TABLE 2. Primers used to amplify the capsid gene of GII.2 NoV strains

Primer	Sequence (5′ to 3′)[a]	Polarity	Location (nt)	Reference or source
COG2F	CARGARBCNATGTTYAGRTGGATGAG	+	5003[b]	19
G2SKF	CNTGGGAGGGCGATCGCAA	+	5058[b]	22
G2SKR	CCRCCNGCATRHCCRTTRTACAT	−	5401[b]	22
N235Rex	GCWANRAAAGCTCCWGCCAT	−	6273[b]	17
MKcap508-524F	CAGAAAGATGATCCCAA	+	508[c]	This study
MKcap524-508R	TTGGGATCATCTTTCTG	−	524[c]	This study
MKcap662-649R	TCCACTGTTGGTGG	−	662[c]	This study
MKcap1109-1125F	TGGGTCAGATTCAAATT	+	1109[c]	This study
MKcap1125-1109R	AATTTGAATCTGACCCA	−	1125[c]	This study
MKcap1304-1290R	AAGAGCAGGCGCTCC	−	1304[c]	This study
MK35R	CAAAAGCTCCAGCCAT	−	1644[c]	This study

[a] Abbreviations for residues in boldface are as follows: B, not A; H, not G. In addition, N is A, C, G, or T; R is A or G; W is A or T; Y is C or T.
[b] Location of the 5′ end of the primer corresponding to the nucleotide position of Lordsdale/93/UK (X86557).
[c] Location of the 5′ end of the primer corresponding to the nucleotide position of ORF2 and ORF3 of Melksham/94/UK (X81879).

and FoyPred3D (25) servers. WHAT IF could not model residues 342, 344, and 345 based on the Norwalk/68/US capsid protein template (PDB ID 1IHM); as they are present in an inserted loop of the P2 subdomain relative to the template structure, but they are predicted to be in the same position as in the 3D model based on the GII.4 genotype capsid protein template (PDB ID 2OBR). A dimer of the GII.2 NoV capsid protein was modeled by superimposing two predicted monomers onto the trimeric template of the Norwalk/68/US capsid protein. The 3D models were visualized by the YASARA view program (version 6.813; http://www.yasara.org/) (24).

Nucleotide sequence accession numbers. The nucleotide sequences determined in this study have been deposited in the DDBJ/EMBL/GenBank with the accession numbers AB281081 to AB281090 (Table 1).

RESULTS

Capsid gene sequence analysis of GII.2 strains collected over 30 years. Sequence data from a total of 36 GII.2 strains showed that the capsid gene was 1,629 nt long and coded for a protein of 542 amino acids. There were no deletions or insertions in the capsid gene among these strains collected over a 30-year period. Sequence comparison showed ≥83.6% nucleotide and ≥93.5% amino acid identities among these GII.2 strains. The phylogenetic tree based on the 1,629 nucleotide sequences of the complete capsid gene confirmed that all strains were characterized as GII.2 genotype (Fig. 2).

Genetic analysis of GII.2 strains from a regional outbreak in Japan in the spring of 2004. Of the 21 GII.2 strains detected in Osaka City, Japan, 14 strains were detected in samples from cases in outbreaks or sporadic cases in the spring epidemic between March and May 2004 (Table 1). These strains were closely related to each other (≥99.2% nucleotide and ≥ 99.1% amino acid identities). To find out if these viruses changed genetically during circulation in the community for a short period of time (about 3 months), the complete nucleotide and amino acid sequences of the capsid gene from these 14 GII.2 strains were compared. A total of 45 nucleotide substitutions were observed (Table 3). The majority were third-base position changes (77.8%) and synonymous substitutions (82.2%). These nucleotide changes resulted in amino acid changes in eight codons, five of which were located in the P2 subdomain. In the alignment of the spring epidemic strains over the 3-month period, of the 45 nucleotide substitutions, only one nucleotide position was fixed (nt 594 in the S domain) at the end of this epidemic. This did not result in an amino acid change. All amino acid changes were sporadic. Eight of the 14 strains had an identical amino acid sequences (Table 1). Of these, strain 04038 was used for further genetic analysis.

Genetic analysis of all GII.2 strains collected between 1976 and 2005. The nucleotide and amino acid sequences of the complete capsid genes were compared for 29 GII.2 strains collected between 1975 and 2005 (30 years), excluding 7 GII.2 strains detected in Osaka City with identical amino acid sequences (10 strains from The Netherlands, 14 from Osaka City, and 5 from GenBank). A total of 488 nucleotide changes were observed (Table 4). Again, the majority of these were third-base changes (83.8%) and synonymous (85.9%). In total, 59 nucleotide changes resulted in amino acid changes (34 in the P2 subdomain) (Table 4). Twenty-five of these were informative changes (19 in the P2 subdomain), but none appeared to be fixed in the genome over time. Of the 25 informative sites, the amino acid position 345 was the most variable (Fig. 3). Nevertheless, statistical analysis showed a significantly higher rate of mutation in the P2 subdomain than in P1 and S, suggesting selective pressure ($P = 0.0018$, chi-square 5.63; and $P < 0.0001$, chi-square 12.9).

Genetic analysis of Melksham-like strains detected between 1994 and 2005. In order to understand the apparent discrepancy between selective changes in the P2 subdomain and the absence of fixation of these mutations, we repeated our anal-

FIG. 1. Phylogenetic analysis of partial RdRp gene sequences of GII.2 NoV strains by the neighbor-joining method (A) and the Bayesian method (B). The genotypes at the RdRp regions that are not assigned numbers are represented as GII-NA. The scale indicates the number of substitutions per site. Reference strains of NoV used in this analysis are given in italics. (A) The bootstrap values are indicated on each branch. (B) Tree topology was evaluated on the basis of 1,500,000 generations. The posterior probabilities are indicated on each branch (≥0.95 of the posterior probability means that the branch has high credibility). The GenBank accession numbers of the additional reference strains used in this analysis are as follows: Hokkaido/133/03/JP, AB212306; Lordsdale/93/UK, X86557; MOH/99/HU, AF397256; Norwalk/68/US, M87661; Pont de Roide 673/04/FR, AY682549; Saitama U1, AB039775; Toronto/93/CA, U02030. For strain abbreviations, see Table 1.

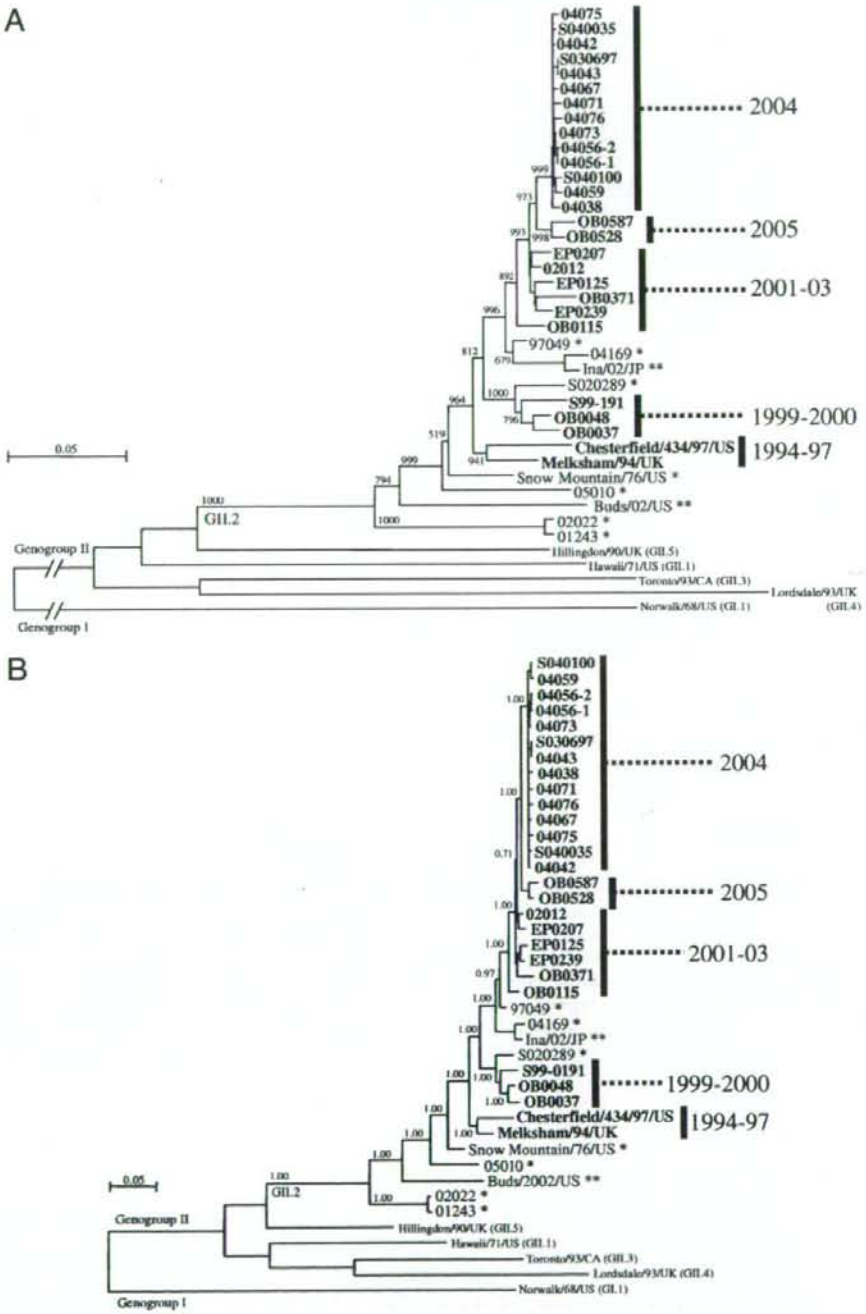

FIG. 2. Phylogenetic analysis of complete capsid gene sequences of GII.2 NoV strains by the neighbor-joining method (A) and the Bayesian method (B). Melksham-like strains, which have a matching (GII.2) RdRp sequence, are shown in boldface. Asterisks indicate the GII.2 strains that have different (*) or unknown (**) genotypes in the RdRp region. The scale indicates the number of substitutions per site. (A) The bootstrap values are indicated on each branch. (B) Tree topology was evaluated on the basis of 500,000 generations. The posterior probabilities are indicated on each branch. The GenBank accession numbers of the additional reference strains used in this analysis are as follows: Hawaii/71/US, U07611; Hillingdon/90/UK, AJ277607; Lordsdale/93/UK, X86557; Norwalk/68/US, M87661; Toronto/93/CA, U02030. For strain abbreviations, see Table 1.

TABLE 3. The numbers of nucleotide and amino acid substitutions in the complete capsid gene among 14 GII.2 strains detected in the spring epidemic of 2004 in Osaka City, Japan

Domain or subdomain[a]	Nucleotide substitutions						Amino acid substitutions	
	No. of substitutions	No. at the indicated position of the changed codon			Synonymous changes (no.)	Nonsynonymous changes (no.)	No. of substitutions	Informative changes (no.)
		1st	2nd	3rd				
N	1	0	0	1	1	0	0	0
S	18	3	2	13	16	2	2	0
P1	7	0	0	7	6	1	1	0
P1-1	4	0	0	4	4	0	0	0
P1-2	3	0	0	3	2	1	1	0
P2	19	3	2	14	14	5	5	0
Total	45	6	4	35	37	8	8	0

[a] Each domain of GII.2 NoV capsid gene was determined according to Chen et al. (7). The region and abbreviation of each domain are as follows: N, 5′ end of ORF2 and N terminal domain (residues 1 to 45); S, shell domain (residues 46 to 216); P1, P1 subdomain (P1-1, residues 217 to 274; P1-2, residues 421 to 542); P2, P2 subdomain (residues 275 to 420).

ysis after removing recombinant genomes from the alignment (Table 5) (nucleotide and amino acid sequences of the complete capsid gene from 20 Melksham-like strains: 10 strains from The Netherlands, 8 from Osaka City, and 2 from GenBank). Sequence comparison showed ≥91.5% nucleotide and ≥97.4% amino acid identities among these Melksham-like strains. A total of 301 nucleotide changes were observed (Table 5), the majority of which were third-base changes (87%) and synonymous (88.7%). These nucleotide changes resulted in amino acid changes in 32 codons, half of which were located in the P2 subdomain. Twelve of 32 amino acid positions were informative (9 in the P2 subdomain) (Fig. 3). In contrast with the previous finding, several mutations were fixed: of the 12 informative sites, 2 amino acid substitutions (amino acid positions 245 and 440) in the P1 subdomain and 3 amino acid substitutions (amino acid positions 342, 344, and 345) in P2 subdomain were cumulative (Table 6), segregating the strains into five genetic groups (1994 to 1997, 1999 to 2000, 2001 to 2003, 2004, and 2005) by the neighbor-joining method (Fig. 2A) and Bayesian method (Fig. 2B).The strains detected in the spring epidemic in Osaka City had a unique sequence, with S or P residues at amino acid position 364 (Fig. 3 and Table 6). The other six informative sites were not fixed.

The 3D structure of the P domain of a monomer of the NoV capsid protein was predicted by WHAT IF, 3D-Jigsaw, and EsyPred3D, based on the known 3D structure of the VA387/98/US GII.4 genotype capsid protein, which has 55% amino acid sequence identity in the P domain to the Melksham capsid protein. A comparison of the positions of the six fixed mutations to the predicted 3D structure indicated that all six residues were predicted to be located at the surface of the capsid protein, with three residues (342, 344, and 345) close to each

TABLE 4. The numbers of nucleotide and amino acid substitutions in the complete capsid gene among 29 GII.2 strains collected in the GenBank, The Netherlands, and Japan over a 30-year period

Domain or subdomain[a]	Nucleotide substitutions						Amino acid substitutions	
	No. of substitutions	No. at the indicated position of the changed codon			Synonymous changes (no.)	Nonsynonymous changes (no.)	No. of substitutions	Informative changes (no.)
		1st	2nd	3rd				
N	20	0	2	18	17	3	3	1
S	137	9	3	125	131	6	6	1
P1	162	17	7	138	143	19	16	4
P1-1	53	4	2	47	49	4	3	2
P1-2	109	13	5	91	94	15	13	2
P2	169	28	13	128	128	41	34	19
Total	488	54	25	409	419	69	59	25

[a] See note to Table 3.

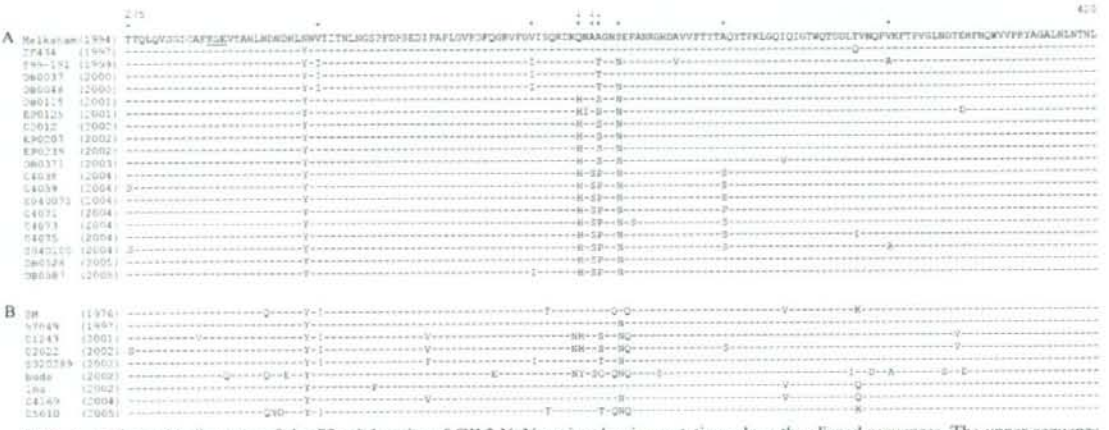

FIG. 3. Amino acid alignment of the P2 subdomains of GII.2 NoV strains showing mutations along the aligned sequences. The upper sequence alignment group (A) includes the Melksham-like strains and the lower group (B)includes the other GII.2 strains, which were recombinant genomes. In each group, sequences are arranged chronologically from top (oldest) to bottom (most recent). The detection years of the strains are indicated in parentheses. The asterisk indicates informative sites among Melksham-like strains. The arrow denotes cumulatively changing amino acid positions (342, 344, and 345) among Melksham-like strains. The numbers above the sequences indicate the sequence position relative to the position in the capsid protein of strain Melksham. Underlined sequences indicate the KGE motif that corresponds to the RGD-like motif of other NoVs and was determined with the amino acid sequence alignment of other NoVs according to Tan et al. (34). For strain abbreviations, see Table 1.

other in the P2 subdomain (Fig. 4). Furthermore, residues 342, 344, 345 of the P2 subdomain and residues 245 and 440 of the P1 subdomain were grouped closely together on the predicted 3D structure of a dimer (3D-modeling by WHAT IF) (Fig. 5).

DISCUSSION

In this study, we analyzed the complete capsid gene of GII.2 NoVs collected over a 30-year period. The collection included a relatively large sample from an epidemic that was observed only in Japan in the spring period of 2004. In this 3-month period, no evolutionary changes were observed, but compared with other GII.2 Melksham-like strains these variants had unique amino acid sequences (S or P) at position 364. One of the recombinant genomes, strain 02022, had the same amino acid residues, suggesting that the presence of this mutation was not causally related to the epidemic pattern. The sequence analysis confirmed that the spring epidemic was an outbreak.

Molecular characterization of the GII.2 capsids over the full study period showed an interesting difference between recombinant genomes and nonrecombinant (Melksham-like) genomes. The Melksham-like strains clearly evolved over time, with accumulation of mutations particularly in the P2 subdomain. In contrast, GII.2 capsids from recombinant genomes did not fit this pattern and had a seemingly erratic pattern of mutations. Melksham-like strains are occasionally observed in molecular surveillance data from The Netherlands and Osaka

TABLE 5. The numbers of nucleotide and amino acid substitutions in the complete capsid gene among 20 Melksham-like strains over a 12-year period

Domain or subdomain[a]	Nucleotide substitutions						Amino acid substitutions	
	No. of substitutions	No. at the indicated position of the changed codon			Synonymous changes (no.)	Nonsynonymous changes (no.)	No. of substitutions	Informative changes (no.)
		1st	2nd	3rd				
N	10	0	2	8	8	2	2	0
S	81	4	3	74	76	5	6	1
P1	98	8	3	87	89	9	8	2
P1-1	34	2	1	31	32	2	2	1
P1-2	64	6	2	56	57	7	6	1
P2	112	13	6	93	94	18	16	9
Total	301	25	14	262	267	34	32	12

[a] See note to Table 3.

TABLE 6. Amino acid substitutions at six positions in the P domain among Melksham-like strains

Strain description			Amino acid and codon at the indicated position of the capsid protein[a]											
			245		342		344		345		364		440	
Name[b]	Country[c]	Detection date (mo/day/yr)	Residue	Codon	Residue	Codon	Residue	Codon	Residue	Codon	Residue	Codon	Residue	Codon
Melksham	UK	1994	P	CCC	Q	CAG	A	GCC	A	GCT	A	GCA	L	CTC
CF434	US	1997	—	CCC	—	CAG	—	GCT	—	GCT	—	GCA	—	CTT
S99-191	NL	1/1999	S	TCT	—	CAA	—	GCC	T	ACT	—	GCA	I	ATC
OB0037	NL	9/2000	S	TCT	—	CAA	—	GCC	T	ACT	—	GCA	I	ATC
OB0048	NL	12/2000	S	TCT	—	CAA	—	GCC	T	ACT	—	GCA	I	ATC
OB0115	NL	2/2001	S	TCT	H	CAC	—	GCC	S	TCT	—	GCA	I	ATC
EP0125	NL	11/2001	S	TCC	H	CAC	—	GCC	S	TCT	—	GCA	I	ATC
02012	JP	1/2002	S	TCC	H	CAC	—	GCC	S	TCT	—	GCA	I	ATC
EP0207	NL	1/2002	S	TCC	H	CAC	—	GCC	S	TCT	—	GCA	I	ATC
EP0239	NL	2/2002	S	TCC	H	CAC	—	GCC	S	TCT	—	GCA	I	ATC
OB0371	NL	10/2003	S	TCC	H	CAC	—	GCC	S	TCT	—	GCA	I	ATC
04038	JP	3/11/2004	S	TCC	H	CAC	S	TCC	P	CCT	S	TCA	I	ATC
04059	JP	4/18–30/2004	S	TCC	H	CAC	S	TCC	P	CCT	S	TCA	I	ATC
S040035	JP	4/21/2004	S	TCC	H	CAC	S	TCC	P	CCT	S	TCA	I	ATC
04071	JP	5/16–23/2004	S	TCC	H	CAC	S	TCC	P	CCT	P	CCA	I	ATC
04073	JP	5/22/2004	S	TCC	H	CAC	S	TCC	P	CCT	S	TCA	I	ATC
04075	JP	5/25/2004	S	TCC	H	CAC	S	TCC	P	CCT	S	TCA	I	ATC
S040100	JP	5/31/2004	S	TCC	H	CAC	S	TCC	P	CCT	S	TCA	I	ATC
OB0528	NL	1/2005	S	TCC	H	CAC	S	AGC	P	CCT	—	GCA	I	ATT
OB0587	NL	4/2005	S	TCC	H	CAC	S	AGC	P	CCT	—	GCA	I	ATT

[a] The position number corresponds to the capsid sequence of the Melksham/94 UK strain; a dash indicates sequence identity with this strain. Residues are indicated by their single-letter codes.

[b] For strain abbreviations, see Table 1.

[c] UK, United Kingdom; US, United States; NL, The Netherlands; JP, Japan.

City, and strains from Japan and The Netherlands could be consistently grouped in the same alignment, suggesting continuous widespread circulation and an ability to cause disease. The recombinant genomes with GII.2 capsids, however, occur sporadically and did not show evidence for evolution, suggesting that their circulation is limited (1, 4, 17). The finding that strains from such widely separated geographic regions were similar shows that evolution of GII.2 NoVs is a global phe-

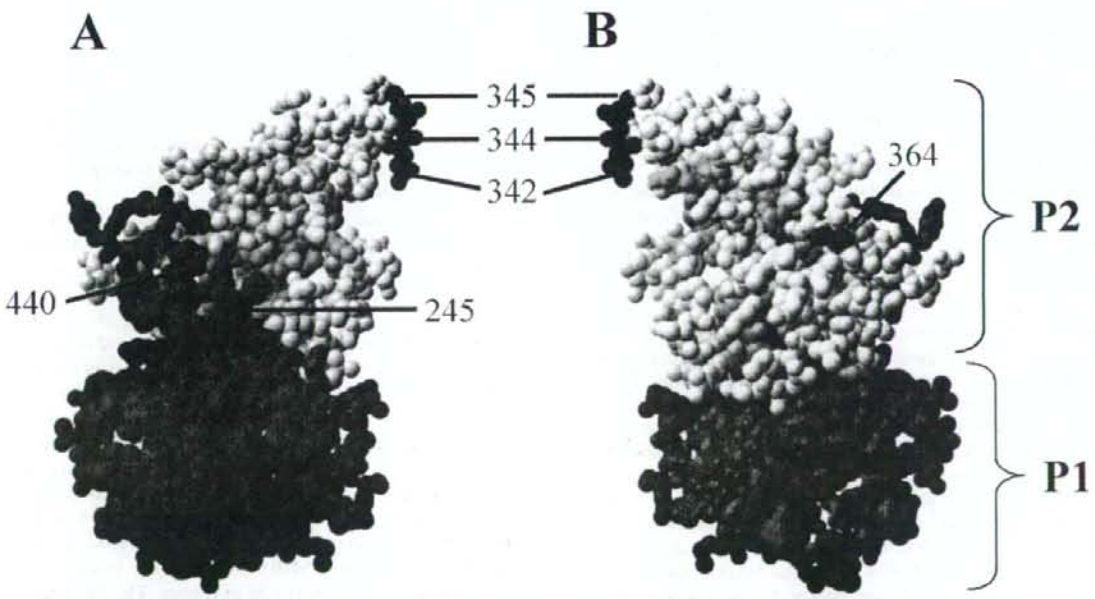

FIG. 4. Location of six fixed amino acid residues (positions 245, 342, 344, 345, 364, and 440, shown in red) on the monomer of the capsid protein. This 3D structure for the monomer P domain of the GII.2 NoV capsid protein was made by WHAT IF. The P1 and P2 subdomains are shown in blue and gray, respectively. The S domain is not shown. The KGE motif in the P2 domain is shown in green. (A) Predicted location of amino acid residues 342, 344, 345, and 364 in the P2 subdomain. (B) A view identical to panel A rotated 180° horizontally showing the location of amino acid residues 245 and 440 in the P1 subdomain.

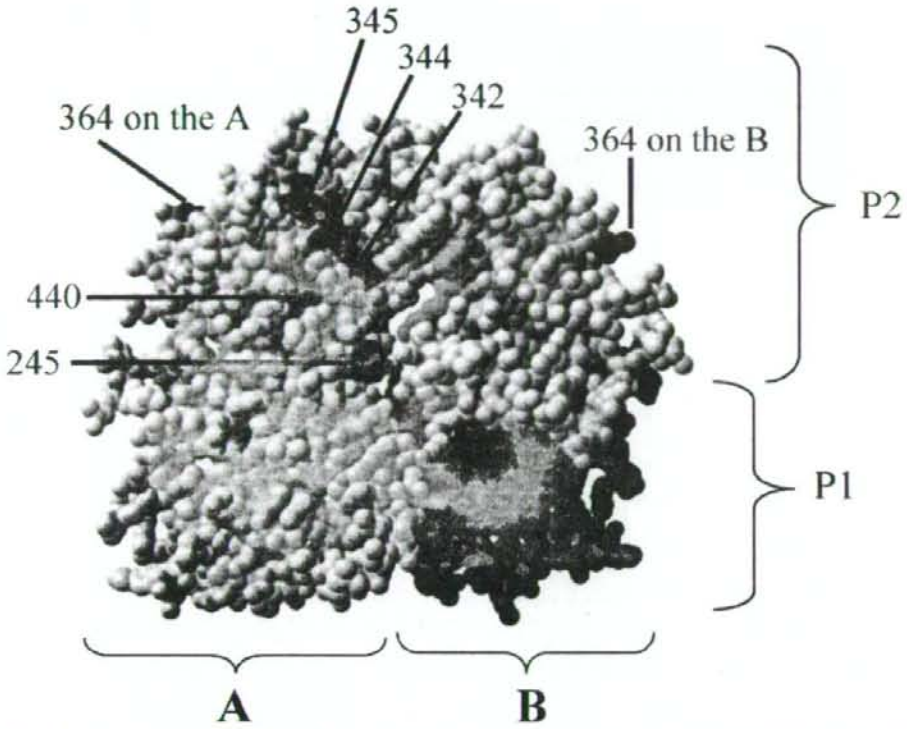

FIG. 5. The 3D structure of a dimer of the GII.2 NoV capsid protein, as predicted by WHAT IF, showing the location of the six informative amino acid residues (red). Monomer A is shown in yellow, while the monomer B is shown in blue (P1) and gray (P2). The S domains are not shown. The KGE motif (green) in the P2 subdomain on monomer B is also shown.

nomenon, similar to what has been described for GII.4 NoVs (5, 27, 33).

The molecular characterization of Melksham-like strains showed accumulation of mutations over a 12-year period, segregating them into five genetic groups. Three of these were supported in phylogenetic analysis with high bootstrap values. Two others (Fig. 2, 1999 to 2000 and 2001 to 2003) were not distinguishable in phylogenetic analysis, possibly because of the small number of isolates in the comparison. The present collection, however, contains all GII.2 strains detected in the two countries in the past 12 years of surveillance. Amino acid position 345 was the most variable in all GII.2 strains. This is suggestive of immune pressure, particularly because the amino acid is predicted to be located on a surface-exposed part of the capsid. The fixed amino acid changes were topologically clustered, judging from the 3D structure prediction, indicating that combined they may form an epitope. Immunity to NoVs has not been studied very systematically, but recent work on GII.4 NoVs has suggested that these viruses evolved under immune pressure (9, 30, 33). The lack of a tissue culture model makes it difficult to corroborate these findings by using cross-neutralization tests with hyperimmune serum directed against specific variants. Evolution of strains in an immunocompromised patient with chronic shedding of NoVs has been demonstrated by Nilsson et al. (28), who suggested that the cumulative amino acid substitutions appearing in the P2 subdomain were immune response driven. To clarify the relations between these amino acid mutations and receptor or antibody binding further immunological studies are needed.

Recently, new variants of the predominant NoV, the GII.4 genotype, appeared in 2002 (27), 2004 to 2005 (5), and 2006 (33) with global distribution. These new GII.4 variants were characterized into different clusters by the phylogenetic analysis based on the capsid gene (5, 9, 30). Okada et al. (30) reported that GII.4 strains had cumulative amino acid changes in the P domain and 5% to 18% amino acid diversity in the P2 subdomain between GII.4 subtypes for 5 years study. Bull et al. (5) also described more than 5% amino acid diversity in the complete capsid gene between past GII.4 strains and variants. Siebenga et al. (33) found 9% fixed amino acid mutations across the capsid gene over a 12-year period, but only five of these consistently delineated subsequent epidemic strains. In contrast, here we found only 2.6% amino acid diversity across the complete capsid gene of Melksham-like strains in a 12-year period, showing a clearly lower rate of change. The difference between these genotypes is intriguing and suggests clear differences in epidemiology that are not easy to explain. This study has been done using a limited number of strains in a rare genotype from a limited number of locations. It is necessary to

continue molecular surveillance of NoV infections to further the evolutional analysis of each genotype.

In conclusion, the present study showed that NoVs belonging to a rare genotype evolved by the accumulation of mutations in the surface-exposed parts of the capsid, suggesting immune response-driven evolution. The pattern of change similar to what has been observed for GII.4 is a global one, suggesting that these viruses circulate much more than is apparent from their presence in surveillance data. The data also showed for the first time that recombinant genomes of NoVs behave differently, even when they share the same capsid genes as nonrecombinant genomes. Understanding how NoVs evolve is necessary for finding more effective ways to control this disease and particularly its impact in healthcare settings.

ACKNOWLEDGMENTS

We are grateful to Erwin Duizer, Erwin de Bruin, and Bas van de Veer (RIVM, Bilthoven, The Netherlands) for supporting our work.

This work was supported by the European Commission, DG Research Quality of Life Program, 6th Framework (EVENT, SP22-CT-2004-502571), and by a grant for Research on Food Safety of the Ministry of Health, Labour and Welfare, Japan.

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Journal of Tropical Pediatrics Advance Access published March 4, 2008

Norovirus and Sapovirus Infections among Children with Acute Gastroenteritis in Ho Chi Minh City during 2005–2006

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Summary

A molecular epidemiological study on common diarrheal viruses was conducted in a children's hospital in Ho Chi Minh City between December 2005 and November 2006. Fecal samples were collected from 502 pediatric patients with acute gastroenteritis, and were screened for the presence of norovirus (NoV) and sapovirus (SaV). NoVs GII and SaVs were detected in 6.4% and 1.2% specimens, respectively, while there was no NoV GI found among studied samples. NoVs could be identified through the year, except in April and July, with the peak of detection rate (62.5%) during the rainy season. Conversely, four out of six (66.7%) of the SaV strains were identified during the dry season. Patients aged between 6 and 23 months were found to be more infected by NoVs. The overall mean severity score of norovirus-positive patients was 9.8 ± 3.6, and no significant difference of severity scores among patients belonged to different age groups, gender and place of living. The results of phylogenetic analysis showed the diversity of caliciviruses circulating in the area, and various types of recombination were identified among NoVs and SaVs detected. These results provide important information on calicivirus infections among Vietnamese children.

Key words: norovirus, sapovirus, clinical manifestations, recombinant, Vietnam.

Introduction

Norovirus (NoV) and sapovirus (SaV) are members of the family *Caliciviridae* (other two genera are *Lagovirus* and *Vesivirus*). The NoV and SaV strains are determined as the major causes of non-bacterial acute gastroenteritis in infants and young children [1, 2].

After being discovered through electron microscope in 1972 [3], NoVs were identified widely in epidemiological studies, and were the cause of outbreaks of gastroenteritis in various settings including hospitals [4, 5], schools [6, 7], cruise ships [8, 9], restaurants [10, 11] and day care centers [12, 13]. Sequence analyses of worldwide NoVs revealed that they are classified into seven distinct genogroups (GI to GVII), of these, GI, GII, GIV, GVI and GVII are known to infect humans [14]. NoV contains a positive-sense single-stranded RNA genome surrounded by an icosahedral capsid. The NoV genome contains three open reading frames (ORFs). The ORF1 encodes non-structural proteins, including NTPase, protease and RNA-dependent RNA polymerase (RdRp), ORF2 encodes the capsid protein (VP1) and ORF3 encodes a minor structural protein (VP2).

SaV infects both children and adults, and have been found to cause outbreaks of gastroenteritis in kindergarten [15], hospital [16] and mental health care facility [17]. SaV- associated diarrhea is usually mild, compared to that caused by NoVs [18].

Acknowledgements

We deeply thank the staff of the Department of Gastroenterology, Children's Hospital 1, Ho Chi Minh City for collecting samples. This research was supported by the Ministry of Education, Culture, Sports, Science, and Technology, Japan and the Ministry of Health, Labor, and Welfare, Japan.

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doi:10.1093/tropej/fmn036

TABLE 1
Primer pairs used to amplify NoVs and SaVs in this study

Primer	Target virus	Polarity	Sequence position (5' to 3') reference strain	Amplicon size	Target region
G1SKF	NoV GI	+	5342-5361 (Norwalk 68)	330bp	Polymerase and capsid junction
G1SKR	NoV GI	−	5653-5671 (Norwalk 68)		
COG2F	NoV GII	+	5003-5028 (Lordsdale)	387bp	Polymerase and capsid junction
G2SKR	NoV GII	−	5367-5389 (Lordsdale)		
SLV5317	SaV	+	5083-5105 (Manchester)	434bp	Polymerase and capsid junction
SLV5719	SaV	−	5494-5516 (Manchester)		

NoV GI, norovirus GI; NoV GII, norovirus GII; SaV, sapovirus.

SaVs can be divided into five genogroups (GI to GV), among which, GI, GII, GIV and GV are identified within humans [19]. The SaV GI, GIV and GV genomes contain three ORFs, whereas the SaV GII genome contains two ORFs. ORF1 encodes all the non-structural proteins, including RdRp, and the major capsid protein (VP1). ORF2 encodes a small protein, and ORF3 encode a protein of unknown function [20].

Normally, in both NoV and SaV, the genogroup genotypes are generally maintained across the three ORFs. A recombinant NoV or SaV can be defined as one that clusters with two distinct groups of strains when two different regions (normally the capsid and polymerase) of the genome are subjected to phylogenetic analysis. Since the first NoV recombinant, Snow Mountain strain [21], was reported, various naturally occurring recombinants in different types have been identified [22–25]. Likewise, the identification of SaV recombinants have been reported elsewhere [26–28].

In Vietnam, NoVs and SaVs were identified from several epidemiological surveillances, and are considered as the important agents of viral gastroenteritis in the country [29, 30]. The first Vietnamese NoV recombinants were reported from a surveillance during 1999–2000 [30], since then, neither data about calicivirus infections nor recombinant virus has been reported. A hospital-based surveillance was conducted in Ho Chi Minh City during 2005–2006 that investigated the presence of common viral agents causing diarrhea in children, has been described elsewhere [31]. In this study, we reported in details the detection of NoVs and SaVs in the surveillance mentioned above, and described the molecular characteristics of NoV and SaV strains detected. The clinical manifestations and the evaluation of disease severity in patients were also included.

Materials and Methods

Patients

Patients with acute gastroenteritis who either visited the out-patient ward or were admitted to the Department of Gastroenterology, Children's Hospital 1, Ho Chi Minh City from December 2005 to November 2006, were recruited in the surveillance. Patients were examined by pediatricians, and the clinical symptoms of dehydration were assessed based on the WHO guideline [32]. A 20-point Vesikari's score was used to evaluate the disease severity in patients [33].

Fecal samples collection and virus detection

A total of 502 fecal samples were collected from studied patients (one specimen from each patient). The fecal specimens from the outpatients were collected at the out-patient ward or from the inpatients within 24 h after admission and stored at −20 C until use. They were prepared as a 10% suspension in distilled water and the viral RNA genomes were extracted from the fecal suspension with a QIAamp Viral RNA Mini kit (QIAGEN®, Hilden, Germany) according to the manufacturer's instruction. The presence of NoVs and SaVs in fecal specimens was determined by RT-multiplex PCR [34]. Three primer pairs, G1SKR-G1SKF, COG2F-G2SKR and SLV5317-SLV5749 [34] were used to amplify NoVs GI, NoVs GII and SaVs, respectively (Table 1). PCR products were electrophoresed in a 1.5% agarose gel, followed by staining with ethidium bromide for 20 min, and then visualized under ultraviolet light. The results were recorded by photography.

Nucleotide sequencing and phylogenetic analysis

All of NoVs and SaVs detected in this study were subjected to nucleotide sequencing by using the Big Dye Terminator Cycle Sequencing kit version 3.1 and an ABI Prism 310 Genetic Analyzer (Applied Biosystems, Inc.) according to the manufacturer's instruction. Primer pairs mentioned above were used as sequencing primers, generating a partial nucleotide sequence, including both polymerase region and the capsid region [34]. Similarities of the sequenced strains with other strains were assessed by BLAST search using the default options (DNA DataBank of Japan). Multiple sequence alignments were

TABLE 2
Monthly distribution of NoVs and SaVs detected from children with acute gastroenteritis in the Children's Hospital 1, Ho Chi Minh City, during 2005–2006

Seasonality	Dry season					Rainy season						Dry	Total (%)
	Dec 05	Jan 06	Feb	Mar	Apr	May	Jun	Jul	Aug	Sep	Oct	Nov	
No of specimens	20	30	43	57	30	53	32	17	30	32	60	98	502 (100)
No (%) of NoVs	2 (10.0)	1 (3.3)	2 (4.7)	4 (7.0)	0 (0)	3 (5.7)	2 (6.3)	0 (0)	6 (20.0)	4 (12.5)	5 (8.3)	3 (3.1)	32 (6.4)
No (%) of SaVs	0 (0)	1 (3.3)	2 (4.7)	0 (0)	1 (3.3)	1 (1.9)	0 (0)	0 (0)	1 (3.3)	0 (0)	0 (0)	0 (0)	6 (1.2)

NoV, norovirus; SaV, sapovirus. 62.5% of NoVs were identified in the rainy season.

calculated using the CLUSTALX program, and the phylogenetic trees were constructed by the neighbor-joining method with the MEGA 3.1 software package [35], and using different NoVs and SaVs sequences available in GenBank for comparison and as outgroups.

Accession numbers
The selected nucleotide sequences of Vietnamese NoVs and SaVs strains described in this study have been deposited in GenBank under accession numbers EU137732–EU137739.

Results

Detection of NoVs and SaVs
Among 502 fecal specimens collected during the 1-year surveillance, NoVs GII were determined in 32 (6.4%) specimens, and SaVs were detected in six (1.2%) specimens. Fifteen and four specimens showing positive with NoV and SaV, respectively, were found to be in mixed infection with other viral pathogens. There was no NoV GI found in this study. Regarding seasonal pattern, NoVs could be identified through the year, except in April and July. Twenty out of 32 (62.5%) of the NoVs were detected during the rainy season, which usually begins in May and ends in October in the southern part of Vietnam, including Ho Chi Minh City. Conversely, four out of six (66.7%) of SaV strains were identified during the dry season (Table 2).

Characteristics of the NoV and SaV-positive patients
The Table 3 showed the characteristics of positive cases with NoVs and SaVs. Twenty-eight and four patients showing positive with NoVs and SaVs, respectively, had adequate medical records for further analyses. To characterize the age distribution, all patients were classified into five different age groups (<6, 6–11, 12–23, 24–35 and >35 months old). NoV patients were neither found in <6 nor in >35 months of age, while 27 out of 28 NoV patients were between 6 and 23 months of age. Similarly, three out of four SaV cases were classified into either 6–11 or 12–23 months age group. Majority of NoV patients (22/28, 78.6%) were male, however, three out of four SaV patients were female. Although the surveillance was conducted in a children's hospital in Ho Chi Minh City, only 11/28 (39.3%) cases lived in the city, the remaining 17/28 (60.7%) of NoV patients came from various provinces in the southern part of Vietnam.

Clinical signs and symptoms of NoV infections
Seventeen patients showing monoinfection with NoV [31] were selected for analysis of the clinical manifestations, among them 15 medical records were enough data for further analyses. The main clinical signs and symptoms observed in children with NoV infection were diarrhea (100%), watery stool (93.3%), vomiting (66.7%), highest temperature ≥38.5°C (33.3%), coughing (26.7%) and coryza (6.7%). The mean duration of diarrhea and vomiting were 4.4 ± 3.9 days and 1.5 ± 1.7 days, respectively, and the maximum episodes of diarrhea and vomiting were 6.5 ± 2.5 times per day and 3.3 ± 2.8 times per day, respectively (Table 4).

Evaluation of severity in patients showing monoinfection with NoV by using a 20-point numerical score showed that the mean severity score of NoV positive patients was 9.8 ± 3.6. The severity scores were analyzed further by age groups, gender, place of living (Ho Chi Minh City and non-Ho Chi Minh City residents), time of collection (during rainy and dry season) and status of patients (hospitalized and non-hospitalized patients) (Table 3). Obviously, the mean severity scores of patients belonging to some groups were observationally lower than those of other groups (e.g., patients who were 12–23 months old, or patients who lived in Ho Chi Minh City); however, the difference was not statistically significant ($p > 0.05$). The only significant difference was observed between inpatients and outpatients, with the mean severity scores in each group being 10.82 ± 3.49 ($N = 11$) and 7.0 ± 2.45 ($N = 4$), respectively ($p < 0.05$). A comparison of the mean severity scores between monoinfection cases and

TABLE 3
Attributes of NoV positive cases[a] and mean severity score of patients in each group

	Distribution of patients by											
	Age (months)					Gender		Place of living		Patient status		
	<6	6–11	12–23	24–35	>35	Male	Female	HCMC[b]	Others	Inpatient	Outpatient
No. (%) of NoV cases	0 (0)	10 (35.7)	17 (60.7)	1 (3.6)	0 (0)	22 (78.6)	6 (21.4)	11 (39.3)	17 (60.7)	21 (75)	7 (25)
Vesikari's score[c]		11.4 ± 4.28 (*N* = 5)	8.67 ± 3.16 (*N* = 9)	12.0 (*N* = 1)		9.7 ± 3.97 (*N* = 10)	10.0 ± 3.16 (*N* = 5)	8.2 ± 1.92 (*N* = 5)	10.6 ± 4.06 (*N* = 10)	10.82 ± 3.49[d] (*N* = 11)	7.0 ± 2.45[d] (*N* = 4)

[a]Data based on 28 complete medical records.
[b]HCMC, Ho Chi Minh City.
[c]Data based on 15 medical records of patients who showed monoinfection with NoV.
[d]$P < 0.05$.

TABLE 4
Clinical signs and symptoms of patients who showed monoinfection with NoV during a one-year surveillance in Ho Chi Minh City, 2005–2006

Signs and symptoms	NoV infection cases
Diarrhea	100%
Watery stool	93.3%
Vomiting	66.7%
Temperature ≥ 38.5°C	33.3%
Coughing	26.7%
Coryza	6.7%
Mean duration of diarrhea	4.4 ± 3.9 days
Mean duration of vomiting	1.5 ± 1.7 days
Maximum episodes of diarrhea day	6.5 ± 2.5 times per day
Maximum episodes of vomit day	3.3 ± 2.8 times per day

mixed infection cases was also performed, however, the difference was not statistically significant (data not shown).

Only one medical record from two patients showing monoinfection with SaV was available, therefore, description of the clinical features of SaV infection in this study was not performed.

Phylogenetic analysis of NoV strains and identification of various recombinations

All of the 32 NoV strains detected in this study were successfully determined nucleotide sequence with the amplified fragments, which included both polymerase and capsid region. Phylogenetic analysis based on the capsid region revealed that 16/32 (50%) NoV strains clustered within the GII.4, and 13/32 (40.6%) strains belonged to the GII.3b cluster, according to the classification reported by Phan *et al.* [14]. One strain, HCMC91, clustered together with GII.12 NoV strains (96% nucleotide identity with the Chitta strain), and other two strains, HCMC204 and HCMC311, belonged to the GII.6 cluster (95% nucleotide identity with the SaitamaU17 strain). Interestingly, these two Vietnamese GII.6 strains did not group with any GII.6 NoV strains from sublineage a to d, therefore, clustered into a novel sublineage, tentatively called GII.6e (Fig. 1).

To verify the sequence identities of the GII strains, an additional phylogenetic analysis of Vietnamese NoV strains and other reference strains based on the polymerase region was performed (Fig. 2). All of the 16 capsid-based GII.4 NoV strains maintained their genotype in the polymerase region, however, other strains bored a different either genotype or subgenotype when polymerase-based grouping was carried out. Twelve out of the 13 capsid-based GII.3b NoV strains clustered into the GII.3a lineage.

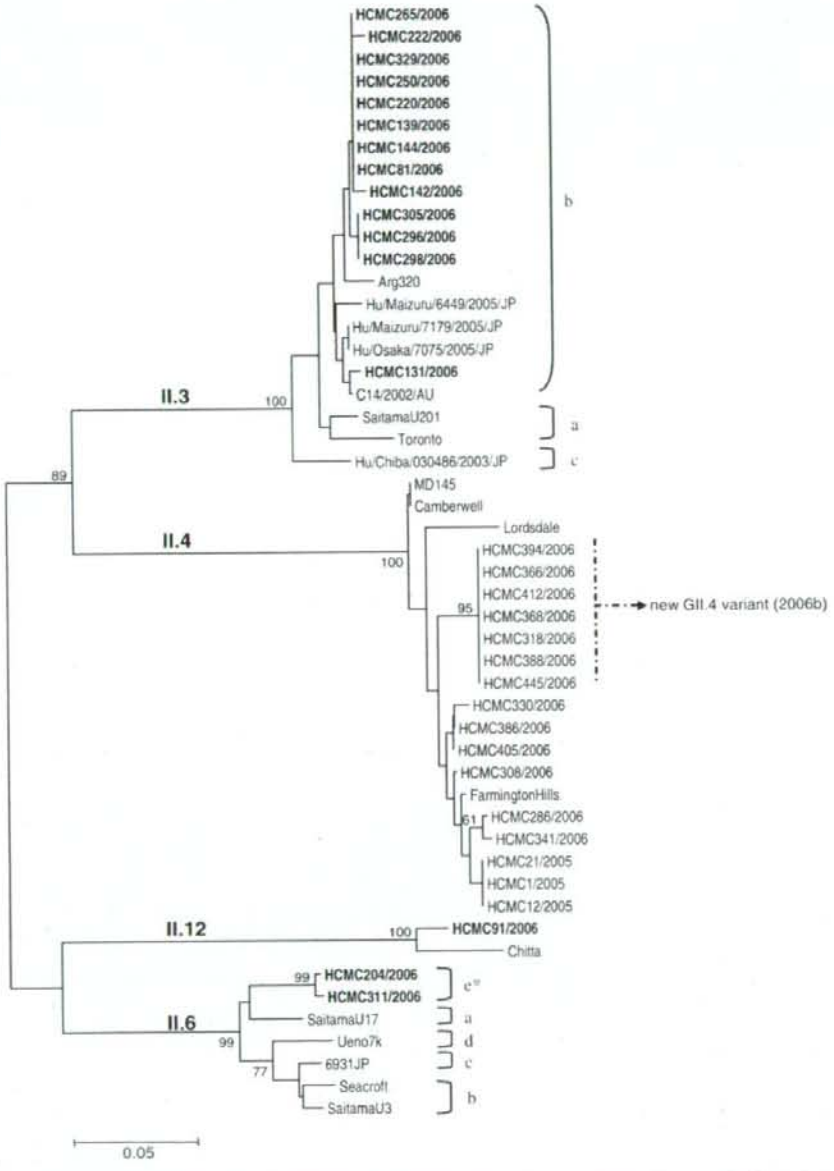

FIG. 1. Phylogenetic tree of the capsid region of 32 Vietnamese NoVs and other reference NoV strains available from GenBank. Vietnamese recombinant strains are in bold face. Genotypes and subgenotypes are indicated. Bootstrap values >75% are shown at the brach nodes.

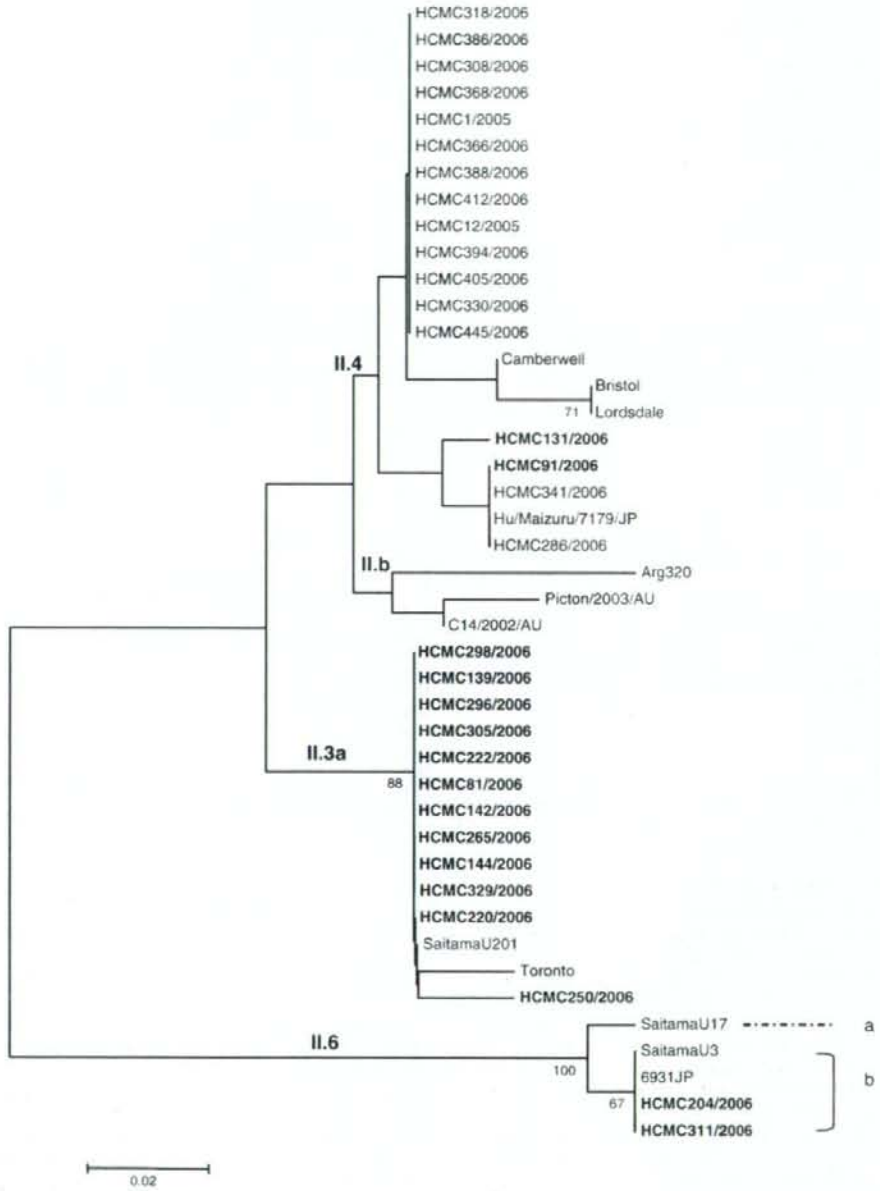

FIG. 2. Phylogenetic tree of the polymerase region of 32 Vietnamese NoVs and other reference NoV strains available from GenBank. Vietnamese recombinant strains are in bold face. Genotypes and subgenotypes are indicated.

TABLE 5
Molecular characteristics of NoVs strains detected among patients with acute gastroenteritis in the Children's Hospital 1, Ho Chi Minh City, during 2005–2006

Strain	Collection date[a]	Polymerase		Capsid		Type of recombination
		Genogroup genotype	Representative	Genogroup genotype	Representative	
HCMC1	Dec	II.4		II.4		
HCMC12	Dec	II.4		II.4		
HCMC21	Jan	II.4		II.4		
HCMC81	Feb	II.3a	Toronto	II.3b	Arg320	Intersubgenotype
HCMC91	Feb	II.4	Lordsdale	II.12	Chitta	Intergenotype
HCMC131	Mar	II.4	Lordsdale	II.3b	Arg320	Intergenotype
HCMC139	Mar	II.3a	Toronto	II.3b	Arg320	Intersubgenotype
HCMC142	Mar	II.3a	Toronto	II.3b	Arg320	Intersubgenotype
HCMC144	Mar	II.3a	Toronto	II.3b	Arg320	Intersubgenotype
HCMC204	May	II.6b	SaitamaU3	II.6e	N/A	Intersubgenotype
HCMC220	May	II.3a	Toronto	II.3b	Arg320	Intersubgenotype
HCMC222	May	II.3a	Toronto	II.3b	Arg320	Intersubgenotype
HCMC250	Jun	II.3a	Toronto	II.3b	Arg320	Intersubgenotype
HCMC265	Jun	II.3a	Toronto	II.3b	Arg320	Intersubgenotype
HCMC286	Aug	II.4		II.4		
HCMC296	Aug	II.3a	Toronto	II.3b	Arg320	Intersubgenotype
HCMC298	Aug	II.3a	Toronto	II.3b	Arg320	Intersubgenotype
HCMC305	Aug	II.3a	Toronto	II.3b	Arg320	Intersubgenotype
HCMC308	Aug	II.4		II.4		
HCMC311	Aug	II.6b	SaitamaU3	II.6e	N/A	Intersubgenotype
HCMC318	Sep	II.4		II.4		
HCMC329	Sep	II.3a	Toronto	II.3b	Arg320	Intersubgenotype
HCMC330	Sep	II.4		II.4		
HCMC341	Sep	II.4		II.4		
HCMC366	Oct	II.4		II.4		
HCMC368	Oct	II.4		II.4		
HCMC386	Oct	II.4		II.4		
HCMC388	Oct	II.4		II.4		
HCMC394	Oct	II.4		II.4		
HCMC405	Nov	II.4		II.4		
HCMC412	Nov	II.4		II.4		
HCMC445	Nov	II.4		II.4		

N/A, not applicable.

whereas the other strain, HCMC131, grouped with other GII.4 strains when polymerase-based grouping was performed. This type of recombination, GII.3b/GII.4, was similar to that of the NoV recombinant strain 5017/04/JP, which was reported formerly [36]. Similarly, the capsid-based GII.12 strain, HCMC91, bored a different genotype, GII.4 when a BLAST search was performed in the polymerase region. This strain also shared best identity, 96%, with the well-known GII.4/GII.12 recombinant strain SaitamaU1 [22] in both the polymerase and capsid region, demonstrating that HCMC91 was also a recombinant virus. Regarding two capsid-based GII.6e strains, HCMC204 and HCMC311, the polymerase-based phylogenetic tree clearly showed that they clustered together with other NoV strains into the GII.6b sublineage, therefore, these two Vietnamese strains were GII.6b/GII.6e recombinant strains. Altogether, half of the NoV (16/32) strains identified in this study were determined as recombinant viruses (Table 5).

Phylogenetic analysis of SaV strains and the identification of a novel recombination

Results of nucleotide sequencing of the 434 bp PCR product allowed us to analyze the molecular characteristics of both polymerase and the capsid region of SaV strains detected. Among six Vietnamese SaV strains, genotype GI.1, GI.2 and GII.1 were identified in two, one and one strain, respectively, and all of these four SaV strains maintained the same genogroup/genotype across polymerase and the capsid region (Fig. 3). However, the remaining two strains, HCMC86 and HCMC180, showed different genotypes when the polymerase-based and capsid-based phylogenetic analyses were conducted. These two SaV strains shared 100% nucleotide identity,

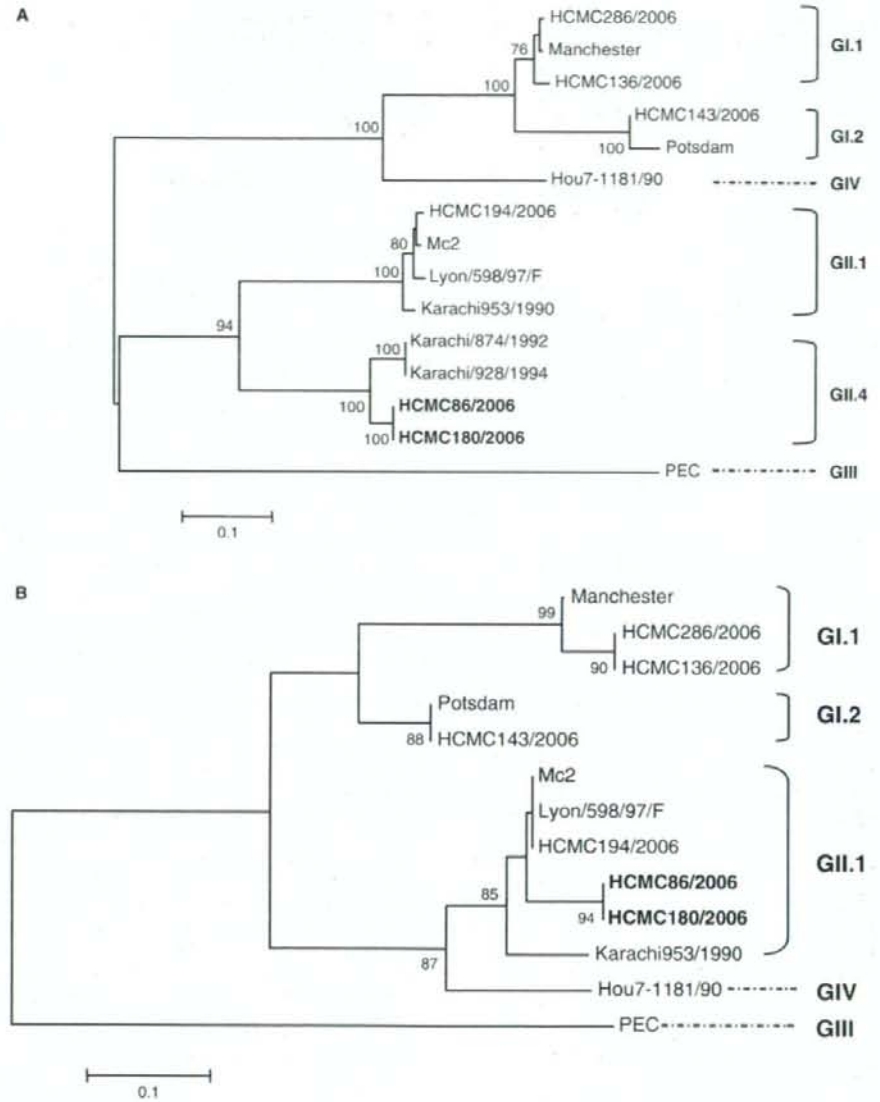

FIG. 3. Phylogenetic tree of the (**A**) capsid and (**B**) polymerase region of 32 Vietnamese SaVs and other reference SaVs. Two Vietnamese SaVs strain, HCMC86 and HCMC180, showed different genotypes when polymerase-based and capsid-based phylogenetic trees were constructed.

which indicates that they are the same strain. Nucleotide comparison showed that HCMC86 and HCMC180 had best identities (89.8–94.2%) with GII.1 strains in the polymerase region; however, they had higher homology with two Pakistani GII.4 SaV strains (Karachi/874 and Karachi/928) than GII.1 strains (94.2% vs. 67.7–68.1%) when a capsid-based comparative analysis was performed (Table 6).

TABLE 6
Percentage nucleotide homology of the polymerase and capsid region[a] of Vietnamese SaV strains and other reference strains

	I.1			I.2		IV	II.1			II.4				III
	Manchester	HCMC286	HCMC136	Potsdam	HCMC143	Hou7-1181	Mc2	HCMC194	Lyon 598	HCMC86	HCMC180	Karachi 874	Karachi 928	PEC
Manchester		99.2	97.4	80.8	81.5	65.7	48.9	49.6	49.6	49.6	49.6	48.5	48.5	45.4
HCMC286	97.1		97.4	81.2	81.9	66.0	48.9	49.6	49.6	49.2	49.2	48.2	48.2	45.4
HCMC136	97.1	100		80.4	80.4	65.3	48.9	49.6	49.6	48.5	48.5	48.2	48.2	45.4
Potsdam	84.0	81.1	81.1		97.1	63.1	46.0	45.3	46.0	44.3	44.3	42.9	42.9	41.4
HCMC143	84.0	81.1	81.1	100		63.8	47.1	46.4	47.1	46.0	46.0	44.6	44.6	40.7
Hou7-1181	73.9	72.4	72.4	73.9	73.9		46.7	47.1	47.5	49.2	49.2	47.1	47.1	40.8
Mc2	72.4	69.5	69.5	76.8	76.8	85.5		98.9	98.2	68.1	68.1	67.3	67.3	43.5
HCMC194	68.1	65.2	65.2	72.4	72.4	81.1	95.6		97.8	68.1	68.1	67.3	67.3	43.2
Lyon 598	72.4	69.5	69.5	76.8	76.8	85.5	100	95.6		67.7	67.7	67.0	67.0	44.6
HCMC86	71.0	71.0	71.0	75.3	75.3	84.0	94.2	89.8	94.2		100	94.2	94.2	45.7
HCMC180	71.0	71.0	71.0	75.3	75.3	84.0	94.2	89.8	94.2	100		94.2	94.2	45.7
Karachi 874[b]	N A	N A	N A	N A	N A	N A	N A	N A	N A	N A	N A		100	45.0
Karachi 928[b]	N A	N A	N A	N A	N A	N A	N A	N A	N A	N A	N A	N A		45.0
PEC	50.7	50.7	50.7	55.0	55.0	50.7	52.1	49.2	52.1	52.1	52.1	N A	N A	

Best identities results of HCMC86 and HCMC180 are in shaded.
[a]Nucleotide homology of polymerase region is shown in the lower left, and capsid region is in the upper right. Genogroups and genotypes are also indicated.
[b]The nucleotide sequences of the polymerase region of Karachi 874 and Karachi 928 are not available.

The phylogenetic analysis also indicated clearly that HCM86 and HCM180 clustered into two different genotypes when polymerase-based and capsid-based nucleotide phylogenetic trees were constructed. Altogether, these two Vietnamese SaV strains are GII.1/GII.4 recombinant strains.

Discussion

In this study, we reported the detection of NoVs and SaVs among diarrheic children in the Children's Hospital 1, Ho Chi Minh City, during 2005–2006. With the overall detection rate of 6.4% and 1.2%, respectively, NoVs and SaVs continued to be viral agents causing acute gastroenteritis in children in the southern part of Vietnam. Although these detection rates were slightly lower than those of the studies in developed countries [37, 38], the results in this study were similar to those of the epidemiological studies conducted previously at the same hospital [29, 30], and also comparative with other surveillances in other developing countries [39, 40]. Despite difference of time, the detection of NoVs and SaVs with similar proportions in the southern part of Vietnam indicated that these viruses have circulated stably in the area. NoV GI was not found in this study, and this result was in agreement with the previous study [29]. The absence of NoV GI in epidemiological surveillance was also reported elsewhere [18, 34]. The primer sets using in this study have been used to screen caliciviruses in other surveys, and they successfully identified NoV GI in the studied samples. Therefore, the inability to detect NoV GI strains in this study might have resulted from the absence of this virus within the collected fecal specimens.

In temperate climate countries, NoVs are usually identified in the winter time [36, 38], whereas in tropical countries, the seasonal pattern of NoVs is not clear. In this survey, NoVs was found all year round, except in April and July. Moreover, 62.5% of NoVs were detected from May to October, indicated that this virus prevailed during the rainy season. This result was concordant with that of the previous study during 2002–2003 [29], and slightly different from the result of the 1999–2000 survey, in which, NoVs prevailed at the end of the rainy season and the first half of the dry season [30]. However, the results of the 1999–2000 survey based on the specimens that were negative for other common viral agents, therefore, the absence of NoVs strains, if any, which were mixed infection with other viruses, might make the feature of monthly distribution of NoVs incomplete.

NoV GII.4 was the most common (50.0%) genotype among NoV strains detected in this study. Previous studies in Ho Chi Minh City also found NoV GII.4 in 78% and 82.1% of samples [29, 30], confirming the predominance of this genotype.

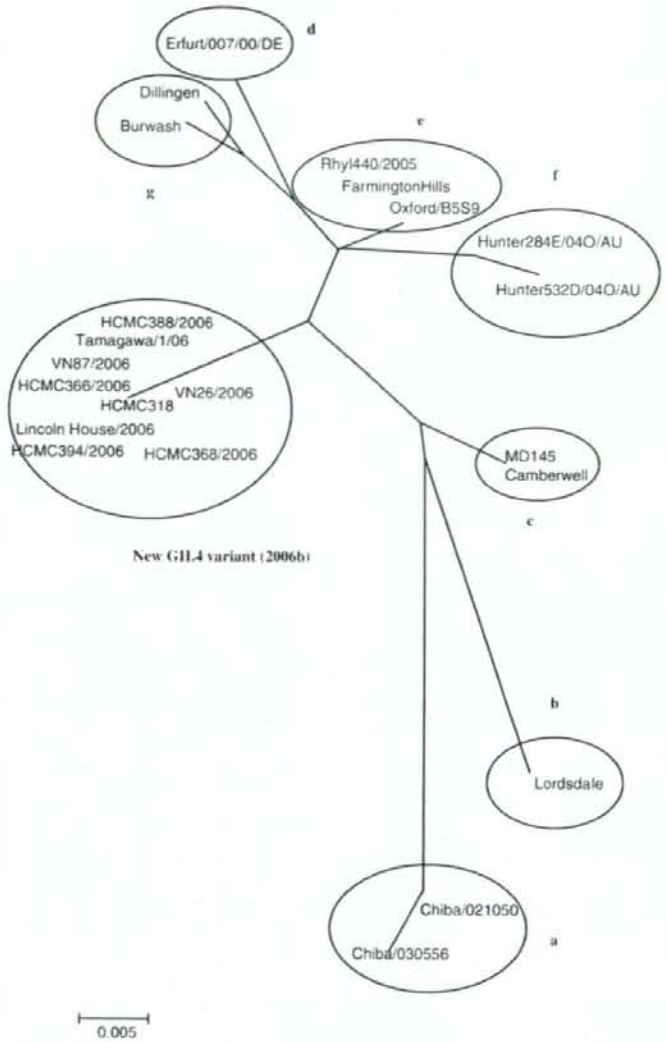

FIG. 4. Unrooted phylogenetic tree of new NoV GII.4 variant identified in this study and other GII.4 lineages. Vietnamese NoVs detected from another study [41], VN26 and VN87, as well as other reference strains, Tamagawa/1/06 and Lincoln House/2006, are included. The classification is based on Phan *et al.* [14].

However, 7 out of 16 Vietnamese GII.4 strains in this study belonged to a distinct cluster which has been determined as a novel GII.4 variant, 2006b [41] (Figs 1 and 4). These strains were firstly identified in September, and continued to be detected until the end of the surveillance, suggesting that these viruses have been continuing to prevail in this area in the coming year. Different genotypes of NoVs and SaVs were determined in this study, and among them, several genotypes have not been reported formerly in Vietnam (NoV GII.6, SaV GI.2 and GII.4). Of interest, SaV GII.4 was only

reported in two unique Pakistani strains collected in 1992 and 1994, respectively [42]. On the other hand, the 'new variant' designated GII.b NoVs, which has been detected in Europe in the beginning of 2000s and then identified in Asia [36, 38, 43, 44], could not be found in this study. A larger number of specimens, as well as an attempt to collect fecal samples from different places in Vietnam is needed for confirming the absence of this virus in the country.

Although detected in several epidemiological studies, and being considered as important viral agent causing acute gastroenteritis in young infants and children, this was the first time, to our knowledge that the clinical manifestations of NoV infections were described in Vietnamese pediatric patients. The clinical features of NoV-associated acute gastroenteritis observed among patients in this study were similar to those of other reports, including diarrhea with watery stool, vomiting and fever [18, 20]. Although the results of this study were comparable to another study conducted in Japan [18], the mean duration of diarrhea and maximum episodes of diarrhea per day in Vietnamese children were observationally higher than those of Finnish children (4.4 days vs. 2 days, 6.5 times/day vs. 4 times/day) [45]. The difference might be explained by the population studied. In this study, we collected samples from patients who sought to the hospital, whereas the survey carried out in Finland was a community-based study. Therefore, although both were classified as moderately severe diseases (8–10 points) [45], the mean severity score in Vietnamese patients was obviously higher than that of Finish children (9.8 vs. 8).

A comparative analysis was performed in order to see the difference in severity among several groups of patients, however, only the mean scores were statistically different between inpatients and outpatients. This situation was also observed among astrovirus positive patients described previously [31].

The clinical manifestation of SaV infection in this study could not be demonstrated because only one medical record among two SaV monoinfection cases was available. This patient suffered from an 8-day diarrhea, with maximum episode of diarrhea was 20 times per day and high fever up to 39°C. This feature was much different from other reports, which described SaV-associated diarrhea to be a mild disease. More clinical data on larger number of patients are needed in order to identify properly the clinical features of SaV infection in Vietnamese children.

RNA recombination plays a key role in virus evolution and it shapes a good deal of the virus diversity [46]. Recombinant NoV strains were increasingly found in epidemiological surveillances throughout the world [22, 23, 38, 43], including Vietnam [30]. In this survey, various types of recombination in NoVs were identified. Of interest, the GII.6b (polymerase)/GII.6e (capsid) recombination was first reported in this study. Similarly, the recombinant GII.1/GII.4 SaV strain detected in this survey has not been described elsewhere. Half of NoV strains, and one out of six SaV strains were identified as recombinant viruses, thus indicates that recombination is not a rare event, and the caliciviruses circulating in Vietnam have a trend to be more diverse.

The results of this study highlight the impact of caliciviruses in diarrheal diseases among children in Ho Chi Minh City, and are the first to describe the clinical manifestations of NoV infections in Vietnamese children. The data of nucleotide analysis from this study could provide useful information for knowledge on caliciviruses characteristics.

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Journal of Medical Virology 80:1749–1755 (2008)

Genetic Diversity of Norovirus, Sapovirus, and Astrovirus Isolated From Children Hospitalized With Acute Gastroenteritis in Chiang Mai, Thailand

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Norovirus (NV), sapovirus (SV), and human astrovirus (HAstV) are important causes of acute gastroenteritis in infants and young children. This study investigated the prevalence of NV, SV, and HAstV infections in children hospitalized with acute gastroenteritis in Chiang Mai, Thailand from May 2000 to March 2002. Fecal specimens were tested for NV, SV, and HAstV by reverse transcription polymerase chain reaction (RT-PCR) using degenerate specific primers. These viruses were characterized further by sequence and phylogenetic analyses of the partial capsid gene. From 296 fecal specimens tested, 13.5% (40 of 296) were positive for NV, SV, and HAstV. Of these, NV most predominant, with a prevalence of 60% (24 of 40), of which 17.5% were NVGI and 42.5% were NVGII. Of note, one specimen was positive for both NVGI and SV. SV was detected in 25%, while HAstV was detected in 17.5%. Analysis of nucleotide and amino acid sequences revealed that NVGI strains comprised GI/3, GI/4, GI/6, GI/7, and GI/13 genotypes. Among NVGII strains, approximately half of them belonged to genotype GII/4 (Lordsdale virus cluster), followed by GII/3, GII/10, GII/1, GII/6, GII/8, and GII/15. Analysis of SV sequences revealed that SVGI (Manchester virus) was more common than SVGII (London virus). The SV genotypes detected in this study belonged to SVGI/1, SVGI/4, SVGI/5, SVGII/1, and SVGII/2, whereas the HAstV belonged to genotypes HAstV-1, HAstV-2, HAstV-3, and HAstV-5. The findings suggest that NV, SV, and HAstV are important enteric viruses cocirculating among hospitalized children in Chiang Mai, Thailand. ***J. Med. Virol. 80:1749–1755, 2008.*** © 2008 Wiley-Liss, Inc.

KEY WORDS: noroviruses; sapoviruses; astroviruses; acute gastroenteritis; Chiang Mai; Thailand

INTRODUCTION

Acute gastroenteritis is one of the most common diseases in infants, children, and adults worldwide. During the first 5 years of life, every child will contract diarrheal disease, which enhances the risk of dehydration and nutritional deficiency [Jiraphongsa et al., 2005]. In addition to rotavirus (RV), human caliciviruses and astrovirus have emerged as etiologic causes of acute gastroenteritis in this age group. Norovirus (NV) and sapovirus (SV) are classified into the family *Caliciviridae* and are members of nonenveloped, positive-sense, single-stranded RNA viruses. Their genome contain approximately 7,300–8,300 nucleotides long, and a genome-linked protein (VPg) at the 5′ terminus and a poly A tail at the 3′ terminus [Bertolotti-Ciarlet et al., 2003]. Human astrovirus (HAstV) is a member of the family *Astroviridae*, and has a small (28–30 nm in diameter), round, nonenveloped characteristic. The genome is a positive-sense, single-stranded RNA of approximately 6,800 nucleotides in length [Schnagl et al., 2002].

NVs can be divided into five distinct genogroups based on the variation in the capsid gene sequences, in which strains belonging to GI, GII, and GIV are found in humans, whereas GIII and GV are found in cows and mice, respectively [Zheng et al., 2006]. Recently, NVs were classified into 8, 17, 1, 1, and 1 genotypes in GI, GII, GIII, GIV, and GV, respectively [Zheng et al., 2006]. SVs are divided into five genogroups (GI to GV) based on the

Grant sponsor: Faculty of Medicine; Grant sponsor: Graduate School of Chiang Mai University, Chiang Mai, Thailand.

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Accepted 7 May 2008
DOI 10.1002/jmv.21244
Published online in Wiley InterScience (www.interscience.wiley.com)