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Wastewater surveillance of enteric viruses in eastern Argentina: High rates of detection and first report of NoV GI.5 and GII.20

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ABSTRACT

Individuals infected with enteric viruses excrete them in their feces for an extended period, whether symptomatic or asymptomatic. This characteristic, combined with the capability of these viruses to persist in the environment, forms the core of our research. The objective of our study was to investigate the presence of viruses associated with diarrhea and hepatitis: Hepatitis A virus (HAV), Hepatitis E virus (HEV), Norovirus GI (NoV GI), Norovirus GII (NoV GII), and Rotavirus (RV) by RT-real time PCR, in untreated wastewater samples (n=100) collected in the period July 2020 to August 2021 from two low-income neighborhoods in the district of La Plata, Buenos Aires, Argentina. Globally, the percentage of positive samples was 25 % for HEV, 27 % for RV, 14 % for NoV GII, 1 % for NoV GI, and no detectable samples were found for HAV. HEV, RV, and NoV GII were detected in most of the studied months, with the highest detection rates of RV and NoV GII during the winter season. Regarding RV positive samples, the gene encoding the VP8* protein of three samples was sequenced and phylogenetic analysis revealed that the detected strains belonged to genotypes P[8] and P[3]. Additionally, four NoV strains were also genetically characterized by amplifying a fragment corresponding to the ORF-1/ORF-2 junction region. The identified strains were NoV GI.5, NoV GII.4, NoV GII.17 and NoV GII.20. Our results provide relevant information and serve as scientific evidence of the importance of considering wastewater analysis as a feasible strategy to determine the circulation of enteric viruses in the population, with the further benefit of predicting emerging strains. Moreover, this study represents the first report of the circulation of NoV GII.20 and GI.5 genotypes in our country.

1. Introduction

The study of enteric viruses in wastewater has gained interest during the last decade, especially after the coronavirus disease 2019 (COVID-19) pandemic (Amahmid et al., 2022; Mohan et al., 2021). Wastewater surveillance (WWS) can be a useful tool for tracking viral pathogens that are transmitted by the fecal-oral route, providing information about viral circulation in a community and complementing the epidemiological data in populations with little or questionable clinical surveillance (Barras, 2018). WWS allows to estimate the viral prevalence, its geographic distribution, and to perform viral molecular characterization, regardless of the clinical cases, since it summarizes the excretion of both symptomatic and asymptomatic infected individuals (Fantilli et al., 2023). This is remarkably important in low-income communities, where

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viral infections are underestimated due to lack of diagnosis and difficult access to health facilities. A significant proportion of individuals infected with enteric transmitted viruses excrete substantial quantities of the virus in their feces for an extended period. Consequently, viruses with a fecal-oral transmission route can be detected in wastewater, serving as sentinels of their circulation in a given population and, in some cases, as an early indicator of outbreaks (Hellmér et al., 2014; Masachessi et al., 2022). Viral wastewater detection may support the public health system and help to make decisions involving human health, like vaccination campaigns or other interventions to prevent viral transmission and potential outbreaks (Diamond et al., 2022; Fantilli et al., 2023).

Human enteric viruses, including norovirus (NoV), rotavirus (RV), hepatitis A virus (HAV), and hepatitis E virus (HEV), are shed in large quantities in the feces of infected individuals for several weeks. In addition, these viruses are highly resistant to environmental conditions (Di Cola et al., 2021; Kotwal and Cannon, 2014). As reported by several authors, enteric viruses are frequently detected in untreated wastewater and their presence in this matrix has been related to the occurrence of diseases within the neighboring community (Farkas et al., 2018; Gholipour et al., 2022; Prado et al., 2021). Furthermore, many enteric viruses may still be infectious after wastewater treatment (Upfold et al., 2021).

In Argentina, there are few studies reporting the detection of enteric viruses in water, particularly wastewater, which were carried out in the central and northern provinces of the country. In the city of Córdoba (central region of the country), HAV, HEV, RV and NoV have been detected in wastewater and river samples. HAV positivity rates of detection ranged between 2.9 % and 56.5 % in sewage and 16.1 % in river samples (Fantilli et al., 2023; Yanez et al., 2014). With respect to HEV, 6.3 % in sewage and 3.2 % in river samples were reported by Martínez Wassaf et al., 2014. For RV, 91.4 % in sewage was reported by Barril et al. (2015) while positive detection rates ranging from 18.7 % to 100 % were described by Prez et al. (2015) in river samples. In a recent study in the city of Mendoza, 39 % of wastewater samples were positive for HAV, while 22.5 % were positive for HEV (Lo Castro et al., 2023). Nevertheless, no studies have been carried out searching for viruses in wastewaters from La Plata, the capital city of Buenos Aires province, at the east of Argentina, which is one of the most populated districts of the country.

According to the last census conducted in Argentina, 26.7 % of households still do not have access to the sewerage system and some of them depend on septic tanks and cesspools (INDEC, 2022). This represents a high risk of infection due to the contamination of groundwater and surface waters with untreated wastewater contaminated with enteric viruses. Results from the 2022 census reported that the population of La Plata grew around 18 %, with a notable increase in population density during the recent years and a continuous development of affordable housing, but with limited sewage infrastructure.

Although WWS studies are scarce in Argentina, they have enabled to demonstrate the circulation of HAV, HEV, RV, and NoV in the population during different periods, even revealing their silent circulation (such is the case of HEV, which produces a high rate of asymptomatic cases), or monitoring viruses under vaccination program. It is important to mention at this point that, in Argentina, vaccination against RV and HAV has been included in the national vaccination schedule since 2015 and 2005, respectively. These two viruses used to have a high incidence of cases until the introduction of the respective vaccines (Degiuseppe and Stupka, 2018; Blanco Fernández et al., 2012). Since then, the epidemiology and circulation pattern of these viruses have changed, and the WWS becomes a key tool for their monitoring.

The objective of this study was to track the circulation of enteric viruses in wastewater from two low-income neighborhoods in La Plata, Argentina and to genetically characterize the detected viruses to gain further insights into their strain diversity and potential public health risks.

2. Materials and Methods

2.1. Study area

La Plata district is located in the east area of Argentina (S34°55′17.22" O57°57′16.31") and has 772618 inhabitants, distributed in an urban area in 23 neighborhoods (INDEC, 2022). Altos de San Lorenzo is one of the most populated neighborhoods located in the southeast of La Plata district, which comprises 13 low-income neighborhoods with 3800 families living there (Fig. 1). None of the houses are connected to the sewage network, but the vast majority of homes throw their waste in a septic tank that can overflow if it fills up or there is heavy rain.

2.2. Samples

A total of 100 untreated wastewater samples were collected from the discharge point of a septic tank intake pipe serving two densely populated low-income neighborhoods with 595 families, in the Altos de San Lorenzo community center, southwest La Plata district. The samples were collected in sterilized 500 ml containers weekly from July 2020 to August 2021 and stored at 4°C until processed. The number of collected samples was reduced during 2020 due to COVID-19 restrictions.

2.3. Viral concentration and RNA extraction

Before processing, samples were subjected to a 90-min treatment at 60°C to ensure biological safety. Viral concentration was carried out from 250 ml of sample by adding 20 grams of PEG 8000 and 4.5 grams of NaCl. Samples were incubated 2 hours at 4°C with constant rocking at approximately 60 oscillations min^{-1}, then they were centrifuged at 12,000 g for 1 hour at 4°C (Iglesias et al., 2021). As wastewater samples were very dirty, a first extraction was carried out with Trizol (Invitrogen) to maintain the integrity of the RNA given its RNase inhibitory activity, destroying cells and dissolving cellular components during sample homogenization. After this treatment, a second extraction was performed with the commercial QiAamp Viral RNA mini kit from QIAGEN according to the manual provided. Purified RNA was stored at -80°C until use.

2.4. Detection of enteric viruses by real-time RT-PCR

RV, NoV GI, NoV GII, HAV, and HEV detections were carried out by real-time RT-PCR. Briefly, cDNA synthesis was first performed using random hexamers (Promega) and M-MLV reverse transcriptase (Promega), according to the manufacturer's instructions, and the cDNA was stored at -20°C until use. Specific real-time RT-PCR assays were performed using iTaq Universal Probes Supermix kit, (BIO-RAD) on a StepOnePlus™ Real-Time PCR System (Applied Biosystems). For RV analysis, prior to reverse transcription, RNA was subjected to denaturation at 95°C for 5 min to separate the RNA strands followed by incubation on ice for 2 min. Table 1 provides a detailed list of the set of primers, probes, and target regions for each PCR, along with corresponding references.

Assays' limit of detection (LOD) for each gene region were: 264 for HEV, 11,38 for NSP3, 63,39 for NoV GI, 31,33 for NoV GII and 250 for HAV genomic copies/µl. Each PCR efficiency ranged from 91–102 % and R^2 values were greater than 0,95. Positive samples for each virus were included as positive controls. A non-template control was also included in each PCR.

2.5. PCRs and Sanger sequencing

Samples that were positive for NoV GI, NoV GII, HEV and RV by real-time RT-PCR were further analyzed by conventional PCR (GoTaq® G2 DNA Polymerase, PROMEGA) to amplify specific fragments for

Fig. 1. Map of the study area in La Plata district, located in the east Buenos Aires, Argentina (S34°55′17.22" O57°57′16.31")

subsequent sequencing and phylogenetic analysis. For RV, VP4 gene was amplified by RT nested-PCR as previously described (Who, 2009), using 4con2/4con3 primers for the first round and VP4F/ VP4R for the second round. The VP7 gene was amplified using primers Gra-5 and Gra-3 (Parreño et al., 2004). NoV GI and GII positive samples were amplified as described by Kitajima et al. (2012). For NoV GII, primers COG2F/G2SKR (390 bp) and G2SKF/G2SKR (340 bp) were used for the first and second rounds, respectively. For NoV GI, a 381 bp fragment was amplified using primers COG1F/G1SKR and G1SKF/G1SKR for the first and second rounds, respectively. Samples that tested positive for HEV, were subjected to two nested RT-Nested PCRs targeting ORF-1 and ORF-2 regions, using protocols previously described, amplifying fragments of 418 bp and 348 bp, respectively (Huang et al., 2002; Wang et al., 1999). Primer sequences are shown in Table 1.

Amplified products were visualized in agarose gels, purified using the PURO gel extraction kit (Bio-Lógicos) according to the manufacturer's instructions and sent to the Unidad de Genómica, Instituto de Biotecnología, CICVyA, INTA for sequencing by Sanger.

The nucleotide sequence data reported in this paper were deposited in GenBank under the following accession numbers: OQ588724; OQ784636, OQ784761 and OQ799914; OQ800859, OQ919406 and OQ919407.

2.6. Rotavirus and Norovirus genotype assignment

The RV genotypes were assigned by the web-based automated rotavirus genotyping tool version 0.1 (https://www.rivm.nl/mpf/typingtool/rotavirusa/). Similar, to assess the NoV genotype, the Norovirus Genotyping Tool version 2.0 (https://www.rivm.nl/mpf/typingtool/norovirus) was used (Kroneman et al., 2011). Subsequently, genotype assignment was confirmed by phylogenetic analyses.

2.7. Phylogenetic analyses

Three datasets were constructed with sequences of NoV GI, NoV GII, and RV VP4, to perform the phylogenetic analysis. Each one consisted of the sequences amplified during this study (for each virus), the 20 most closely related strains obtained by the Basic Local Alignment Search Tool (BLAST) on the NCBI website (https: //blast.ncbi.nlm.nih.gov/), and related strains that circulate in Argentina. The multiple sequence alignments were performed with ClustalX (Thompson et al., 2002) implemented in BioEdit 7.0.5.3 sequence Alignment Editor (Hall, 1999) using default parameters. The alignments were reviewed, edited, and trimmed using Bioedit. The phylogenetic analysis was carried out using the MEGA software version 11.0.13, with the Neighbor-Joining (NJ) method in the Kimura 2-parameter model in the case of the tree made for NoV GI and GII and the 3-parameter model for Tamura for RV (Tamura et al., 2021). Bootstrap values were determined with 1000 new samples from the data sets.

3. Results

3.1. Prevalence of enteric viruses

The overall prevalence for each virus in the studied period was: 27 % (27/100) for RV, 1 % (1/100) for NoV GI, 14 % (14/100) for NoV GII, 0 % (0/100) for HAV and 25 % (25/100) for HEV. Globally, 50 % of samples were positive for enteric viruses, 36 % were positive for a single virus and 14 % were positive for more than one virus. During 2020, 35 samples were collected, among them, 11 (31.4 %) were positive for HEV, 5 (13.3 %) for NoV GII and 12 (34.3 %) for RV. Both NoV GI and HAV were not detected. Out of the 65 samples collected in 2021, 14 (21.5 %) were detectable for HEV, 1 (1.5 %) for NoV GI, 9 (13.8 %) for NoV GII and 15 for RV (23.1 %). (Table 2). HEV was detected throughout the whole period except for September 2020; RV prevalence peaked in August 2021 and NoV GII was detected during the entire period, except for July, August, and November 2020, as well as August 2021. In contrast, NoV GI was only detected in July 2021 (Fig. 2). In each evaluated month at least two viruses were detected. Furthermore, in October and December 2020; February, March, April, and July 2021, more than 3 viruses were found.

3.3. Genotyping and phylogenetic analysis

From the total positive samples for NoV GII, only three of them could be successfully amplified through heminested PCR and subsequently sequenced (samples 122, 194, and 212). In the case of NoV GI the only positive sample was amplified and sequenced (sample 340). According to Norovirus Genotyping Tool version 2.0 (https://www.rivm.nl/mpf/typingtool/norovirus) (Kroneman et al., 2011), the detected strains were

Table 1
PCRs used in this study to detect and genotype enteric viruses in sewage water. The sets of primers, probe and target regions are specified for each viral target and molecular assay.

Viral target	Molecular Method	Primers, Probe	Sequence 5'–>3'[a]	Target	PCR product Lenght	Reference
RVA	Real Time	NSP3-F	ACCATCTWCACRTRACCCTCTATGAG	NSP3	73bp	Zeng *et al.*, 2008
		NSP3-R	GGTCACATAACGCCCCTATAGC			
		NSP3-P	[b]FAM-AGTTAAAAGCTAACACTGTCAAA-BHQ1[c]			
	Nested-PCR	4CON2	ATTTCGGACCATTATAAC	VP4	876bp	*Manual of rotavirus detection and characterization methods*, 2009
		4CON3	TGGCTTCGCTCATTTATAGACA			
		VP4FW	TAT GCT CCA GTN AAT TGG		663 bp	
		VP4REV	ATT GCA TTT CTT TCC ATA ATG			
	PCR	GRA5	GGCTTTAAAAGCGAGAATTT	VP7	1062 bp	Chang *et al.*, 1996; Parreño *et al.*, 2004
		GRA3	GGTCACATCATACAACTCTA			
HEV	Real Time	HEV-F	GGTGGTTTCTGGGGTGAC	ORF-3	69 bp	Jothikumar *et al.*, 2006
		HEV-R	AGGGGTTGGTTGGATGAA			
		HEV-P	FAM-TGATTCTCAGCCCTTCGC-BHQ1			
	Nested-PCR	Primer+1 (E01ES)	CTG GCA TYA CTA CTG CYR TWG AGC	ORF-1	418 bp	Wang *et al.*, 1999
		Primer-1 (E01EA)	CCA TCR ARR CGA TAW GTG CGG TC			
		Primer+2 (E01IS)	CTG CCY TKG CGA ATG CTG TGG			
		Primer-2 (E01IA)	GGC AGW RTA CCA RCG CTG RAC RTC			
		Primer+1 (B09ES)	AAYTATGCMCAGTACCGGGTTG	ORF-2	348 bp	Huang *et al.*, 2002
		Primer-1 (B10EA)	CCCTTATCCTGCTGAGCATTCTC			
		Primer+2 (B11IS)	GTYATGYTYTGCATACATGGCT			
		Primer-2 (B12IA)	AGCCGACGAAATY}AATTCTGTC			
NoV GI	Real Time	COG1F	CGYTGGATGCGNTTYCATGA	ORF1-ORF2 junction region	84 bp	Kageyama *et al.*, 2003
		COG1R	CTTAGACGCCATCATCATTYAC			
		RING 1C	[d]HEX-AGATYGCGITCICCTGTCCA-BHQ1			Hill *et al.*, 2010
	Heminested-PCR	COG1F	CGYTGGATGCGNTTYCATGA	ORF1-ORF2 junction region	381 bp	Kojima *et al.*, 2002; Kageyama *et al.*, 2003
		G1SKR	CCAACCCARCCATTRTACA			
		G1SKF	CTGCCCGAATTYGTAAATGA		330 bp	
NoV GII	Real Time	COG2F	CARGARBCNATGTTYAGRTGGATGAG	ORF1-ORF2 junction region	97 bp	Kageyama *et al.*, 2003
		COG2R	TCGACGCCATCTTCATTCACA			
		RING 2C	FAM-TGGGAGGGCGATCGCAATCT-BHQ1			
	Heminested-PCR	COG2F	CARGARBCNATGTTYAGRTGGATGAG	ORF1-ORF2 junction region	390 bp	Kojima *et al.*, 2002; Kageyama *et al.*, 2003
		G2SKR	CCRCCNGCATRHCCRTTRTACAT			
		G2SKF	CNTGGGAGGGCGATCGCAA		340 bp	
HAV	Real Time	HAV68 (FW)	TCACCGCCGTTTGCCTAG	5'-NCR	173 bp	Costafreda *et al.*, 2006
		HAV240(REV)	GGAGAGCCCTGGAAGAAAG			
		HAV150(-)	FAM-CCTGAACCTGCAGGAATTAA-MGB[e]-NFQ[f]			

[a] IUPAC codes used to indicate degenerate positions.
[b] FAM: 6-carboxyfluorescein reporter dye.
[c] BHQ: Black Hole Quencher.
[d] HEX Hexachlorofluorescein reporter dye.
[e] MGB: minor groove binder
[f] NFQ: Non-fluorescent quencher

Table 2
Positive samples for various viral targets during the testing period (July 2020-August 2021).

Virus Detected	2020 Number of positive samples (%)	2021 Number of positive samples (%)
HEV	11 (31.4)	14 (21.5)
HAV	0 (0)	0 (0)
NoV GI	0 (0)	1 (1.5)
NoV GII	5 (13.3)	10 (15.4)
RVA	12 (34.3)	15 (23.1)
Total samples analyzed	35	65

typed as: GI.5, GII.4, GII.17 and GII.20. Phylogenetic analyses confirmed this genotype assignment, with the presence of 4 different monophyletic groups. The sample 212, showed a similarity of 99.97 % with NoV GII.4-Camberwell and 99.95 % with other strains detected in Argentina during 2010, 2011 and 2015. The strain of NoV GII.17, sample 194, also showed to be related to strains previously isolated from humans in Argentina in 2015 and 2018. The NoV GII.20 detected strain, sample 122, grouped with sequences from the United Kingdom, South Africa, and Ethiopia, while NoV GI.5 grouped with viruses detected in Brazil, Russia, and Korea from 2018, 2022 and 2019, respectively (Fig. 3).

Three out of 27 RV positive samples could be amplified and sequenced comprising the VP4 region. The results obtained with the

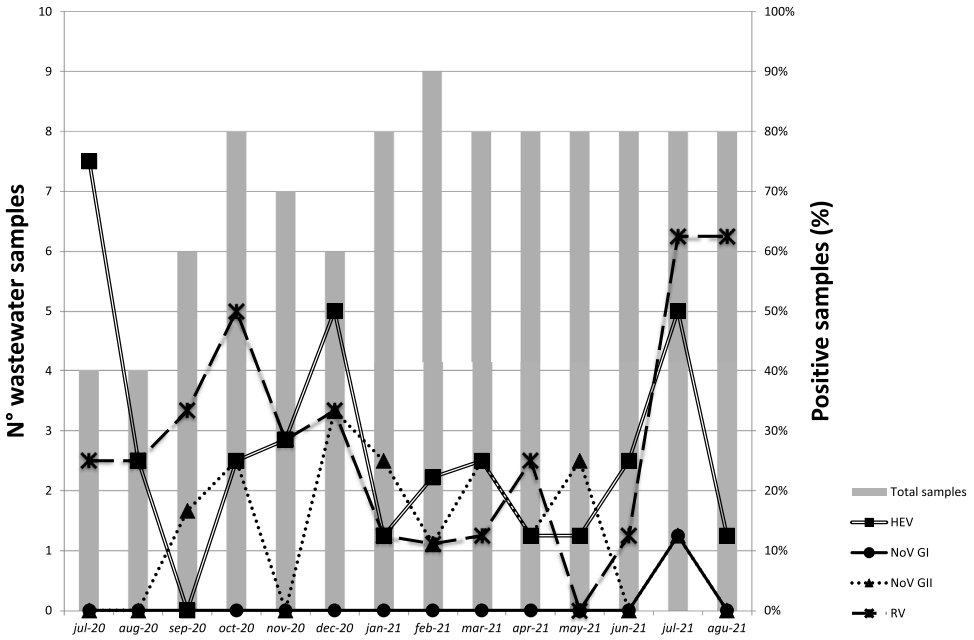

Fig. 2. Distribution of enteric viruses detected in wastewater samples collected from July 2020 to August 2021.

rotavirus genotyping tool (https://www.rivm.nl/mpf/typingtool/rotavirusa/) showed that the genotypes were P[8] and P[3]. Phylogenetic analyses confirmed this genotype assignment and showed a high similarity between strains OQ800059 and OQ 919406 (99.99 %), and with circulating strains in humans in Argentina. The strain OQ919407/RVA/sewage/ARG/2021/346/P[3] showed a 70 % similarity with a horse strain, JX036368/RVA/Horse-wt/ARG/E3198/2008/P[3], found in Argentina in 2008, clustered with sequences detected in other countries, which had been isolated from felines and canines, and show 91,72 % similarity with a sewage strain found in China (MW254170/RVA/Sewage/CHN/2019/B23-R1/P3) (Fig. 4).

Of the 25 HEV-positive samples, only 5 showed amplification for the ORF2 region by RT nested-PCR. Low viral load, suggested by faint bands, likely prevented successful sequencing. Additionally, all attempts at HEV ORF1 amplification were negative.

4. Discussion

In recent years, there has been increasing focus on environmental virology research, specifically on monitoring and understanding the dynamics of enteric virus circulation within populations. Human actions can contaminate water, which serves as a source for viral infections to humans. One of the advantages of wastewater monitoring is that it provides information about settings with reduced individual-based disease surveillance data, where clinical testing is underutilized or unavailable. In the context of the pandemic, the importance of monitoring wastewater became very useful to detect waves of COVID-19. As a result, wastewater monitoring programs experienced a global expansion in the wake of COVID-19, reflecting a substantial paradigm shift towards recognizing the importance of wastewater monitoring not only for SARS-CoV-2 but also for other viruses (Corpuz et al., 2020).

This study investigated the presence of enteric viruses in wastewater from the effluent discharge of two low-income neighborhoods in La Plata, Argentina, lacking sewage systems. HAV, HEV, NoV, and RV were analyzed in 100 wastewater samples collected between July 2020 and August 2021. Our results indicate a high frequency of detection of these viruses in wastewater, except for HAV, which has not been detected in any of the samples. This is probably consistent with the implementation of the HAV vaccine in 2005, which has led to a decrease in virus circulation and reported cases in the last years in our country. Likewise, during 2020 and 2021, there was a marked decrease in reported clinical cases of HAV nationwide (Ministerio de salud Argentina, 2021). Our results contrast with the data previously reported in other regions of Argentina, such as Córdoba and Mendoza, where HAV was detected in sewage samples in the last years, with detection rates of 20.8 % (years 2009-2020) and 23 % (years 2017-2022) for Córdoba and 39 % (years 2016-2017) for Mendoza, evidencing the circulation of the virus in those localities, probably due to introductions by non-vaccinated people (over 18 years of age and/or foreigners) (Fantilli et al., 2023; Lo Castro et al., 2023; Yanez et al., 2014). It is worth mentioning that during 2020-2021, in Córdoba, low HAV detection rates were registered in wastewaters, which correlated with the lack of clinical case reports of HAV in the region (Fantilli et al., 2023), confirming a decrease in the viral circulation during that period. A similar situation occurred in Chile, where HAV was not detected among the studied wastewater treatment plants during 2021 (Plaza-Garrido et al., 2023). In other countries from Latin America, HAV was found in wastewater at rates of 76.9 % in Brazil (Prado et al., 2021) and 13.3 % in Colombia (Báez, 2016). The variations among different countries may be attributed to differences in vaccination schemes, effluent discharge systems and methodologies used for viral concentration.

Regarding HEV, it was detected for the first time in La Plata district, in both years studied. This virus had been previously reported in sewage in Córdoba and Mendoza, Argentina, with detection rates of 6.3 % and 22.5 %, respectively (Lo Castro et al., 2023; Martínez Wassaf et al., 2014), and all corresponding to genotype 3 (HEV-3). Aqueous matrices, additionally, constitute a source of viral contamination for nearby waters, used recreationally or for animal consumption, constituting a possible source of virus to exposure populations. At the same time, the presence of HEV has been reported in our country in animal reservoirs, such as pigs and wild boars, with high infection rates, although it has not been detected in derived foods, which would indicate that the food transmission route would not be the main one in our area (Di Cola et al., 2023; Pisano et al., 2019). Unfortunately, none of the HEV positive samples yielded sequences due to insufficient viral load, as indicated by the faint bands, so the viral genotype and subtype could not be assessed. Despite the relatively high percentages of HEV detection obtained in this work (31.4 % in 2020 and 21.5 % in 2021) and the fact that notification of viral hepatitis is mandatory in Argentina, only 4 cases of HEV have been reported in Buenos Aires during the studied period, according to

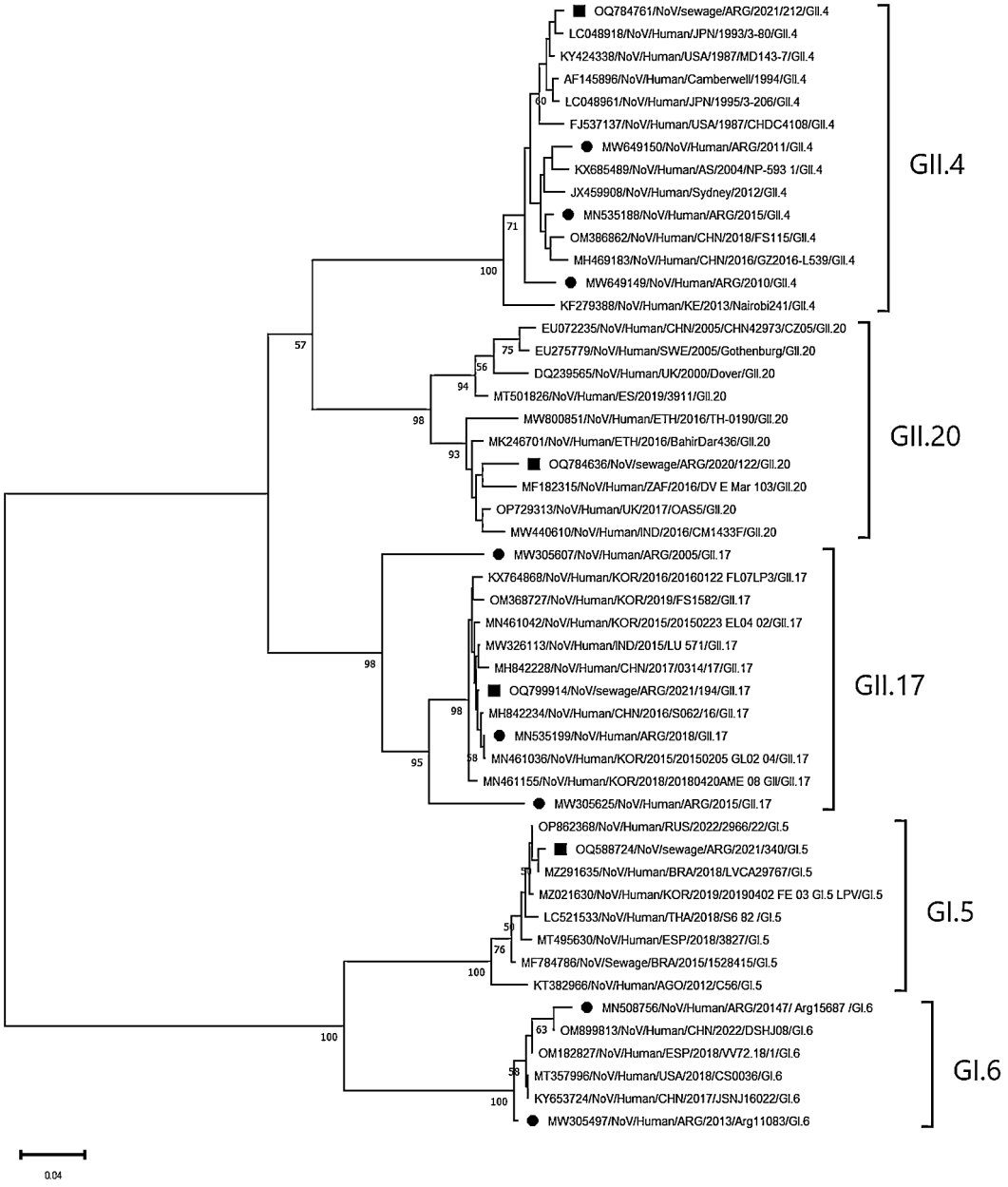

Fig. 3. Neighbor-joining phylogenetic tree of partial nucleotide sequences of ORF1-ORF2 junction region derived from NoV GI/GII strains detected in wastewater samples. Bootstrap values above 70 % are given at branch nodes. The NoV GI/GII strains found in this study are represented by the black square while the Argentine strains circulating in recent years are represented by a black circle

the Epidemiological Bulletin (Patricia Angeleri, María de los Ángeles Pando and Vidiella, 2022). This could be attributed to a high incidence of asymptomatic cases, limited reporting of clinical cases, a lack of awareness about the virus among the medical community and/or limited diagnostic availability. In any case, the significant importance and utility of surveillance for this virus in wastewater is evident, as it allows to monitor its presence within a population even in the absence of case notifications.

In this study, NoV GI detection rates were 0 % and 1,5 % during 2020 and 2021, respectively. In the case of NoV GII, detection rates were 13,3 % and 15,4 % during 2020 and 2021, respectively. Our results are in agreement with previous reports, which demonstrated that NoV GII was the predominant strain worldwide (Eftim et al., 2017; Hoa Tran et al., 2013; Parra et al., 2017). In this regard, Blanco Fernández et al. (2011) reported that 50 % of sewage samples analyzed from Córdoba were positive for NoV GII, with no detections for NoV GI. This pattern was also observed in other South American regions. In a study in Rio de Janeiro, Brazil, carried out in 2018, the authors described the detection of both NoV GI and NoV GII in 38.5 % and 96.1 % of raw sewage samples, respectively (Fumian et al., 2019). In Uruguay, NoV GI was detected only in 7.3 % of the samples, while NoV GII was detected in 51 % of them (M. Victoria et al., 2014). Our findings showed that both NoV GI and GII circulated in the district of La Plata, evidencing the infection in humans during the studied years.

It is important to consider that NoV infections are underestimated in our country as it is not a notifiable virus. Moreover, clinicians do not always search for the cause of enteric diseases. In this regard, viral monitoring in sewage constitutes a valuable tool for the detection of NoV strains that circulate in the population.

It is well known that NoV exhibits a strong seasonality in winter months (Ahmed et al., 2013). In our work NoV GI was only detected in July 2021, while NoV GII was detected in September, October, and

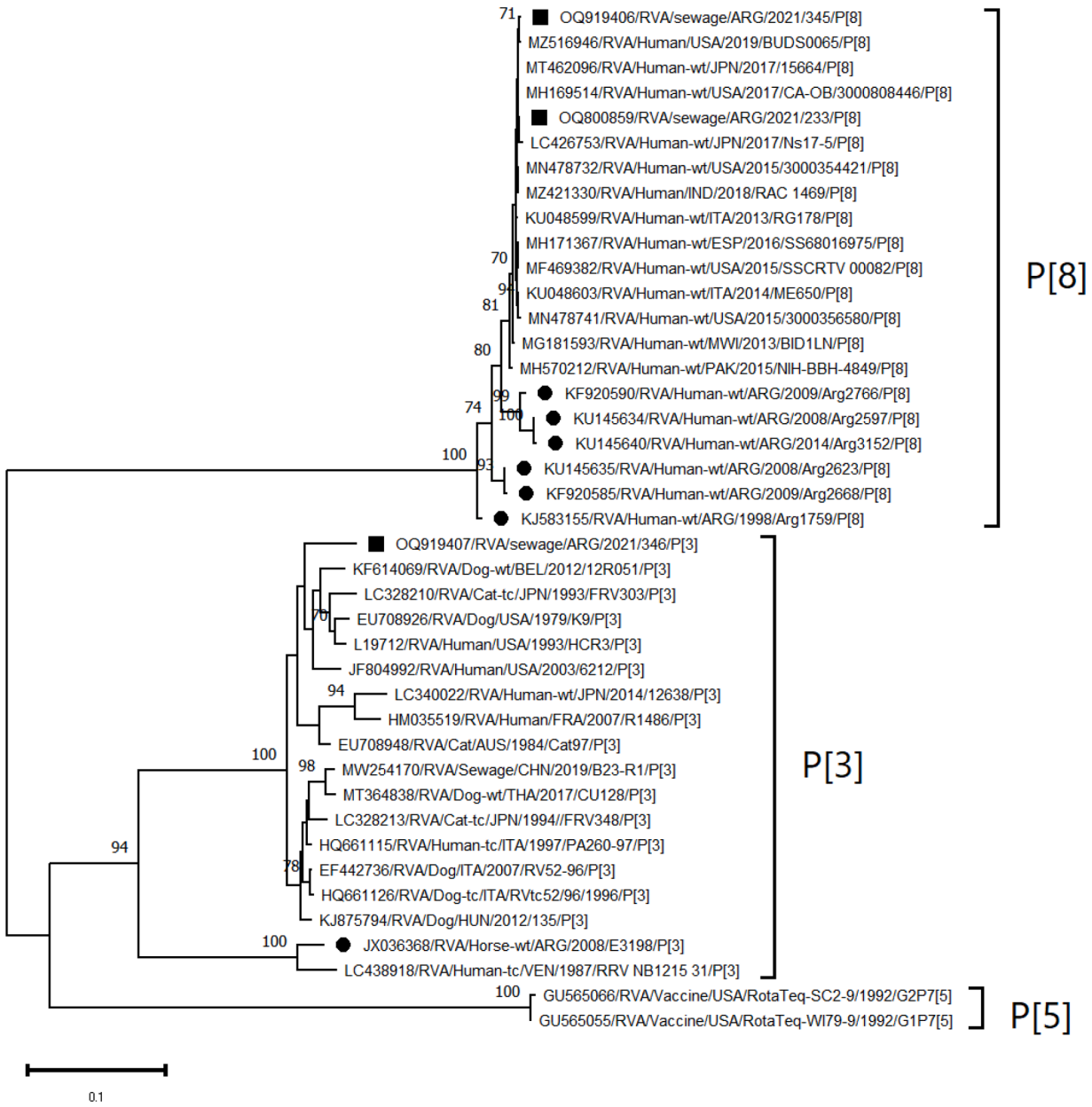

Fig. 4. Neighbor-joining phylogenetic tree based on nucleotide sequences of VP4. Bootstrap values above 70 % are given at branch nodes. The VP4 strains found in this study are represented by the black square while the Argentine strains circulating in recent years are represented by a black circle

December 2020, and almost during the whole period evaluated during 2021. These results are consistent with previous studies that described that NoV was detected in both cold and warm months (Masachessi et al., 2018; Teixeira et al., 2017; M. Victoria et al., 2014). As described by Rohayem (2009), the differences observed among results from our work and other studies could be explained by climate change, rainfall patterns, human activities, sampling methods and frequency, and analytical techniques that may affect the seasonality pattern observed (Ahmed et al., 2013;Celik et al., 2015; Haramoto et al., 2018).

We were only able to sequence three strains of NoV GII (GII.4, GII.17 and GII.20) and the only detected strain of NoV GI (GI.5). Some of the NoV GII detected strains have already been described in our country. Degiuseppe et al. (2017) reported the presence of NoV GII.17 from a 3-year-old girl from the city of San Martin de los Andes, in the south of Argentina in 2015. Same authors reported that NoV GII.4 was the most prevalent strain detected between 2010-2017 in children from our country with acute gastroenteritis (Degiuseppe et al., 2020). Although NoV GII.17 was once thought to replace the GII.4 strain, the latter remains the most frequently detected genotype worldwide and it is still associated with nearly 70 % of NoV infections. With respect to NoV GI.5 and NoV GII.20, to our knowledge, this is the first study to report their circulation in our country. These genotypes have been previously informed in wastewater from Paris without previous clinical cases reported in the country (Prevost et al., 2015). According to another study in which the NoroSurv database (https://www.norosurv.org/login) was analyzed, NoV GII.20 and GI.5 were classified as rarely detected (Cannon et al., 2021). NoV GII.20 has been reported in recent years in countries such as Ethiopia (Gelaw et al., 2019), Thailand (Supadej et al., 2017) and Brazil (Aragão et al., 2013), while NoV GI.5 has been detected in countries such as Mexico, (García et al., 2006), Peru (Saito et al., 2014) and China (Li et al., 2018).

Considering RV, our study showed that 27 % of the samples were positive for this virus. This prevalence was slightly lower than that previously reported in Argentina, and other countries where RV

positivity rates ranged from 49 % to 91.4 % in wastewater samples (Barril et al., 2015; Prevost et al., 2015; M Victoria et al., 2014). Since 2006, two RV vaccines were authorized in Argentina, Rotarix (monovalent, RV1) and RotaTeq (pentavalent, RV5) which were included in the vaccination programs in 2015. Despite the implementation of RV vaccination in Argentina, which has resulted in a significant reduction in cases of acute diarrhea, the prevalence of RV RNA in wastewater remained high, as observed in recent years (Areso et al., 2018). This could be attributed to the detection of animal rotaviruses in addition to human RV and to the excretion of RV from the vaccine since it is a live attenuated vaccine (Hassine-Zaafrane et al., 2015).

In this study, RV was detected during the whole analyzed period except for January and May 2021, with a high percentage of detection observed in October 2020, and July and August 2021. This is consistent with previous findings in wastewaters in which RV was detectable in all seasons but with high positive rates of detection in autumn and winter seasons (Barril et al., 2015). Given that Argentina introduced the RV vaccine into the schedule in 2015 (Ministerio de Salud, 2014), RV seasonality should be studied in two periods: before the introduction of the vaccine and after the introduction. Degiuseppe et al. (2017) described a seasonal pattern prior to 2015, with higher detection rate during the winter months. Nevertheless, after the introduction of the vaccine in 2015, a loss of seasonality was observed (Marti et al., 2023). This is consistent with our results, as wastewater samples were collected after vaccine introduction.

Variations observed among enteric virus detections during this study (and previous works), could be attributed to external factors, such as temperature, rainfall, sample processing methodologies, viral concentration methods and nucleic acid extraction techniques. Also, it is to mention that this study lacks a sample process control potentially leading to an underestimation of the frequency of viral detection. Despite this limitation, the primary objective of this work was to identify, rather than quantify, the presence of enteric viruses in wastewater from two low-income neighborhoods that have not been studied before. This qualitative approach successfully detected several enteric viruses, revealing their occurrence and providing information for future quantitative studies with important public health implications.

It is worth noting that samples were collected during pandemic. In this regard, contrasting observations have been reported about the impact of the pandemic on other enteric viruses. Abd-Elshafy et al. (2022) described a decrease in some viruses in Egypt, while Hoque et al. (2023) found no significant change. Our results align with the latter and suggest that overall enteric virus prevalence might not be significantly impacted by restriction measures, warranting further investigation.

The genome of enteric viruses has the ability to continuously mutate, giving rise to novel variants that may exhibit different transmissibility and severity. The importance of genetic characterization of viruses within wastewater monitoring lies in the fact that it provides a basis for studying how individual mutations occur over time and the detection of new emerging variants. In this regard, molecular characterization of viruses detected in wastewater may contribute to public health systems to make measures to prevent potential outbreaks by identifying emerging strains that circulate within a community, even in the absence of clinical samples that confirm their presence (Diamond et al., 2022).

In our study, attempts were made to perform genetic characterization of all strains found, however, not all samples could be sequenced possibly due to low viral load.

With regards to RV positive samples, only the VP4 gene could be sequenced. Phylogenetic analyses revealed that positive samples belonged to P[8] and P[3]. During 2012 and 2013 in Argentina (pre-vaccination period), RV strains carrying P[4] and P[8] genotypes circulated in the population with a high predominance of P[4] genotype strains (Degiuseppe et al., 2017). However, since 2014 there has been a decrease in the genotype P[4] and an increase in the presence of the P[8] genotype (Degiuseppe and Stupka, 2021; Mandile et al., 2020). In the case of the P[3] genotype, it is usually associated with animal hosts such as cats and dogs, although in some studies it was also detected in humans, as previously described by Okitsu et al. (2018) and Matthijnssens et al. (2011). It is important to mention that it was not possible to confirm the origin of these strains since the entire genome could not be sequenced.

Our study provides a comprehensive understanding of the epidemiology and circulation of enteric viruses within two low-income neighborhoods in Buenos Aires that had not been previously monitored. These results highlight the importance of wastewater-based epidemiology as a valuable tool for monitoring enteric pathogens within a population, thereby overcoming limitations associated with traditional case detection.

5. Conclusion

The present study provides information regarding the epidemiology of enteric viruses through the analysis of wastewater samples collected in two low-income neighborhoods from La Plata district, Buenos Aires, Argentina, between July 2020 and August 2021. Furthermore, this study is the first to report the detection of NoV GII.20 and NoV GI.5 in the Argentine population, which have not been described before. The identification of the viral strains and genotypes was also assessed, which may be associated with asymptomatic or unreported infections in the general community. Surveillance of enteric viruses in wastewater may contribute with Public Health authorities to the early detection of viral diseases and the prevention of future outbreaks.

CRediT authorship contribution statement

C Frydman: Methodology, Investigation, Writing – original draft, Visualization, Formal analysis, Data curation. **S Miño:** Formal analysis, Validation, Writing – review & editing, Data curation. **NG Iglesias:** Resources, Writing – review & editing. **JM Carballeda:** Resources, Writing – review & editing. **M Simari:** Resources. **MB Pisano:** Writing – review & editing, Formal analysis. **MJ Dus Santos:** Formal analysis, Funding acquisition, Writing – review & editing, Supervision. **M Mozgovoj:** Conceptualization, Formal analysis, Writing – review & editing, Visualization, Supervision, Project administration, Funding acquisition.

Declaration of competing interest

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

Data availability

Data will be made available on request.

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Supplementary materials

Supplementary material associated with this article can be found, in the online version, at doi:10.1016/j.envadv.2024.100501.

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