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Norovirus contamination in the restrooms of markets in Bangkok, Thailand

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Abstract. *Rupprom K, Phumisantiphong U, Wongsuk T, Hirunwong K. 2025. Norovirus contamination in the restrooms of markets in Bangkok, Thailand. Biodiversitas 26: 1406-1413.* Norovirus is among the most important causative agents of acute nonbacterial gastroenteritis. The transmission of norovirus can occur through the fecal-oral route, including person-to-person contact and contaminated food, water, and environmental surfaces. This study first investigated norovirus contamination in the restrooms of local markets in Bangkok, Thailand, using Reverse Transcription-quantitative Polymerase Chain Reaction (RT-qPCR) and subsequent semi-nested RT-PCR. An RT-qPCR tested on 211 samples found norovirus GII in 9.5%, GI in 4.3%, and a mix of GI and GII in 1.4% of samples. The urinal flush valves had a higher contamination rate for norovirus than the doorknobs (18.7% vs. 12.5%). Meanwhile, doorknobs had a greater quantity of noroviruses than urinal flush valves ($1.68 \times 10^3 \pm 3.83 \times 10^3$ RNA copies vs. $6.07 \times 10^2 \pm 8.71 \times 10^2$ RNA copies). Further, the female restrooms and doorknobs were more significantly contaminated with GII than GI ($p = 0.048$). Using semi-nested RT-PCR, only GII was detected in 31.4% of RT-qPCR-positive samples, and GII.4 was identified as the dominant genotype. Our results revealed a high prevalence of norovirus contamination on frequently touched surfaces in non-outbreak situations. This is significantly beneficial for norovirus outbreak prevention, health risk assessment, and molecular epidemiological investigation of norovirus infection.

Keywords: Bangkok, environmental contamination, market, norovirus, RT-qPCR

INTRODUCTION

Norovirus is a highly contagious gastrointestinal virus and significant public health threat worldwide. Characterized by its rapid and widespread transmission, norovirus outbreaks can cause significant disruptions in communities and healthcare settings. Norovirus is classified within the Caliciviridae family. The virus is a non-enveloped, single-stranded, positive-sense RNA, and its genome is approximately 7.5 kb with three major Open Reading Frames (ORF). ORF1 encodes the nonstructural viral proteins, including RNA-dependent RNA polymerase (RdRp). ORF2 encodes the major capsid protein (VP1), and ORF3 codes for the minor structural proteins (VP2) (Buesa and Rodriguez-Díaz 2016; Fang et al. 2022). Norovirus can be classified into 10 Genogroups (G) (from GI to GX) based on the phylogenetic analysis of the whole VP1 gene and subdivided into more than 49 genotypes. Among the 10 genogroups, GI and GII are significantly interesting as they are the most common genogroups that infect humans (Chhabra et al. 2019). These viruses exhibit remarkable genetic diversity and contribute to the ongoing evolution of the virus, enabling it to evade host immunity and maintain its infectious potential.

Human norovirus is an important causative pathogen of sporadic and outbreak-associated acute gastroenteritis and foodborne disease across all age groups, particularly children, worldwide (Lucero et al. 2021; Chuchaona et al. 2023; Lu et al. 2023). Norovirus transmission is well-documented to occur through close contact with infected individuals, via person-to-person spread, or indirectly through contaminated food, water, and surfaces (Cardemil et al. 2017). Other studies have reported the burden of norovirus disease in Thailand (Khamrin et al. 2022; Phengma et al. 2022, 2023; Chuchaona et al. 2023). According to recent studies, norovirus GII was the most common virus detected in human fecal outbreak samples in the community in Eastern Thailand (Chuchaona et al. 2023; Udompat et al. 2024) and clinical samples in Southern Thailand (Chuchaona et al. 2024) and Northern Thailand (Phengma et al. 2023). By contrast, norovirus GI and recombinant strains may also be circulating in pediatric patients hospitalized due to acute gastroenteritis in Northern Thailand (2015-2021) (Khamrin et al. 2024). Recently, Kumthip et al. (2024) investigated the prevalence and diversity of norovirus contamination in the environmental water in Chiang Mai, Thailand, from July 2020 to December 2022. They found GII.17 was the most predominant genotype, followed by GII.2, GI.3, and GI.4. The contamination of objects in environmental surfaces in human impact areas, such as fresh markets, by infected people excreting in the toilet may facilitate the transmission of norovirus. Several studies have revealed that surface contamination leads to the spread of norovirus outbreaks (Jayasekara et al. 2016; Maunula et al. 2017; Aprea et al. 2021). Norovirus is persistent in the environment. Moreover, it is resistant to freezing/thawing (at least 14

cycles), drying, low pH (gastric pH 3-4), and common chemical disinfectants (Liu and Moore 2020). People can touch contaminated surfaces. If hand hygiene is not effective, the norovirus spreads to other surfaces or persons. Outbreaks can be controlled by eliminating norovirus from environmental surfaces that may facilitate transmission (Rico et al. 2020).

Currently, there are no data on the prevalence of norovirus within the markets in Bangkok, Thailand. This study aimed to determine the presence of noroviruses on toilet surfaces in local markets using Reverse Transcription-quantitative Polymerase Chain Reaction (RT-qPCR), a highly sensitive and specific molecular technique, under non-norovirus outbreak conditions in Bangkok, Thailand. Moreover, the molecular characterization of norovirus genotypes using gold-standard RT-PCR and the genogroup circulations in the study area were investigated. To the best of our knowledge, this study first monitored noroviruses in environmental toilet surfaces in local markets in Thailand. The findings of this study have significant implications for public health. By identifying the prevalence of norovirus on toilet surfaces within local markets, public health officials can implement targeted interventions to improve hygiene practices and reducing the transmission risks, such as implementing regular cleaning and disinfection protocols, promoting proper hand hygiene, and educating the norovirus infection (Barclay et al. 2014; Winder et al. 2022).

MATERIALS AND METHODS

Ethics approval

The Ethical Review Board of the Faculty of Medicine, Vajira Hospital, Navamindradhiraj University (Bangkok, Thailand) approved this study. The study protocol was in accordance with "the Research with Exemption" category (approval ID: COE009/2021).

Environmental swab samples

A total of 211 archived environmental surface samples were determined in this study. The samples were collected from toilets within 57 local markets across six districts (A-F) in North and West Bangkok, Thailand, between March and April 2021 (Figure 1). The frequently touched surfaces, including doorknobs from inside individual female and male toilets and urinal flush valve handles from a communal male toilet, were selected as sampling sites and collected between 9 AM and 11 AM each day. The samples were swabbed from the surfaces of doorknobs (n = 120) and urinal flush valve handles (n = 91) in male (n = 95) and female (n = 116) toilets. The viscose swab was pre-wet with a Viral Transport Medium (VTM). The whole surface of the doorknobs (60 × 40 mm) and urinal flush valve handles (10 × 65 mm) was swabbed and then immersed into 2 mL of VTM. Swabs were kept in a cool place at 4-6°C during transport to the laboratory and stored at -80°C until use for virus detection.

Procedures

Preparation of RNA transcript

Archived fecal samples positive for norovirus genogroups GI.2 and GII.4 served as the source material for RNA transcript preparation (Kittigul et al. 2010). Viral RNA was extracted from these fecal samples. The ORF1-ORF2 junction of the norovirus was amplified using RT-PCR. Forward primers incorporated a T7 promoter sequence fused to the 3' end of a gene-specific sequence. Reverse primers were gene-specific sequences (Kojima et al. 2002). Primer sets employed were T7COG1F (5'-AAT TCT AAT ACG ACT CAC TAT AGG GAG ACG YTG GAT GCG NTT YCA TGA-3') (this study) and G1-SKR for GI and JJV2F T7comp (5'-CAC GTA ATA CGA CTC ACT ATA GGG CAA GAG TCA ATG TTT AGG TGG ATG AG-3') (Gregory et al. 2011) and G2-SKR for GII.

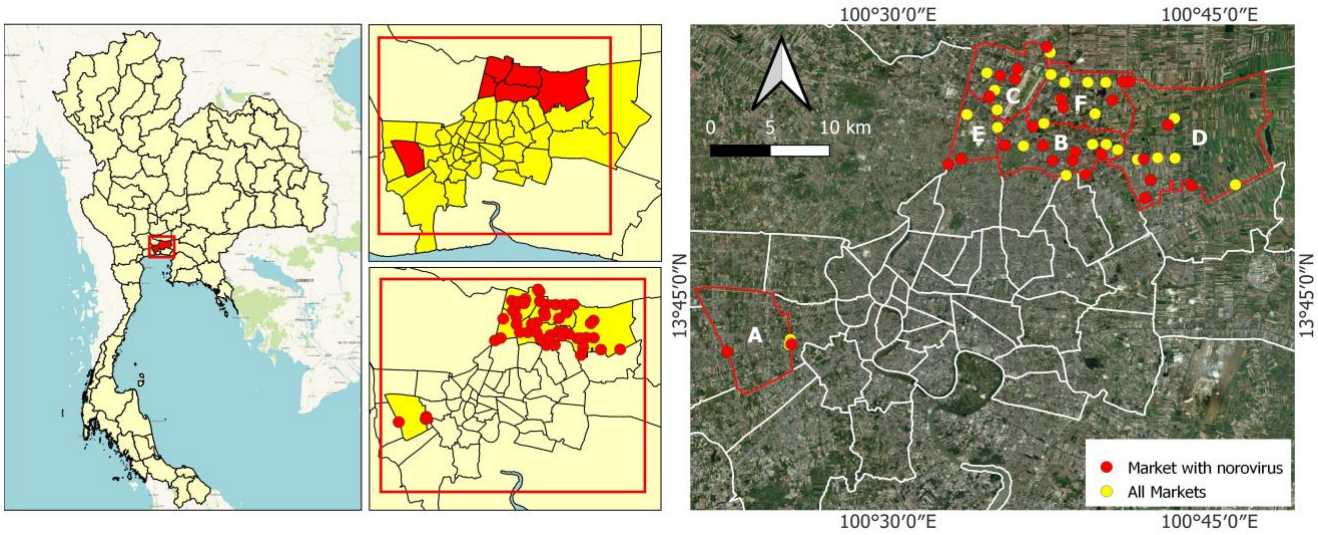

Figure 1. Location of environmental surface sampling in six districts (A-F) in North and West Bangkok, Thailand. Local market areas are presented with the yellow points. The markets found norovirus contamination are presented with red points

Amplicons of the expected size (421 bp for GI and 416 bp for GII) containing the T7 RNA polymerase promoter were purified using a QIAquick gel extraction kit (QIAGEN, USA). Purified DNA amplicons were then in vitro transcribed into RNA using the MegaScript T7 Transcription Kit (Applied Biosystems/Ambion, USA) following the manufacturer's protocol. Following DNase treatment with TURBO DNase, the RNA transcripts were purified using a MEGAclear RNA purification kit (Ambion, USA) and quantified using a NanoDropTM 2000 spectrophotometer (Thermo Fisher Scientific, USA). The concentration of RNA transcripts was calculated using the formula: RNA copies/µL = (g/µL RNA) / (length of RNA × 340) × (6.022×10^{23}) (Troxler et al. 2011). Finally, RNA transcript stocks for GI and GII noroviruses were adjusted to a concentration of approximately 10^{12} RNA copies/µL.

Viral RNA extraction

Environmental swab samples were thawed at room temperature before RNA extraction. Viral RNA was extracted directly from 400 µL of VTM. RNA extraction based on the magnetic bead method was performed using the Zybio Nucleic Acid Extraction Kit (Zybio Inc., China) on the EXM3000 Nucleic Acid Isolation System (Zybio Inc., China), according to the manufacturer's instructions.

RT-qPCR

The one-step RT-qPCR is an efficient method, was performed using primers and probes targeting the ORF1-ORF2 overlapping regions for the detection and quantification of noroviruses GI and GII (Kageyama et al. 2003; Leone et al. 2018). In total, 5 µL of RNA was added to 20 µL of the reaction mixture using the QuantiTect® Probe RT-PCR Kit (QIAGEN GmbH, Germany). The reaction was performed with a separate tube for GI or GII. The RT-qPCR mixture comprised 1X QuantiTect Probe RT-PCR Master Mix, 0.4 µM each of primer COG1F and COG1R, 0.2 µM probe RING1E for GI or 0.4 µM each of primer COG2F and COG2R, 0.2 µM probe RING2 for GII, QuantiTect RT Mix, and RNase-free water. The reaction was conducted in the CFX96 Touch Real-Time PCR Detection System (BioRad, USA). The cycling conditions included reverse transcription at 50°C for 30 min, initial denaturation at 95°C for 15 min, then 45 cycles of denaturation at 94°C for 15 s, and annealing/extension at 60°C for 1 min. Noroviruses GI and GII DNA products were 85 and 98 bp, respectively. Norovirus-positive samples were considered if the quantification cycle (Cq) value was <45 and the fluorescence signal could be distinguished from the background. The norovirus genome copy numbers were quantified by comparing Cq values obtained from the standard curve created from the 10-fold serial dilution of the norovirus GI or GII RNA transcript, ranging from 10^3 to 10^7 RNA copies/mL. The results expressed as the RNA copy number/sample were calculated using the following formula:

$$\text{RNA copies/sample} = \frac{N \times R}{S_1} \times S_0$$

Where:

N : RNA copies
R : RNA extract volume
S_1 : Sample volume for detection
S_0 : Total sample volume

Semi-nested RT-PCR

The samples tested positive for noroviruses based on RT-qPCR were further confirmed by semi-nested RT-PCR. The specific primers targeting capsid genes (region C) for noroviruses GI and GII previously described by Kojima et al. (2002) and Kageyama et al. (2003) were used. The one-step RT-PCR reaction was performed in a 25 µL reaction volume using the QIAGEN OneStep RT-PCR Kit (QIAGEN GmbH, Germany) with a separate tube for GI or GII. RNA extract (2 µL) was added with the 23 µL reaction mixture comprising 1X Qiagen RT-PCR Buffer, 0.4 mM dNTP mix, QIAGEN OneStep RT-PCR Enzyme Mix, 20U RNase inhibitor, 0.4 µM primer COG1F, G1SKR for GI or 0.4 µM primer COG2F, G2SKR for GII, and nuclease-free water. The reaction tube was then placed into a thermocycler (Thermo Hybaid, USA). The thermocycling profiles were performed using the following steps: reverse transcription at 42°C for 30 min, initial denaturation at 95°C for 15 min, PCR with 40 cycles at 94°C for 30 s, 50°C for 30 s, 72°C for 1 min, and final extension at 72ºC for 7 min.

For semi-nested PCR, the RT-PCR amplification product (2 µL) was added to the reaction mixture (48 µL) comprising 1X PCR Buffer (20 mM Tris-HCl, pH 8.4, 50 mM KCl), 2.5 mM $MgCl_2$, 0.2 mM dNTP, 2.5U *Taq* DNA polymerase, 0.4 µM each primer for GI (G1-SKF and G1-SKR) or GII (G2-SKF and G2-SKR), and nuclease-free water. The thermocycling profiles were as follows: initial denaturation at 94°C for 3 min, PCR with 30 cycles at 94°C for 1 min, 50°C for 1 min, and 72°C for 2 min, and final extension at 72°C for 15 min. Amplicon was analyzed using 1.5% agarose gel electrophoresis in 1X Tris-borate-EDTA (TBE) buffer containing the 1X SERVA DNA Stain G solution (SERVA Electrophoresis GmbH, Germany) and visualized on the UV transilluminator using the Gel Doc XRþ System (BioRad, USA). Noroviruses GI and GII generated DNA fragments of 330 and 344 bp, respectively.

Phylogenetic analysis

The norovirus PCR products from semi-nested RT-PCR were purified using the QIAquick® Gel Extraction Kit (QIAGEN GmbH, Germany), according to the manufacturer's instructions. Purified PCR products were submitted for Sanger DNA sequencing. The nucleotide sequences of norovirus were compared with those of reference strains available in the GenBank database using the Basic Local Alignment Search Tool (BLAST) server. Phylogenetic analysis of norovirus was performed using Molecular Evolutionary Genetic Analysis (MEGA) version 6.06. The nucleotide sequences of norovirus-positive samples were submitted to the GenBank database with accession numbers PP780644-PP780654.

Data analysis

The chi-square test or Fisher's exact test was used to compare the distribution of norovirus GI and GII in district areas, restrooms, and other environmental surfaces. Fisher's exact test was used if the cell count (>20%) was expected

to be <5. The concentration of noroviruses on the surfaces was compared using the Mann-Whitney U test. A p-value of <0.05 was considered statistically significant, and the Statistical Package performed the statistical analysis for the Social Sciences (SPSS) software version 28.0.0.0 **(**IBM Armonk, USA).

RESULTS AND DISCUSSION

Market locations and frequency of norovirus RNA contamination on restroom surfaces

The presence of noroviruses GI and GII on toilet surfaces in local markets was determined. In total, 211 environmental swab samples from 57 local markets located in six districts were quantified using RT-qPCR. Figure 2 shows the detection rate of noroviruses in six district areas. The highest detection rate of noroviruses was found in district C (23.1%), followed by B (17.9%), D (15.8%), F (12.2%), E (11.1%), and A (8.4%). As shown in Table 1, 32 (15.2%) tested positive for noroviruses from environmental surface samples. The norovirus GII detected in 20 (9.5%) samples collected from 6 districts was higher than norovirus GI detected in 9 (4.3%) samples from 4 districts. Mixed GI and GII were found in 3 (1.4%) samples from 3 districts. The norovirus RNA detected in urinal flush valve samples (18.7%) was higher than in doorknob samples (12.5%). The norovirus genome was found in 27 (47.4%) of 57 local markets composed of 2/6 (33.3%), 8/15 (53.3%), 4/7 (57.1%), 5/10 (50.0%), 2/5 (40.0%), and 6/14 (42.8%) markets from districts A through F, respectively (Figure 1).

The correlation between norovirus genogroups and environmental factors was analyzed. Table 2 shows the characteristics of noroviruses GI and GII contamination in toilet surfaces. There were no statistically significant differences (p = 0.962) between the presence of noroviruses GI and GII on environmental surfaces in six districts in Bangkok. Nevertheless, norovirus GII was significantly more prevalent than GI in female toilets (40.0%) and doorknobs (40.0%) (p = 0.044).

Norovirus quantification

The standard curve for noroviruses GI and GII was plotted based on the average Cq value of the RNA transcript for GI or GII against the amount of RNA copies/mL. The amplification efficiency was 90.25% (slope = -3.58, R^2 = 0.98) and 107.71% (slope = -3.15, R^2 = 0.99) for GI and GII, respectively (Figure 3). The analytical sensitivity of both GI and GII detection was 1×10^3 RNA copies/mL or 5 RNA copies/reaction.

The concentration of viruses on the toilet surface samples significantly ranged from 95.5 to 950 RNA copies for GI and from 18.9 to 16,400 RNA copies for GII. As shown in Figure 4, the mean concentration of total noroviruses (GI and GII) on doorknobs was significantly higher than that on urinal flush valves (1,680 ± 3,830 RNA copies vs. 607 ± 871 RNA copies, p = 0.048). Similarly, when analyzing each genogroup, doorknobs showed higher concentrations of both GI (567 ± 23.4 vs. 397 ± 271 RNA copies, p = 0.110) and GII (1,910 ± 4,210 vs. 817 ± 1,200 RNA copies, p = 0.019) than urinal flush valves.

Norovirus genotype

Of 35 norovirus-positive environmental swab samples assessed using RT-qPCR, only 11 samples tested positive for norovirus GII based on semi-nested RT-PCR. Further, this finding was confirmed via sequencing. The phylogenetic trees of norovirus GII were constructed using the partial capsid sequences (233 bp). The nucleotide sequences of GII were similar to the norovirus strains found in fecal samples from Brazil (accession no. KX722437), with nucleotide identity ranging from 95% to 99%, and GII strains found in Korea (accession no. MG548737), with a nucleotide identity of 88.2%. All norovirus sequences were identified as GII.4 variant Den Haag 2006b, as shown in Figure 5.

Table 1. The presence of noroviruses GI and GII on the environmental surfaces in the markets located in Bangkok, Thailand

Surface type	Total	No. of norovirus-positive samples (%)			
		GI	GII	GI+GII	Total
Doorknob	120	1 (0.8)	12 (10)	2 (1.7)	15 (12.5)
Urinal flush valve	91	8 (8.8)	8 (8.8)	1 (1.1)	17 (18.7)
Total	211	9 (4.3)	20 (9.5)	3 (1.4)	32 (15.2)

Table 2. Characteristics of factors for the presence of norovirus GI and GII according to district, restroom type, and surface type

Characteristics	No. of norovirus-positive samples (%) by genogroup		p-value
	GI	GII	
	12	23	
District			
A	1 (2.9)	2 (5.7)	0.962
B	4 (11.4)	7 (20.0)	
C	2 (5.7)	4 (11.4)	
D	3 (8.6)	3 (8.6)	
E	0	2 (5.7)	
F	2 (5.1)	5 (14.3)	
Restroom type			
Men	9 (25.7)	9 (25.7)	0.044
Women	3 (8.6)	14 (40.0)	
Surface type			
Doorknob	3 (8.6)	14 (40.0)	0.044
Urinal flush valve	9 (25.7)	9 (25.7)	

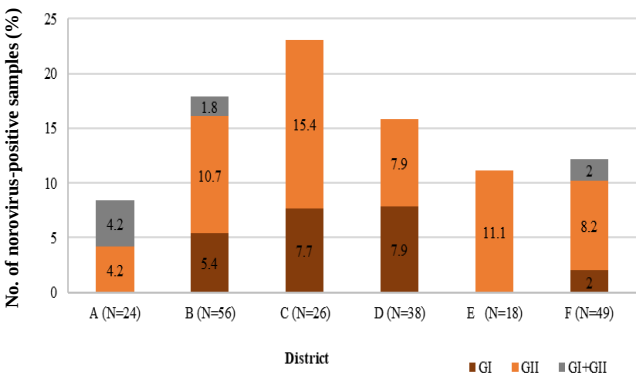

Figure 2. The detection rate of norovirus GI, GII, and mixed GI and GII in districts A to F in Bangkok, Thailand

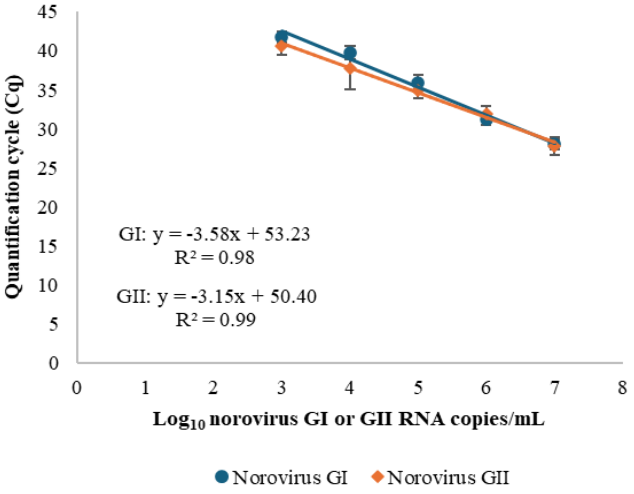

Figure 3. Linearity of real-time RT-PCR assays for the detection of norovirus GI and GII

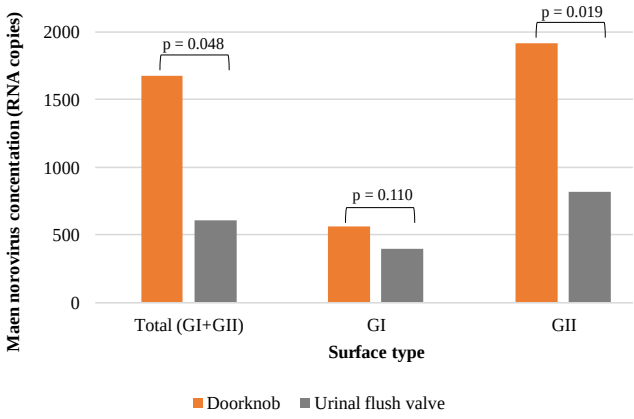

Figure 4. Quantification of noroviruses GI and GII on doorknobs and urinal flush valves in toilets from local markets in Bangkok, Thailand

Discussion

To the best of our knowledge, this study first investigated the presence of norovirus on environmental toilet surfaces in local markets under non-norovirus outbreak conditions in Bangkok, Thailand. The sampling sites for this study were selected from the northern regions of Bangkok. The samples are archived samples from a prior investigation into Severe Acute Respiratory syndrome Coronavirus 2 (SARS-CoV-2) that mentioned areas that had a high prevalence of Coronavirus Disease 2019 (Covid-19) cases at that time. All markets categorized by size as small, medium, and large (35-1,500 venders) were selected to explore. Mostly, norovirus was detected in nearby markets in the same districts. Among six districts, District C has the highest population density and exhibited the highest norovirus detection rate. Thus, higher population density increases the risk of contamination. Norovirus RNA was detected in 15.2% of toilet surface samples from 27 of 57 local markets. The detection rate of norovirus in this study was higher than that in other studies, which showed that the norovirus contamination rate on toilet surfaces in different locations such as food establishments (Leone et al. 2018; Elviss et al. 2022) and garrisons (Oristo et al. 2017) ranged from 1.5% to 9.4%. Even though sample collection occurred during the Covid-19 pandemic, noroviruses were detected on environmental surfaces.

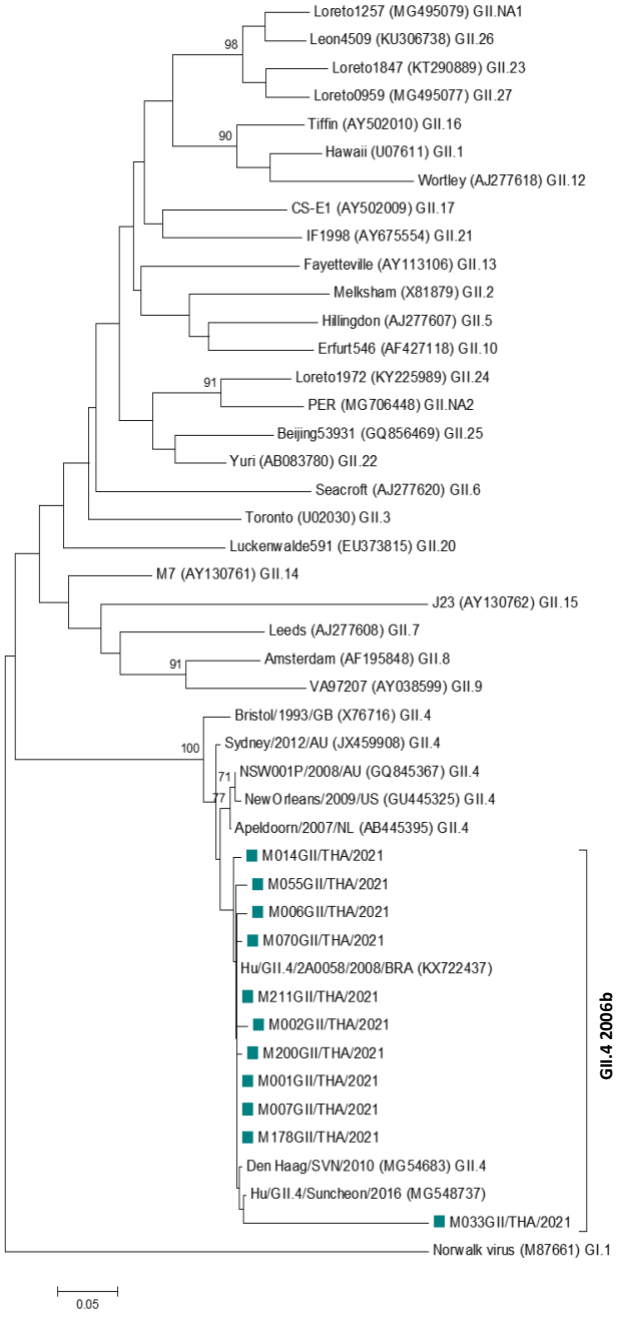

Figure 5. Phylogenetic analysis of partial capsid sequences of norovirus GII detected on 11 environmental samples from local markets in Bangkok (M001-M211). The tree was constructed by the neighbor-joining method by MEGA version 6.06. The norovirus strains detected in the present study are presented with the square symbol

While 15.2% of samples were positive for norovirus, none of the samples were positive for SARS-CoV-2 RNA (Phumisantiphong et al. 2025). This observation supports the notion of norovirus's environmental resistance. Moreover, norovirus is relatively resistant to standard disinfectants, including ammonium base and alcohol (Liu and Moore 2020). Only sodium hypochlorite was sufficiently effective against norovirus (Winder et al. 2022; Jeon et al. 2024). The local markets in Bangkok are most important for the distribution of fresh products and ready-to-eat food, and all markets are located in community areas. Based on this finding, norovirus contamination on toilet surfaces in local markets can be a source of norovirus spread in Bangkok.

The detection rate of norovirus GII (10.9%) was higher than that of GI (5.7%) on toilet surface samples. This finding is in accordance with that of other studies (Boxman et al. 2015; Aprea et al. 2021). Similarly, norovirus GII is the most common cause of acute gastroenteritis outbreaks, and it infects a higher number of patients than the GI (Winder et al. 2022; Chuchaona et al. 2024). Noroviruses GI and GII were found on urinal flush valves and doorknobs. However, in the study of Leone et al. (2018) and Stobnicka et al. (2018), norovirus GII was significantly present on doorknob samples, the same as in the present study. The presence of norovirus on commonly touched surfaces indicated that contaminated hands could spread them. Symptomatic or asymptomatic individuals with norovirus infection excrete the virus in their feces at high concentrations (10^5-10^9 particles/g) (Barclay et al. 2014). The virus can survive for a long period (up to 7 days) (Wu et al. 2019). Moreover, it can attach to stainless steel surfaces such as doorknobs and flush valves and persist on them at room temperature for 42 days (Tung-Thompson et al. 2015). Other studies showed that environmental surfaces such as door handles and toilet seats in restrooms were a source of norovirus outbreaks (Rico et al. 2020; Aprea et al. 2021; Abney et al. 2024). Hence, it is our responsibility to control norovirus outbreaks through effective hand hygiene, eliminating the transmission of norovirus to other surfaces or individuals.

As few as 10-100 norovirus particles can infect a healthy individual (Teunis et al. 2008). The concentration of norovirus particles or genome copies can be used to evaluate the risk of norovirus infection. The detection of a viral genome using RT-qPCR does not always indicate the presence of an infectious virus. However, the number of viral genomes on surfaces may be associated with the chance of infection and stability (Overbey et al. 2021). A high number of norovirus RNA in this study, particularly GII on doorknobs, were detected on contact objects, indicating that there is a significant risk of norovirus transmission in these areas. Then, to further our understanding and potentially mitigate this risk, it is crucial that we conduct additional research to refine the method for detecting infectious viral particles.

Only 11 of 23 norovirus GII-positive samples by RT-qPCR were positive by semi-nested RT-PCR. This could be because 1) RNA breaks down or sheds, 2) the primers used in RT-qPCR (ORF1-ORF2 junction) and semi-nested RT-PCR (ORF2 region) target different gene regions of norovirus, and 3) the target size for RT-qPCR for GII is 98 bp, while the target size for semi-nested RT-PCR is 344 bp. Semi-nested RT-PCR failed to detect norovirus GI in any samples. Potential primer mismatches may have contributed to this result, suggesting the need for alternative primer sets in future research. Norovirus GII.4 is the common genotype in outbreaks and sporadic cases worldwide (Winder et al. 2022). The norovirus GII.4 variant Den Haag 2006b was predominant from 2006 to 2008 and was still detected between 2010 and 2019 (Khamrin et al. 2017; Xue et al. 2019; Fu et al. 2021). In Thailand, GII.4 2006b was detected in infected patients and various environments, including water and oyster habitats (Kittigul et al. 2010, 2022). The current study found GII.4 2006b on toilet surface samples in 2021, which corresponds to GII.4 2006b identified in fecal samples collected from patients with acute gastroenteritis in Uttaradit Province, Thailand, in the same period (unpublished data). Based on this finding, norovirus GII.4 2006b is still circulating in humans and the environment.

In conclusion, this study provides the first evidence of norovirus contamination on environmental surfaces in local markets in Bangkok, Thailand, during non-outbreak periods. The study employed RT-qPCR and subsequent semi-nested RT-PCR to investigate the presence of noroviruses GI and GII on toilet surfaces. Our findings revealed a high prevalence of norovirus contamination on frequently touched surfaces, including doorknobs and urinal flush valves. Notably, these contaminated surfaces exhibited high norovirus concentrations. Norovirus GII.4 2006b emerged as the predominant strain. The contaminated surfaces can serve as potential vectors for norovirus transmission. These findings highlight the critical need for routine environmental monitoring and the implementation of effective cleaning protocols to minimize the risk of norovirus spreading. Finally, our data are useful for molecular epidemiology surveillance, prevention of norovirus outbreaks, and health risk analysis of norovirus infection.

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REFERENCES

Abney SE, Higham CA, Wilson AM, Ijaz MK, McKinney J, Reynolds KA, Gerba CP. 2024. Transmission of viruses from restroom use: A quantitative microbial risk assessment. Food Environ Virol 16 (1): 65-78. DOI: 10.1007/s12560-023-09580-1.

Aprea G, Di Bartolo I, Monini M, D'Angelantonio D, Scattolini S, Boni A, Truglio G, Di Giacobbe S, Serio A, Antoci S, Di Marzio V, Migliorati G, D'Alterio N, Pomilio F. 2021. The role of staff and contaminated environmental surfaces in spreading of norovirus infection in a long-term health care facility in Italy. Vet Ital 57 (4): 311-318. DOI: 10.12834/VetIt.2479.15150.1.

Barclay L, Park GW, Vega E, Hall A, Parashar U, Vinjé J, Lopman B. 2014. Infection control for norovirus. Clin Microbiol Infect 20 (8): 731-740. DOI: 10.1111/1469-0691.12674.

Boxman ILA, Verhoef L, Hägele G, Klunder K, te Loeke NAJM, Vennema H, Jansen CCC, Koopmans M. 2015. Environmental testing for norovirus in various institutional settings using catering companies as sentinels for norovirus prevalence among the general population. Food Control 47: 98-102. DOI: 10.1016/j.foodcont.2014.06.026.

Buesa J, Rodriguez-Díaz J. 2016. The Molecular Virology of Enteric Viruses. In: Goyal S, Cannon J (eds). Viruses in Foods. Food Microbiology and Food Safety. Springer, Cham. DOI: 10.1007/978-3-319-30723-7_3

Cardemil CV, Parashar UD, Hall AJ. 2017. Norovirus infection in older adults: epidemiology, risk factors, and opportunities for prevention and control. Infect Dis Clin North Am 31 (4): 839-870. DOI: 10.1016/j.idc.2017.07.012.

Chhabra P, de Graaf M, Parra GI, Chan MC-W, Green K, Martella V, Wang Q, White PA, Katayama K, Vennema H, Koopmans MPG, Vinjé J. 2019. Updated classification of norovirus genogroups and genotypes. J Gen Virol 100: 1393-1406. DOI: 10.1099/jgv.0.001318.

Chuchaona W, Khongwichit S, Luang-On W, Vongpunsawad S, Poovorawan Y. 2023. Norovirus GII.3[P25] in patients and produce, Chanthaburi Province, Thailand, 2022. Emerg Infect Dis 29 (5): 1067-1070. DOI: 10.3201/eid2905.221291.

Chuchaona W, Vongpunsawad S, Lawtongkum W, Thepnarong N, Poovorawan Y. 2024. Acute gastroenteritis associated with norovirus GII.8[P8], Thailand, 2023. Emerg Infect Dis 30 (1): 194-197. DOI: 10.3201/eid3001.231264.

Elviss NC, Allen DJ, Kelly D, Akello JO, Hau S, Fox AJ, Hopkins M, Derrick J, O'Brien S, Iturriza-Gomara M. 2022. Norovirus attribution study: Detection of norovirus from the commercial food preparation environment in outbreak and non-outbreak premises. J Appl Microbiol 133 (6): 3391-3403. DOI: 10.1111/jam.15761.

Fang Y, Zhang Y, Wang H, Shi O, Wang W, Hou M, Wang L, Wu J, Zhao Y. 2022. Molecular epidemiology of norovirus infections in children with acute gastroenteritis in 2017-2019 in Tianjin, China. J Med Virol 94 (2): 616-624. DOI: 10.1002/jmv.27340.

Fu J, Ai J, Bao C, Zhang J, Wu Q, Zhu L, Hu J, Xing Z. 2021. Evolution of the GII.3[P12] Norovirus from 2010 to 2019 in Jiangsu, China. Gut Pathog 13 (1): 34. DOI: 10.1186/s13099-021-00430-8.

Gregory JB, Webster LF, Griffith JF, Stewart JR. 2011. Improved detection and quantitation of norovirus from water. J Virol Methods 172 (1-2): 38-45. DOI: 10.1016/j.jviromet.2010.12.011.

Jayasekara L, Leone CM, Sharp J, Getty M. 2016. Environmental factors associated with norovirus transmission in long-term care facilities in South Carolina. J Environ Health 79 (2): 22-29.

Jeon EB, Roy A, Park SY. 2024. Application of sodium hypochlorite for human norovirus and hepatitis A virus inactivation in groundwater. Appl Environ Microbiol 90: e0140524. DOI: 10.1128/aem.01405-24.

Kageyama T, Kojima S, Shinohara M, Uchida K, Fukushi S, Hoshino FB, Takeda N, Katayama K. 2003. Broadly reactive and highly sensitive assay for Norwalk-like viruses based on real-time quantitative reverse transcription-PCR. J Clin Microbiol 41 (4): 1548-1557. DOI: 10.1128/jcm.41.4.1548-1557.2003.

Khamrin P, Kumthip K, Supadej K, Thongprachum A, Okitsu S, Hayakawa S, Ushijima H, Maneekarn N. 2017. Noroviruses and sapoviruses associated with acute gastroenteritis in pediatric patients in Thailand: Increased detection of recombinant norovirus GII.P16/GII.13 strains. Arch Virol 162 (11): 3371-3380. DOI: 10.1007/s00705-017-3501-3.

Khamrin P, Kumthip K, Yodmeeklin A, Jampanil N, Phengma P, Yamsakul P, Okitsu S, Kobayashi T, Ushijima H, Maneekarn N. 2022. Changing predominance of norovirus recombinant strains GII.2[P16] to GII.4[P16] and GII.4[P31] in Thailand, 2017 to 2018. Microbiol Spectr 10 (3): e0044822. DOI: 10.1128/spectrum.00448-22.

Khamrin P, Kumthip K, Yodmeeklin A, Okitsu S, Motomura K, Sato S, Ushijima H, Maneekarn N. 2024. Genetic recombination and genotype diversity of norovirus GI in children with acute gastroenteritis in Thailand, 2015-2021. J Infect Public Health 17 (3): 379-385. DOI: 10.1016/j.jiph.2024.01.009.

Kittigul L, Pombubpa K, Rupprom K, Thasiri J. 2022. Detection of norovirus recombinant GII.2[P16] strains in oysters in Thailand. Food Environ Virol 14 (1): 59-68. DOI: 10.1007/s12560-022-09508-1.

Kittigul L, Pombubpa K, Taweekate Y, Diraphat P, Sujirarat D, Khamrin P, Ushijima H. 2010. Norovirus GII-4 2006b variant circulating in patients with acute gastroenteritis in Thailand during a 2006-2007 study. J Med Virol 82 (5): 854-860. DOI: 10.1002/jmv.21746.

Kojima S, Kageyama T, Fukushi S, Hoshino FB, Shinohara M, Uchida K, Natori K, Takeda N, Katayama K. 2002. Genogroup-specific PCR primers for detection of Norwalk-like viruses. J Virol Methods 100 (1-2): 107-114. DOI: 10.1016/s0166-0934(01)00404-9.

Kumthip K, Khamrin P, Thongprachum A, Malasao R, Yodmeeklin A, Ushijima H, Maneekarn N. 2024. Diverse genotypes of norovirus genogroup I and II contamination in environmental water in Thailand during the Covid-19 outbreak from 2020 to 2022. Virol Sin 39 (4): 556-564. DOI: 10.1016/j.virs.2024.05.010.

Leone CM, Dharmasena M, Tang C, DiCaprio E, Ma Y, Araud E, Bolinger H, Rupprom K, Yeargin T, Li J, Schaffner D, Jiang X, Sharp J, Vinjé J, Fraser A. 2018. Prevalence of human noroviruses in commercial food establishment bathrooms. J Food Prot 81 (5): 719-728. DOI: 10.4315/0362-028x.jfp-17-419.

Liu L, Moore MD. 2020. A survey of analytical techniques for noroviruses. Foods 9 (3): 318. DOI: 10.3390/foods9030318.

Lu L, Ao Y, Jia R, Zhong H, Liu P, Xu M, Su L, Cao L, Xu J. 2023. Changing predominance of norovirus strains in children with acute gastroenteritis in Shanghai, 2018-2021. Virol Sin 38 (5): 671-679. DOI: 10.1016/j.virs.2023.08.005.

Lucero Y, Matson DO, Ashkenazi S, George S, O'Ryan M. 2021. Norovirus: Facts and reflections from past, present, and future. Viruses 13 (12): 2399. DOI: 10.3390/v13122399.

Maunula L, Rönnqvist M, Åberg R, Lunden J, Nevas M. 2017. The presence of norovirus and adenovirus on environmental surfaces in relation to the hygienic level in food service operations associated with a suspected gastroenteritis outbreak. Food Environ Virol 9 (3): 334-341. DOI: 10.1007/s12560-017-9291-7.

Oristo S, Rönnqvist M, Aho M, Sovijärvi A, Hannila-Handelberg T, Hörman A, Nikkari S, Kinnunen PM, Maunula L. 2017. Contamination by norovirus and adenovirus on environmental surfaces and in hands of conscripts in two Finnish garrisons. Food Environ Virol 9 (1): 62-71. DOI: 10.1007/s12560-016-9262-4.

Overbey KN, Hamra GB, Nachman KE, Rock C, Schwab KJ. 2021. Quantitative microbial risk assessment of human norovirus infection in environmental service workers due to healthcare-associated fomites. J Hosp Infect 117: 52-64. DOI: 10.1016/j.jhin.2021.08.006.

Phengma P, Khamrin P, Jampanil N, Yodmeeklin A, Ukarapol N, Maneekarn N, Kumthip K. 2022. Molecular epidemiology and characterization of norovirus and sapovirus in pediatric patients with acute diarrhea in Thailand, 2019-2020. J Infect Public Health 15 (9): 1013-1019. DOI: 10.1016/j.jiph.2022.08.006.

Phengma P, Khamrin P, Jampanil N, Yodmeeklin A, Ushijima H, Maneekarn N, Kumthip K. 2023. The emergence of recombinant norovirus GII.12[P16] and predominance of GII.3[P12] strains in pediatric patients with acute gastroenteritis in Thailand, 2019-2020. J Med Virol 95 (1): e28321. DOI: 10.1002/jmv.28321.

Phumisantiphong U, Rupprom K, Wongsuk T, Manomaipiboon A, Maneerit J, Vimonvattana A, Chantratita W, Sensorn I, Hansirisathit T, Thongsopa W, Phutthanu C, Dongphooyao S, Thongnak C. 2025. Severe acute respiratory syndrome coronavirus 2 variants in patients with coronavirus disease 2019 and environmental sampling from the hospital and market during the coronavirus disease 2019 pandemic in Thailand. Diagn Microbiol Infect Dis 111 (2): 116604. DOI: 10.1016/j.diagmicrobio.2024.116604.

Rico E, Pérez C, Belver A, Sabaté S, Razquin E, de Benito J, Coronas L, Domínguez A, Jané M. 2020. Norovirus detection in environmental samples in norovirus outbreaks in closed and semi-closed settings. J Hosp Infect 105 (1): 3-9. DOI: 10.1016/j.jhin.2020.02.011.

Stobnicka A, Gołofit-Szymczak M, Wójcik-Fatla A, Zając V, Korczyńska-Smolec J, Górny RL. 2018. Prevalence of human parainfluenza viruses and noroviruses genomes on office fomites. Food Environ Virol 10 (2): 133-140. DOI: 10.1007/s12560-017-9327-z.

Teunis PFM, Moe CL, Liu P, Miller SE, Lindesmith L, Baric RS, Le Pendu J, Calderon RL. 2008. Norwalk virus: How infectious is it?. J Med Virol 80 (8): 1468-1476. DOI: 10.1002/jmv.21237.

Troxler S, Marek A, Prokofieva I, Bilic I, Hess M. 2011. TaqMan real-time reverse transcription-PCR assay for universal detection and quantification of avian hepatitis E virus from clinical samples in the presence of a heterologous internal control RNA. J Clin Microbiol 49 (4): 1339-1346. DOI: 10.1128/jcm.01626-10.

Tung-Thompson G, Gentry-Shields J, Fraser A, Jaykus L-A. 2015. Persistence of human norovirus RT-qPCR signals in simulated gastric fluid. Food Environ Virol 7: 32-40. DOI: 10.1007/s12560-014-9170-4.

Udompat P, Srimuang K, Doungngern P et al. 2024. An unusual diarrheal outbreak in the community in Eastern Thailand caused by Norovirus GII.3[P25]. Virol J 21 (1): 21. DOI: 10.1186/s12985-024-02296-z.

Winder N, Gohar S, Muthana M. 2022. Norovirus: An overview of virology and preventative measures. Viruses 14 (12): 2811. DOI: 10.3390/v14122811.

Wu Q-S, Xuan Z-L, Liu J-Y, Zhao X-T, Chen Y-F, Wang C-X, Shen X-T, Wang Y-X, Wang L, Hu Y. 2019. Norovirus shedding among symptomatic and asymptomatic employees in outbreak settings in Shanghai, China. BMC Infect Dis 19: 592. DOI: 10.1186/s12879-019-4205-y.

Xue L, Cai W, Gao J, Zhang L, Dong R, Li Y, Wu H, Chen M, Zhang J, Wang J, Wu Q. 2019. The resurgence of the norovirus GII.4 variant associated with sporadic gastroenteritis in the post-GII.17 period in South China, 2015 to 2017. BMC Infect Dis 19: 696. DOI: 10.1186/s12879-019-4331-6.