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Short Communication

Monthly Distribution of Norovirus and Sapovirus Causing Viral Gastroenteritis in Thailand

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SUMMARY: A total of 1,141 rotavirus-negative stool specimens collected from diarrheic children in 4 distinct regions under sentinel surveillance in Thailand between 2006 and 2008 were examined by reverse-transcription (RT)-PCR for norovirus (NoV) and sapovirus (SaV). Three hundred 3 specimens (26.6%) were positive for NoV, with 34 and 269 belonging to genogroup I (GI) and genogroup II (GII), respectively. Twelve specimens (1.1%) were positive for SaV. Mixed infections were found in 5 specimens: 3 samples indicated the presence of both NoV GI and GII, and 2 samples indicated the presence of both NoV GII and SaV. Analysis of the monthly distribution of NoV and SaV revealed that NoV GII was clustered between September and February, while NoV GI was detected mainly in June and July; SaV was found in May, June, and July. In addition, 3 outbreaks of acute gastroenteritis at 2 junior high schools in Phichit and Bangkok, and at a university in Phitsanulok, Thailand in 2006 were found to have been caused by NoV infection. Sequence analysis of NoVs from sporadic cases and outbreaks showed them to be genotypes GII.4 and GII.6.

Norovirus (NoV), a member of the family *Caliciviridae*, is the dominant cause of sporadic and epidemic viral acute gastroenteritis in humans of all ages worldwide. NoV may be responsible for more than 1.1 million hospitalizations and 200,000 deaths in children under 5 years of age in developing countries (1). NoV can be divided into 5 distinct genogroups, GI to GV: the two major ones, GI and GII, and GIV have been shown to infect humans. The GI and GII strains consist of at least 9 and 22 genotypes (GI.1–GI.9 and GII.1–GII.22), respectively (2,3). GII.4 has been the most prevalent genotype in recent years, although the emergence and predominant distribution of GII.17 have been reported recently in Asia (4). Sapovirus (SaV), another member of the family *Caliciviridae*, is also a causative agent of gastroenteritis and is detected at a higher frequency in children than in adults. In this study, we examined the monthly distributions of NoV and SaV in hospitalized children in 4 distinct surveillance regions in Thailand between 2006 and 2008. The genotypes of some of the NoVs from sporadic cases and from 3 gastroenteritis outbreaks in 2006 were also determined.

A total of 1,141 rotavirus-negative stool specimens from hospitalized patients with acute gastroenteritis in 4 distinct sites in Thailand (Songkhla, Chanthaburi, Tak, and Nongkhai) between 2006 and 2008 were examined for NoV and SaV.

In 2006, 662 specimens were examined; 73 were collected from Songkhla, 312 from Chanthaburi, 186 from Tak, and 91 from Nongkhai. In 2007, 303 specimens were examined; 16 were from Songkhla, 121 from Chanthaburi, 85 from Tak, and 81 from Nongkhai. In 2008, 176 specimens were examined; 6 were from Songkhla, 107 from Chanthaburi, 4 from Tak, and 59 from Nongkhai. Approximately 10% suspensions of fecal samples were prepared in phosphate-buffered saline.

For detection of NoV and SaV, RNA extracted from the stool suspensions was subjected to nested reverse transcription-PCR (RT-PCR) in 2 steps (first and second amplifications), as described previously (5). The PCR-generated amplicons from the first or second PCR amplifications were excised from the gel and purified using a QIAquick gel extraction kit (Qiagen, Hilden, Germany). The purified PCR products were sequenced using ABI Prism BigDye Terminator Cycle Sequencing Ready Reaction kits (Applied Biosystems, Foster City, CA, USA) with an ABI Prism 3100 Genetic Analyzer (Applied Biosystems).

As shown in Fig. 1, from a total of 1,141 of stool specimens, NoV GI, NoV GII, and SaV were detected in 34 (2.9%), 269 (23.6%), and 12 (1.1%) samples, respectively. Most NoV GII (195/269: 72.5%) was detected during the dry seasons (January, February, and September to December). In contrast, most NoV GI-positive samples (19/34: 55.9%) were detected in June and July, and almost all SaV-positive samples (11/12: 91.7%) were detected from May to July (Fig. 1). Mixed infections were detected in 5 cases; 3 had both NoV GI and GII, and 2 had both NoV GII and SaV.

In addition to the sporadic child cases, there were three outbreaks associated with NoV at 2 junior high schools in Phichit and Bangkok, and at a university in

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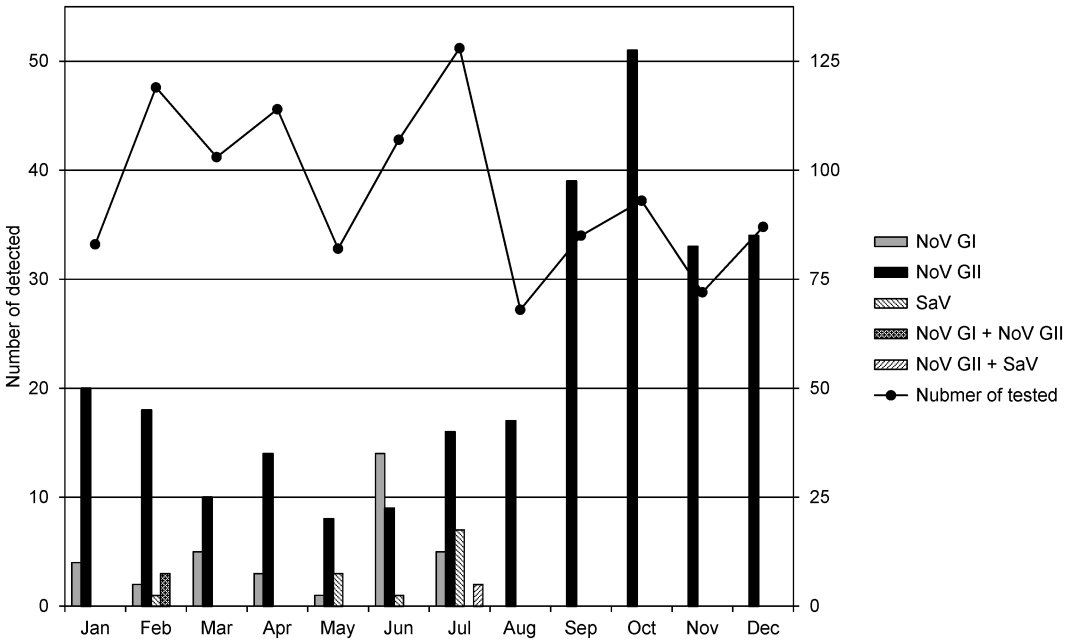

Fig. 1. Monthly distributions of NoV and SaV infections in Thailand between 2006 and 2008. Left vertical axis, the number of cases of NoV GI, NoV GII, SaV, NoV GI + NoV GII, and NoV GII + SaV detected by RT-PCR. Right vertical axis, the number of stool specimens tested for NoV and SaV from rotavirus-negative gastroenteritis patients.

Phitsanulok, Thailand in March, October, and September 2006, respectively. NoV GII was detected in 3 of 10 specimens (30%) from the outbreak in Phichit, 6 of 9 (66.7%) from the outbreak in Bangkok, and 24 of 28 (85.7%) from the university outbreak in Phitsanulok.

The partial nucleotide sequences of a total of 24 NoV strains including 8 strains randomly selected from among sporadic cases in each of 4 different districts, as well as 1 strain from the Phichit outbreak and 15 strains from the Phitsanulok outbreak were determined by direct sequencing of the cDNA products following RT-PCR. Sequence analysis showed that the genotypes of the NoVs from the sporadic cases and Phitsanulok outbreak were all GII.4, and that of NoVs from the Phichit outbreak was GII.6 (data not shown).

The age distribution of antibodies to Norwalk virus corresponding to NoV GI.1 was examined in Thailand in 1983: the antibodies were acquired between 2 and 5 years of age (6), suggesting a high prevalence of Norwalk virus in Thailand. However, surveys on NoV distribution have been performed since 2000. We have conducted a long-term survey on viral gastroenteritis in Thailand. Detection of rotavirus, NoV, SaV, and picobirnavirus has been reported (7–9). Guntapong et al. (7) reported that 11 (13.8%), 9 (11.3%), and 3 (3.8%) of 80 stool specimens from sporadic cases of acute gastroenteritis negative for rotavirus between November 2002 and April 2003 were positive for NoV, SaV, and both NoV and SaV, respectively. In this study, we examined a much higher number of stool specimens in 4 distinct regions in Thailand. The prevalence of GI and GII of NoV and SaV infections in Thailand was clearly shown.

This study has some limitations, including the lack of examination for NoV in rotavirus-positive samples, genotyping of only some NoV specimens, and sequencing of only a part of the genome. However, our results showed distinct monthly distributions of NoV GI, NoV GII, and SaV in Thailand based on a large number of rotavirus-negative stool samples from gastroenteritis patients. It has been well established that most NoV infections occur in the winter season in temperate countries, although the reason for this is not well known. In this study, uneven monthly distributions of NoV GI and SaV were also observed: they were detected only in the months between January and July. In contrast, NoV GII was detected year-round in Thailand, although it was observed mainly in the dry and cool season, between September and February. Several reports have described the seasonality of NoV and SaV in Thailand (10–12). Bodhidatta et al. (10) also reported clear peaks of NoV GII infections between October and February, corresponding to the dry and cool season. Chaimongkol et al. (11) reported a year-round distribution of NoV with a higher frequency between December and June. Malasao et al. (12) described that almost all NoV GI, NoV GII, and SaV infections in Thailand tended to occur in the first 7 months of the year, except that NoV GII was also observed in October. Climate change has been suggested to affect the seasonality of NoV infections by influencing transmissibility, host susceptibility, and resistance to environmental conditions (13,14). Precise analysis of the relation of these factors is required.

A variety of NoV and SaV genotypes have been detected in Thailand (7,10–12,15,16), while GII.4 has been reported to be remarkably predominant in Thailand, as were as worldwide. In this study, all of the randomly selected samples were GII.4. There have been very few reports on outbreaks due to NoV infection in Thailand (17). In this study, we detected GII.4 and GII.6 NoV in outbreaks in junior high schools and a

university in Thailand, respectively. Long-term surveillance in various districts including Thailand is necessary to better understand the NoV epidemiology in the tropics, including seasonal variations in infections.

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Conflict of interest None to declare.

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