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Persistent Circulation and Predominant P-types of Norovirus GII Identified through Wastewater Surveillance in a Mexican High-Tourist Region

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Abstract

This study aimed to detect norovirus GII (NoV GII) genome copies in wastewater and identify the predominant genotypes in Cancun, a major and rapidly growing tourist destination in Mexico. Primary-treated wastewater was collected from five urban Wastewater treatment plants (WWTPs) between March and October 2022 (n = 40). To investigate the presence of NoV GII genome copies in the aquatic environment, groundwater was collected from continental (n = 10) and island sinkholes (n = 10) in the karst aquifer system of the Yucatan peninsula. Viral particles were concentrated by adsorption-elution followed by ultrafiltration. Total RNA was isolated and used for cDNA synthesis, which was then subjected to quantitative PCR (qPCR) using primers targeting the ORF1/ORF2 junction. Additionally, cDNA from selected wastewater samples was subjected to semi-nested PCR to generate NoV GII amplicons of 269 nucleotides (nt) from the ORF1 (RdRp). For NoV GII typing, amplicons from 9 samples were cloned and Sanger-sequenced (n = 37). In addition, amplicons from 3 samples were sequenced using the Illumina high-throughput platform, yielding 9,846 good-quality sequences. NoV GII was detected in 90% (36/40) of wastewater samples, at concentrations ranging from 10^4 to 10^7 genome copies per liter (GC/L). In groundwater samples, NoV GII was detected in 95% (19/20) of the samples, at concentrations from 10^2 to 10^4 GC/L. Results from the Sanger sequence analysis showed a prevalence of GII.P8, followed by GII.P16 and GII.P31. In contrast, next-generation sequencing (NGS) identified GII.P16 as the most prevalent P-type, followed by GII.P31 and GII.P8. Notably, GII.P4 was detected exclusively by NGS, highlighting its potential significance for wastewater monitoring. These findings demonstrate the persistent circulation of NoV GII in Cancun and nearby tourist areas, including Valladolid and Cozumel Island, and underscore the importance of wastewater-based surveillance in high-tourism regions.

Introduction

Enteric viruses (EVs) are among the leading causes of acute viral gastroenteritis worldwide. Noroviruses (NoVs), members of the *Caliciviridae* family, are considered the primary etiological agents of viral gastroenteritis worldwide (Bresee et al., 2012; Carlson et al., 2024). NoVs are classified into ten genogroups (GI–GX), five of which infect humans, with GI and GII being the most prevalent (Chhabra et al., 2019; Carlson et al., 2024). Their genomic classification is based on the analysis of VP1 (ORF2) and RdRp (NS7-ORF1) sequences or a dual typing system (Chhabra et al., 2019; Carlson et al., 2024), with at least 49 genotypes and 60 P-types described (Chhabra et al., 2019). Moreover, high genetic diversity and recombination contribute to the emergence of epidemiologically relevant variants (Kendra et al., 2022; Vakulenko et al., 2023).

In Mexico, NoV genogroup GII has been reported at a higher prevalence than GI, particularly among pediatric patients hospitalized with acute gastroenteritis (Cervantes-Izaguirre et al., 2019). Other studies have also documented considerable genetic diversity among NoVs circulating in asymptomatic pediatric populations, with multiple GI (GI.1, GI.3, GI.5, GI.7, and GI.14) and GII (GII.1, GII.2, GII.7, and GII.17) genotypes identified (García et al., 2006). More recently, recombinant variants have also been reported in

Mexican pediatric cohorts (Cortés-Trigueros et al., 2025), underscoring the dynamic evolution and diversity of NoVs in the region. However, systematic information on NoV prevalence and genetic diversity in Mexico remains limited, as gastroenteritis cases lack molecular confirmation, hindering both viral detection and the monitoring of emerging variants.

Because enteric viruses are excreted in high concentrations in feces (Atmar et al., 2008), wastewater-based surveillance and environmental monitoring provide powerful tools for assessing viral prevalence and identifying the diversity of the viral population or the predominant circulating genotypes (Medema et al., 2020; Hoque et al., 2023; Malla et al., 2024; Izquierdo-Lara et al., 2025). These approaches have successfully correlated NoV concentrations with clinical cases, identifying dominant genotypes and revealing seasonal trends (Huang et al., 2022; Verani et al., 2024). Furthermore, aquatic environments exposed to intense anthropogenic activity have been proposed as complementary sources for enteric virus surveillance (Espinosa et al., 2009; Hata et al., 2021), providing a non-invasive and cost-effective means for viral monitoring (Cavadas et al., 2022; Huang et al., 2022).

The occurrence of NoVs in aquatic environments, including marine and surface water, has been documented in Mexico (Hernández-Morga et al., 2009; León-Félix et al., 2010; Delgado-Gardea et al., 2017). Both GI and GII have been reported in aquatic environments in high-tourism regions, such as Cancun and the Riviera Maya (Rosiles-González et al., 2019), as well as in the Mexican Caribbean (Muñoz-Cortes et al., 2023), with NoV GII showing greater prevalence and genotypic diversity (Rosiles-González et al., 2019). However, most studies have focused on environmental matrices rather than wastewater, leaving gaps in our understanding of viral prevalence and diversity at the population level. Moreover, most genotypes described in Mexico originate from clinical (García et al., 2006) rather than environmental samples, despite the well-recognized value of water-based surveillance as a complementary epidemiological tool.

Therefore, this study aimed to determine the occurrence of NoV GII in wastewater and to identify the predominant circulating genotypes in Cancún, one of Mexico’s major tourist destinations. To broaden the environmental perspective, groundwater from sinkholes in tourist areas across the continent and on a major island was also analyzed, as the presence of these viruses could pose health risks through recreational water activities.

Methods

Study area

Wastewater samples were obtained from five Wastewater treatment plants (WWTP) located in the City of Cancún, municipality of Benito Juárez, in Quintana Roo, Mexico (Fig. 1). Cancún has a total population of 888,797 (INEGI, 2020) and is the central urban area in the Benito Juárez municipality. The state of Quintana Roo is one of Mexico's high-tourism areas, receiving more than 20 million tourists in 2025, with the majority visiting Cancun (over 7.2 million) and the Riviera Maya (over 7.9 million) (SEDETUR, 2025).

Groundwater samples were collected from sinkholes in the municipalities of Cozumel, Quintana Roo, and Valladolid, Yucatán (Fig.1). Cozumel has a total population of 88,626 (INEGI, 2020) and is one of the major islands in the Mexican Caribbean, attracting almost 1 million tourists in 2025 (SEDETUR, 2025). Valladolid is a historic city on the Yucatán Peninsula, designated a Pueblo Mágico for its cultural and tourist significance, with a population of 74,217 inhabitants (INEGI, 2020). It hosts several tourist attractions, including the Zací sinkhole, one of the largest open-air sinkholes in the peninsula (Secretaría de Turismo, 2014).

Water collection

Primary-treated wastewater samples (2 L) were collected from five WWTPs in Cancún, Quintana Roo, between March and October 2022 (n = 1-40) (Table 1). Sample collection was carried out by laboratory personnel and WWTP staff wearing appropriate personal protective equipment (PPE), including long pants, long-sleeved shirts, steel-toed boots, safety vests, hard hats, and safety glasses. All these samples were further subjected to NoV GII quantification by RT-qPCR.

In addition, total RNA isolated from samples collected in 2020 (Rosiles-González et al., 2021) from P3 (n=46-51) and P4 (n=52-57) was subjected to seminested PCR.

Groundwater samples (10 L) were collected in 2023 from sinkholes in the municipality of Valladolid, Yucatán (n = 10). Additionally, total RNA previously extracted from sinkholes in Cozumel, Quintana Roo, in 2022 (n = 10) (Cejudo et al., 2024), and stored in the laboratory archive was also used for the quantification of NoV GII by RT-qPCR (Table 2).

All samples were collected manually using the grab sampling method as described by Haramoto et al. (2020). Sterile, submersible plastic bottles were used, and samples were transported to the laboratory on the same day for further processing.

Viral concentration and nucleic acid isolation

Water samples were concentrated using the adsorption-elution method, followed by a second concentration step with a centrifugal device (Centriprep YM-50 filter), as described in Rosiles-González et al. (2021). Briefly, a final concentration of 25 mM $MgCl_2$ was added to each wastewater sample, which was then filtered through a cellulose-ester membrane (0.45 µm) under vacuum until the membrane clogged. Cations were removed from the filter using 200 mL of H_2SO_4 0.5 M (pH 3.0), and viruses were eluted with 10 mL of NaOH 1 mM pH 10 in a centrifuge tube containing a neutralizing solution (50 µL of H_2SO_4 100 mM and 100 µL of TE 1X). For the second concentration step, a Centriprep® YM-50 filter was used as described by Kitajima et al. (2013). Final volumes of 0.9–5.4 ml were obtained and stored at −20 °C.

An aliquot of 280 µL from the final concentrated sample was used for RNA isolation with the QIAmp Viral RNA Mini Kit (Qiagen, Hilden, Germany) following the manufacturer´s instructions. RNA was stored at

−20 °C.

Quantification of NoV GII by RT-qPCR

The quantification of NoV GII was performed using primers targeting the ORF1-ORF2 junction region of human NoV GII, specifically COG2F and COG2R, in conjunction with the RING2-TP probe (Kageyama et al., 2023). The cDNA was synthesized from an aliquot of 5 µL (approximately 150-200 ng) of RNA using the i-Script Advanced cDNA Synthesis Kit for RT-qPCR (Bio-Rad, Hercules, CA, USA) according to the manufacturer's instructions. Quantification of NoV GII genomes was conducted in a 96-well plate format in a CFX96 Touch^{TM} Real-Time PCR Detection System (Bio-Rad, Hercules, CA, USA) using the Platinum Taq polymerase (Invitrogen, Carlsbad, CA, USA) following the manufacturer's instructions. Reactions were carried out in a final volume of 25 µL containing: 5 µL of cDNA, 2.5 µL of 10x reaction buffer, 50 nmol/L $MgCl_2$, 10nmol/L dNTPs, 500 nmol/L forward and reverse primers (each), 250nmol/L of the probe, and 5 U/ µL of DNA polymerase. qPCR cycling conditions were one cycle of 95°C for 2 min, followed by 50 cycles of 95 °C for 30 seconds, 56°C for 1 min, and 72°C for 30 seconds (Muñoz-Cortes et al., 2023). PCR-grade water was used as the negative control template in each experiment, and all standard and sample amplifications were performed in triplicate. A standard curve was generated for each experiment using 10-fold serial dilutions of plasmid DNA (containing 10^0-10^5 DNA genome copies) with the target NoV GII genome fragment. NoV GII genome copies were determined as described previously (Muñoz-Cortés et al., 2023). The limit of detection (LoD) was 0.6-2.9 copies, calculated following the method described by Houghs et al. (2017).

Semi-nested PCR and cloning

Randomly selected wastewater samples were subjected to semi-nested PCR using primers NKIIF and NKIIR for the first round and primer NKIIR2 for the second round, as described previously by Kong et al. (2015). The resulting amplicon corresponded to a 269 base-pair (bp) fragment of the ORF1 (RdRp). Amplifications were performed using GoTaq DNA polymerase (Promega, Madison, WI) and Platinum™ Taq DNA Polymerase (Invitrogen, Carlsbad, CA, USA) in a final volume of 25 µL per reaction. Each reaction (either for the first or second semi-nested PCR) consisted of 10 µl Green GoTaq buffer or 2.5 µl 10x reaction buffer of Platinum, 20 µmol L−1 of each forward and reverse primer, 10 nmol L−1 of each dNTP, and 1.25 U µL −1 of DNA polymerase or 0.1 U µl-1 of Platinum. Amplicons were cloned into the pGEM-Teasy vector (Promega, Madison, WI) following the manufacturer´s instructions. Ligated products were used to transform competent DH5α cells by heat shock (Sambrook and Russell, 2001). A total of 60 recombinant clones were sequenced at Macrogen, Seoul, Korea, using M13 forward universal primer.

Amplicon library construction and Illumina sequencing

For the first PCR round, the RT-PCR SuperScriptTM III Platinum One-Step qRT-PCR (Thermo Fisher Scientific, Waltham, MA, USA) was used. The reaction consisted of 5 µL of total RNA, 2x Reaction mix, 10 µM each of NKIIF and NKIIR primer (Kong et al., 2015), SuperScript™ III RT/Platinum™ Taq Mix, and DEPC-water to a total volume of 25 µL per reaction. Cycling conditions for the first round were as

follows: 50° C for 15 minutes (for cDNA synthesis), followed by one cycle of 95° C for 2 min, and 40 cycles of 95° C for 30 sec, 55° C for 30 sec, and 72° C for 1 min. The product from the first PCR (2 µl) was used as a template for the second PCR, with gene-specific primers NKIIF and NKIIR2 (Kong et al., 2015). These primers included the Illumina overhang adapter sequences (NKIIF-NGS 5-TCGTCGGCAGCGTCAGATGTGTATAAGAGACAGCTYAGGCARATGTACTGGACY-3´ and NKIIR2-NGS 5-GTCTCGTGGGCTCGGAGATGTGTATAAGAGACAGGGAGCCAGATTGCG

ATCGC-3´). Amplifications were conducted using TaqPlatinum DNA polymerase (Thermo Fisher Scientific, Waltham, MA, USA) in 25 µL reactions. Reactions contained 1µl of the PCR product, 10x PCR Buffer, 50 mM MgCl2, 10 mM dNTP mix, 10 µM each forward and reverse primer, Taq Platinum DNA polymerase 5 U µl-1, and nuclease-free water. Cycling conditions were as follows: 1 cycle at 95° C for 2 min, followed by 40 cycles at 95° C for 30 sec, 55° C for 30 sec, and 72° C for 1 min.

Amplicons were indexed using the Nextera XT Index kit version 2 (Illumina, San Diego, CA, USA), following the Illumina 16S Metagenomic Sequencing Library Preparation protocol. After indexing, barcoded fragments were purified a second time and quantified using the Qubit 3.0 fluorometer (Life Technologies, Malaysia). Barcoded PCR-amplicons were diluted in 10 mM Tris (pH 8.5) and pooled in equimolar concentrations (9 pM). Paired-end sequencing was performed on the MiSeq platform (Illumina, San Diego, CA, USA) with a MiSeq Reagent Kit V3 (600 cycles), at the Aquatic Pathology Laboratory at Cinvestav-Mérida. All sequence data is publicly available in the NCBI Sequence Read Archive under the BioProject PRJNA1405142.

Bioinformatic analysis for Norovirus GII genotyping

Sanger-obtained sequences were manually edited using Finch TV 1.4.0 (Geospiza, Inc.; Seattle, WA, USA; http://www.geospiza.com) and analyzed in batch using the Norovirus Typing Tool Version 2.0 available at https://mpf.rivm.nl/mpf/typingtool/norovirus/. Demultiplexed Illumina reads were analyzed using the QIIME2 (2017.11) pipeline (Caporaso et al., 2010). Sequences were trimmed and denoised using the "consensus" method for chimera removal, and amplicon sequence variants (ASVs) were obtained using the DADA2 plugin. Representative ASVs were taxonomically assigned using the Norovirus Typing Tool Version 2.0 available at https://mpf.rivm.nl/mpf/typingtool/norovirus/ database as reference.

The R environment was used for data analysis using the phyloseq (McMurdie et al., 2013) and ggplot2 (Wickham, 2016) libraries. After removing unassigned reads, the remaining reads were standardized to the lowest sequencing depth. Subsequently, relative abundance graphs were generated.

Phylogenetic reconstruction

The 36 Sanger-obtained sequences and the 17 representative Illumina-obtained sequences were used to construct a matrix that also included all available reference sequences for NoV GII (Chabbra et al., 2019). Sequences were aligned using MUSCLE (Edgar, 2004) implemented in the Molecular Evolutionary Genetic Analysis version 11.0 software (Tamura et al., 2021). Phylogenetic reconstruction was

conducted using the neighbor-joining method available in MEGA 11.0 (Tamura et al., 2021) with 1000 bootstrap iterations.

Results

Occurrence of NoV GII

NoV GII was detected in 90% (36/40) of the wastewater samples in concentrations from 1.6 x 10^4 GC/L to 1.3 x 10^7 GC/L (Table 1). NoV GII average genome copies varied across WWTPs (Fig. 2), with P3 showing the highest average concentration at 3.3 x 10^6 GC/L (Fig. 2).

Table 1. Genome copies per liter (CG/L) of Norovirus GII (NoV GII) detected in primary-treated wastewater from five wastewater treatment plants (WWTP) located in the city of Cancún, México

Wastewater treatment plant (WWTP)	Sample number	Collection date	Norovirus GII (NoV GII) Genome copies per liter (CG/L)
P1	3	03/2022	<LoD
	8	04/2022	1.6×10^4
	18	05/2022	2.2×10^5
	23	06/2022	1.8×10^6
	28	07/2022	8.4×10^5
	33	08/2022	4.8×10^5
	38	09/2022	6.1×10^5
	43	10/2022	1.8×10^6
P2	4	03/2022	9.3×10^4
	9	04/2022	2.7×10^4
	19	05/2022	1.3×10^5
	24	06/2022	1.0×10^6
	29	07/2022	1.4×10^6
	34	08/2022	ND
	39	09/2022	2.4×10^5
	44	10/2022	ND
P3	1	03/2022	3.2×10^6
	6	04/2022	2.0×10^5
	16	05/2022	5.4×10^4
	21	06/2022	1.9×10^6
	26	07/2022	7.2×10^6
	31	08/2022	1.3×10^7

	36	09/2022	6.5×10^5
	41	10/2022	4.7×10^5
P4	2	03/2022	7.9×10^4
	7	04/2022	2.7×10^4
	17	05/2022	1.7×10^6
	22	06/2022	1.8×10^5
	27	07/2022	3.6×10^6
	32	08/2022	3.1×10^6
	37	09/2022	ND
	42	10/2022	4.6×10^5
P5	5	03/2022	2.4×10^4
	10	04/2022	1.2×10^5
	20	05/2022	1.8×10^5
	25	06/2022	2.2×10^5
	30	07/2022	2.8×10^6
	35	08/2022	9.8×10^5
	40	09/2022	5.9×10^5
	45	10/2022	5.2×10^5

<LoD = below the limit of detection; ND = non-determined, negative samples by the qPCR method described

In groundwater, NoV GII was detected in 95% (19/20) of the samples, with concentrations ranging from 1.2×10^2 GC/L in site C9 to 8.1×10^4 GC/L in site V8 (Table 2). Groundwater from the continental sinkholes (samples V1 to V10) showed an average NoV GII concentration of 2.3×10^4 GC/L, whereas island sinkholes (C1 to C10) showed a lower average concentration of 8.77×10^2 GC/L (Fig. 2).

Table 2. Genome copies per liter (CG/L) of Norovirus GII (NoV GII) detected in groundwater from sinkholes in Quintana Roo.

Norovirus GII (NoV GII)			
Sample name	**Water type/Use**	**Collection date**	**Genome copies per liter (GC/L)**
V1	Groundwater from sinkhole/recreational	2023	$9.9x10^3$
V2	Groundwater from sinkhole/urban	2023	$1.1x10^3$
V3	Groundwater from sinkhole/urban	2023	$1.6x10^4$
V4	Groundwater from sinkhole/urban	2023	$1.1x10^3$
V5	Groundwater from sinkhole/urban	2023	$3.1x10^4$
V6	Groundwater from sinkhole/urban	2023	$7.5x10^4$
V7	Groundwater from sinkhole/urban	2023	$1.7x10^3$
V8	Groundwater from sinkhole/urban	2023	$8.1x10^4$
V9	Groundwater from sinkhole/urban	2023	$5.3x10^3$
V10	Groundwater from sinkhole/urban	2023	$7.6x10^3$
C1	Groundwater from sinkhole/urban	2022	$6.8x10^2$
C2	Groundwater from sinkhole/recreational	2022	$1.1x10^3$
C3	Groundwater from sinkhole/urban	2022	$8.4x10^2$
C4	Groundwater from sinkhole/urban	2022	ND
C5	Groundwater from sinkhole/recreational	2022	$1.8x10^3$
C6	Groundwater from sinkhole/recreational	2022	$6.5x10^2$
C7	Groundwater from sinkhole/recreational	2022	$1.9x10^3$
C8	Groundwater from sinkhole/recreational	2022	$3.9x10^2$
C9	Groundwater from sinkhole/urban	2022	$1.2x10^2$
C10	Groundwater from sinkhole/urban	2022	$4.1x10^2$

ND = non-determined, negative samples by the qPCR method described

Genotypic diversity of NoV GII

Genotyping of Sanger sequences isolated from selected wastewater samples showed that GII.P8 was the most prevalent, at 75% (27/36), followed by GII.P16 at 14% (5/36) and GII.P31 at 11% (4/36) (Table 3).

Table 3. List of Norovirus GII genotypes identified in selected wastewater by using Sanger sequencing of amplicons produced by semi-nested PCR.

Sample number	Clone number	GenBank accession number	Norovirus GII genotype
1	1	PX854015	GII.P16
26	1	PX854016	GII.P8
26	2	PX854017	GII.P8
26	3	PX854018	GII.P8
26	4	PX854019	GII.P8
26	5	PX854020	GII.P8
26	6	PX854021	GII.P8
26	7	PX854022	GII.P8
26	8	PX854023	GII.P8
26	9	PX854024	GII.P8
26	10	PX854025	GII.P8
29	1	PX854026	GII.P31 (GII.Pe)
29	2	PX854027	GII.P31 (GII.Pe)
29	3	PX854028	GII.P31 (GII.Pe)
29	4	PX854029	GII.P31 (GII.Pe)
46	1	PX854030	GII.P8
47	1	PX854031	GII.P8
47	2	PX854032	GII.P8
47	3	PX854033	GII.P8
47	4	PX854034	GII.P8
47	5	PX854035	GII.P8
47	6	PX854036	GII.P8
49	1	PX854037	GII.P8
51	1	PX854038	GII.P16
51	2	PX854039	GII.P8
51	3	PX854040	GII.P8
51	4	PX854041	GII.P8
51	5	PX854042	GII.P8

51	6	PX854043	GII.P16
51	7	PX854044	GII.P16
51	8	PX854045	GII.P16
51	9	PX854046	GII.P8
53	1	PX854047	GII.P8
53	2	PX854048	GII.P8
53	3	PX854049	GII.P8
56	1	PX854050	GII.P8

Analysis of Illumina sequencing yielded 9,846 reads after denoising, with a range of 3,198-3,336 reads across three wastewater samples. A total of 14 ASVs were identified, of which six were typed as GII.P16, one as GII.P31, one as GII.P4, and six were not assigned. Based on their relative abundance, GII.P16 was detected in samples 21 and 27 at 44% and 70%, respectively. A polymerase not assigned genotype (PNA) or a not assigned genotype was present in an abundance of 48% and 29% in samples 21 and 27, respectively. Genotype GII.P4 was detected at only 7% abundance in sample 21. Interestingly, sample 29 showed only a single genotype, GII.P31 (Fig. 3).

Phylogenetic reconstruction

Phylogenetic analysis of the NoV sequences revealed three major clades containing the sequences obtained in this study (36 Sanger, 14 Illumina representative sequences) and the other containing GenBank references, with only one major clade showing high bootstrap support of 94% (Fig. 4). One subclade included all sequences assigned to the GII.P4 and GII.P31 (GII.Pe) genotypes (highlighted in green), although it lacked bootstrap support. Another subclade encompassed all GII.P16-assigned genotypes (highlighted in brown), but with bootstrap support below 70%. The GII.PNA (unassigned) subclade contained a group of representative Illumina sequences (highlighted in blue) with high bootstrap support (86%) and was most closely related to the GII.P23 genotype (Fig. 4). The GII.P8 subclade (highlighted in purple) contained most of the sequences (Sanger) obtained in this study (Fig. 4).

Discussion

Noroviruses are well-known fecal contaminants of aquatic environments (Wyn-Jones et al., 2011; Mortari et al., 2023; Desdouits et al., 2022; Rosiles-González et al., 2025) and can be shed in feces of infected individuals (Zheng et al., 2025). As a result, NoVs can reach aquatic environments primarily through the discharge of raw and treated wastewater, and their presence in recreational waters can pose a public health risk to swimmers and other users (Sinclair et al., 2009; Uijtewaal et al., 2025). In this study, a high prevalence of NoV GII was observed not only in wastewater but also in groundwater from

sinkholes in continental and island high-tourism areas of the Yucatan Peninsula, Mexico, indicating a more widespread occurrence than previously reported in the region (Rosiles-González et al., 2019; Muñoz-Cortes et al., 2023). This finding is particularly concerning, as sinkholes in the Yucatan Peninsula are popular tourist sites and, in rural areas, are commonly used as sources of domestic water when piped water is unavailable.

Although NoV are reported to occur in aquatic environments in Mexico (Hernández-Morga et al., 2009; Delgado-Gardea et al., 2017; Rosiles-González et al., 2019 and 2025; Muñoz-Cortes et al., 2023), little is known about their genotypic diversity in these samples. Environmental monitoring of wastewater and aquatic environments can provide valuable insights into community-level viral prevalence and diversity of enteric viruses such as NoVs (Medema et al., 2020; Hoque et al., 2023; Malla et al., 2024; Izquierdo-Lara et al., 2025). This approach has been effective for correlating NoV concentrations with clinical cases, identifying dominant genotypes, and assessing seasonal trends (Huang et al., 2022; Verani et al., 2024). One of the most comprehensive studies on NoV surveillance through wastewater using next-generation sequencing revealed 17 GII genotypes over eight years, with variations in prevalence across years and the identification of persistent variants and emerging genotypes (Bonanno-Ferraro et al., 2025). In this study, a small number of wastewater samples collected over less than a year enabled the identification of four confirmed NoV GII P-types, along with rare types, suggesting substantial NoV diversity in the region and warranting further exploration to understand the dynamics and prevalence of these pathogens in high-tourist areas.

A previous study conducted in the area used traditional Sanger sequencing to examine NoV diversity and identified GII.Pe, GII.P16, and GII.P17 P-types in wastewater and groundwater in Mexico (Rosiles-González et al., 2019), which were most likely the most abundant types at the time of the sampling. Although the present study was not intended to compare sequencing methods, it was notable that Sanger sequencing identified GII.P8 as the most prevalent P-type. In contrast, high-throughput Illumina sequencing identified GII.P16 as the most prevalent. In addition, Illumina sequencing enabled the detection of additional types, including GII.P4 and several unassigned P-types phylogenetically close to GII.P23. Therefore, the results of this study contribute to current knowledge of the temporal genetic diversity of circulating NoVs in the region and help establish a foundational baseline for the use of high-throughput NoV amplicon sequencing in wastewater in Mexico.

Regarding the genetic diversity of NoVs in Mexico, studies of fecal samples from children with acute diarrhea more than two decades ago showed that GII.4 was the most abundant genotype (Gómez-Santiago et al., 2012). In this study, the GII.P4 P-type was detected in one wastewater sample. Although GII.4 was once a globally dominant circulating genotype, it has recently been displaced by recombinant strains (Kendra et al., 2022). Although to detect recombinant strains, a dual typing should be performed (Chhabra et al., 2019), the P-types reported in this study, GII.P31 and GII.P16, are most likely the currently widespread genetic variants of GII.4 Sydney that have been recognized as predominant strains associated with outbreaks in the USA (Cannon et al., 2017), Brazil (Barreira et al., 2017), Japan (Ushijima et al., 2024), and elsewhere (Choi et al., 2017; Lun et al., 2018; Ai et al., 2021).

Moreover, in Mexico, significant norovirus diversity has also been observed in asymptomatic children, among whom GII.1, GII.2, GII.7, and GII.17 genotypes have been reported (García et al., 2006). And a more recent study that used dual typing reported the GII.P2, GII.P31, GII.P4, GII.P7, GII.P40, and GI.P14 P-types, together with capsid genotypes GI.3, GII.2, GII.4, GII.5, GII.14, and GII.17, associated with diarrhea in children and adults (Félix-Valenzuela et al., 2020). Interestingly, this study revealed additional P-types (e.g., GII.P8, GII.P16, and other rare types phylogenetically close to GII.P23), underscoring the value of wastewater-based surveillance as a complementary approach for mapping the genetic diversity of circulating NoV strains.

This study has two main limitations: only a few samples were used to retrieve GII sequences, and these did not span different years; therefore, seasonal shifts in prevalence cannot be identified. In addition, no recombinant strains were identified because dual typing was not performed. Despite these limitations, this study demonstrates that wastewater and groundwater surveillance in a high-tourism region of the Yucatan Peninsula can reveal a diverse and dynamic population of NoV GII types. Moreover, the study identified additional P-types distinct from those previously reported in clinical pediatric samples in Mexico, thereby highlighting shifts in circulating strains and the added resolution provided by high-throughput sequencing in wastewater samples.

Continued, expanded environmental monitoring, coupled with increased sampling frequency, broader geographic coverage, and integration with clinical surveillance, would improve detection of emerging genotypes, help track transmission pathways linked to recreational and potable water exposures, and inform targeted public-health interventions to mitigate norovirus risk for both residents and visitors.

Declarations

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Data availability statement

The datasets generated during and/or analyzed during the current study are available from the corresponding author on reasonable request.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have influenced the work reported in this paper.

CRediT authorship contribution statement

C.H.Z Conceptualization, Project Administration, Methodology, Investigation, Resources, Supervision, Writing- original draft, Formal analysis.

G.R.G. Field work, Formal analysis, Methodology, Supervision, Validation

Y.C.B. Formal analysis

L.A.M.D. Formal analysis

M.C.D. Formal analysis, Methodology

J.Q.G.M. Funding acquisition, Resources, Formal analysis

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Figures

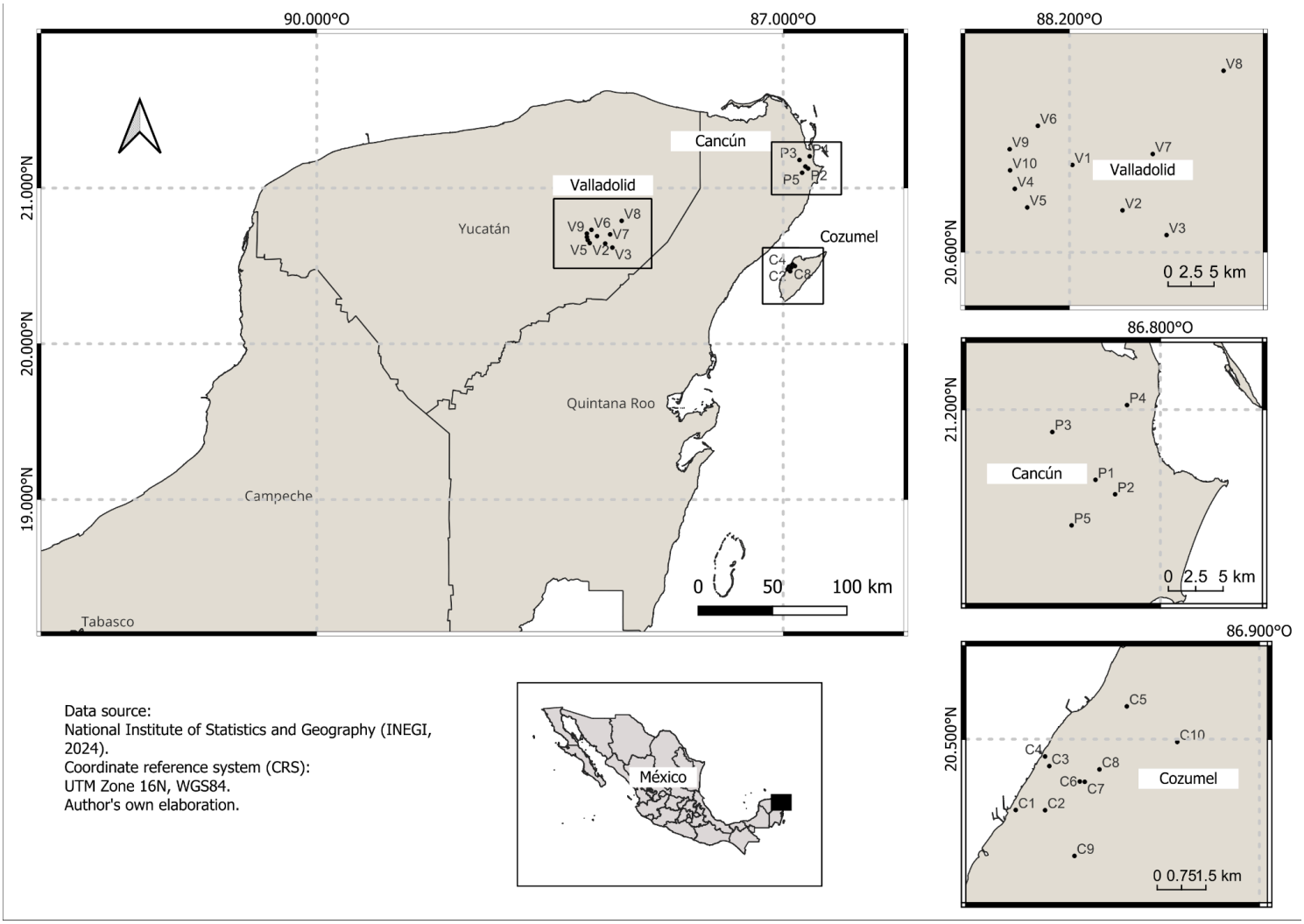


Figure 1

Map showing the location of sampling sites in the municipalities of Cancún (Benito Juárez), Cozumel, and Valladolid, in the Yucatan peninsula

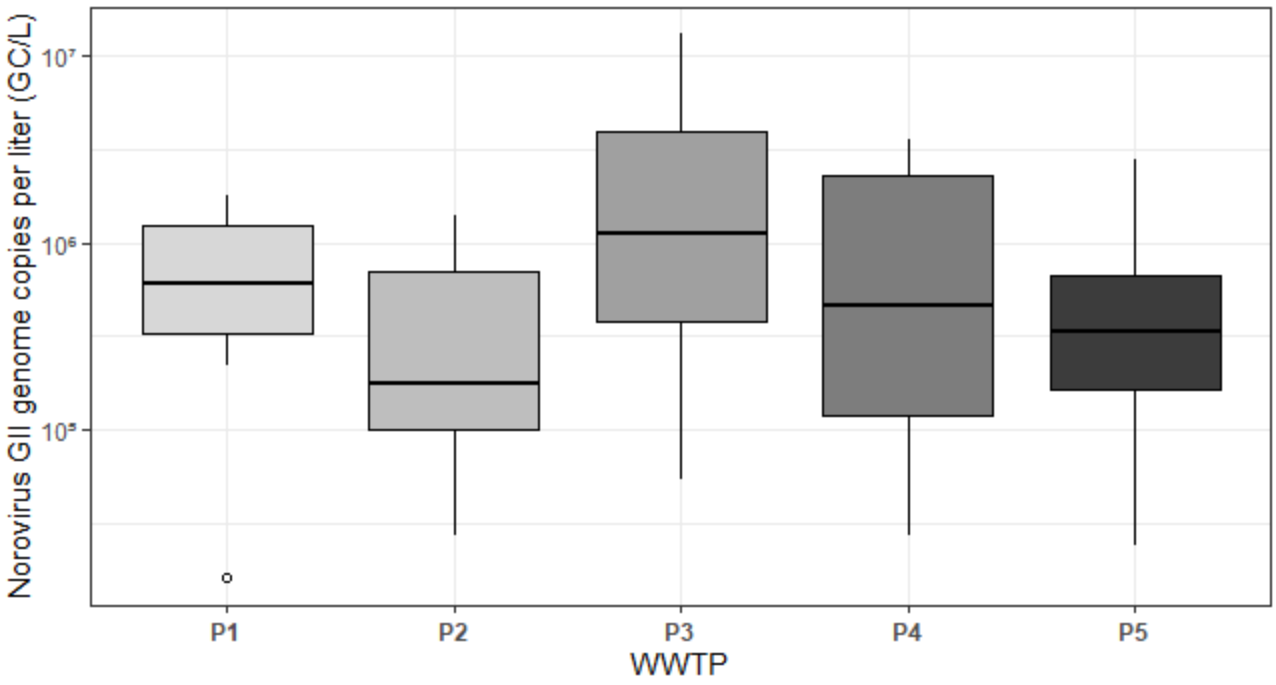


Figure 2

Distribution of NoV GII genome concentrations among five WWTP in Cancun, Quintana Roo, Mexico

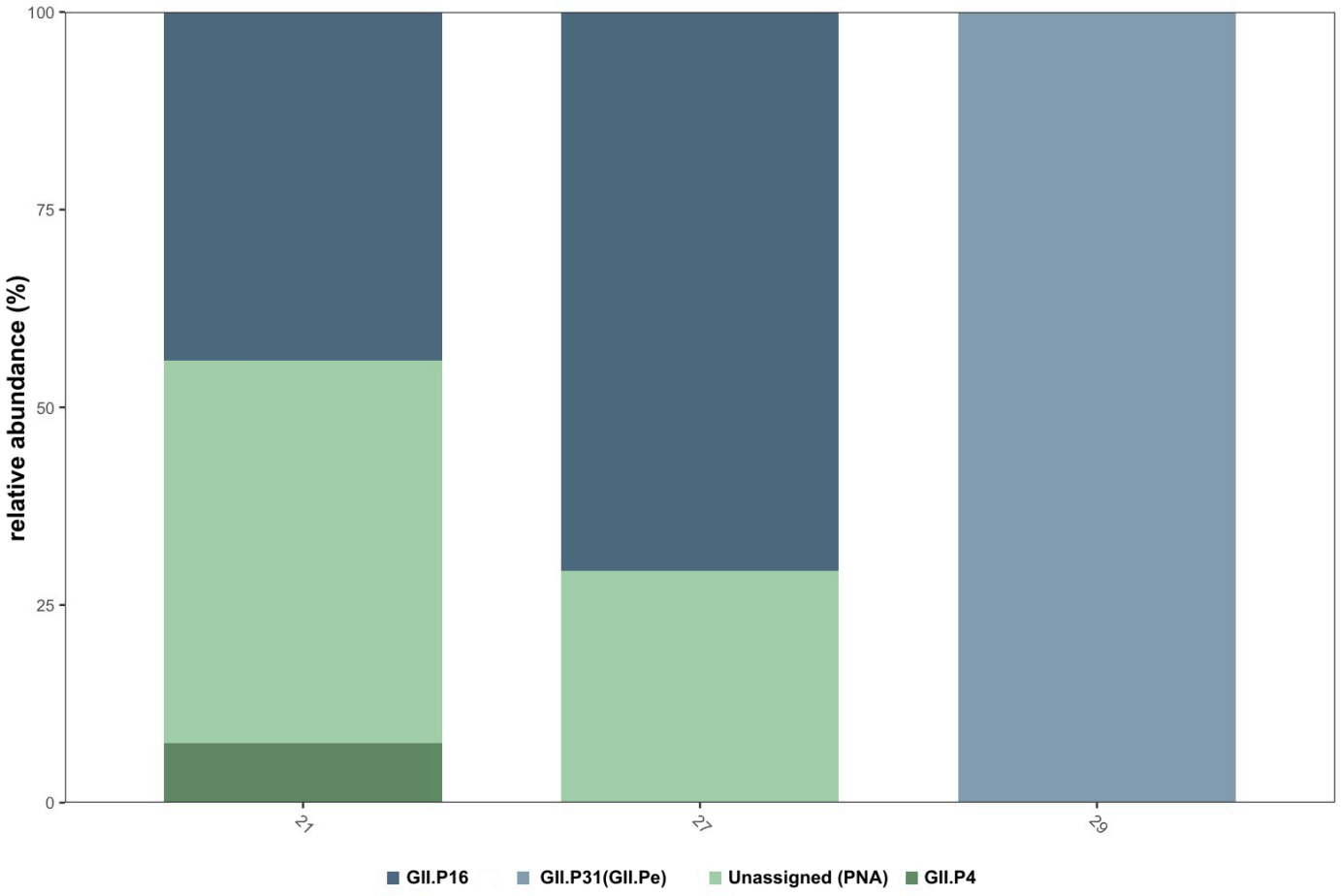


Figure 3

Norovirus GII polymerase genotype distribution in three wastewater samples by ORF1(RdRp) amplicon sequencing using next-generation sequencing

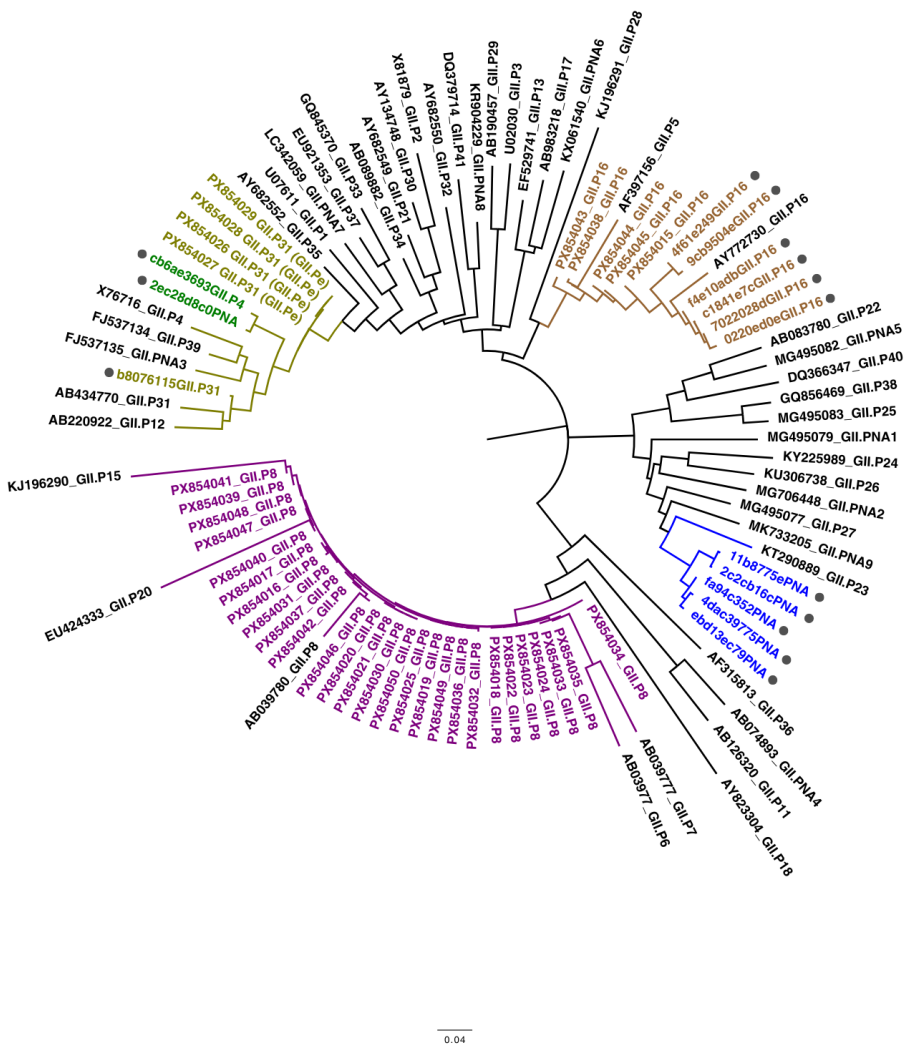


Figure 4

Phylogenetic reconstruction (Neighbor-joining tree) for the NoV GII P-types, based on a 269-base pair partial fragment of the ORF1 (RNA-dependent RNA polymerase region). The viral types obtained in this study comprise 14 representative Illumina sequences (marked with a circle beside the name) and 36 Sanger sequences (colored names with accession numbers from PX854015 to PX854050), together with 45 reference sequences available from GenBank. To name each taxon, the GenBank accession numbers together with the NoV GII assigned type were used to name each taxon, except for the representative sequences.