GII-6; Saitama U3 (AB039776), GII-7; Leeds (AJ277608), GII-8; Saitama U3 (AB067543), GII-9; Idaho Fall (AY004299), GII-10; Mc37 (AY237415), GII-11; Saitama T29GII (AB112221), GII-12; Saitama U1 (AB039775), GII-13; MT (AY130761), GII-14; Kashiwa47 (AB078334), GII-15; Saitama KU80aGII (AB058582), GII-16; Saitama T53GII (AB112260), GII-17; Alphatron (AF189547), GII-18; Chiba/040502/2004 (AJ844470), and for GI-1; Norwalk (M87661) as outgroup. The tree was generated based on the neighbor-joining method and the numbers on each branch indicate the bootstrap values.

by Jiraphongsa et al. [2005]. NoV GI infection took place in October and NoV GII had a peak in August and September, which was earlier than the peak of RV infection, which could be found throughout the year. SaV and AdV infections, however, were only observed in children and in early summer. The seasonal pattern should be interpreted carefully since this study has some limitations regarding the collection of stool samples; the distribution of gastroenteritis patients in age groups and in each month is very different and a number of limitations regarding the collection of stool samples; the distribution of gastroenteritis patients in age groups and in each month is very different and so the presence of human RV genotypes circulating in gastroenteritis patients and bivalve shellfish. Therefore, shellfish is likely to be one of the potential vehicles for rotavirus transmission as well as environmental water, as reported by Kittigul et al. [2005].

The predominance of NoVs GII-4 in this study (60%) is similar to other studies in Thailand [Guntapong et al., 2004; Khamrin et al., 2007a]. NoV GII-4 strains cause 80% of gastroenteritis outbreaks and emergence of the GII-4 variants in The Netherlands has been reported [Siebenga et al., 2007]. In 2006, the emergence of two GII-4 variants (2006a and 2006b) were reported worldwide as having caused outbreaks of gastroenteritis in cruise ships [Koopmans et al., 2006], and healthcare settings [Buesa et al., 2008]. NoV 2006a and 2006b have been implicated as the etiologic agents in acute gastroenteritis in Europe and also in Australia and New Zealand [Tu et al., 2008]. The present study carried out during the year 2006–2007, identified both NoV GII-4 strains; two samples similar to NoV Hu/GII.4/Terneuzen70/2006/NL (2006a variant) and seven samples similar to NoV Hu/GII.4/Nijmegen115/2006/NL (2006b variant). Therefore, these NoV GII-4 strains might be associated with 2006a and 2006b variants, respectively. Further characterization of the viruses would provide information of outbreak strains circulating globally. Using the screening RT-multiplex PCR, NoVs GI were identified in one child (4 years old) and one adult (47 years old). The child infected with NoV GI had the GI-2 genotype. NoVs GI were detected in the year 2000–2001 [Hansman et al., 2004b], but disappeared during 2002–2004 [Khamrin et al., 2007b] and reemerged in this study (year 2006–2007); two NoV GI-positive stool samples were collected in October 2006. NoVs GI in patients with acute gastroenteritis are detected rarely in Japan [Yan et al., 2003] or Vietnam [Hansman et al., 2004].

8. AdV type 41 is the predominant serotype in Asian countries and associated with acute gastroenteritis in infants and children [Li et al., 2005].

In this study, RV G1 strains were the most prevalent, followed by G3 and G9. RV G9 strains were predominant in Thailand during the year 2001–2003 [Jiraphongsa et al., 2005] and declined with a reemergence of G1 and G2 during 2002–2004 [Khamrin et al., 2007c]. In 2006, RV G1 strains were found to be predominant both in this study and in other countries such as Australia [Kirkwood et al., 2007], Germany [Mas Marques et al., 2007], and Japan [Phan et al., 2007a]. One RV G1 strain showed 98% nucleotide sequence identity with human RV G1 (Chi-87), and belonged to lineage III [Trinh et al., 2007]. Other RV G1 strains showed 98–99% identity with rotavirus A strain (7265/JP) which was found in Japan during 2005–2006 and belonging to lineage II; sublineage IIC [Phan et al., 2007a].

Interestingly, a stool sample was obtained from an infant, with a RV G9 associated with a nucleotide sequence that was a close match to the reference strain GH 3574 (AY 211068). This was in accord with a previous report on the detection of group A RV in raw oysters [Kittigul et al., 2008]. These findings confirmed the presence of human RV genotypes circulating in gastroenteritis patients and bivalve shellfish. Therefore, shellfish is likely to be one of the potential vehicles for rotavirus transmission as well as environmental water, as reported by Kittigul et al. [2005].

Further characterization of the viruses would provide information of outbreak strains circulating globally. Using the screening RT-multiplex PCR, NoVs GI were identified in one child (4 years old) and one adult (47 years old). The child infected with NoV GI had the GI-2 genotype. NoVs GI were detected in the year 2000–2001 [Hansman et al., 2004b], but disappeared during 2002–2004 [Khamrin et al., 2007b] and reemerged in this study (year 2006–2007); two NoV GI-positive stool samples were collected in October 2006. NoVs GI in patients with acute gastroenteritis are detected rarely in Japan [Yan et al., 2003] or Vietnam [Hansman et al., 2004].
2004a; Nguyen et al., 2007). In Thailand, NoVs are circulating in humans, oysters, and environmental water, as with RVs. Of note, NoV GI is predominant genogroup in the environment (manuscript in preparation). Further studies on the ecology of NoVs GI and GII need to be elucidated.

SaVs were found at a lower frequency than NoVs. One SaV strain belonged to GI-3, with an almost 100% nucleotide sequence identity with the cruise ship reference strain. Two previous studies have demonstrated the presence of SaV GI/1, GI/2, GIV [Khamrin et al., 2007a] and GI, GII, GV strains [Hansman et al., 2004b] with genetic diversity. The differences in SaV genotypes circulating depend on the different study sites.

The hospital-based study reported above demonstrate the importance of enteric viruses as causes of gastroenteritis in Thailand. An accurate diagnosis of acute gastroenteritis would facilitate appropriate management of patients and reduce excessive or unnecessary use of antibiotics for treatment. The use of a short conserved sequence is valuable for diagnosis of enteric virus infections, however, genotyping and phylogenetic analyses using the short sequence should be determined with caution. In conclusion, by screening acute gastroenteritis using molecular methods including RT-multi-plex PCR and confirmation by DNA sequencing, an etiologic identification and genetic characterization of enteric viruses can be achieved, leading to a comprehensive investigation of the disease burden caused by RVs, NoVs, SaVs, and AdVs.

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