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Research Article

Diverse genotypes of norovirus genogroup I and II contamination in environmental water in Thailand during the COVID-19 outbreak from 2020 to 2022

Kattareeya Kumthip [a,b], Pattara Khamrin [a,b], Aksara Thongprachum [b,c], Rungnapa Malasao [b,d], Arpaporn Yodmeeklin [a,b], Hiroshi Ushijima [e], Niwat Maneekarn [a,b,*]

[a] Department of Microbiology, Faculty of Medicine, Chiang Mai University, Chiang Mai, Thailand
[b] Center of Excellence (Emerging and Re-emerging Diarrheal Viruses), Chiang Mai University, Chiang Mai, Thailand
[c] Faculty of Public Health, Chiang Mai University, Chiang Mai, Thailand
[d] Department of Community Medicine, Faculty of Medicine, Chiang Mai University, Chiang Mai, Thailand
[e] Department of Pathology and Microbiology, Nihon University School of Medicine, Tokyo, Japan

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ABSTRACT

Noroviruses (NoVs) are the most significant viral pathogens associated with waterborne and foodborne outbreaks of nonbacterial acute gastroenteritis in humans worldwide. This study aimed to investigate the prevalence and diversity of NoVs contaminated in the environmental water in Chiang Mai, Thailand. A total of 600 environmental water samples were collected from ten sampling sites in Chiang Mai from July 2020 to December 2022. The presence of NoV genogroups I (GI), GII, and GIV were examined using real-time RT-PCR assay. The genotype of the virus was determined by nucleotide sequencing and phylogenetic analysis. The results showed that NoV GI and GII were detected at 8.5% (51/600) and 11.7% (70/600) of the samples tested, respectively. However, NoV GIV was not detected in this study. NoV circulated throughout the year, with a higher detection rate during the winter season. Six NoV GI genotypes (GI.1-GI.6) and eight NoV GII genotypes (GII.2, GII.3, GII.7, GII.8, GII.10, GII.13, GII.17, and GII.21) were identified. Among 121 NoV strains detected, GII.17 was the most predominant genotype (24.8%, 30 strains), followed by GII.2 (21.5%, 26 strains), GI.3 (17.4%, 21 strains), and GI.4 (16.5%, 20 strains). Notably, NoV GII.3, GII.7, GII.8, and GII.10 were detected for the first time in water samples in this area. This study provides insight into the occurrence and seasonal pattern of NoV along with novel findings of NoV strains in environmental water in Thailand during the COVID-19 outbreak. Our findings emphasize the importance of further surveillance studies to monitor viral contamination in environmental water.

1. Introduction

Human noroviruses (NoVs) are important enteric pathogens that cause acute gastroenteritis in humans around the world and responsible for foodborne and waterborne viral infections (Kirk et al., 2015; Wittler, 2023). NoVs are highly infectious and can be transmitted from person to person via fecal-oral route in various ways, including direct close contact with an infected person, consumption of contaminated food or water, or exposure to contaminated environments (Teunis et al., 2008; Yezli and Otter, 2011). Several surveillance studies have demonstrated that NoVs contaminate various types of food and environmental waters such as shellfish, fresh vegetables, wastewater, river water, and sewage water (Kitajima et al., 2010; Kittigul et al., 2019; La Rosa et al., 2017; Lowmoung et al., 2017; Suffredini et al., 2018; Thongprachum et al., 2018).

NoVs, members in the family *Caliciviridae*, genus *Norovirus*, are classified into 10 genogroups (GI to GX) based on phylogenetic analysis of the entire VP1 sequence, and each genogroup is further subdivided into at least 48 genotypes. GI is classified into 9 genotypes (GI.1–GI.9) and GII is classified into 26 genotypes (GII.1–GII.14, and GII.16–GII.27). In addition, GIII is classified into 3 genotypes (GIII.1–GIII.3) whereas GIV, GV, and GVI each are classified into 2 genotypes (GIV.1–GIV.2, GV.1–GV.2, GVI.1–GVI.2). Furthermore, each of GVII, GVIII, GIX, and GX has only 1 genotype. NoV GI, GII, GIV, GVIII, and GIX have been reported to infect humans (Chhabra et al., 2019). Additionally, based on the

* Corresponding author.
E-mail address: niwat.m@cmu.ac.th (N. Maneekarn).

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partial RNA-dependent RNA-polymerase (RdRp) nucleotide sequence, NoV is divided into 8 polymerase (P)-groups (GI.P–GVII.P and GX.P), and each P-group is further subdivided into a total of 60 P-types (Parra, 2019). Among these, NoV GII is the most prevalent enteric pathogens causing acute gastroenteritis in human worldwide and is frequently detected in environmental waters (Farahmand et al., 2022). NoV GI is less commonly detected in the human population than GII, even though GI may sometime cause outbreaks in some countries and is often identified in water samples (Boonchan et al., 2017; Inoue et al., 2016; Khamrin et al., 2020; Liao et al., 2021; Nenonen et al., 2012; Suffredini et al., 2018; Cannon et al., 2017).

Although NoVs contribute to 16% of acute gastroenteritis cases globally (Liao et al., 2021), their prevalence in environmental water is much higher than in humans, ranging from 23.8% to 73.9% (Boonchan et al., 2017; Khamrin et al., 2020; Kitajima et al., 2010; Kittigul et al., 2019). The high prevalence of NoV contamination in environmental waters raises concerns about the potential transmission of the virus to humans, underscoring the essence of monitoring NoV presence in the environment. In this study, our objective was to investigate the prevalence, genetic diversity, and seasonal patterns of NoV contamination in environmental water sources over a 30-month period, from July 2020 to December 2022, during the COVID-19 outbreak in Chiang Mai, Thailand.

2. Materials and methods

2.1. Water sample collection and virus precipitation

Water samples for this study were collected from 10 different locations in Chiang Mai, Thailand at two-week intervals from July 2020 to December 2022. Each month, 4 samples were collected from the environmental reservoir (2 sites), 8 from irrigation water (4 sites), 4 from river water (2 sites), and 4 from wastewater (2 sites) as shown in Fig. 1. A total of 20 samples were collected per month, resulting in 240 samples were collected per year. Altogether, a total of 600 water samples were obtained over the two-and a-half-year (30 months) study period. In each location, 100 mL of water samples were randomly collected, placed in clean plastic bottles, and transported on ice to the laboratory. To concentrate the samples, we employed the polyethylene glycol (PEG) precipitation method (Lewis and Metcalf, 1988) with slightly modified from the original procedure, as previously described (Kumthip et al., 2020). In brief, 100 mL of the water sample were mixed with 8 g of PEG-6000 and 2.3 g of NaCl. This mixture was then stirred using a magnetic stirrer at 4 °C overnight. Afterward, the sample was pelleted by centrifugation at 10,000 ×*g* at 4 °C for 30 min. The pellet was then resuspended in 200 μL of nuclease-free water.

2.2. Viral RNA genome extraction and reverse transcription (RT)

Viral nucleic acid was extracted from 200 μL of the concentrated water using the QIAGEN Viral Nucleic Acid Extraction Kit (QIAGEN, Germany) following the manufacturer's instructions. A final volume of 50 μL of nucleic acid extract was eluted and used directly for cDNA synthesis. The cDNA was synthesized from 10 μL of the nucleic acid extract using the Maxima First Strand cDNA Synthesis Kit (Thermo Scientific, USA) in the final reaction volume of 20 μL. The reverse transcription process was performed by thermocycling at 25 °C for 10 min, 50 °C for 30 min, and 85 °C for 5 min. The synthesized cDNA was subsequently used in the real-time PCR analysis.

2.3. Screening of NoV GI, GII, and GIV

Real-time polymerase chain reaction (real-time PCR) was carried out using the Thunderbird Probe qPCR assay Kit (TOYOBO, USA) to detect NoV GI, GII, and GIV. The primer and probe sets used in this study were described previously (Kageyama et al., 2003; Kojima et al., 2002; Yan et al., 2013) and are listed in Supplementary Table S1. Each 20 μL

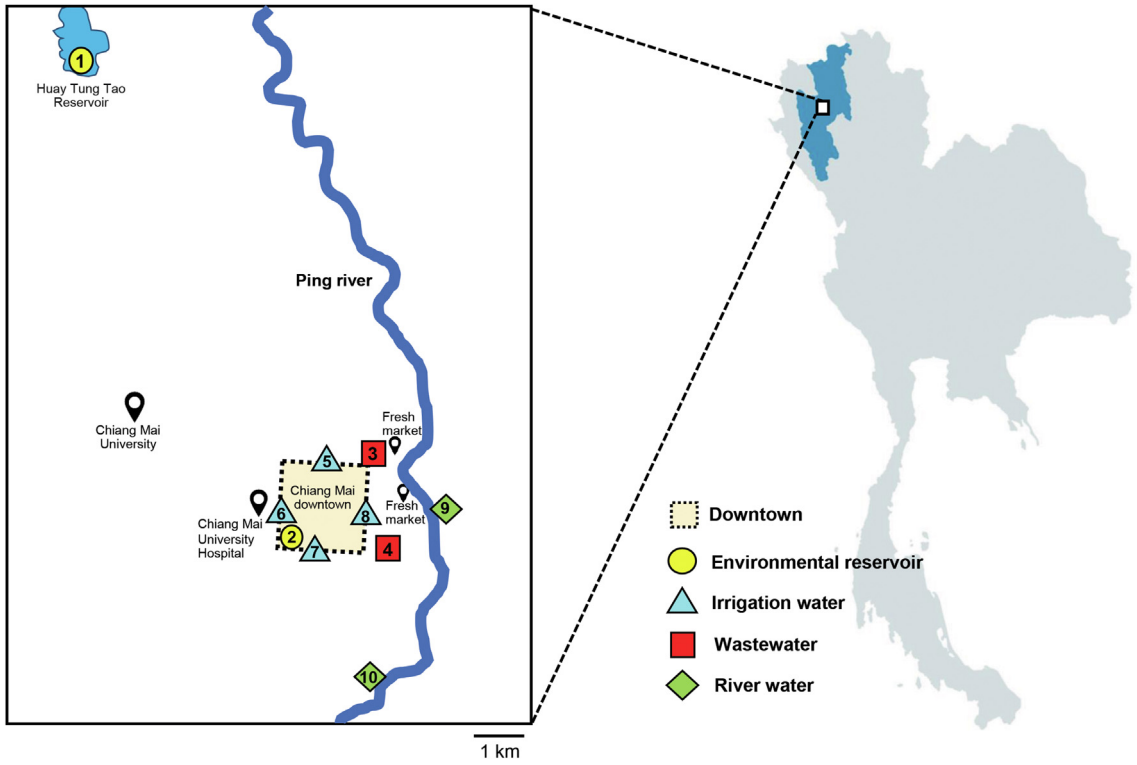

Fig. 1. A map of sampling sites at 10 different locations in the city of Chiang Mai, Thailand. The country map was generated by using mapchart.net, and the sampling sites 1–10 are marked on their respective locations on Google Maps.

real-time PCR reaction mixture consisted of 2.0 μL of cDNA, 10 μL of 2x Thunderbird Probe qPCR Master Mix, 10 μM each of forward primer, reverse primer, and probe, 0.1 μL of 10x ROX reference Dye, and 6.2 μL of nuclease-free water. The reactions were performed using an ABI7500 Real-Time PCR instrument (Applied Biosystems, USA). The thermocycling conditions included an initial step of 1 min at 95 °C for pre-denaturation, followed by 40 cycles of 15 s at 95 °C for denaturation and 60 s for annealing and extension. During the process of viral genome detection in environmental water samples, archival known norovirus GI, GII, and GIV positive and negative stool samples from patients with acute gastroenteritis were run in parallel with test samples through the same process of viral genome extraction, reverse transcription, and real-time PCR to ensure that the entire process of virus detection was reliable and no contamination occurred throughout the process of viral detection. The NoV GI, GII, and GIV cDNAs were used as positive controls, cDNA of NoV negative sample and nuclease-free water were used as a negative control/no template control for validation of the method in each real-time PCR assay. The cut off value of Ct $<$ 40 was considered as potential positive samples based on the number of cycles used for performing real-time PCR. Samples that showed a Ct value less than 40 were considered for further amplification of the partial *VP1* gene by semi-nested PCR. In contrast, samples with a Ct value more than 40 were considered negative.

2.4. Amplification of the partial VP1 gene

To identify NoV genotypes, we conducted semi-nested PCR to amplify the partial *VP1* gene of NoV GI and GII, following previously established protocols (Kageyama et al., 2003; Kojima et al., 2002) using GoTaq DNA Polymerase (Promega, USA), and specific primers are listed in Supplementary Table S1. Specifically, we employed primers COG1F, G1SKF, and G1SKR for NoV GI and primers COG2F, G2SKF, and G2SKR for NoV GII. The PCR products were subsequently analyzed by electrophoresis in 1.5% agarose gels and visualized under ultraviolet light.

2.5. Nucleotide sequencing and phylogenetic analysis

The PCR product was purified using a Gel/PCR DNA Fragment Extraction Kit (Promega, USA) according to the manufacturer's instructions. Nucleotide sequences of the *VP1* gene were directly determined using the Sanger sequencing method, utilizing an automatic genetic analyzer (Applied Biosystems®) provided by First BASE Laboratories (APICAL SCIENTIFIC SDN. BHD, Malaysia). To identify and confirm the genotype of the virus, the nucleotide sequences of NoV strains detected in this study were initially compared with those of the reference strains available in the NCBI GenBank database using the BLAST server (http://blast.ncbi.nlm.nih.gov). Further genotyping was confirmed using the web-based tools such as the Norovirus typing tool (https://www.rivm.nl/mpf/typingtool/norovirus/) and the Human Calicivirus typing tool (https://norovirus.ng.philab.cdc.gov).

Phylogenetic analysis was conducted using the Maximum likelihood method with MEGA X software (Tamura et al., 2021), and the analysis was supported by a Bootstrap value of 1000 replicates. The reference sequences that showed high nucleotide sequence identity to those of the norovirus strains detected in the present study, as analyzed by BLAST, and representative norovirus sequences of different genotypes from human and water samples collected in the same area as well as those in other regions in Thailand, were included in the phylogenetic analysis. The phylogenetic tree was visualized using an online tool called the Interactive Tree Of Life (iTOL). Nucleotide sequences of the partial capsid gene of NoV GI and GII identified in this study in water samples are accessible in the GenBank database under accession numbers OR618152-OR618202 and OR618204-OR618273, respectively.

3. Results

3.1. Prevalence and genotypes of norovirus detected in environmental water samples

A total of 600 environmental water samples included four distinct types of water sources: environmental reservoirs (120 samples), wastewater (120 samples), irrigation water (240 samples), and river water (120 samples) (Fig. 1). Overall, NoVs were detected in 85 out of 600 samples collected (14.2%). NoV GI and GII were detected in 51 (8.5%) and 70 (11.7%) out of 600 samples, respectively (Table 1). Additionally, both NoV GI and GII were co-detected in 36 (6.0%) out of 600 samples. Notably, NoV GIV was not detected in this study. Among various types of water samples, wastewater exhibited highest degree of NoV contamination. Nearly all NoV positive samples of GI (50/51) and GII (66/70) strains were identified in wastewater samples. Of these, 63 (52.1%) and 53 (43.8%) out of 121 NoV-positive samples were detected at sites 3 and 4 of wastewater collection, respectively (Table 1), and the virus was detected all year round at these two sites. Additionally, two each of NoV-positive samples were detected at sites 5 and 6 of irrigation water, respectively; and one NoV-positive sample was detected at site 9 of river water. A map of water sampling sites is shown in Fig. 1. Only one NoV GI and three NoV GII were detected in irrigation water and one NoV GII was found in river water. Remarkably, none of the NoV was detected in environmental reservoirs. In term of NoV genotypes, six distinct genotypes of NoV GI (GI.1 to GI.6) and eight different genotypes of NoV GII (GII.2, GII.3, GII.7, GII.8, GII.10, GII.13, GII.17, and GII.21) were detected in the present study (Fig. 2A). Among NoV GI, GI.3 was the most prevalent genotype, accounting for 41.2% (21/51) of the NoV GI detected, followed by GI.4 (39.2%, 20/51), GI.2 (7.8%, 4/51), GI.6 (5.9%, 3/51), GI.5 (3.9%, 2/51), and GI.1 (2.0%, 1/51). For NoV GII, GII.17 was the most prevalent genotype (42.9%, 30/70), followed by GII.2 (37.1%, 26/70), GII.3 (5.7%, 4/70), GII.21 (4.3%, 3/70), GII.7, GII1.10, and GII.13 (each at 2.9%, 2/70), and GII.8 (1.4%, 1/70) genotypes.

In this study, we analyzed the influence of the COVID-19 pandemic on the occurrence and genotype diversity of NoV detected in environmental waters by comparing the data from our previous study conducted during 2016–2018 (Khamrin et al., 2020) with the data from this study conducted during 2020–2022. Our findings revealed that the prevalence of NoV detected in environmental waters in this area before the COVID-19 outbreak (2016–2018) was 31.7% (40/126) which was about 2.2 times higher than those detected in this study at 14.2% (85/600) during 2020–2022. However, we could not identify a clear influence of COVID-19 outbreak on the variation of NoV genotypes. The dominance of NoV GII.2 and GII.17 was observed in both studies, before and during

Table 1
Prevalence of norovirus detected in different types of water samples.

Type of water	Total samples tested	No. of NoV GI positive	No. of NoV GII positive	No. of NoV GI+GII co-detected
Environmental reservoir (sites 1–2)	120	0 (0.0%)	0 (0.0%)	0
Wastewater (sites 3–4)	120	[a]50 (41.7%)	[a]66 (55.0%)	36 (30.0%)
Irrigation water (sites 5–8)	240	1 (0.4%)	3 (1.2%)	0
River water (sites 9–10)	120	0 (0.0%)	1 (0.8%)	0
Total	600	51 (8.5%)	70 (11.7%)	36 (6.0%)

[a] Number of positive samples included NoV GI and GII co-detection.

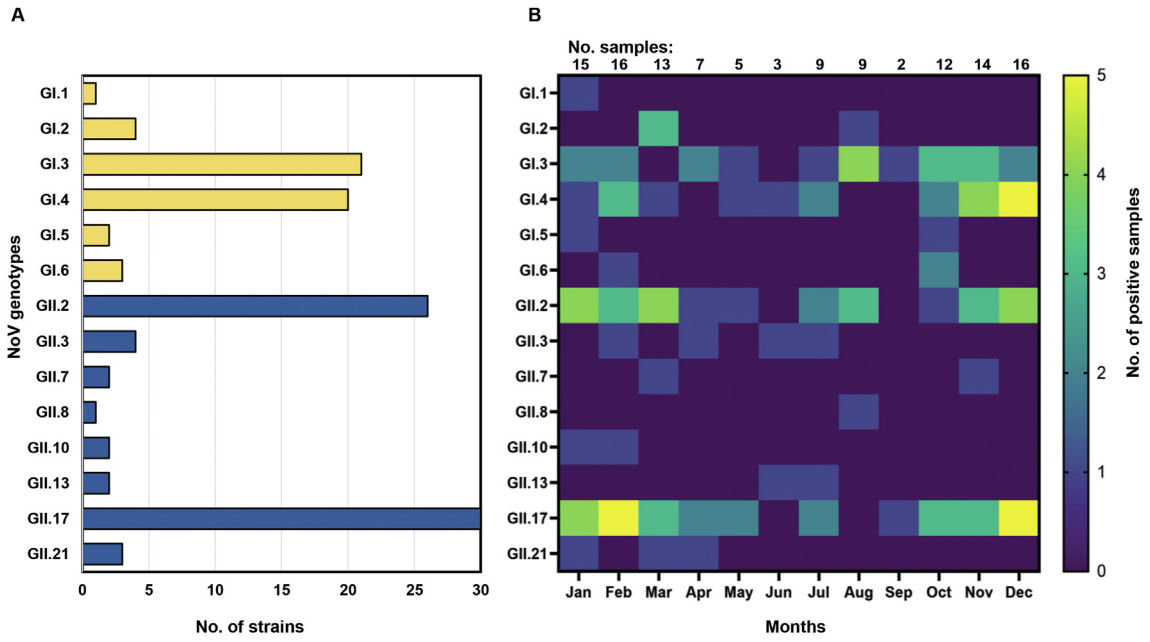

Fig. 2. Distribution of norovirus genotypes (**A**) and seasonality of different norovirus genotypes detected in this study (**B**).

the COVID-19 pandemic, although there was a difference in the predominance of NoV GI genotype.

3.2. Seasonality of norovirus contamination

A total of 20 water samples were collected each month over the entire 30-month study period from July 2020 to December 2022. Our study demonstrated that the high detection rate of NoV contamination in environmental water were found in two periods, between October 2020 and March 2021, and from December 2021 to February 2022 (Figs. 2B and 3). It should be noted that no NoV was detected in June and September 2021 (Fig. 3). The data indicated that contamination of NoV in environmental water was observed almost throughout the year with a long period of high prevalence spanning from October–November (end of rainy season in Thailand) to December–February (all winter long) and March (beginning of summer in Thailand). The seasonal distribution of different NoV genotypes in environmental waters is shown in Fig. 2B. The NoV GI.3 and GI.4 genotypes were detected nearly throughout the year with high prevalence in October–December and January–March. In addition, NoV GII.2 and GII.17 genotypes were also detected almost throughout the year with high prevalence in October–December and January–March. All other genotypes including GI.1, GI.2, GI.5, GI.6, GII.3, GII.7, GII.8, GII.10, GII.13, and GII.21 were detected only in a few months of the year with very low prevalence.

3.3. Phylogenetic analysis of norovirus contamination in environmental water samples

To investigate the genetic relationship between NoV strains detected in this study and the reference strains previously reported in this area and in the other parts of the world, phylogenetic trees of NoV GI and GII were constructed as shown in Figs. 4 and 5, respectively. The phylogenetic analysis of 51 NoV GI strains detected in this study revealed that all 21 NoV GI.3 strains were closely related to the NoV GI.1 reference strains isolated from clam, wastewater, and sewage in Japan, water in Thailand, and human stool samples in Japan, China, Brazil, Spain, and USA (Fig. 4).

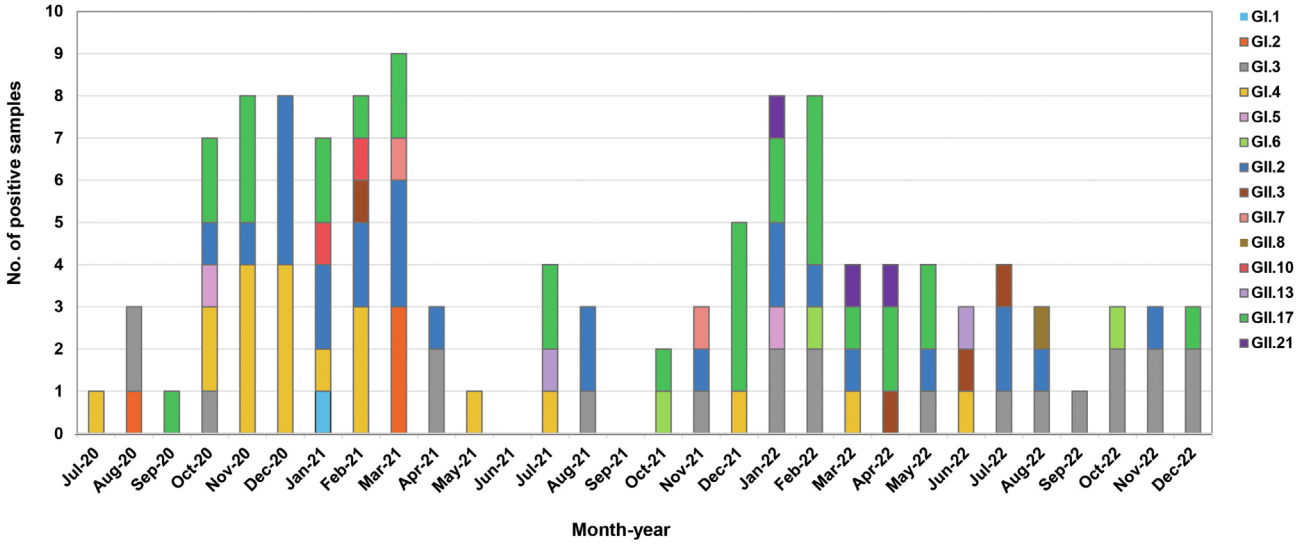

Fig. 3. Monthly detection of different norovirus genotypes in environmental waters in Chiang Mai, Thailand from July 2020 to December 2022.

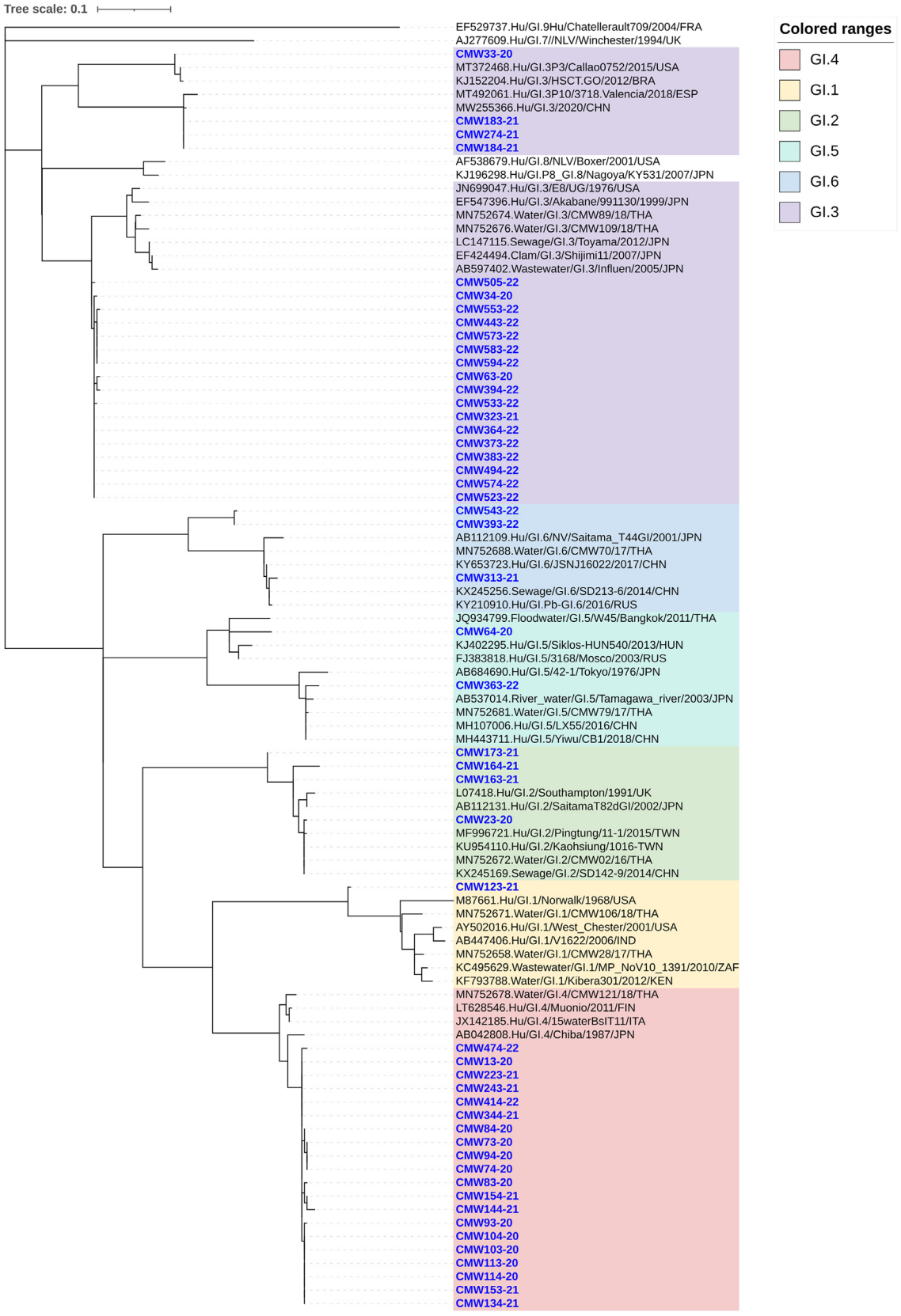

Fig. 4. Phylogenetic tree of the partial *VP1* sequences of norovirus GI strains detected in environmental waters in this study (blue color letter) and the norovirus GI reference strains detected in human stool samples and water samples worldwide. The tree was constructed by MEGA X software using Maximum likelihood method with best fit model TN93 + G + I. Bootstrap value of 1000 replicates was set. The branch length indicates the number of substitutions per site.

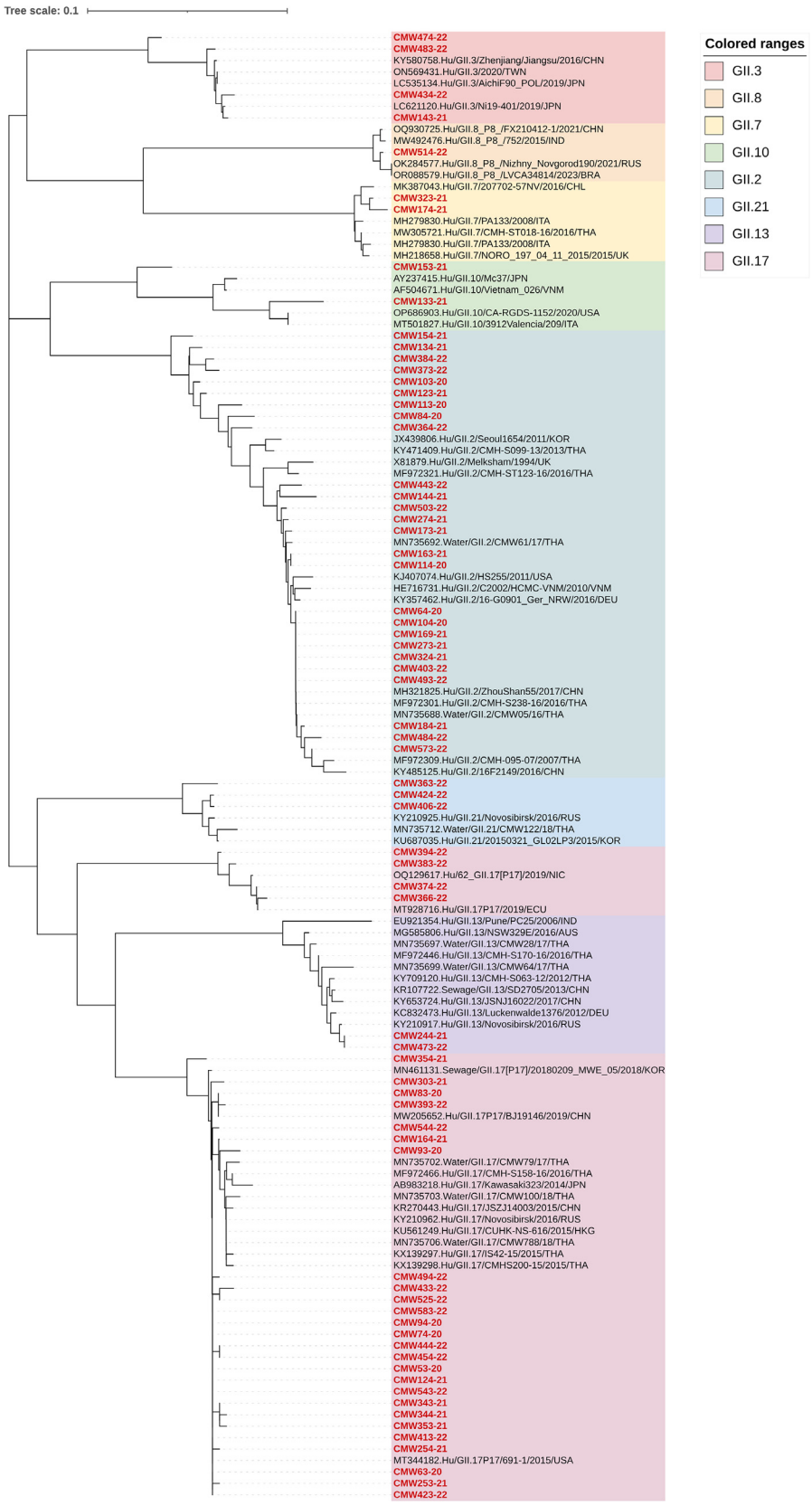

Fig. 5. Phylogenetic tree of the partial *VP1* sequences of norovirus GII strains detected in environmental waters in this study (red color letter) and norovirus GII reference strains detected in human stool samples and water samples worldwide. The tree was constructed by MEGA X software using Maximum likelihood method with Best fit model K2 + G. Bootstrap value of 1000 replicates was set. The branch length indicates the number of substitutions per site.

All 20 NoV GI.4 strains were clustered closely together and formed a single branch with NoV GI.3 reference strains detected in human stool samples from Japan, Italy, and Finland, and water sample in Thailand. Four NoV GI.2 strains showed close genetic relationship with human NoV GI.2 reference strains reported from the regions of United Kingdom, Japan, and China's Taiwan, and GI.2 strain detected in sewage in China and in water in Thailand. Three strains of NoV GI.6 were closely related to human NoV GI.6 isolated in China, Japan, and Russia, and in sewage in China and in water in Thailand. Two NoV GI.5 strains were clustered with NoV GI.5 reference strains detected in human stool samples and river water samples from China, Japan and Thailand. The only one NoV GI.1 strain was closely related with human NoV GI.1 strains detected in USA and India and NoV GI.1 isolated in water samples from Kenya, Thailand, and South Africa.

The phylogenetic analysis of 70 NoV GII strains detected in this study revealed that 30 strains of NoV GII.17 were closely related to the NoV GII.17 reference strains detected in human stool samples and water samples worldwide (Fig. 5). Twenty-six NoV GII.2 strains were clustered together with human NoV GII.2 reference strains reported from several countries worldwide as well as NoV GII.2 reference strains detected in water in Thailand. Four NoV GII.3 strains were closely related to human NoV GII.3 reported from Japan, China's mainland and Taiwan region. Three NoV GII.21 strains were clustered with the GII.21 reference strains detected in human stool samples from Korea and Thailand, and in water sample in Thailand. Two GII.7 and GII.10 strains showed close genetic relationship to corresponding NoV reference strains detected in human stool samples reported from several countries worldwide. Two NoV GII.13 strains showed close genetic relationship with NoV GII.13 reference strains detected in water in China and Thailand and in human stool samples in Australia, China, Germany, India, and Russia. Finally, the only one NoV GII.8 strain was clustered in the same branch with human NoV GII.8 strains detected in China, India, Russia, and Brazil.

4. Discussion

NoVs are one of the most important viral pathogens associated with foodborne and waterborne outbreaks of nonbacterial acute gastroenteritis in humans worldwide (Kirk et al., 2015; Wittler, 2023). The contamination of NoVs in various types of food and environmental waters has been well documented (Khamrin et al., 2020; Kitajima et al., 2010; Kittigul et al., 2016, 2022; La Rosa et al., 2017; Lowmoung et al., 2017; Suffredini et al., 2018; Thongprachum et al., 2018). The co-circulation of NoVs in contaminated environmental water sources and in human population raises concerns about the potential transmission of the virus to humans, thereby affecting human health. In the present study, we investigated the prevalence, genotype diversity, and seasonal patterns of NoV contamination in various sources of environmental water in Chiang Mai, Thailand from July 2020 to December 2022, during the period of COVID-19 outbreak. We collected four distinct types of environmental water including river water, environmental reservoirs, irrigation water, and wastewater and screened for NoV in those water samples. In this study, we observed varying levels of NoV contamination among four different types of water samples tested. Wastewater exhibited the highest degree of NoV contamination, with 116 (95.9%) NoV strains detected, whereas only four (3.3%) and one (0.8%) NoV strains were detected in the irrigation water and river water, respectively. None of the NoV was detected in the water collected from environmental reservoirs. This data indicates that wastewater from homes and business parks can contain a high degree of NoV contamination shedding from human sources. Inadequately treated or untreated wastewater before discharging into the environment, or use for any purposes, can introduce the viruses into surface water and groundwater, potentially leading to virus transmission through waterborne routes. Proper wastewater treatment processes are essential to reduce the risk of virus transmission from wastewater. Additionally, irrigation water used in agriculture can also serve as a vehicle for virus transmission. Contaminated water can introduce the viruses into crops and aquatic animals, leading to NoV foodborne outbreaks when people consume uncooked food. This is a crucial need for immediate treatment of wastewater before draining into the canal in the Chiang Mai City.

Over the course of two and a half years investigation, NoVs were frequently detected in environmental waters during the cool months in Thailand. The finding aligns with previous reports from Thailand and other countries such as Japan and Sweden, which also demonstrated the high prevalence of NoVs during the winter season (Haramoto et al., 2006; Khamrin et al., 2020; Kitajima et al., 2010; Westrell et al., 2006). Similarly, the prevalence of NoV infection in patients with acute gastroenteritis was also high in the winter season in Chiang Mai, Thailand (Khamrin et al., 2017; Kumthip et al., 2018; Phengma et al., 2022; Supadej et al., 2019). The high prevalence of NoVs during the same seasonal period both in contaminated environmental water and in patients with acute gastroenteritis in the same geographical area indicates a correlation between NoV contamination in the environment and NoV infection in human population. This suggests a potential risk of NoV infection when people are exposed to environmental water or use water for agricultural purposes.

In this study, the NoV was undetectable in June and September of 2021. We don't know the fact underlying this observation, however, it is possible that several environmental factors can influence pathogen abundance, including the dilution effect of heavy rain fall during the rainy season and changes in environmental conditions such as temperature, humidity, pH, and turbidity as well as increased water flow rates (Rohayem, 2009). Heavy rain fall can increase water volume and flow, leading to the dilution of virus concentration in water samples. Moreover, rain fall can alter environmental parameters, potentially affect virus survival and stability, making them more difficult to be detected.

Our previous report described epidemiological data regarding the occurrence of NoV GI, GII, and GIV in environmental water, in Chiang Mai, Thailand, over a 21-month study period from 2016 to 2018, which provided valuable information about the contamination of NoV in environmental water for the first time in this area (Khamrin et al., 2020). In this study, we expanded the study period to 30 months and extended our sampling sites to cover the downtown area. The results revealed a wide variety of NoV genotypes, with a total of 14 different genotypes identified, including GI.1 to GI.6, GII.2, GII.3, GII.7, GII.8, GII.10, GII.13, GII.17, and GII.21. The most prevalent genotypes included GII.17 (24.8%), GII.2 (21.5%), GI.3 (17.4%), and GI.4 (16.5%). These results differ from the epidemiological data obtained from our previous surveillance (Khamrin et al., 2020), where NoV GI.1 strains (25.4%) were the most predominant, followed by GII.17 (17.9%) and GII.2 (16.4%). It should be noted that only one NoV GI.1 strain (0.8%) was detected in the present study. Furthermore, the current study identified various rarely detected NoV genotypes, including GII.3, GII.7, GII.8, GII.10, for the first time, in Chiang Mai City, Thailand. Our findings indicate that multiple NoV strains are circulating in the environmental water in this area and the predominance of NoV genotypes has changed over time. It is interesting to point out that NoV GI was more frequently detected than NoV GII in environmental water (Khamrin et al., 2020; Kitajima et al., 2010). In other words, NoV GII was more widely spread among humans than NoV GI. Specifically, NoV GII.4 is the most predominant genotype detected in patients with acute gastroenteritis, while NoV GI is rarely detected in human cases. This discrepancy may be explained, at least in part, by the higher stability of NoV GI in environmental condition compared to GII. In fact, it has been demonstrated that NoV GI exhibited greater persistence through wastewater treatment processes compared to GII (da Silva et al., 2007; Li et al., 2013; Nordgren et al., 2009).

Our studies demonstrated the declining of NoV detection rate during the COVID-19 pandemic in 2020–2022 (this study) compared to our previous study in the same geographical area in 2016–2018 before the pandemic of COVID-19 (Khamrin et al., 2020). Similar to other studies conducted in the clinical settings during the COVID-19 pandemic which indicate that the infection control measures, including the enforcement

of social distancing protocols, lockdowns of outbreak areas, childcare and school closures, and face masking, resulted in a significant decrease in the incidence of several infectious diseases including NoV infection (Douglas et al., 2021; Pham et al., 2023; Sarmento et al., 2023). However, information about the impact of COVID-19 on the level of viral contamination in the environment is very limited. Hoque and colleagues reported a high abundance of gastroenteritis viruses in raw sewages during the COVID-19 pandemic of 2020–2021, while acute gastroenteritis cases decreased dramatically in clinics (Hoque, Kotaki, et al., 2023a, 2023b). The discrepancy between this report and our study could be explained, at least in part, by differences in number of samples tested and virus concentration methods. Further studies are needed to better understand the impact of COVID-19 pandemic on the level of viral contamination in the environment.

Phylogenetic analyses clearly demonstrated that NoV strains detected in environmental water in this study were closely related to the NoV strains previously reported in human stool samples and water samples from the same geographical area as well as other reference strains detected worldwide. The findings suggest a close genetic relationship between the virus found in environmental water and the virus circulating in human population, again underscore the potential transmission of the virus from the environment to humans. In this study, we employed the direct sequencing method for sequence analysis. The direct sequencing method is primarily capable of identifying the most prevalent sequence type (genotype) in each test sample, which could be a limitation of this study. Next generation sequencing and cloning-sequencing methods are recommended to be used for environmental samples that may contain multiple NoV genotypes.

5. Conclusions

This study provides insights into the occurrence and seasonal patterns of NoVs in environmental water in Thailand, along with genetic diversity of rarely detected NoV strains, including GII.3, GII7, GII.8, and GII.10. Furthermore, our findings emphasize the importance of further surveillance studies to monitor the viral contamination in environmental water in conjunction with clinical study in patients at the same period of time. This integrated approach will contribute to a better understanding of the potential transmission of NoVs to humans and the circulation of the virus in both environment and human populations.

Data availability

Nucleotide sequences of partial *VP1* gene of NoVs detected in environmental water have been deposited in the GenBank database under accession numbers OR618152-OR618202 and OR618204-OR618273. The datasets of norovirus detected in this study are available in the Science Data Bank: https://doi.org/10.57760/sciencedb.08947.

Ethics statement

This article does not contain any studies with human or animal subjects performed by any of the authors.

Author contributions

Kattareeya Kumthip: conceptualization, data curation, visualization, investigation, writing-original draft preparation. Pattara Khamrin: conceptualization, data curation, visualization, investigation. Aksara Thongprachum, Rungnapa Malasao, Arpaporn Yodmeeklin: methodology, data curation, investigation. Hiroshi Ushijima: methodology, validation. Niwat Maneekarn: conceptualization, supervision, validation, writing-reviewing and editing.

Declaration of competing interest

The authors declare no conflict of interest relevant to this article.

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Appendix A. Supplementary data

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