

Short Communication

Norovirus and Sapovirus Infections in Thailand

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SUMMARY: Stool specimens collected between November 2002 and April 2003 from hospitalized infants with acute gastroenteritis from four distinct geographical regions in Thailand were examined for norovirus (NoV) and sapovirus (SaV) by reverse transcription-PCR and sequence analysis. Of the 80 specimens examined, we identified 11 NoV and 9 SaV single infections, and 3 NoV/SaV mixed infections. The majority of NoV strains (64%) belonged to genogroup II/genotype 4 (GII/4; Lordsdale cluster). Other NoV strains co-circulating belonged to GII/1, GII/3, GII/6, and one new genotype cluster (GII/New). The majority of SaV strains (83%) were from the Manchester cluster. One isolated SaV strain represented a recently discovered novel genogroup within the SaV genus (SG-V), and another isolated SaV strain represented a novel SaV genogroup II cluster.

Norovirus (NoV) is the most important cause of outbreaks of gastroenteritis in the United States, infecting all age groups (1). Human NoVs have been divided into two genogroups, genogroup I (GI) and genogroup II (GII). A recent study indicated that NoV GI and GII strains consist of 14 and 17 genotypes, respectively (2). Sapovirus (SaV) is also a causative agent of gastroenteritis, though more frequent in young children than in adults (3). SaVs can be divided into four genogroups (SG-I to SG-IV) (4), though only SG-I, SG-II, and SG-IV are known to infect humans, whereas SG-III affects pigs. Human SaV genogroups tentatively comprise four SG-I clusters, three SG-II clusters, and one SG-IV cluster (4). Other viruses causing gastroenteritis include rotavirus, astrovirus, and enteric adenovirus. These viruses can be transmitted by the fecal-oral route through person-to-person contact, and food- and water-borne infections (5-7).

We recently reported the genetic diversity of NoV and SaV in hospitalized infants in the Northeastern region of Thailand (8) and identified a diversity of NoV and SaV strains, including one strain that belonged to a new NoV genotype (GII/10).

In this study, 80 of 321 stool specimens from hospitalized infants with sporadic cases of acute gastroenteritis, all of which had previously been found negative for rotavirus, astrovirus, enteric adenovirus, and bacterial agents (unpublished data), were examined for NoV and SaV using RT-PCR and sequence analysis (Table 1). Specimens were collected between November 2002 and April 2003 from four distinct geographical regions in Thailand: Northern, Tak province; Northeastern, Nong Khai province; Central, Sa Kaeo and Chanthaburi provinces; and Southern, Songkhla province. RNA was extracted and purified as described elsewhere (8). The purified RNA (12.5 μ l) was added to 2.5

μ l of the reaction mixture containing DNase I buffer and 1 unit of RQ1 DNase (Promega, Madison, Wis., USA). This reaction mixture was incubated for 30 min at 37°C, then for 5 min at 75°C to inactivate RQ1 DNase. The reaction mixture was then added to 15 μ l of a mixture containing 100 mM Tris-HCl (pH 8.3), 150 mM KCl, 6 mM MgCl₂, 1 mM of each dNTPs, 10 mM DTT, 75 pmol of random hexamers (pdN6; Amersham, Life Science, Buckinghamshire, England), 30 units of RNase inhibitor (Promega) and 200 units of SuperScript II RNaseH (-) reverse transcriptase (Invitrogen, Carlsbad, Calif., USA). Reverse transcription was performed for 1 h at 37°C, and inactivation of the enzyme was performed for 5 min at 94°C. The cDNA was kept at -20°C until used in PCR. The NoV PCR was carried out according to the method described by Kojima et al. (9). For NoV GI, G1SKF and G1SKR primers were used. For NoV GII, G2SKF and G2SKR primers were used. The SaV PCR was performed according to the method described by Okada et al. (4). For the first PCR, SV-F11 and SV-R1 primers were used. For the nested PCR, SV-F21 and SV-R2 primers were used. The PCR products were analyzed with 2% agarose gel electrophoresis and visualized by ethidium bromide staining. The PCR-generated amplicons of either the first or nested PCR were excised from the gel and purified using a QIAquick gel extraction kit (Qiagen, Hilden, Germany). Nucleotide sequences were prepared with the terminator cycle sequence kit (version 3.1) and determined with the ABI 3100 avant sequencer (Perkin-Elmer ABI, Boston, Mass., USA). Nucleotide sequences were aligned with Clustal X and the distances were calculated by Kimura's two-parameter method (10). Phylogenetic trees with bootstrap analysis from 1,000 replicas were generated by the neighbor-joining method as described elsewhere (10).

In total, 23 of 80 infants (29%) were positive for NoV and/or SaV (Table 1). We identified 11 (14%) NoV and 9 (11%) SaV single infections, and 3 (4%) NoV/SaV mixed infections.

Fourteen NoV sequences were used for phylogenetic analy-

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Table 1. Details of positive NoV and SaV specimens in Thailand between November 2002 and April 2003

Province	No. of specimens					
	Total	selected randomly	Positive ¹⁾	Single positive ¹⁾		Mixed positive ¹⁾
				NoV	SaV	NoV/SaV
Sa Kaeo	48	15	8	5	2	1
Chanthaburi	57	16	5	1	4	0
Songkhla	97	23	4	3	1	0
Nong Khai	69	13	4	1	2	1
Tak	50	13	2	1	0	1
Total	321	80	23 (29%)	11 (14%)	9 (11%)	3 (4%)

¹⁾: from the randomly selected specimens.

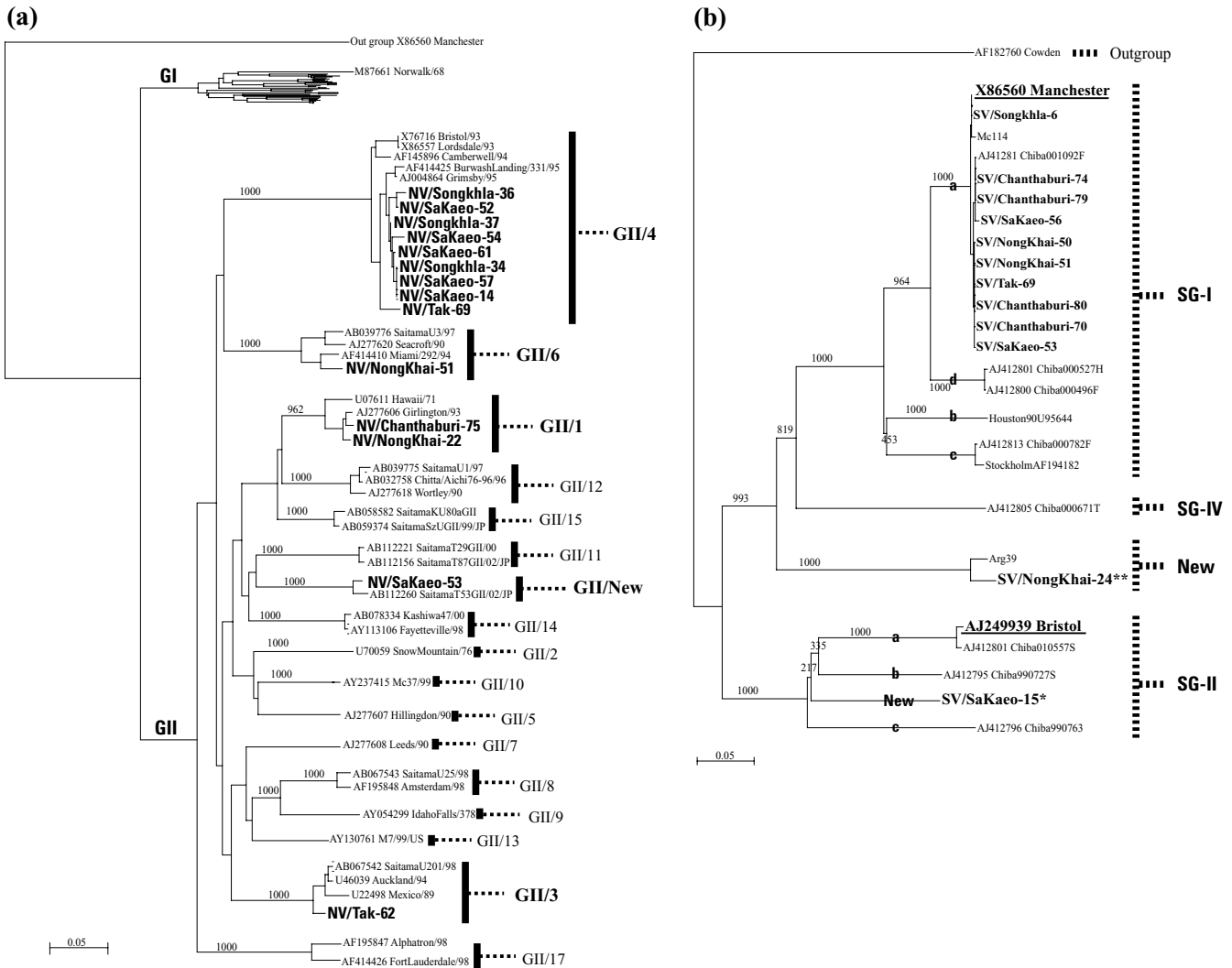


Fig. 1. Phylogenetic trees of the Thai sequences isolated in this study (represented in bold). The numbers on the branches indicate the bootstrap values for the clusters. Bootstrap values of 950 or higher were considered statistically significant for the grouping (10). The scale represents nucleotide substitutions per site. (a) The NoV sequences were constructed with the partial N/S capsid region, using SaV Manchester strain as an outgroup. The genotypes are indicated on each genotype cluster according to the method described by Kageyama et al. (2). (b) The SaV sequences were constructed with the partial N-terminal capsid region, using SaV Cowden strain as an outgroup. The genogroups and genotypes are indicated on each branch according to the method described by Okada et al. (4). Underlined bold letters showed SG-I-a and SG-II-a reference strains. The asterisks indicate new strains.

sis and classified according to the method described by Kageyama et al. (2). All NoV sequences were grouped in GII (Fig. 1a). Nine of 14 NoV sequences (64%) were clustered in GII/4. These nine isolates were derived from each distinct geographical region. These results not only showed that GII/4 strains were dominant but that they were circulating through-

out Thailand. Strains belonging to GII/4 have been reported as a major cause of global outbreaks (Fig. 1a) (11-13). Several other NoV strains were also found to be co-circulating in these four regions. We detected one GII/3 strain (isolate NV/Tak-62), two GII/1 strains (isolates NV/NongKhai-22 and NV/Chanthaburi-75), one GII/6 strain (isolate NV/NongKhai-

51), and one newly identified genotype strain (GII/New; isolate NV/SaKaeo-53) (2). All genotype clusters, including the newly found GI/new cluster were statistically supported by the bootstrap value (Fig. 1a).

Twelve SaV sequences were used for phylogenetic analysis and classified according to Okada et al. (4). Ten of 12 SaV sequences (83%) were grouped in genogroup-I-a cluster (SG-I-a) (Fig. 1b). The remaining two SaV sequences (isolates SV/NongKhai-24 and SV/SaKaeo-15) were grouped in two novel SaV clusters (Fig. 1b). NongKhai-24 represented a novel genogroup within the SaV genus (new cluster), whereas SaKaeo-15 represented a novel SG-II genetic cluster (SG-II-new cluster). The NongKhai-24 cluster was statistically supported by the bootstrap value (bootstrap value = 1,000). The bootstrap value of SaKaeo-15 was low (bootstrap value = 217) (Fig. 1b), though the branch length (distance) between the Bristol strain and the SaKaeo-15 strain was the same as that between the Bristol strain and the Chiba990727S strain. Therefore, SaKaeo-15 was considered a distinct genetic cluster. Arg39 (AF405715) containing similar sequence to NongKhai-24 (97% nucleotide identity) was recently detected in the US. This finding suggests the emergence and circulation of a novel human SaV genogroup.

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