Heliyon 7 (2021) e08250

Contents lists available at ScienceDirect

Heliyon

journal homepage: www.cell.com/heliyon

Research article

Spread of genetically similar noroviruses in Bangkok, Thailand, through symptomatic and asymptomatic individuals

Patcharaporn Boonyos [a,1], Michittra Boonchan [a,1], Benjarat Phattanawiboon [a,1], Nutthawan Nonthabenjawan [a,1], Ratana Tacharoenmuang [b], Ratigorn Gunpapong [b], Phakapun Singchai [b], Sompong Upchai [b], Pimpha Rungnobhakhun [c], Jutarat Mekmullica [c], Worakarn Towayunanta [d], Kobkool Chuntrakool [d], Karn Ngaopravet [d], Kriangsak Ruchusatsawat [b], Somchai Sangkitporn [b], Ballang Uppapong [b], Eisuke Mekada [e], Yoshiharu Matsuura [f], Masashi Tatsumi [a], Hiroto Mizushima [a,*]

[a] Thailand–Japan Research Collaboration Center on Emerging and Re-emerging Infections, Nonthaburi, Thailand
[b] National Institute of Health, Department of Medical Sciences, Ministry of Public Health, Nonthaburi, Thailand
[c] Bhumibol Adulyadej Hospital, Bangkok, Thailand
[d] Public Health Center 41, Bangkok, Thailand
[e] Research and Education Promotion Foundation, Bangkok, Thailand
[f] Research Institute for Microbial Diseases, Osaka University, Osaka, Japan

HIGHLIGHTS

- GII.4 Sydney predominated in gastroenteritis patients of Bangkok during 2017–2019.
- Common norovirus genotypes spread in symptomatic and asymptomatic individuals.
- Noroviruses in symptomatic and asymptomatic individuals share genetical similarity.

ARTICLE INFO

Keywords:
Norovirus
Symptomatic infection
Asymptomatic infection

ABSTRACT

Norovirus infection is a major cause of acute gastroenteritis, although some infected individuals are asymptomatic. GII.4 is the predominant genotype worldwide and, since 2000, has been the most prevalent in patients in Thailand with acute gastroenteritis. We screened stool samples for norovirus in 786 patients with acute gastroenteritis who were admitted to a hospital in Bangkok from 2017 to early 2019 and detected it in 136 specimens (17.3%). Eight and 124 specimens were positive for the GI and GII genogroups, respectively, and the remaining 4 specimens were double-positive. Nine genotypes (GI.3, GI.5, GII.2, GII.3, GII.4, GII.6, GII.8, GII.13, and GII.17) were identified from 140 strains, and 72 strains (51.4%) were GII.4. We had previously conducted a one-year survey of norovirus infection in residents of a community in Bangkok from May 2018 to April 2019 and found that a substantial portion of the residents were infected asymptomatically. The 9 genotypes identified in the patients were also commonly identified in the community residents. To investigate the relationship between noroviruses identified in the acute gastroenteritis patients and those identified in the community residents, phylogenetic tree analysis was conducted. Of the 9 genotypes, 8 showed similarities in both their genomic sequences and their deduced amino acid sequences. In addition, strain replacement of GI.3 was observed in both the patients and the community residents within the overlapping period. These results suggested that norovirus spreads efficiently to the community by simultaneously causing symptomatic and asymptomatic infections.

* Corresponding author.
E-mail address: hmizushi@biken.osaka-u.ac.jp (H. Mizushima).
[1] These authors contributed equally to this work.

https://doi.org/10.1016/j.heliyon.2021.e08250
Received 23 July 2021; Received in revised form 16 September 2021; Accepted 21 October 2021

1. Introduction

Acute gastroenteritis (AGE) caused by norovirus (NV) infection is a public health concern. It is estimated that approximately 20% of diarrhea cases are associated with NV infections (Lopman et al., 2016). NV is thought to be a perfect pathogen whose properties, such as a low infectious dose and high stability in the environment, allow persistent prevalence in human communities (Hall, 2012). The high viability of NV in the environment facilitates transmission through indirect contact mediated by substances such as contaminated food and water (Kotwal and Cannon, 2014). In addition, the existence of more than 40 genotypes that are presumed to have distinct antigenicities may increase infection frequency by allowing NVs to avoid herd immunity (Ford-Siltz et al., 2020). It has also been shown that genomic mutation drives the emergence of strains with antigenicities (Lindesmith et al., 2011, 2012).

NV's diversity has been attributed to genomic mutation and recombination, and 49 genotypes belonging to 10 genogroups (GI to GX) have been identified thus far (Chhabra et al., 2019). In Thailand, GII.4 followed by GII.3 were the most prevalent genotypes in symptomatic individuals from 2000 to 2016 (Kumthip et al., 2018). A study reported that GII.4 was still dominant in AGE cases until 2018 (Chuchaona et al., 2019), although the dominance of GII.2 and GII.17 in sporadic cases was reported (Thanusuwannasak et al., 2018). Recombinant viruses of GII.2, GII.3, GII.4, GII.6, GII.12, GII.13, and GII.14 have been detected (Kumthip et al., 2018). Recombinant strains of GII.2 (Supadej et al., 2019; Thanusuwannasak et al., 2018) and GII.4 (Charoenkul et al., 2020; Chuchaona et al., 2019) have been detected in AGE patients since 2015. We recently identified a GII.14 recombinant strain from a post-symptomatic individual (Nonthabenjawan et al., 2020).

A meta-analysis of epidemiological studies of healthy individuals showed that asymptomatic NV infections occur in a population of individuals (Qi et al., 2018). In our recent study of residents of a community in Bangkok, nearly 90% of NV infections detected were derived from individuals who had not experienced an episode of diarrhea within the past month (Phattanawiboon et al., 2020). The study identified GII.4 as the most common genotype in asymptomatic individuals. The predominance of GII.4 in asymptomatic individuals has been reported in Tanzanian and American Indian children (Grant et al., 2017; Moyo et al., 2014), while the dominance of non-GII.4 genotypes in asymptomatic individuals has also been reported in other countries (Wang et al., 2018; Bucardo et al., 2017; Utsumi et al., 2017; Koo et al., 2016). These findings suggest that genotype distributions in symptomatic and asymptomatic individuals vary depending on the setting or country. Since a difference between symptomatic and asymptomatic individuals in the duration of viral shedding has been reported, this difference may be the cause of the differential prevalence of genotypes (Wu et al., 2019; Teunis et al., 2015). In addition, the relationship between NVs identified in symptomatic individuals and those found in asymptomatic individuals has not been well elucidated.

To investigate the relationship between NVs identified in AGE patients and those found in asymptomatic individuals, we carried out an epidemiological study of NV in AGE patients admitted to Bhumibol Adulyadej Hospital, Bangkok, and compared the NVs identified in AGE patients with those found in community residents. Here we demonstrate that the genomic sequences of 8 genotypes identified in the AGE patients show high similarity to those in the community residents. The maintenance and transmission relationships between NVs identified in symptomatic and asymptomatic individuals are also discussed.

2. Materials and methods

2.1. Stool specimen collection

The protocol of this study was reviewed and approved by the Ethics Committee of the Institute for the Development of Human Research Protections in Thailand. Diarrhea stool specimens were collected from patients aged 0–14 years who were hospitalized for AGE in the pediatric ward of Bhumibol Adulyadej Hospital, Bangkok. AGE patients with only symptoms other than diarrhea, such as vomiting, were not included in this study. The hospital is located approximately 30 km from the Klong Toei district, where the community survey was conducted (Phattanawiboon et al., 2020). Specimens from minors were obtained with the consent of their parent(s) or guardian(s). Specimens were stored in a -80 °C freezer until use.

2.2. Detection and genotyping of NV

Stool specimens were suspended in phosphate-buffered saline to yield a 10% (w/v) suspension. NV was detected from RNA extracted from the stool suspension using the Viral RNA Mini Kit (Qiagen, Hilden, Germany) according to the manufacturer's instructions. Reverse transcription of RNA and the first PCR were performed using the OneStep RT-PCR Kit (Qiagen) and NV-specific primers. Semi-nested PCR was performed using ExTaq polymerase (Takara, Shiga, Japan) and NV-specific primers. NV-specific primers were as follows. The primer sets used for the first PCR were COG1F and GISKR for GI genotypes and COG2F and G2SKR for GII genotypes (Kojima et al., 2002). The primer sets used for the second PCR were G1SKF and G1SKR for GI genotypes and G2SFK and G2SKR for GII genotypes (Kojima et al., 2002). The second PCR products were run on an agarose gel and purified using NucleoSpin Gel and PCR Clean-up (Macherey-Nagel, Dueren, Germany). The primer sequences used were described previously (Phattanawiboon et al., 2020). The nucleotide sequence of the PCR product was determined using sequencer Model 310 (Applied Biosystems, Foster City, CA, USA). The genotype was determined by using the Norovirus Typing Tool (Kroneman et al., 2011). The nucleotide sequences of the strains determined in this study are available in the GenBank database (accession numbers: LC573290-LC573429). The genotype of each strain appears in Supplementary Table 1.

2.3. Determination of copy number

The copy numbers of NV in the stool specimens were determined by real-time RT-PCR as described previously (Phattanawiboon et al., 2020).

2.4. Phylogenetic tree analyses

The neighbor-joining method (Saitou and Nei, 1987) was used to infer evolutional history as follows. The percentage of replicate trees in which the associated taxa were clustered together in the bootstrap test (1000 replicates) is shown next to the branches (Felsenstein, 1985). The tree is drawn to scale, with branch lengths in the same units as those of the evolutionary distances used to infer the phylogenetic tree. The evolutionary distances were computed using the maximum composite likelihood method (Tamura et al., 2004) for nucleotide sequences or the Poisson correction method for amino acid sequences. Analyses were conducted using MEGA7 software (Kumar et al., 2016). Phylogenetic trees were inferred using 294 and 282 bases of the 5′ region of the open reading frame (ORF) 2 sequence of the GI and GII strains, respectively.

3. Results

3.1. NV epidemics in AGE patients from 2017 to early 2019 in Bangkok

To investigate epidemics of NV in symptomatic individuals in Bangkok, we determined the NV genotypes in specimens collected from 2017 to early 2019 in AGE patients. A total of 786 specimens were collected over nine periods (Table 1). NVs belonging to the GI or GII genogroup were detected by RT-PCR, and their genotypes were determined. Of the 786 specimens, 136 (17.3%) were NV-positive: single infections with GI and GII were detected in 8 and 124 specimens, respectively, and co-infection with GI and GII was detected in 4 specimens. As a result, a total of 140 strains were detected. The genotype and copy number of each strain are shown in Supplementary Table 1.

Table 1. NV infection in AGE patients. Number of NV-positive specimens is shown for each genotype.

Date	May 30 2017	Oct 4 2017	Dec 17 2017	Jan 10 2018	Feb 7 2018	Feb 27 2018	Aug 9 2018	Oct 30 2018	Jan 24 2019	Total	%[a]
GI.3			2	3				1	3	9	6.4
GI.5							2		1	3	2.2
GII.2	8									8	5.7
GII.3	15		1					4	2	22	15.7
GII.4	4	1	3	7	15	8	5	2	27	72	51.4
GII.6									1	1	0.8
GII.8					2	2		1	1	6	4.3
GII.13							3	7		10	7.1
GII.17		3	1	1	2	1	1			9	6.4
Total	113	57	103	110	98	61	107	80	57	786	
NV	27	4	7	11	19	11	11	15	35	136	
NV %	23.9	7	4.9	10	19.4	18	10.3	18.8	61.4	17.3	

Co-infection with GI and GII was counted as independent infections.
[a] Percentage of detection frequencies of each genogroup among NV-positive specimens.

Sequencing of the 140 strains identified 2 GI genotypes (GI.3 and GI.5) and 7 GII genotypes (GII.2, GII.3, GII.4, GII.6, GII.8, GII.13, and GII.17). GII.4 was detected in all periods, while other genotypes were detected sporadically (Table 1). GII.4 was the most frequently detected (51.4%) among the identified genotypes, and all detected GII.4 strains were assigned as the Sydney 2012 variant. These results suggested that GII.4 Sydney 2012 was the most prevalent genotype in AGE patients in Bangkok during the overall study period.

3.2. NV genotypes in AGE patients and community residents in Bangkok

We recently conducted a survey of NV infection in community residents of a district of Bangkok and identified 14 genotypes (Phattanawiboon et al., 2020). Approximately 90% of the stool samples analyzed in the study were collected from individuals who had experienced no episode of diarrhea within the past month. Therefore, it was assumed that the data mostly represented the actual condition of NV infections in asymptomatic individuals.

To investigate the relationship between NVs in symptomatic and asymptomatic individuals, NVs identified in the AGE patients and the community residents were analyzed. A comparison of the NV genotypes revealed that all 9 identified in the AGE patients were also found in the community residents. GII.4 Sydney 2012 was the most frequently detected also in the community residents (Phattanawiboon et al., 2020). GII.3 was the second most common genotype in AGE patients (15.7%), while 2.9% of NV-positive specimens in the community residents had GII.3 (Phattanawiboon et al., 2020) (Supplementary Table 2). These results suggested a difference in genotype distribution between the AGE patients and the community residents. In our previous study, we detected an uncommon genotype, GII.8, in the community residents (Phattanawiboon et al., 2020). GII.8 was detected in the community residents after October 2018 (Phattanawiboon et al., 2020) and in AGE patients after February 2018 (Table 1). The detection rates of GII.8 in the community residents and in the AGE patients were 9.7% and 4.3%, respectively (Supplementary Table 2). The genotype distribution in the community residents was not significantly affected by the presence or absence of a diarrhea episode (Supplementary Table 2). These results indicated that the various NV genotypes had spread to Bangkok through both symptomatic and asymptomatic infections.

3.3. Phylogenetic tree analyses of NVs detected in AGE patients and community residents

To clarify the relationship between the NVs identified in the AGE patients and those found in the community residents, phylogenetic tree analysis was conducted based on the 5′-portion of the ORF2 gene and its deduced amino acid sequence corresponding to the amino-terminal portion of the VP1 protein. Since GI.3, GII.2, GII.4, and GII.8 were each detected in 6 or more specimens of AGE patients as well as in 6 or more community residents, these 4 genotypes were selected for this detailed analysis. The NV strains derived from AGE patients and those derived from community residents are represented by GenBank accession numbers LC573290 to LC573429 and LC521401 to LC521603, respectively. The genotype of each strain derived from AGE patients is shown in Supplementary Table 1.

3.3.1. GI.3

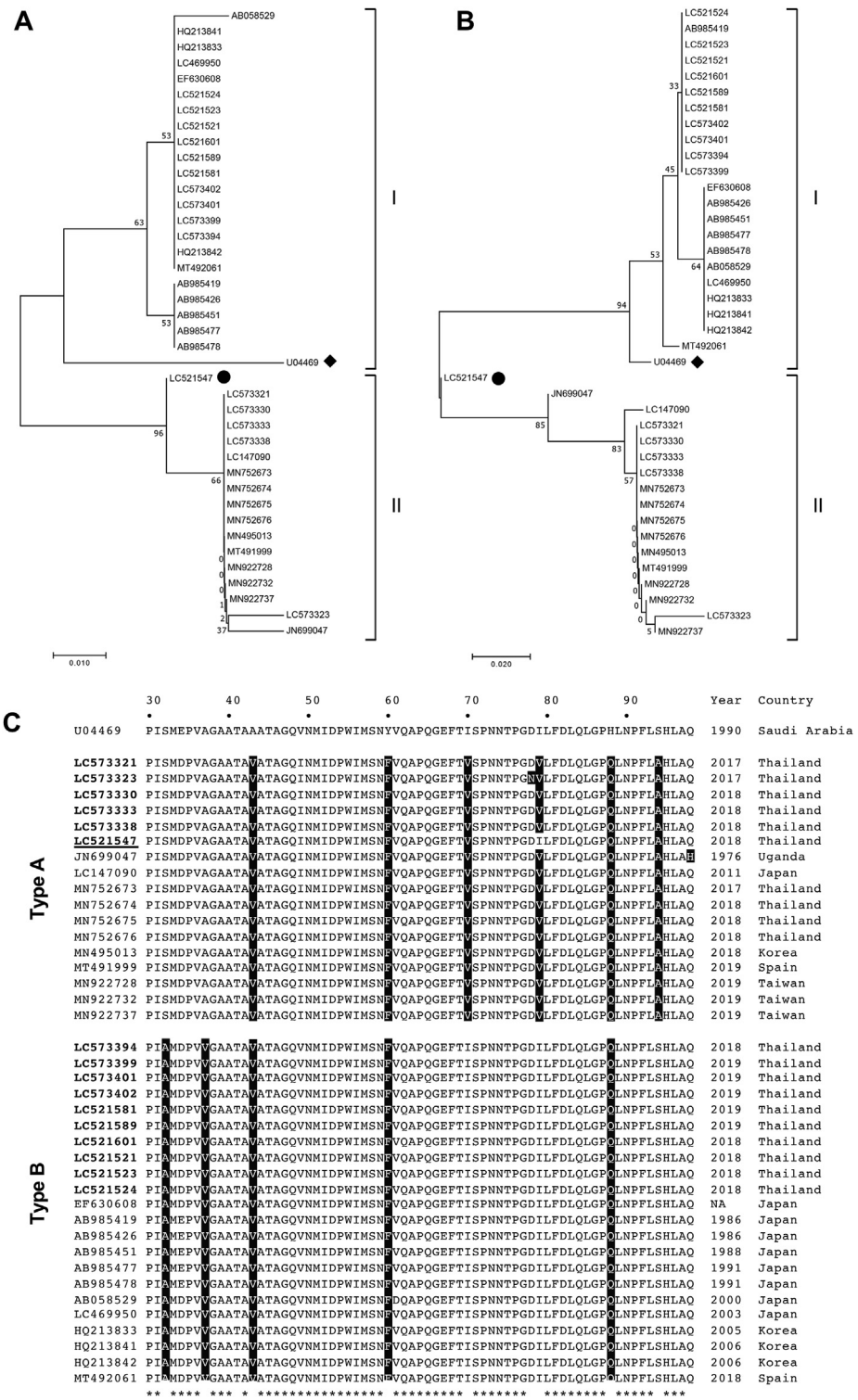
Nine and 7 strains were identified in AGE patients and community residents, respectively. Phylogenetic tree analyses of these strains, together with the reference strain U04469 and 23 related strains, led to the classification of these strains into two clusters with subclusters when analyzed based on nucleotide (Figure 1A) or amino acid (Figure 1B) sequences. Common amino acid substitutions (A43V, Y60F, and H88Q) were found compared with the reference strain (Figure 1C). Other amino acid substitutions (S32A, A37V, I70V, I79V, and S94A) were also identified in parts of the strains. These strains could be largely divided into two types: strains with the A43V, Y60F, I70V, I79V, H88Q, and S94A substitutions (tentatively referred to as Type A), and strains with S32A, A37V, A43V, Y60F, and H88Q substitutions (tentatively referred to as Type B). Types A and B fell into Cluster II and Cluster I, respectively (Fig. 1A and B). Each type included strains from both AGE patients and community residents. Type A strains were detected in 2017 and 2018, while Type B strains were identified in 2018 and 2019. This observation suggested that the strains were replaced around 2018.

3.3.2. GII.2

Eight and 7 strains were identified in AGE patients and community residents, respectively. These 15 strains were analyzed, as were 13 strains identified in Thailand by other groups from 2015 to 2017. All strains identified in our studies and 9 strains identified by other groups belonged to the same cluster, Cluster I, when analyzed based on nucleotide sequence (Figure 2A) or amino acid sequence (Figure 2B). Alignment of amino acid sequences showed a high similarity of strains identified in our studies (Figure 2C). Four strains identified from water samples by another group were placed into another cluster, Cluster II. These results suggested that at least two strains spread in Thailand from 2015 to 2019 and that strains having similar origins spread in Bangkok.

3.3.3. GII.8

Six and 10 strains were identified in AGE patients and community residents, respectively. These 16 strains were analyzed, as were 5 strains with complete or nearly complete 5′ sequences identified in Thailand by



C

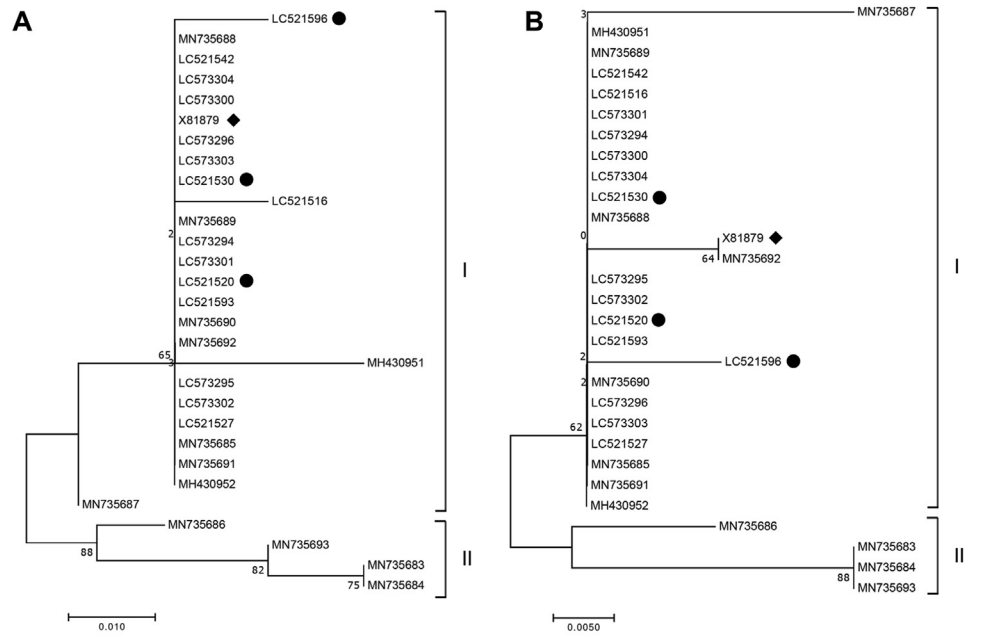
```
                    30        40        50        60        70        80        90          Year  Country
                    •         •         •         •         •         •         •
U04469              PISMEPVAGAATAAATAGQVNMIDPWIMSNYVQAPQGEFTISPNNTPGDILFDLQLGPHLNPFLSHLAQ  1990  Saudi Arabia

LC573321            PISMDPVAGAATAVATAGQINMIDPWIMSNFVQAPQGEFTVSPNNTPGDVLFDLQLGPQLNPFLAHLAQ  2017  Thailand
LC573323            PISMDPVAGAATAVATAGQINMIDPWIMSNFVQAPQGEFTVSPNNTPGNVLFDLQLGPQLNPFLAHLAQ  2017  Thailand
LC573330            PISMDPVAGAATAVATAGQINMIDPWIMSNFVQAPQGEFTVSPNNTPGDVLFDLQLGPQLNPFLAHLAQ  2018  Thailand
LC573333            PISMDPVAGAATAVATAGQINMIDPWIMSNFVQAPQGEFTVSPNNTPGDVLFDLQLGPQLNPFLAHLAQ  2018  Thailand
LC573338            PISMDPVAGAATAVATAGQINMIDPWIMSNFVQAPQGEFTVSPNNTPGDVLFDLQLGPQLNPFLAHLAQ  2018  Thailand
LC521547            PISMDPVAGAATAVATAGQINMIDPWIMSNFVQAPQGEFTVSPNNTPGDILFDLQLGPQLNPFLAHLAQ  2018  Thailand
JN699047            PISMDPVAGAATAVATAGQINMIDPWIMSNFVQAPQGEFTVSPNNTPGDVLFDLQLGPQLNPFLAHLAH  1976  Uganda
LC147090            PISMDPVAGAATAVATAGQINMIDPWIMSNFVQAPQGEFTVSPNNTPGDVLFDLQLGPQLNPFLAHLAQ  2011  Japan
MN752673            PISMDPVAGAATAVATAGQINMIDPWIMSNFVQAPQGEFTVSPNNTPGDVLFDLQLGPQLNPFLAHLAQ  2017  Thailand
MN752674            PISMDPVAGAATAVATAGQINMIDPWIMSNFVQAPQGEFTVSPNNTPGDVLFDLQLGPQLNPFLAHLAQ  2018  Thailand
MN752675            PISMDPVAGAATAVATAGQINMIDPWIMSNFVQAPQGEFTVSPNNTPGDVLFDLQLGPQLNPFLAHLAQ  2018  Thailand
MN752676            PISMDPVAGAATAVATAGQINMIDPWIMSNFVQAPQGEFTVSPNNTPGDVLFDLQLGPQLNPFLAHLAQ  2018  Thailand
MN495013            PISMDPVAGAATAVATAGQINMIDPWIMSNFVQAPQGEFTVSPNNTPGDVLFDLQLGPQLNPFLAHLAQ  2018  Korea
MT491999            PISMDPVAGAATAVATAGQINMIDPWIMSNFVQAPQGEFTVSPNNTPGDVLFDLQLGPQLNPFLAHLAQ  2019  Spain
MN922728            PISMDPVAGAATAVATAGQINMIDPWIMSNFVQAPQGEFTVSPNNTPGDVLFDLQLGPQLNPFLAHLAQ  2019  Taiwan
MN922732            PISMDPVAGAATAVATAGQINMIDPWIMSNFVQAPQGEFTVSPNNTPGDVLFDLQLGPQLNPFLAHLAQ  2019  Taiwan
MN922737            PISMDPVAGAATAVATAGQINMIDPWIMSNFVQAPQGEFTVSPNNTPGDVLFDLQLGPQLNPFLAHLAQ  2019  Taiwan

LC573394            PIAMDPVVGAATAVATAGQVNMIDPWIMSNFVQAPQGEFTISPNNTPGDILFDLQLGPQLNPFLSHLAQ  2018  Thailand
LC573399            PIAMDPVVGAATAVATAGQVNMIDPWIMSNFVQAPQGEFTISPNNTPGDILFDLQLGPQLNPFLSHLAQ  2019  Thailand
LC573401            PIAMDPVVGAATAVATAGQVNMIDPWIMSNFVQAPQGEFTISPNNTPGDILFDLQLGPQLNPFLSHLAQ  2019  Thailand
LC573402            PIAMDPVVGAATAVATAGQVNMIDPWIMSNFVQAPQGEFTISPNNTPGDILFDLQLGPQLNPFLSHLAQ  2019  Thailand
LC521581            PIAMDPVVGAATAVATAGQVNMIDPWIMSNFVQAPQGEFTISPNNTPGDILFDLQLGPQLNPFLSHLAQ  2019  Thailand
LC521589            PIAMDPVVGAATAVATAGQVNMIDPWIMSNFVQAPQGEFTISPNNTPGDILFDLQLGPQLNPFLSHLAQ  2019  Thailand
LC521601            PIAMDPVVGAATAVATAGQVNMIDPWIMSNFVQAPQGEFTISPNNTPGDILFDLQLGPQLNPFLSHLAQ  2018  Thailand
LC521521            PIAMDPVVGAATAVATAGQVNMIDPWIMSNFVQAPQGEFTISPNNTPGDILFDLQLGPQLNPFLSHLAQ  2018  Thailand
LC521523            PIAMDPVVGAATAVATAGQVNMIDPWIMSNFVQAPQGEFTISPNNTPGDILFDLQLGPQLNPFLSHLAQ  2018  Thailand
LC521524            PIAMDPVVGAATAVATAGQVNMIDPWIMSNFVQAPQGEFTISPNNTPGDILFDLQLGPQLNPFLSHLAQ  2018  Thailand
EF630608            PIAMDPVVGAATAVATAGQVNMIDPWIMSNFVQAPQGEFTISPNNTPGDILFDLQLGPQLNPFLSHLAQ  NA    Japan
AB985419            PIAMEPVVGAATAVATAGQVNMIDPWIMSNFVQAPQGEFTISPNNTPGDILFDLQLGPQLNPFLSHLAQ  1986  Japan
AB985426            PIAMEPVVGAATAVATAGQVNMIDPWIMSNFVQAPQGEFTISPNNTPGDILFDLQLGPQLNPFLSHLAQ  1986  Japan
AB985451            PIAMEPVVGAATAVATAGQVNMIDPWIMSNFVQAPQGEFTISPNNTPGDILFDLQLGPQLNPFLSHLAQ  1988  Japan
AB985477            PIAMEPVVGAATAVATAGQVNMIDPWIMSNFVQAPQGEFTISPNNTPGDILFDLQLGPQLNPFLSHLAQ  1991  Japan
AB985478            PIAMEPVVGAATAVATAGQVNMIDPWIMSNFVQAPQGEFTISPNNTPGDILFDLQLGPQLNPFLSHLAQ  1991  Japan
AB058529            PIAMDPVVGAATAVATAGQVNMIDPWIMSNFDQAPQGEFTISPNNTPGDILFDLQLGPQLNPFLSHLAQ  2000  Japan
LC469950            PIAMDPVVGAATAVATAGQVNMIDPWIMSNFVQAPQGEFTISPNNTPGDILFDLQLGPQLNPFLSHLAQ  2003  Japan
HQ213833            PIAMDPVVGAATAVATAGQVNMIDPWIMSNFVQAPQGEFTISPNNTPGDILFDLQLGPQLNPFLSHLAQ  2005  Korea
HQ213841            PIAMDPVVGAATAVATAGQVNMIDPWIMSNFVQAPQGEFTISPNNTPGDILFDLQLGPQLNPFLSHLAQ  2006  Korea
HQ213842            PIAMDPVVGAATAVATAGQVNMIDPWIMSNFVQAPQGEFTISPNNTPGDILFDLQLGPQLNPFLSHLAQ  2006  Korea
MT492061            PIAMDPVVGAATAVATAGQVNMIDPWIMSNFVQAPQGEFTISPNNTPGDILFDLQLGPQLNPFLSHLAQ  2018  Spain
                    ** **** *** * ********************* ********* ********  ******** ***** ***

Type A
Type B
```

Figure 1. Phylogenetic tree analyses of GI.3 strains. (A) Phylogenetic tree based on nucleotide sequences. (B) Phylogenetic tree based on amino acid sequences. The reference strain U04469 and the strain identified in a community resident with an episode of diarrhea are indicated by a diamond and a circle, respectively. Clusters are indicated by square brackets with Roman numerals. (C) Alignment of the amino acid sequence of the amino-terminal region of the VP1 protein. Amino acid residues distinct from that of the reference strain are shown in white letters on a black background. Strains identified in our studies and in community residents with an episode of diarrhea are shown in bold and underline, respectively. The year and country in which the virus was detected are shown. NA, not applicable. Amino acid residues conserved in the listed strains are indicated by asterisks.

other groups (Malasao et al., 2008; Chuchaona et al., see GenBank accession numbers MK590675-MK590678). A phylogenetic tree based on amino acid sequences showed that all strains, including EU363874, which was first identified in Thailand from a specimen collected in 2000 (Malasao et al., 2008), exhibited high similarity to reference strain AF195848 (Figure 3B). However, the reference strain was classified into a distinct cluster, Cluster II, when analyzed based on the nucleotide sequence (Figure 3A). Consistent with the results of the phylogenetic tree analysis based on the amino acid sequence, the amino acid sequence was almost entirely conserved in the strains analyzed (Figure 3C). Although GII.8 was rarely detected in Thailand until recently, it was detected by our and another group in 2018 and 2019. Therefore, it was assumed that GII.8 with a genetically similar origin is currently spreading in Thailand.

3.3.4. GII.4

Seventy-two and 42 strains were identified in AGE patients and community residents, respectively. Reference strain X76716, which is classified into the Bristol 1993 variant, fell into a subcluster when



C

```
                    10        20        30        40        50        60        70        80        90      Year
X81879    MKMASNDAAPSTDGAAGLVPESNNEVMALEPVAGAALAAPVTGQTNIIDPWIRANFVQAPNGEFTVSPRNAPGEVLLNLELGPELNPYLAHLAR 1989

LC573294  MKMASNDAAPSTDGAAGLVPESNNEVMALEPVAGAALAAPVTGQTNIIDPWIRANFVQAPNGEFTVSPRNAPGEVLLNLELGPELNPYLAHLAR 2017
LC573295  MKMASNDAAPSTDGAAGLVPESNNEVMALEPVAGAALAAPVTGQTNIIDPWIRANFVQAPNGEFTVSPRNAPGEVLLNLELGPELNPYLAHLAR 2017
LC573296  MKMASNDAAPSTDGAAGLVPESNNEVMALEPVAGAALAAPVTGQTNIIDPWIRANFVQAPNGEFTVSPRNAPGEVLLNLELGPELNPYLAHLAR 2017
LC573300  MKMASNDAAPSTDGAAGLVPESNNEVMALEPVAGAALAAPVTGQTNIIDPWIRANFVQAPNGEFTVSPRNAPGEVLLNLELGPELNPYLAHLAR 2017
LC573301  MKMASNDAAPSTDGAAGLVPESNNEVMALEPVAGAALAAPVTGQTNIIDPWIRANFVQAPNGEFTVSPRNAPGEVLLNLELGPELNPYLAHLAR 2017
LC573302  MKMASNDAAPSTDGAAGLVPESNNEVMALEPVAGAALAAPVTGQTNIIDPWIRANFVQAPNGEFTVSPRNAPGEVLLNLELGPELNPYLAHLAR 2017
LC573303  MKMASNDAAPSTDGAAGLVPESNNEVMALEPVAGAALAAPVTGQTNIIDPWIRANFVQAPNGEFTVSPRNAPGEVLLNLELGPELNPYLAHLAR 2017
LC573304  MKMASNDAAPSTDGAAGLVPESNNEVMALEPVAGAALAAPVTGQTNIIDPWIRANFVQAPNGEFTVSPRNAPGEVLLNLELGPELNPYLAHLAR 2017
LC521516  MKMASNDAAPSTDGAAGLVPESNNEVMALEPVAGAALAAPVTGQTNIIDPWIRANFVQAPNGEFTVSPRNAPGEVLLNLELGPESNPYLAHLAR 2017
LC521520  MKMASNDAAPSTDGAAGLVPESNNEVMALEPVAGAALAAPVTGQTNIIDPWIRANFVQAPNGEFTVSPRNAPGEVLLNLELGPELNPYLAHLAR 2018
LC521527  MKMASNDAAPSTDGAAGLVPESNNEVMALEPVAGAALAAPVTGQTNIIDPWIRANFVQAPNGEFTVSPRNAPGEVLLNLELGPELNPYLAHLAR 2018
LC521530  MKMASNDAAPSTDGAAGLVPESNNEVMALEPVAGAALAAPVTGQTNIIDPWIRANFVQAPNGEFTVSPRNAPGEVLLNLELGPELNPYLAHLAR 2018
LC521542  MKMASNDAAPSTDGAAGLVPESNNEVMALEPVAGAALAAPVTGQTNIIDPWIRANFVQAPNGEFTVSPRNAPGEVLLNLELGPELNPYLAHLAR 2018
LC521593  MKMASNDAAPSTDGAAGLVPESNNEVMALEPVAGAALAAPVTGQTNIIDPWIRANFVQAPNGEFTVSPRNAPGEVLLNLELGPELNPYLAHLAR 2019
LC521596  MKMASNDAAPSTDGAAGLVPESNNEVMALEPVAGAALAAPVTGQTNIIDPWIRANFVQAPNGEFTVSPRDAPGEVLLNLELGPELNPYLAHLAR 2019

MN735683  MKMASNDAAPSNDGAAGLVPESNNEVMAFEPVAGAAFAAPVTGQTNIIDPWIRNNFVQAPNGEFTVSPRNAPGEMLLNLELGPELNPYLAHLAR 2017
MN735684  MKMASNDAAPSNDGAAGLVPESNNEVMAFEPVAGAAFAAPVTGQTNIIDPWIRNNFVQAPNGEFTVSPRNAPGEMLLNLELGPELNPYLAHLAR 2017
MN735685  MKMASNDAAPSTDGAAGLVPESNNEVMALEPVAGAALAAPVTGQTNIIDPWIRANFVQAPNGEFTVSPRNAPGEVLLNLELGPELNPYLAHLAR 2017
MN735686  MKMASNDAAPSNDGAAGLVPESNNEVMALEPVAGAAMAAPVTGQTNIIDPWIRANFVQAPNGEFTVSPRNAPGEMLLNLELGPELNPYLAHLAR 2016
MN735687  MKMASNDAAPSNDGAAGLVPESNNEVMALEPVAGAALAAPVTGQTNIIDPWIRANFVQAPNGEFTVSPRNAPGEVLLNLELGPELNPYLAHLAR 2016
MN735688  MKMASNDAAPSTDGAAGLVPESNNEVMALEPVAGAALAAPVTGQTNIIDPWIRANFVQAPNGEFTVSPRNAPGEVLLNLELGPELNPYLAHLAR 2016
MN735689  MKMASNDAAPSTDGAAGLVPESNNEVMALEPVAGAALAAPVTGQTNIIDPWIRANFVQAPNGEFTVSPRNAPGEVLLNLELGPELNPYLAHLAR 2016
MN735690  MKMASNDAAPSTDGAAGLVPESNNEVMALEPVAGAALAAPVTGQTNIIDPWIRANFVQAPNGEFTVSPRNAPGEVLLNLELGPELNPYLAHLAR 2016
MN735691  MKMASNDAAPSTDGAAGLVPESNNEVMALEPVAGAALAAPVTGQTNIIDPWIRANFVQAPNGEFTVSPRNAPGEVLLNLELGPELNPYLAHLAR 2017
MN735692  MKMASNDAAPSTDGAAGLVPESNNEVMALEPVAGAALAAPVTGQTNIIDPWIRANFVQAPNGEFTVSPRNAPGEVLLNLELGPELNPYLAHLAR 2017
MN735693  MKMASNDAAPSNDGAAGLVPESNNEVMAFEPVAGAALAAPVTGQTNIIDPWIRTNFVQAPNGEFTVSPRNAPGEMLLNLELGPELNPYLAHLAR 2017
MH430951  MKMASNDAAPSTDGAAGLVPENNTEVMALEPVAGAALAAPVTGQTNIIDPWIRANFVQAPNGEFTVSPRNAPGEVLLNLELGPELNPYLAHLAR 2015
MH430952  MKMASNDAAPSTDGAAGLVPESNNEVMALEPVAGAALAAPVTGQTNIIDPWIRANFVQAPNGEFTVSPRNAPGEVLLNLELGPELNPYLAHLAR 2017
          ********** ********* ****************************** *************** ************** *********
```

Figure 2. Phylogenetic tree analyses of GII.2 strains identified in Thailand. (A) Phylogenetic tree based on nucleotide sequences. (B) Phylogenetic tree based on amino acid sequences. The reference strain X81879 and the strains detected in community residents with an episode of diarrhea are indicated by a diamond and circles, respectively. Clusters are indicated by square brackets with Roman numerals. (C) Alignment of amino acid sequence of the amino-terminal region of the VP1 protein. Amino acid residues distinct from that of the reference strain are shown in white letters on a black background. Strains identified in our studies and in community residents with an episode of diarrhea are shown in bold and underline, respectively. Amino acid residues conserved in the listed strains are indicated by asterisks.

analyzed with both nucleotide and amino acid sequences (Fig. 4A and B). Some subclusters contained only strains derived from AGE patients in the tree based on nucleotide sequence, but two large subclusters included most of the strains derived from both AGE patients and community residents (Figure 4A). When the amino acid sequence of the reference strain was compared with those of the strains identified in our studies, common amino acid substitutions (N6S and S93A) were identified in all strains except for LC573370, which had the S93G substitution (Supplementary Figure 1). Another amino acid substitution, A8V, was identified in 8 strains from AGE patients and 1 strain from a community resident. These 9 strains with the A8V substitution also had the N6S and S93A substitutions, and these strains fell into a subcluster containing strains identified in Thailand by another group (MK928496, MK928497, MK928498, and MK928499) (Figure 4B). By searching the GenBank database, we identified strains with several combinations of the 3 amino acid substitutions (Figure 5). Accumulation of amino acid substitutions at the 3 positions seemed to be roughly associated with the chronological emergence of variants. Phylogenetic tree analyses of the strains listed in Figure 5, together with strains having the A8V substitution in our studies, suggested that almost all strains with the 3 amino acid substitutions are similar in genomic sequence and deduced amino acid sequence (Supplementary Figure 2).

Comparison of the amino acid sequences derived from short genome sequences of the strains identified in our studies with those of the reference strains identified common or related amino acid substitutions in the remaining genotypes: GI.5, GII.3, GII.6, GII.13, and GII.17 (Supplementary Material 1, Supplementary Figures 3, 4, 5, 6, and 7).

4. Discussion

Symptomatic and asymptomatic NV infections often occur simultaneously from the same virus source. In a study of NV administration in

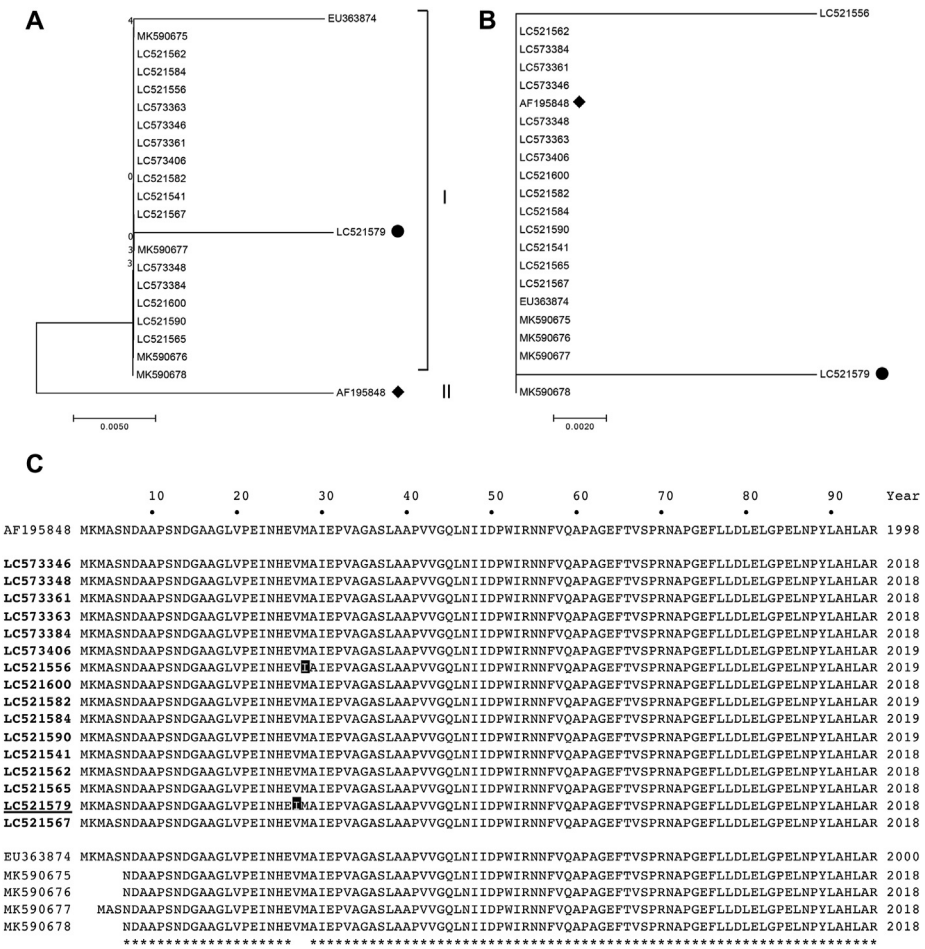


Figure 3. Phylogenetic tree analyses of GII.8 strains identified in Thailand. (A) Phylogenetic tree based on nucleotide sequences. (B) Phylogenetic tree based on amino acid sequences. The reference strain AF195848 and the strain identified in community residents with an episode of diarrhea are indicated by a diamond and a circle, respectively. Clusters are indicated by square brackets with Roman numerals. (C) Alignment of the amino acid sequence of the amino-terminal region of the VP1 protein. Amino acid residues distinct from that of the reference strain are shown in white letters on a black background. Strains identified in our studies and in community residents with an episode of diarrhea are shown in bold and underline, respectively. Amino acid residues conserved in the listed strains are indicated by asterisks.

volunteers, about 80% of the volunteers were infected with NV, of whom about 30% were asymptomatic (Graham et al., 1994). Studies of outbreaks caused by the same NV sources showed that asymptomatic infection occurred in varying percentages of cases (Wu et al., 2019; Bucardo, 2018; He et al., 2016; Ozawa et al., 2007). In a meta-analysis, it was estimated that NV is pooled by approximately 7% of individuals without AGE symptoms (Qi et al., 2018). A statistical model based on a survey of outbreaks estimated that approximately 30% of NV infections were asymptomatic (Miura et al., 2018). A recent household-based study reported that nearly 50% of NV infections from symptomatic individuals resulted in asymptomatic infections (Quee et al., 2020). These results suggest that NVs with the same genetic origins spread through human communities via both symptomatic and asymptomatic infections. In addition, asymptomatic individuals could be a major reservoir of prevalent viruses. However, the relationship between NVs identified in symptomatic vs. asymptomatic individuals in distinct settings had not been analyzed in detail. In this study, we analyzed NVs identified in AGE patients admitted to hospital and in community residents, most of whom had experienced no recent episode of diarrhea, to clarify the relationship between symptomatically and asymptomatically infected NVs in Bangkok. We initially speculated that the two geographically nonoverlapping settings would not share any genotypes in common without commonality in diarrhea episodes. However, our results demonstrated that 8 of the 9 genotypes commonly identified in AGE patients and community residents shared genetic similarities during the period of partial overlap. We also showed that the replacement of the GI.3 strain occurred simultaneously in symptomatic and asymptomatic individuals (Figure 1C). As far as we know, this is the first example of a strain being replaced simultaneously in both symptomatic and asymptomatic individuals during an overlapping period in the same municipality. Moreover, we identified the minor genotype GII.8 in symptomatic and asymptomatic individuals. These data supported that genetically similar NVs have spread widely in communities regardless of the induction of illness. The similarity between the NVs identified in the present study and those identified by another group in an area other than Bangkok (Charoenkul et al., 2020) appears to support this possibility. The data on asymptomatic individuals analyzed in this study were based on our previous study (Phattanawiboon et al., 2020), in which episodes of symptoms other than diarrhea, such as vomiting, were not investigated. Therefore, the relationship between NVs identified in symptomatic individuals and those identified in asymptomatic individuals shown in this study remains to be elucidated. Further investigation with a more appropriate experimental design will be necessary.

A retrospective study showed that a population of NV circulates in a community via asymptomatic infections (Bucardo et al., 2017). Our recent study demonstrated that asymptomatic individuals contribute to the maintenance of NV in a community (Phattanawiboon et al., 2020). NVs transmitted from asymptomatic individuals are occasionally involved in the occurrence of outbreaks and sporadic symptomatic infections (Phattanawiboon et al., 2020; Hardstaff et al., 2018; Chen, 2016). We here provided evidence that NVs prevalent in AGE patients and NVs latent in community residents around 2018 in Bangkok shared genetic similarities. Therefore, NVs maintained in asymptomatic individuals are presumed to contribute to the spread of viruses and the induction of AGE in communities. However, since few analyses have been done on NV transmission mediated by asymptomatic individuals in communities, it remains unclear to what extent asymptomatic individuals contribute to overall NV transmission or to the incidence of

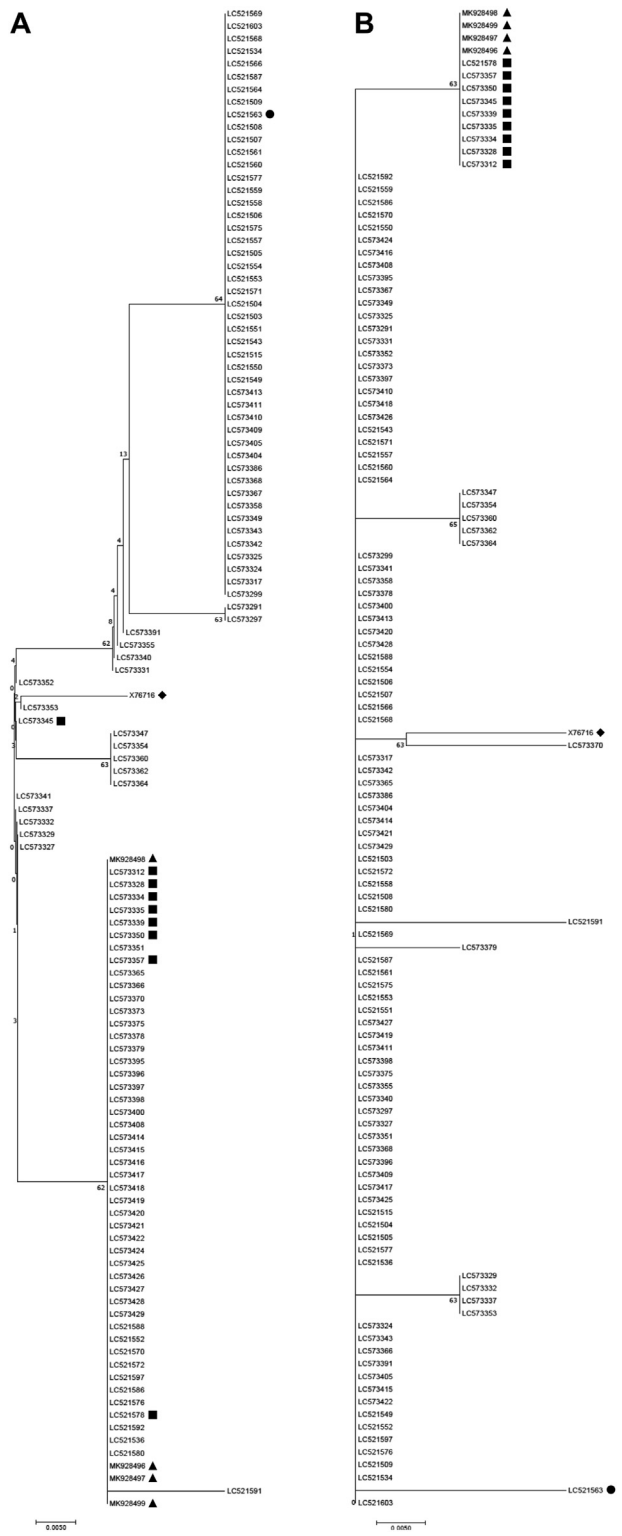


Figure 4. Phylogenetic tree analyses of GII.4 strains. (A) Phylogenetic tree based on nucleotide sequences. (B) Phylogenetic tree based on amino acid sequences. The reference strain X76716 and a strain identified in a community resident with an episode of diarrhea are indicated by a diamond and a circle, respectively. The strains with the A8V substitution identified by our group and another group are indicated by squares and triangles, respectively.

AGE. In our 1-year survey conducted in Bangkok, 35 of the 38 community residents analyzed (92%) experienced NV infection during the survey, and nearly 90% of NVs detected in the community residents were not

	10	20	80	90	Year	Country	Variant
X76716	MKMASNDANPSDGSAANLVP		-----PLGPDLNPYLSHLSR		1993	U.K.	Bristol 1993
LC048884	MKMASSDANPSDGSAANLVP		-----PLGPDLNPYLSHLSR		1993	Japan	Camberwell 1994
KX722414	MKMASNDVNPSDGSAANLVP		-----PLGPDLNPYLSHLSR		2003	Brazil	Unassigned
KU756292	MKMASNDVNPSDGSAANLVP		-----PLGPDLNPYLSHLSR		2003	Brazil	Unassigned
JF909047	MKMASNDVNPSDGSAANLVP		-----PLSPDLNPYLSHLSR		2009	Ethiopia	Unassigned
EF630555	MKMASNDVNPSDGSAANLVP		-----PLGPDLNPYLSHLSR		NA	Japan	Kaiso 2003
DQ078820	MKMASNDASPSDGSTANLVP		-----PLGPDLNPYLSHIAR		NA	Australia	Lanzou 2002
DQ078814	MKMASNDATPSDGSTANLVP		-----PLGPDLNPYLSHIAR		NA	Australia	Hunter 2004
DQ078801	MKMASNDATPSDGSTANLVP		-----PLGPDLNPYLSHIAR		NA	Australia	Hunter 2004
DQ078794	MKMASNDATPSDGSTANLVP		-----PLGPDLNPYLSHIAR		NA	Australia	Hunter 2004
MH540347	MKMASNDANPSDGSAANLVP		-----PLGPDLNPYLSHIAR		2006	France	Den Haag 2006b
KC911680	MKMASNDANPSDGSAANLVP		-----PLGPDLNPYLSHIAR		2006	Thailand	Den Haag 2006b
EU814458	MKMASNDANPSDGSAANLVP		-----PLGPDLNPYLSHIAR		2007	Korea	Den Haag 2006b
HM635181	MKMASNDANPSDGSAANLVP		-----PLGPDLNPYLSHIAR		2008	Korea	Den Haag 2006b
KM245072	MKMASSDANPSDGSTANLVP		-----PLGPDLNPYLSHIAR		2008	Taiwan	Apeldoorn 2007
HM362778	MKMASNDANPSDGSAANLVP		-----PLGPDLNPYLSHIAR		2009	Taiwan	Den Haag 2006b
KP784692	MKMASSDANPSDGSTANLVP		-----PLGPDLNPYLSHIAR		2011	South Africa	Apeldoorn 2007
FJ788317	MKMASSDANPSDGSAANLVP		-----PLGPDLNPYLSHIAR		2006	Singapore	Den Haag 2006b
AB541200	MKMASSDANPSDGSAANLVP		-----PLGPDLNPYLSHIAR		2008	Japan	Den Haag 2006b
JN595867	MKMASSDANPSDGSTANLVP		-----PLGPDLNPYLSHIAR		2010	U.S.A.	New Orleans 2009
JX416413	MKMASSDANPSDGSAANLVP		-----PLGPDLNPYLSHIAR		2010	Burkina Faso	Sydney 2012
KP244320	MKMASSDANPSDGSTANLVP		-----PLGPDLNPYLPHIAR		2011	Italy	New Orleans 2009
KM462612	MKMASSDANPSDGSAANLVP		-----PLGPDLNPYLSHIAR		2013	China	Sydney 2012
KP868614	MKMASSDANPSDGSAANLVP		-----PLGPDLNPYLSHIAR		2014	U.S.A.	Sydney 2012
FJ788332	MKMASNDVNPSDGSAANLVP		-----PLGPDLNPYLSHIAR		2006	Singapore	Den Haag 2006b
EU814449	MKMASNDVNPSDGSAANLVP		-----PLGPDLNPYLSHIAR		2007	Korea	Den Haag 2006b
MN494614	MKMASNDVNPSDGSAANLVP		-----PLGPDLNPYLSHIAR		2016	Korea	Sydney 2012
KU963483	MKMASSDVNPSDGSTANLVP		-----PLGPDLNPYLSHIAR		2011	Lebanon	Unassigned
MG023177	MKMASSDVNPSDGSAANLVP		-----PLGPDLNPYLSHIAR		2013	Brazil	Unassigned
MN308008	MKMASSDVNPSDGSAANLVP		-----PLGPDLNPYLSHIAR		2013	Brazil	Sydney 2012
MH393663	MKMASSDVNPSDGSAANLVP		-----PLGPDLNPYLSHIAR		2014	Brazil	Unassigned
MK907785	MKMASSDVNPSDGSAANLVP		-----PLGPDLNPYLSHIAR		2014	France	Sydney 2012
MK907788	MKMASSDVNPSDGSAANLVP		-----PLGPDLNPYLSHIAR		2014	France	Sydney 2012
MK408520	MKMASSDVNPSDGSAANLVP		-----PLGPDLNPYLSHIAR		2015	Indonesia	Unassigned
KX764831	MKMASSDVNPSDGSAANLVP		-----PLGPDLNPYLSHIAR		2016	Korea	Sydney 2012
KY407166	MKMASSDVNPSDGSAANLVP		-----PLGPDLNPYLSHIAR		2016	China	Sydney 2012
MG763293	MKMASSDVNPSDGSAANLVP		-----PLGPDLNPYLSHIAR		2017	China	Sydney 2012
MG763310	MKMASSDVNPSDGSAANLVP		-----PLGPDLNPYLSHIAR		2017	China	Sydney 2012
MG763347	MKMASSDVNPSDGSAANLVP		-----PLGPDLNPYLSHIAR		2017	China	Sydney 2012
MG763348	MKMASSDVNPSDGSAANLVP		-----PLGPDLNPYLSHIAR		2017	China	Sydney 2012
MG763349	MKMASSDVNPSDGSAANLVP		-----PLGPDLNPYLSHIAR		2017	China	Sydney 2012
MH229940	MKMASSDVNPSDGSAANLVP		-----PLGPDLNPYLSHIAR		2017	China	Sydney 2012
MH469207	MKMASSDVNPSDGSAANLVP		-----PLGPDLNPYFSHIAR		2017	China	Sydney 2012
MH469208	MKMASSDVNPSDGSAANLVP		-----PLGPDLNPYFSHIAR		2017	China	Sydney 2012
MH842240	MKMASSDVNPSDGSAANLVP		-----PLGPDLNPYLSHIAR		2017	China	Unassigned
MH842243	MKMASSDVNPSDGSAANLVP		-----PLGPDLNPYLSHIAR		2017	China	Unassigned
MK213821	MKMASSDVNPSDGSAANLVP		-----PLGPDLNPYLSHIAR		2017	China	Sydney 2012
MK213823	MKMASSDVNPSDGSAANLVP		-----PLGPDLNPYLSHIAR		2017	China	Sydney 2012
MK213828	MKMASSDVNPSDGSAANLVP		-----PLGPDLNPYLSHIAR		2017	China	Sydney 2012
MK213834	MKMASSDVNPSDGSAANLVP		-----PLGPDLNPYLSHIAR		2017	China	Sydney 2012
MK213835	MKMASSDVNPSDGSAANLVP		-----PLGPDLNPYLSHIAR		2017	China	Sydney 2012
MK213836	MKMASSDVNPSDGSAANLVP		-----PLGPDLNPYLSHIAR		2017	China	Sydney 2012
MK928496	MKMASSDVNPSDGSAANLVP		-----PLGPDLNPYLSHIAR		2018	Thailand	Sydney 2012
MK928497	MKMASSDVNPSDGSAANLVP		-----PLGPDLNPYLSHIAR		2018	Thailand	Sydney 2012
MK928498	MKMASSDVNPSDGSAANLVP		-----PLGPDLNPYLSHIAR		2018	Thailand	Sydney 2012
MK928499	MKMASSDVNPSDGSAANLVP		-----PLGPDLNPYLSHIAR		2018	Thailand	Sydney 2012
MN494659	MKMASSDVNPSDGSAANLVP		-----PLGPDLNPYLSHIAR		2018	Korea	Sydney 2012
MN764311	MKMASSDVNPSDGSAANLVP		-----PLGPDLNPYLSHIAR		2018	China	Sydney 2012
MT032004	MKMASSDVNPSDGSAANLVP		-----PLGPDLNPYLSHIAR		2018	U.S.A.	Sydney 2012
MN854085	MKMASSDVNPSDGSAANLVP		-----PLGPDLNPYLSHIAR		2019	Spain	Unassigned
MT031821	MKMASSDVNPSDGSAANLVP		-----PLGPDLNPYLSHIAR		2019	U.S.A.	Sydney 2012

Figure 5. Alignment of VP1 protein amino acid sequences of previously identified GII.4 strains with amino acid substitutions. Amino acid residues distinct from those of the reference strain X81879 are shown in white letters on a black background. The year and country of the sample in which the virus was detected and the variant name are listed next to the sequence.

associated with an incidence of diarrhea (Phattanawiboon et al., 2020). Therefore, the total burden of NV pooled in asymptomatic individuals may be higher than that of NV pooled in symptomatic individuals, depending on the setting. Further investigation into the role of asymptomatic individuals in the maintenance and transmission of NV may reveal the mechanism underlying the persistence of NV. Since short genome sequences were used for the analyses in this study, a more detailed analysis using longer genome sequences will be necessary.

GII.4 has predominated in Thailand since 2000 (Kumthip et al., 2018). Three GII.4 variants (Asia 2003, New Orleans 2009, and Sydney 2012) were identified from 2015 to 2016, after which Sydney 2012 has predominated (Supadej et al., 2019). Recent studies found that recombinant GII.4 Sydney 2012[P31] and GII.4 Sydney 2012[P16] predominated from 2017 to 2018 (Chuchaona et al., 2019). Our results also showed that GII.4 Sydney 2012 predominated among the 9 genotypes identified in AGE patients until recently (Table 1). Since the ORF1 genotype and the complete ORF2 sequence were not determined in this study, the precise relationships between GII.4 strains identified by our group and those identified by other groups remain unknown. In this study, GII.4 strains with the A8V substitution were identified from symptomatic and asymptomatic individuals, although the strains were

minor (9 of 118 GII.4-positive specimens). Charoenkul et al. also identified 4 strains of GII.4 Sydney 2012[P31] with the A8V substitution from children and canines who lived on the same premises in Thailand (Charoenkul et al., 2020) (Figure 5). Although there was no cluster or subcluster with statistical significance in the phylogenetic trees of GII.4 (Figure 4), the 4 strains identified in the previous study (Charoenkul et al., 2020) and 8 of the 9 strains identified in our studies fell into a subcluster of the nucleotide sequence-based tree (Figure 4A). This suggested that GII.4 strains with the A8V substitution originating from a common ancestor may have spread in Thailand recently. Further analysis of the recombination statuses of the GII.4 strains identified in our studies will be necessary to clarify their relationships with the GII.4 strains that can infect both humans and canines.

In summary, our results demonstrated that various genotypes of genetically similar NVs spread in Bangkok through symptomatic and asymptomatic infections around 2018. The simultaneous occurrence of symptomatic and asymptomatic infections has the potential to maintain NV efficiently and sustainably in a community by increasing the frequency of infection. To prevent the persistent spread of NV, hygiene management that addresses the potential for transmission of viruses from both symptomatic and asymptomatic individuals will be necessary. It will also be important to elucidate the mechanism underlying the establishment of asymptomatic NV infections.

Declarations

Author contribution statement

Patcharaporn Boonyos: Performed the experiments; Analyzed and interpreted the data; Wrote the paper.

Michittra Boonchan: Performed the experiments; Analyzed and interpreted the data.

Benjarat Phattanawiboon, Nutthawan Nonthabenjawan, Ratana Tacharoenmuang, Ratigorn Gunpapong, Phakapun Singchai, Sompong Upchai: Performed the experiments.

Pimpha Rungnobhakhun, Jutarat Mekmullica, Worakarn Towayunanta, Kobkool Chuntrakool, Karn Ngaopravet: Contributed reagents, materials, analysis tools or data.

Kriangsak Ruchusatsawat, Somchai Sangkitporn, Ballang Uppapong: Analyzed and interpreted the data.

Eisuke Mekada, Masashi Tatsumi: Conceived and designed the experiments; Analyzed and interpreted the data; Wrote the paper.

Yoshiharu Matsuura: Conceived and designed the experiments.

Hiroto Mizushima: Conceived and designed the experiments; Performed the experiments; Analyzed and interpreted the data; Wrote the paper.

Funding statement

This work was supported by Japan Agency for Medical Research and Development (AMED) under Grant number JP21wm0125010h0002 (to YM).

Data availability statement

Nucleotide sequences of the strains determined in this study are available in the GenBank database (accession numbers: LC573290-LC573429).

Declaration of interests statement

The authors declare no conflict of interest.

Additional information

Supplementary content related to this article has been published online at https://doi.org/10.1016/j.heliyon.2021.e08250.

Acknowledgements

We thank the participants for their cooperation in this study and the staff members of Bhumibol Adulyadej Hospital and Public Health Center 41 for the recruitment of participants and for supporting the sample collection. We thank Drs. Opart Karnkawinpong and Tadahiro Kanda for their comments and encouragement. We thank KN International for English language editing.

References

Bucardo, F., Reyes, Y., Becker-Dreps, S., Bowman, N., Gruber, J.F., Vinjé, J., Espinoza, F., Paniagua, M., Balmaseda, A., Svensson, L., Nordgren, J., 2017. Pediatric norovirus GII.4 infections in Nicaragua, 1999-2015. Infect. Genet. Evol. 55, 305–312.

Bucardo, F., 2018. Understanding asymptomatic norovirus infections. EClinicalMedicine 2–3, 7–8.

Charoenkul, K., Nasamran, C., Janetanakit, T., Tangwangvivat, R., Bunpapong, N., Boonyapisitsopa, S., Suwannakarn, K., Theamboonler, A., Chuchaona, W., Poovorawan, Y., Amonsin, A., 2020. Human Norovirus infection in dogs, Thailand. Emerg. Infect. Dis. 26, 350–353.

Chen, M.Y., Chen, W.C., Chen, P.C., Hsu, S.W., Lo, Y.C., 2016. An outbreak of norovirus gastroenteritis associated with asymptomatic food handlers in Kinmen, Taiwan. BMC Publ. Health 16, 372.

Chhabra, P., de Graaf, M., Parra, G.I., Chan, M.C., Green, K., Martella, V., Wang, Q., White, P.A., Katayama, K., Vennema, H., Koopmans, M.P.G., Vinjé, J., 2019. Updated classification of norovirus genogroups and genotypes. J. Gen. Virol. 100, 1393–1406.

Chuchaona, W., Chansaenroj, J., Wanlapakorn, N., Vongpunsawad, S., Poovorawan, Y., 2019. Recombinant GII.Pe-GII.4 norovirus, Thailand, 2017-2018. Emerg. Infect. Dis. 25, 1612–1614.

Felsenstein, J., 1985. Confidence limits on phylogenies: an approach using the bootstrap. Evolution 39, 783–791.

Ford-Siltz, L.A., Wales, S., Tohma, K., Gao, Y., Parra, G.I., 2020. Genotype-specific neutralization of norovirus is mediated by antibodies against the protruding domain of the major capsid protein. J. Infect. Dis.

Graham, D.Y., Jiang, X., Tanaka, T., Opekun, A.R., Madore, H.P., Estes, M.K., 1994. Norwalk virus infection of volunteers: new insights based on improved assays. J. Infect. Dis. 170, 34–43.

Grant, L.R., O'Brien, K.L., Weatherholtz, R.C., Reid, R., Goklish, N., Santosham, M., Parashar, U., Vinjé, J., 2017. Norovirus and sapovirus epidemiology and strain characteristics among Navajo and Apache infants. PLoS One 12, e0169491.

Hall, A.J., 2012. Noroviruses: the perfect human pathogens? J. Infect. Dis. 205, 1622–1624.

Hardstaff, L.J., Clough, H.E., Lutje, V., McIntyre, K.M., Harris, J.P., Garner, P., O'Brien, S.J., 2018. Foodborne and food-handler norovirus outbreaks: a systematic review. Foodb. Pathog. Dis. 15, 589–597.

He, Y., Jin, M., Zhang, H., Yang, H., Zhou, F., Zhao, D., Zhen, H., Yao, X., Zhang, Z., Chen, L., Zhou, Y., Duan, D.J., 2016. Gastroenteritis outbreaks associated with the emergence of the new GII.4 Sydney norovirus variant during the epidemic of 2012/13 in Shenzen City, China. PLoS One 11, e0165880.

Koo, H.S., Lee, M.O., Ku, P.T., Hwang, S.J., Park, D.J., Baik, H.S., 2016. Molecular epidemiology of norovirus in asymptomatic food handlers in Busan, Korea, and emergence of genotype GII.17. J. Microbiol. 54, 686–694.

Kojima, S., Kageyama, T., Fukushi, S., Hoshino, F.B., Shinohara, M., Uchida, K., Takeda, N., Katayama, K., 2002. Genogroup-specific PCR primers for detection of Norwalk-like viruses. J. Virol. Methods 100, 107–114.

Kotwal, G., Cannon, J.L., 2014. Environmental persistence and transfer of enteric viruses. Curr. Opin. Virol. 4, 37–43.

Kroneman, A., Vennema, H., Deforche, K.V.D., Avoort, H., Peñaranda, S., Oberste, M.S., Vinjé, J., Koopmans, M., 2011. An automated genotyping tool for enteroviruses and noroviruses. J. Clin. Virol. 51, 121–125.

Kumar, S., Stecher, G., Tamura, K., 2016. MEGA7: molecular evolutionary genetics analysis version 7.0 for bigger datasets. Mol. Biol. Evol. 33, 1870–1874.

Kumthip, K., Khamrin, P., Maneekarn, N., 2018. Molecular epidemiology and genotype distributions of noroviruses and sapoviruses in Thailand 2000-2016: a review. J. Med. Virol. 90, 617–624.

Lindesmith, L.C., Donaldson, E.F., Baric, R.S., 2011. Norovirus GII.4 strain antigenic variation. J. Virol. 85, 231–242.

Lindesmith, L.C., Beltramello, M., Donaldson, E.F., Corti, D., Swanstrom, J., Debbink, K., Lenzavecchia, A., Baric, R.S., 2012. Immunogenetic mechanisms driving norovirus GII.4 antigenic variation. PLoS Pathog. 8, e1002705.

Lopman, B.A., Steele, D., Kirkwood, C.D., Parashar, U.D., 2016. The vast and varied global burden of norovirus: prospects for prevention and control. PLoS Med. 13, e1001999.

Malasao, R., Maneekarn, N., Khamrin, P., Pantip, C., Tonusin, S., Ushijima, H., Peerakome, S., 2008. Genetic diversity of norovirus, sapovirus, and astrovirus isolated from children hospitalized with acute gastroenteritis in Chiang Mai, Thailand. J. Med. Virol. 80, 1749–1755.

Miura, F., Matsuyama, R., Nishiura, H., 2018. Estimating the asymptomatic ratio of norovirus infection during foodborne outbreaks with laboratory testing in Japan. J. Epidemiol. 28, 382–387.

Moyo, S., Hanevik, K., Blomberg, B., Kommedal, O., Vainio, K., Maselle, S., Langeland, N., 2014. Genetic diversity of norovirus in hospitalised diarrhoeic children and asymptomatic controls in Dar es Salaam, Tanzania. Infect. Genet. Evol. 26, 340–347.

Nonthabenjawan, N., Boonyos, P., Phattanawiboon, B., Towayunanta, W., Chuntrakool, K., Ngaopravet, K., Ruchusatsawat, K., Uppapong, B., Sangkitporn, S., Mekada, E., Matsuura, Y., Tatsumi, M., Mizushima, H., 2020. Identification of GII.14 [P7] norovirus and its genomic mutations from a case of long-term infection in a post-symptomatic individual. Infect. Genet. Evol. 86, 104612.

Ozawa, K., Oka, T., Takeda, N., Hansman, G.S., 2007. Norovirus infections in symptomatic and asymptomatic food handlers in Japan. J. Clin. Microbiol. 45, 3996–4005.

Phattanawiboon, B., Nonthabenjawan, N., Boonyos, P., Jetsukontorn, C., Towayunanta, W., Chuntrakool, K., Ngaopravet, K., Ruchusatsawat, K., Uppapong, B., Sangkitporn, S., Mekada, E., Matsuura, Y., Tatsumi, M., Mizushima, H., 2020. Norovirus transmission mediated by asymptomatic family members in households. PLoS One 15, e0236502.

Qi, R., Huang, Y.T., Liu, J.W., Sun, Y., Sun, X.F., Han, H.J., Qin, X.R., Zhao, M., Wang, L.J., Li, W., Li, J.H., Chen, C., Yu, X.J., 2018. Global prevalence of asymptomatic norovirus infection: a meta-analysis. EClinicalMedicine 2–3, 50–58.

Quee, F.A., de Hoog, M.L.A., Schuurman, R., Bruijning-Verhagen, P., 2020. Community burden and transmission of acute gastroenteritis caused by norovirus and rotavirus in The Netherlands (RotaFam): a prospective household-based cohort study. Lancet Infect. Dis. 20, 598–606.

Saitou, N., Nei, M., 1987. The neighbor-joining method: a new method for reconstructing phylogenetic trees. Mol. Biol. Evol. 4, 406–425.

Supadej, K., Khamrin, P., Kumthip, K., Malasao, R., Chaimongkol, N., Saito, M., Oshitani, H., Ushijima, H., Maneekarn, N., 2019. Distribution of norovirus and sapovirus genotypes with emergence of NoV GII.P16/GII.2 recombinant strains in Chiang Mai, Thailand. J. Med. Virol. 91, 215–224.

Tamura, K., Nei, M., Kumar, S., 2004. Prospects for inferring very large phylogenies by using the neighbor-joining method. Proc. Natl. Acad. Sci. U.S.A. 101, 11030–11035.

Teunis, P.F.M., Sukhrie, F.H.A., Vennema, H., Bogerman, J., Beersma, M.F.C., Koopmans, M.P.G., 2015. Shedding of norovirus in symptomatic and asymptomatic infections. Epidemiol. Infect. 143, 1710–1717.

Thanusuwannasak, T., Puenpa, J., Chuchaona, W., Vongpunsawad, S., Poovorawan, Y., 2018. Emergence of multiple norovirus strains in Thailand, 2015-2017. Infect. Genet. Evol. 61, 108–112.

Utsumi, T., Lusida, M.I., Dinana, Z., Wahyuni, R.M., Yamani, L.N., Juniastuti, Soetjipto, Matsui, C., Deng, L., Abe, T., Doan, Y.H., Fujii, Y., Kimura, H., Katayama, K., Shoji, I., 2017. Occurrence of norovirus infection in an asymptomatic population in Indonesia. Infect. Genet. Evol. 55, 1–7.

Wang, A., Huang, Q., Qin, L., Zhong, X., Li, H., Chen, R., Wan, Z., Lin, H., Liang, J., Li, J., Zhuang, Y., Zhang, Y., 2018. Epidemiological characteristics of asymptomatic norovirus infection in a population from oyster (Ostrea rivularis Gould) farms in southern China. Epidemiol. Infect. 146, 1955–1964.

Wu, Q.S., Xuan, Z.L., Liu, J.Y., Zhao, X.T., Chen, Y.F., Wang, C.,X., Shen, X.T., Wang, X.Y., Wang, L., Hu, Y., 2019. Norovirus shedding among symptomatic and asymptomatic employees in outbreak settings in Shanghai, China. BMC Infect. Dis. 19, 592.