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Surveillance of SARS-CoV-2, rotavirus, norovirus genogroup II, and human adenovirus in wastewater as an epidemiological tool to anticipate outbreaks of COVID-19 and acute gastroenteritis in a city without a wastewater treatment plant in the Peruvian Highlands

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Abstract:	The pandemic of coronavirus disease 2019 (COVID-19) has shown that Wastewater Based Epidemiology is a fast and economical alternative for monitoring severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) at the community level in high-income countries. In the present study, wastewater from a city in the Peruvian Highlands without a wastewater treatment plant was monitored for one year to evaluate the relationship between the concentration of SARS-CoV-2 and the reported cases of COVID-19 in the community. Additionally, the relationship between rotavirus (RV), norovirus genogroup II (NoV GGII), and human adenovirus (HAdV) were also compared to the number of reported cases of acute gastroenteritis. Before starting the analysis of the samples, the viral recovery efficacy of three processing methods was tested in spiked wastewater with SARS-CoV-2. The results demonstrated a higher performance of direct analysis (72.2%) compared to ultrafiltration (50.8%) and skimmed milk flocculation (5.6%). Wastewater monitoring showed that 72.0% (36/50) of the samples were positive for SARS-CoV-2 and the direct analysis obtained the highest detection frequency and the highest quantifications of SARS-CoV-2. In addition, a strong level of correlation was observed between the concentration of SARS-CoV-2 in wastewater and the reported cases of COVID-19, which made it possible to anticipate, by up to 2 weeks, the start of the fourth and fifth waves of the pandemic in Peru. All the samples processed by skimmed milk flocculation method were positive and showed high concentrations for RV, NoV GGII, and HAdV. In fact, the highest RV concentrations were registered up to 4 weeks before outbreaks of acute gastroenteritis reported in children under 4 years of age. In conclusion, the results of this study suggest that periodic monitoring of wastewater is an excellent epidemiological tool to surveillance and would anticipate outbreaks of infectious

	diseases, such as COVID-19, in low- and middle-income countries.
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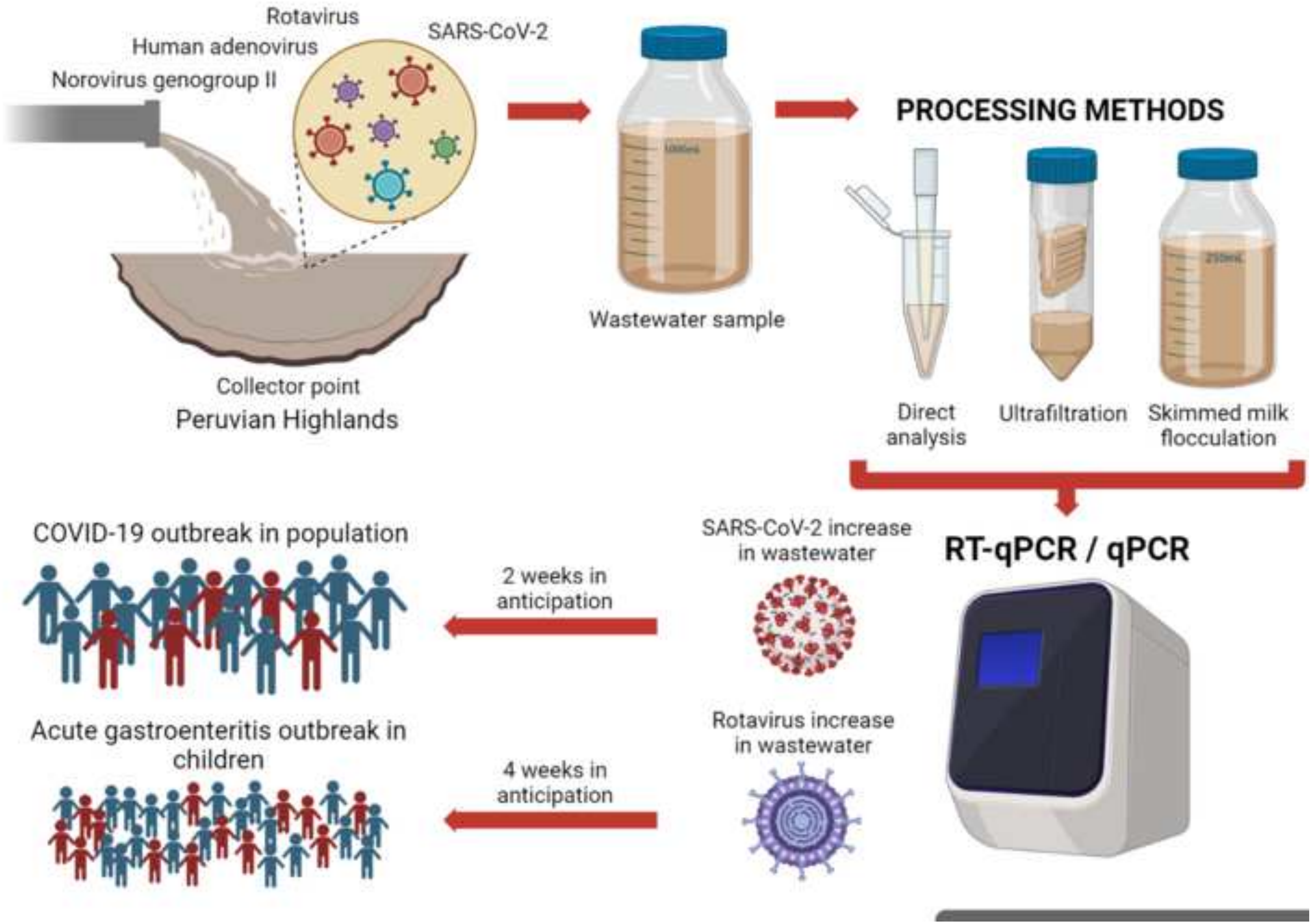
Lima, April 24th, 2023

Dear Science of the Total Environment Journal,

I am pleased to submit the final version of our paper entitled “Surveillance of SARS-CoV-2, rotavirus, norovirus genogroup II, and human adenovirus in wastewater as an epidemiological tool to anticipate outbreaks of COVID-19 and acute gastroenteritis in a city without a wastewater treatment plant in the Peruvian Highlands” for publication in Science of the Total Environment. This manuscript describes the surveillance of wastewater from a year to monitor the concentration of SARS-CoV-2, rotavirus, norovirus genogroup II and Human adenovirus. These fascinating results also show a relationship with the presence of active cases of COVID-19 and acute gastroenteritis in children. Finally, our study concludes with the importance of Wastewater Based Epidemiology as a tool to monitor viral epidemics in low- & middle-income countries. All the authors have contributed to, seen, and approved the final, submitted version of this manuscript.

Eloy Gonzales-Gustavson

Graphical Abstract



Highlights

- SARS-CoV-2 surveillance in wastewater anticipated waves of COVID-19 in Peru.
- RV surveillance in wastewater would anticipate acute gastroenteritis outbreaks.
- Skimmed milk flocculation detected high concentrations of RV, NoV GGII, and HAdV.
- Direct analysis of wastewater allows SARS-CoV-2 surveillance in pandemic conditions.

Surveillance of SARS-CoV-2, rotavirus, norovirus genogroup II, and human adenovirus in wastewater as an epidemiological tool to anticipate outbreaks of COVID-19 and acute gastroenteritis in a city without a wastewater treatment plant in the Peruvian Highlands

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Abstract

The pandemic of coronavirus disease 2019 (COVID-19) has shown that Wastewater Based Epidemiology is a fast and economical alternative for monitoring severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) at the community level in high-income countries. In the present study, wastewater from a city in the Peruvian Highlands without a wastewater treatment plant was monitored for one year to evaluate the relationship between the concentration of SARS-CoV-2 and the reported cases of COVID-19 in the community. Additionally, the relationship between rotavirus (RV), norovirus genogroup II (NoV GGII), and human adenovirus (HAdV) were also compared to the number of reported cases of acute gastroenteritis. Before starting the analysis of the samples, the viral recovery efficacy of three processing methods was tested in spiked wastewater with SARS-CoV-2. The results demonstrated a higher performance of direct analysis (72.2%) compared to ultrafiltration (50.8%) and skimmed milk flocculation (5.6%). Wastewater monitoring showed that 72.0% (36/50) of the samples were positive for SARS-CoV-2 and the direct analysis obtained the highest detection frequency and the highest quantifications of SARS-CoV-2. In addition, a strong level of correlation was observed between the concentration of SARS-CoV-2 in wastewater and the reported cases of COVID-19, which made it possible to anticipate, by up to 2 weeks, the start of the fourth and fifth waves of the pandemic in Peru. All the samples processed by skimmed milk flocculation method were positive and showed high concentrations for RV, NoV GGII, and HAdV. In fact, the highest RV concentrations were registered up to 4 weeks before outbreaks of acute gastroenteritis reported in children under 4 years of age. In conclusion, the results of this study suggest that periodic monitoring of wastewater is an excellent epidemiological tool to surveillance and would anticipate outbreaks of infectious diseases, such as COVID-19, in low- and middle-income countries.

Keywords:

SARS-CoV-2, rotavirus, direct analysis, skimmed milk flocculation, Peruvian Highlands, Wastewater Based Epidemiology

Abbreviations:

COVID-19: coronavirus disease 2019.

DNA: deoxyribonucleic acid.

EW: epidemiologic week.

GC: genomic copies.

HAdV: human adenovirus.

NoV GGII: norovirus genogroup II.

qPCR: real-time polymerase chain reaction.

RNA: ribonucleic acid.

RT-qPCR: reverse transcription real-time polymerase chain reaction.

RV: rotavirus.

SARS-CoV-2: severe acute respiratory syndrome coronavirus 2.

WBE: Wastewater Based Epidemiology.

1. Introduction

At the beginning of the coronavirus disease 2019 (COVID-19) pandemic, caused by the severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), the health authorities massively applied individual clinical diagnostic tests in order to determine prevalence in the population (Guerrero-Latorre et al., 2022; Rusiñol et al., 2021). However, this approach was economically unsustainable in the long term for most countries (Polo et al., 2020; Tandukar et al., 2022). In addition, the high demand for medical supplies and the limited production capacity of suppliers quickly caused a shortage of clinical tests. Therefore, only people with clinical manifestations compatible with COVID-19 were tested, which in turn led to a considerable misreporting of the number of reported cases in the community (Rusiñol et al., 2021). All these facts exacerbated the global health crisis and evidenced the need of having a tool that allows a rapid and economical epidemiological surveillance at a community level (Daughton, 2020).

During the course of the pandemic, many studies reported the presence of SARS-CoV-2 RNA in fecal samples from infected persons (Tang et al., 2020; Wölfel et al., 2020) and in wastewater from affected communities in various countries (Medema et al., 2020; Rusiñol et al., 2021). These findings evidenced the possibility of using wastewater as an epidemiological surveillance tool to estimate the magnitude and distribution of SARS-CoV-2 in the community under a denominated Wastewater Based Epidemiology (WBE) approach (Kitajima et al., 2020; Polo et al., 2020). WBE is an environmental science tool that allows the surveillance of infectious disease, at the community level, through the periodic monitoring of wastewater (Ahmed et al., 2020a). Its application in the pandemic achieved important results allowing to anticipate the increase in clinical cases of COVID-19 (Medema et al., 2020; Randazzo et al., 2020; Rusiñol et al., 2021), evaluate the effect of public health interventions (Rusiñol et al., 2021), optimize and focus individual clinical diagnostic tests (Randazzo et al., 2020), complement clinical epidemiological surveillance (Randazzo et al., 2020; Tandukar et al., 2022), and identify asymptomatic, paucisymptomatic and presymptomatic individuals (Hart and Halden, 2020; Polo et al., 2020). Based on these precedents, applying the WBE arises as an alternative surveillance strategy for human enteric viruses, the main causal agent responsible for gastroenteritis, such as rotavirus (RV), norovirus genogroup II (NoV GGII), and human adenovirus (HAdV), that represent a constant concern for public health in low- and middle-income countries (Dey et al., 2011; Mans, 2019; PAHO, 2007). In addition, it is important to consider that previous studies have successfully monitored enteric viruses in different environmental matrices (Gonçalves et al., 2018; Hellmér et al., 2014; Kazama et al., 2017) and suggest that a person with acute gastroenteritis may excrete 10^7 to 10^{12} viral particles per gram of feces daily (Rusiñol and Girones, 2017).

In Latin America, it is estimated that 65% of the wastewater does not receive any treatment and that its disposal in the environment causes deterioration of the microbiological quality of rivers and allows the introduction of different pathogenic microorganisms into the community (Guerrero-Latorre et al., 2018; Rodriguez et al., 2018). This situation is observed in Huancayo City, considered one of the main agricultural regions of the Peruvian Highlands. Likewise, poverty, the lack of drinking water, and the absence of wastewater treatment plants force farmers to widely reuse wastewater for irrigation of agricultural products intended for human and animal consumption. Therefore, public health and environmental health in Huancayo City are greatly affected due to the absence of a wastewater treatment plant.

In the present study, the wastewater from the main districts of Huancayo City was monitored for one year to evaluate the relationship between genomic copies (GC) concentration of SARS-CoV-2 and the reported cases of COVID-19 in the community and also the relationship between RV, NoV GGII, and HAdV with the number of reported cases of acute gastroenteritis in order to determine the feasibility of monitoring the outbreaks of infectious diseases in low- and middle-income countries and in regions that lack a wastewater treatment plant.

2. Materials and methods

2.1. Study site

The study was carried out in the main districts of Huancayo City: Huancayo and El Tambo. Both districts concentrate 65% of the population in Huancayo City (286,352 people out of a total of 438,369). Huancayo City is located at 3,200 meters above sea level, has a temperate climate (5-20°C), with an average relative humidity of 75%, and is characterized as one of the main agricultural regions of the Peruvian Highlands (CENEPRED, 2021), where its intense agricultural activity is based on the use of the water from the Mantaro river. Nevertheless, the lack of a treatment plant causes the wastewater generated in Huancayo City to be discharged, without any prior treatment, into the river.

2.2. Wastewater sampling

The interval and type of sampling were adapted to the recommendations of the Pan American Health Organization. Moreover, the sampling personnel strictly followed the biosafety protocol indicated in the Guide for the analysis and quantification of SARS-CoV-2 in wastewater (PAHO, 2021). A total of 65 wastewater samples were collected every two weeks from September 2021 to December 2022. Samples (1 L) were manually collected in sterile glass bottles during the morning (9-11 am) and in two collector points responsible for eliminating wastewater generated in the districts of Huancayo and El Tambo. Immediately after collecting the samples, the main physicochemical parameters of the wastewater were determined: pH, temperature, electrical conductivity, and total dissolved solids. Subsequently, the samples were transported at 4°C to the laboratory and processing was performed within 2 hours of collection.

2.3. Processing of wastewater samples

2.3.1. Recovery efficacy from spiked samples

Before starting the sampling and to determine the best option, the recovery efficacy of each processing method was evaluated. For this purpose, four wastewater samples of 200 mL each, previously evaluated and negative for SARS-CoV-2, were spiked with 400 µL (7.13E+03 GC/mL) of a nasopharyngeal swab sample positive for SARS-CoV-2 and were homogenized at 200 rpm for 30 minutes at room temperature. Finally, 100 mL of each spiked sample were used for the skimmed milk flocculation method (Calgua et al., 2013), 50 mL for the ultrafiltration method (Ahmed et al., 2020b), and 200 µL for the direct analysis.

The recovery efficacy for SARS-CoV-2 was calculated for each processing method and based on the quantification of GC by reverse transcription real-time polymerase chain reaction (RT-qPCR) through the following equation:

$$\text{Recovery efficacy (\%)} = \frac{\text{Amount of GC recovered}}{\text{Amount of GC spiked}} * 100$$

The nasopharyngeal swab samples positive for SARS-CoV-2 used for the evaluation of the recovery efficacy were obtained from another research project, which was approved under certificate No. 676-24-21 by the “Comité Institucional de Ética en Investigación” from the “Universidad Peruana Cayetano Heredia” in Lima, Peru.

2.3.2. Direct analysis

Based on the recovery efficacy results, the direct analysis was used to detect and quantify SARS-CoV-2 in the wastewater samples collected from January to December 2022. In the laboratory, 200 µL of each previously homogenized wastewater sample were taken to immediately extract the viral RNA.

2.3.3. Viral concentration methods

The evaluation of SARS-CoV-2 was also carried out with two concentration methods: skimmed milk flocculation (Calgua et al., 2013) and ultrafiltration (Ahmed et al., 2020b). Skimmed milk flocculation was used in samples collected from January to December 2022, while ultrafiltration was only used from February to December 2022 due to logistical issues and a shortage of these devices due to the pandemic. The evaluation of RV, NoV GGII, and HAdV was carried out from September 2021 to September 2022 and only with the skimmed milk flocculation method because previous studies have used it successfully in the concentration of enteric viruses in wastewater samples (Calgua et al., 2013; Gonzales-Gustavson et al., 2019, 2017; Hellmér et al., 2014).

2.3.3.1. Skimmed milk flocculation

Before starting the sample processing, a pre-flocculated skimmed milk solution (1%, w/v) was prepared by dissolving 1 g of skimmed milk powder (Difco, Le Pont de Claix, France) in 100 mL of artificial seawater (33.33 gr/L) (Sigma-Aldrich Inc., St. Louis, USA) and pH was adjusted to 3.5 (± 0.02) with HCl 1N. The wastewater sample (100 mL) was transferred to a 250 mL sterile glass bottle, stirred for 10 minutes and the pH was adjusted to 3.5 (± 0.02). Additionally, 3 mL of the pre-flocculated skimmed milk solution was added to the sample and the pH was adjusted to 3.5 (± 0.02). The sample was stirred at room temperature for 8 hours to facilitate the adsorption of the viral particles to the milk flocs and then allowed to stand for 8 hours to allow the flocs to precipitate. The supernatant was discarded, and the precipitate was centrifuged at 8000 g for 30 minutes at 4°C. Finally, the remained supernatant was discarded, and the pellet was resuspended in 10 mL of phosphate buffer at pH 7.5 (1:2, v/v Na_2HPO_4 0.2 M and NaH_2PO_4 0.2 M). The concentrated sample (10 mL) was stored at -80°C.

2.3.3.2. Ultrafiltration

Prior to sample processing, Amicon® Ultra-15 30 kDa devices (Merck Millipore Ltd., Ireland) were rinsed with nuclease-free water to remove the glycerin coating of the filtration membrane. The wastewater sample (50 mL) was centrifuged at 4500 g for 10 minutes at 4°C to precipitate the larger particles. Subsequently, the supernatant was transferred to 4 Amicon® Ultra-15 30 kDa devices and centrifuged at 4750 g for 10 minutes at 4°C. The concentrated sample (~2 mL) was stored at -80°C.

2.4. Extraction of nucleic acids and viral quantification

Nucleic acids extraction was performed from 200 µL of each processed sample following the instructions of the MagMAX™ Viral/Pathogen Nucleic Acid Isolation Kit

(Applied Biosystems, Thermo Fisher Scientific, TX, USA). In addition, 200 µL of nuclease-free water was used as a negative control to validate the nucleic acids extraction. At the end of the extraction, 50 µL of eluate were obtained from each sample. Molecular analysis was performed immediately after to avoid nucleic acids degradation associated with the freezing and thawing of the sample.

Specific RT-qPCR and qPCR assays described above were used to detect and quantify SARS-CoV-2 (Center for Disease Control, 2019; Whitney et al., 2020), RV (Zeng et al., 2008), NoV GGII (Loisy et al., 2005), and HAdV (Hernroth et al., 2002). Reaction mixtures were prepared using RNA UltraSense™ One-Step Quantitative RT-PCR System (Invitrogen, Thermo Fisher Scientific, MD, USA) and TaqMan® Environmental Master Mix 2.0 (Applied Biosystems, Thermo Fisher Scientific, WA, UK) for RNA and DNA viruses, respectively. The Supplementary Material (Tables S1-S2) details the sequences of the primers and probes along with the temperature and time conditions for each assay. All RT-qPCR and qPCR assays were performed in a QuantStudio™ 3 Real-Time PCR System (Thermo Fisher Scientific, USA), using automatic settings for threshold and baseline. Standard curves were made from gBlocks® synthetic DNA fragments (Integrated DNA Technologies, Inc., Coralville, IA, USA). In the case of SARS-CoV-2, a plasmid containing the N1 and N2 regions of the viral nucleocapsid was used (Integrated DNA Technologies, Inc., Coralville, IA, USA). A Qubit™ 4 fluorometer (Invitrogen™, Thermo Fisher Scientific, USA) was used to determine the concentration of the gBlocks® synthetic DNA. The presence of inhibitors in the assay were analyzed by performing a double dilution of each sample. In addition, each sample was analyzed in sextuplicate (two replicates for the undiluted sample and two replicates for each of the two dilutions) and a standard curve and negative controls were included in each assay. Samples showing cycle threshold values greater than 40 were considered negative for SARS-CoV-2 (Medema et al., 2020; Philo et al., 2021).

Nucleic acids extraction and preparation of reaction mixtures were performed in different environments to avoid cross-contamination.

2.5. Government report cases of COVID-19 and acute gastroenteritis

The number of new reported cases of COVID-19 and acute gastroenteritis in the districts of Huancayo and El Tambo were routinely registered from the records of the Junin health authority: Dirección Regional de Salud. This information is publicly available in the official website (http://www.diresajunin.gob.pe/grupo_ordenado/nombre/20190122050243_epidemiologa/). The distribution of the number of cases was adjusted to the sampling interval and registered in a database as cumulative cases every two weeks. Sometimes, the health authority published the records of COVID-19 as accumulated cases after a certain number of days. Therefore, the number of cases was distributed proportionally by the number of days in which there was no daily record. In addition, the reported cases of acute gastroenteritis were classified into 4 groups: under 1 year of age, between 1 to 4 years of age, over 5 years of age, and total.

2.6. *Data analysis*

QuantStudio™ Design & Analysis software (Version 1.5.1, Applied Biosystems, Thermo Fisher Scientific, USA) was used to evaluate the performance of each RT-qPCR and qPCR assay. All analyses were performed using R Statistical Software (v4.2.2; R Core Team 2022). Spearman's correlation coefficient was calculated to determine the level of correlation between concentrations of viral GC in wastewater and the number of cumulative cases every two weeks of COVID-19 and acute gastroenteritis. Data collection and results management were performed using Microsoft Excel (Microsoft Corp., Redmond, WA, USA).

3. Results

3.1. Recovery efficacy of spiked SARS-CoV-2 in wastewater samples

The direct analysis obtained the highest average recovery with 72.2 ± 5.9%, followed by ultrafiltration with the Amicon® Ultra-15 30 kDa device at 50.8 ± 4.3%. Skimmed milk flocculation reached the lowest recovery efficacy with only 5.6 ± 0.5%. All individual values of the recovery efficacy for each replicate are included in Table S3 of the Supplementary Material.

3.2. Viral detection and quantification in wastewater

Wastewater monitoring showed that 72.0% (36/50) of the analyzed samples were positive for SARS-CoV-2. The proportions of positive samples by district were 80.0% (20/25) and 64.0% (16/25) in Huancayo and El Tambo, respectively. The direct analysis reached the highest detection frequency and the highest quantifications for SARS-CoV-2 (Table 1). Furthermore, the CDC-N1 assay was able to consistently detect and quantify SARS-CoV-2 ARN in all positive samples, while the CDC-N2 assay was able to detect SARS-CoV-2 in only 8.3% (3/36) of the positive samples for the CDC-N1 assay. On the other hand, all wastewater samples processed by skimmed milk flocculation method were positive and showed high concentrations for RV, NoV GGII, and HAdV. Table 2 indicates the median, minimum, and maximum values for the analyzed enteric viruses by district. Bi-weekly variation quantified in each epidemiologic week (EW) by each district for SARS-CoV-2, RV, and HAdV are represented in Fig. 1, Fig 2A-B, and Fig. 2C, respectively. Complete information is provided in the Supplementary Material, including the bi-weekly variation of NoV GGII in wastewater (Fig. S3-S4).

3.3. Cases of COVID-19 and acute gastroenteritis

Currently, 5 waves of the COVID-19 pandemic have been reported in Peru, and the occurrence of the last three matches with the development of this study. The third wave (December 2021-February 2022) reached the peak of the epidemic curve in EW 2/2022 and 3/2022 with 1,379 cases in Huancayo District and 1,602 cases in El Tambo District, respectively. The fourth wave (June 2022-September 2022) reached the peak of infections in EW 27/2022 and 600 cases were registered in Huancayo District and 609 cases in El Tambo District. Finally, the beginning of the fifth wave (December 2022-January 2023) coincided with the final stage of the study and a constant increase in the number of reported cases was observed since EW 44/2022 in both districts (Fig. 1).

In general, the number of reported cases of acute gastroenteritis remained within the estimated values throughout the study, except for the three outbreaks identified in both districts. In El Tambo District, only one outbreak of 64 cases of acute gastroenteritis was reported in the group under 1 year of age during EW 13/2022 (Fig. 2A). In Huancayo District, the first outbreak occurred in the initial week of the study (39/2021) and 49 cases were reported in the group over 5 years of age. In the second, 18 cases were registered in the group between 1 to 4 years of age during EW 35/2022 (Fig. 2B-C).

3.4. Relationship between the concentration of SARS-CoV-2 in wastewater and the number of reported cases of COVID-19

Overall, a similar pattern of behavior was observed between the concentrations of SARS-CoV-2 in wastewater samples and the evolution of the COVID-19 pandemic. The highest quantifications recorded in this study for SARS-CoV-2 in wastewater were 9.32E+06 GC/100 mL (Huancayo District) and 8.42E+06 GC/100 mL (El Tambo District) (Table 1) and coincided with the peak of infections of the third wave (EW

2/2022 and 3/2022) in both districts (Fig. 1). However, due to the lack of wastewater quantifications before January 2022, we were not able to anticipate the occurrence of the third wave. During EW 21/2022 and 23/2022, were registered increases of 3.04E+04 GC/100 mL in Huancayo District and 2.25E+04 GC/100 mL in El Tambo District, respectively. These quantifications managed to anticipate the start of the fourth wave by up to 2 weeks. The highest concentrations of SARS-CoV-2 in the fourth wave occurred in EW 25/2022 for El Tambo District (2.41E+06 GC/100 mL) and in EW 29/2022 for Huancayo District (1.06E+06 GC/100 mL) (Fig. 1). Finally, the quantifications of 5.48E+05 GC/100 mL in Huancayo District and of 1.21E+05 GC/100 mL in El Tambo District, registered in EW 45/2022, also preceded by 2 weeks the start of the fifth wave (Fig. 1).

Spearman´s correlation coefficients between the concentrations of SARS-CoV-2 and reported cases of COVID-19 were 35.9% (p>0.05) in Huancayo District and 60.8% (p<0.05) in El Tambo District. Meanwhile, a 2-week lag in the concentration of SARS-CoV-2 was considered to anticipate the increase in reported cases of COVID-19 in the community. With this lag, it was possible to improve the level of correlation to 55.2% (p<0.05) in Huancayo District and 71.4% (p<0.05) in El Tambo District. Therefore, these results confirm our findings in the monitoring of wastewater and suggest that the increase in GC concentrations of SARS-CoV-2 has the capacity to anticipate, by up to 2 weeks, the start of a COVID-19 outbreak in the community. Likewise, it was observed that the progressive decrease of SARS-CoV-2 in wastewater to levels below the detection limit preceded the decrease in the number of reported clinical cases (Fig. 1).

3.5. Relationship between the concentration of RV, NoV GGII, and HAdV in wastewater and the number of reported cases of acute gastroenteritis

The concentrations of RV and HAdV in wastewater presented important variations in some EW that managed to anticipate outbreaks of acute gastroenteritis in the community and the highest concentrations were detected 4 weeks before the start of the outbreaks. In El Tambo District, the highest quantification for RV (2.75E+08 GC/100 mL) in this study was recorded during EW 9/2022 and 4 weeks before the start of an outbreak of 64 cases in children under 1 year of age (Fig. 2A). Subsequently, in Huancayo District, two considerable increases of 2.38+08 GC/100 mL for RV (second highest quantification) (Fig. 2B) and 5.03+07 GC/100 mL for HAdV (highest quantification) (Fig. 2C) were registered in EW 31/2022. Both concentrations also anticipated in 4 weeks an outbreak of 18 cases in children between 1 to 4 years of age (EW 35/2022). On the other hand, an outbreak of 49 cases was detected in the group over 5 years of age in Huancayo District but it was not possible to anticipate because it occurred in the initial week of the study (EW 39/2021).

Spearman's correlation coefficients showed a moderate level of correlation between RV concentrations in wastewater and the number of reported cases of acute gastroenteritis in children under 4 years of age in both districts. A moderate correlation was also observed between HAdV and reported cases in children between 1 to 4 years of age in Huancayo District. Considering a 2-week lag in viral concentration, results like those of SARS-CoV-2 were obtained (Table S9 of the Supplementary Material).

3.6. Physicochemical parameters of wastewater

The variations of the physicochemical parameters remained within the normal ranges and were not associated with any considerable increase in the viral load in the wastewater. Table 3 describes the average values of the physicochemical parameters including minimum and maximum values. Table 10 and Fig. S7 of the Supplementary

Material detail the measurements and variations of the physicochemical parameters in each district by EW, respectively.

4. Discussion

In the present study, the concentration of SARS-CoV-2 in the wastewater of the main districts of Huancayo City was monitored for one year to establish a relationship with the number of reported cases of COVID-19 in the community. Likewise, the relationship between RV, NoV GGII, and HAdV with reported cases of acute gastroenteritis was also evaluated. Our results showed that periodic monitoring of SARS-CoV-2 was able to anticipate, by up to 2 weeks, the start of the fourth and fifth waves of the COVID-19 pandemic in Peru (Fig. 1). This fact coincided with studies carried out in Brazil, Netherlands, and Spain where they managed to detect SARS-CoV-2 in wastewater between 1 to 2 weeks before the report of the first case of COVID-19 in the population and the start of the first wave (Claro et al., 2021; Medema et al., 2020; Randazzo et al., 2020; Rusiñol et al., 2021). In addition, it has been suggested that the concentration of SARS-CoV-2 in wastewater represents an adequate predictor of the number of reported cases of COVID-19 up to one week after sampling (Rusiñol et al., 2021; Stehlík et al., 2021). In our case, we observed a strong level of correlation between findings in the wastewater and reported cases of COVID-19 up to 2 weeks after sampling. Although the sampling interval every 2 weeks was a useful and economical option to monitor for SARS-CoV-2 and to anticipate the imminent start of a COVID-19 outbreak in the community, we considered that a one-week interval would increase the sensitivity to detect outbreaks under our conditions.

In South America, the first report of SARS-CoV-2 in wastewater was carried out in Brazil in April 2020 (Prado et al., 2020). However, there is evidence to suggest that SARS-CoV-2 may have been circulating undetected in the population before the first cases of COVID-19 were officially reported in the region (Fongaro et al., 2021). Subsequently, more studies were developed in Brazil, Argentina, and Chile that helped monitor the spread of SARS-CoV-2 (Barrios et al., 2021; Plaza-Garrido et al., 2023) and complemented the containment and mitigation measures implemented by health authorities in the community (Prado et al., 2021). Our research in the Peruvian Highlands contributes to the collective efforts of these countries to promote large-scale WBE implementation in the region.

On the other hand, RV concentrations in wastewater peaked 4 weeks before the start of acute gastroenteritis outbreaks in children under 4 years of age in both districts (Fig. 2). This fact may also prove the feasibility of the WBE to identify different other potential scenarios in the same city. Similarly, a study in Sweden was able to anticipate, by up to 3 weeks, an outbreak of diarrheal disease due to NoV GGII (Hellmér et al., 2014). However, and unlike the previous case, a limitation of our study was that the local health authority does not have the resources to identify the etiological agent of the cases of acute gastroenteritis.

High RV concentrations detected in wastewater previous an acute gastroenteritis outbreak in children under 1 year of age would be considered a spurious relationship. Nevertheless, it is important to indicate that RV is the main cause of diarrhea in children (PAHO, 2007) and that in Peru vaccination coverage in children under 1 year of age is low (~45%) (MINSA, 2022). Moreover, the use of cloth diapers is frequent in the Peruvian Highlands hence baby feces would inevitably end into the wastewater. Likewise, close contact between children and their parents would cause asymptomatic infection in adults, which would increase the viral load in the wastewater.

Although there is an misreporting of clinical cases by the health authority of Peru due to the fact that a high proportion of the population does not seek medical attention as well as the insufficient number of clinical tests in health centers and that we had to resort to manual sampling at a specific time of the day due to the lack of an automatic monitoring system that collects a sample throughout the day as implemented in treatment plants, our results showed that wastewater monitoring would anticipate the increase in reported cases of COVID-19 and acute gastroenteritis in the population. Therefore, this fact suggests that the WBE would be an excellent complement to the surveillance of endemic infectious diseases and to anticipate epidemic outbreaks in regions where there are limitations in epidemiological records.

The concentrations of RV, NoV GGII, and HAdV in the wastewater from Huancayo City remained relatively constant throughout the study, except for the peaks described before. The results of NoV GGII and HAdV revealed the highest quantifications recorded in the region, surpassing the reports in Chile (Plaza-Garrido et al., 2023), Brazil (Espinosa et al., 2022; Fumian et al., 2019), and Uruguay (Lizasoain et al., 2018). These results were also higher than those reported in high-income countries on different continents: United States (McCall et al., 2020), Spain (Silva-Sales et al., 2020), Sweden (Hellmér et al., 2014), Japan (Kobayashi et al., 2017), China (Zhou et al., 2016), and South Africa (Mabasa et al., 2018). On the other hand, RV concentrations were similar to those obtained in Brazil (Prado et al., 2019) and Spain (Silva-Sales et al., 2020), and higher than those in Argentina (Barril et al., 2015), Uruguay (Lizasoain et al., 2018), United States (Kiulia et al., 2021), Sweden (Hellmér et al., 2014), Japan (Kobayashi et al., 2017), and Egypt (Elmahdy et al., 2020). It is important to highlight that the results of this study must be analyzed with caution and consider that the viral quantifications may vary in each region due to the geographical and population characteristics of the place of study, the physicochemical particularities of the wastewater, and the viral load excreted by people, including the sampling methodology and the recovery efficacy of the concentration method.

Previous studies strongly recommend the application of concentration methods in the analysis of wastewater samples to increase the limits viral detection and quantification (Gonçalves et al., 2021; Hellmér et al., 2014). However, in our study, the direct analysis obtained the highest performance for SARS-CoV-2, both in evaluating recovery efficacy and in the wastewater surveillance for this virus (Table 1). These findings would be due to the high number of people infected during the pandemic, which increased the viral load in wastewater and facilitated the detection and quantification of SARS-CoV-2. In addition, it is likely that the acidification of the sample during skimmed milk flocculation degrades SARS-CoV-2 (an enveloped virus), which would explain its lower performance compared to direct analysis. In fact its performance has been accurately described in enveloped viruses, such bovine viral diarrhea virus (Gonzales-Gustavson et al., 2017). However, skimmed milk flocculation is one of the main methods for the concentration of non-enveloped viruses in wastewater, such as RV, NoV GGII, and HAdV (Calgua et al., 2013; Gonzales-Gustavson et al., 2019; Hellmér et al., 2014). Finally, and considering the transition of COVID-19 to endemicity, we recommend using ultrafiltration with the Amicon® Ultra-15 30 kDa device in a periodic surveillance of SARS-CoV-2 in wastewater because it was the method with the second highest performance in the study. Although it has been reported that this method would reduce the efficacy of viral recovery (Ahmed et al., 2020b; Ye et al., 2016), it is highly reproducible and presents low method intravariability (Forés et al., 2021).

5. Conclusions

In general, the results of the present investigation support the implementation of the WBE as an early warning tool to anticipate outbreaks of SARS-CoV-2 and other viruses, such as RV, in low- and middle-income countries. Our results also suggest that the WBE is an excellent complement in the surveillance of infectious diseases in regions where there are limitations in epidemiological records. Direct analysis of wastewater, without the prior concentration stage, would save time and resources in SARS-CoV-2 surveillance under pandemic conditions. However, it is necessary to evaluate the direct analysis in different contexts. On the other hand, all the wastewater samples processed by the skimmed milk flocculation method showed high concentrations of RV, NoV GGII, and HAdV. This fact reveals the alarming health situation suffered by a city that lacks a wastewater treatment plant.

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Bibliographic references

Ahmed, W., Angel, N., Edson, J., Bibby, K., Bivins, A., O'Brien, J.W., Choi, P.M., Kitajima, M., Simpson, S.L., Li, J., Tscharke, B., Verhagen, R., Smith, W.J.M., Zaugg, J., Dierens, L., Hugenholtz, P., Thomas, K. V, Mueller, J.F., 2020a. First confirmed detection of SARS-CoV-2 in untreated wastewater in Australia: A proof of concept for the wastewater surveillance of COVID-19 in the community. Sci. Total Environ. 728, 138764. https://doi.org/10.1016/j.scitotenv.2020.138764

Ahmed, W., Bertsch, P.M., Bivins, A., Bibby, K., Farkas, K., Gathercole, A., Haramoto, E., Gyawali, P., Korajkic, A., McMinn, B.R., Mueller, J.F., Simpson, S.L., Smith, W.J.M., Symonds, E.M., Thomas, K. V., Verhagen, R., Kitajima, M., 2020b. Comparison of virus concentration methods for the RT-qPCR-based recovery of murine hepatitis virus, a surrogate for SARS-CoV-2 from untreated wastewater. Sci. Total Environ. 739, 139960. https://doi.org/10.1016/j.scitotenv.2020.139960

Barril, P.A., Fumian, T.M., Prez, V.E., Gil, P.I., Martínez, L.C., Giordano, M.O., Masachessi, G., Isa, M.B., Ferreyra, L.J., Ré, V.E., Miagostovich, M., Pavan, J. V, Nates, S. V, 2015. Rotavirus seasonality in urban sewage from Argentina: effect of meteorological variables on the viral load and the genetic diversity. Environ. Res. 138, 409–415. https://doi.org/10.1016/j.envres.2015.03.004

Barrios, M.E., Díaz, S.M., Torres, C., Costamagna, D.M., Blanco Fernández, M.D., Mbayed, V.A., 2021. Dynamics of SARS-CoV-2 in wastewater in three districts of the Buenos Aires metropolitan region, Argentina, throughout nine months of surveillance: A pilot study. Sci. Total Environ. 800, 149578. https://doi.org/10.1016/j.scitotenv.2021.149578

Calgua, B., Rodriguez-Manzano, J., Hundesa, A., Suñen, E., Calvo, M., Bofill-Mas, S., Girones, R., 2013. New methods for the concentration of viruses from urban sewage using quantitative PCR. J. Virol. Methods 187, 215–221. https://doi.org/10.1016/j.jviromet.2012.10.012

CENEPRED, 2021. COVID-19 risk scenario (Escenario de riesgo por COVID-19). CENEPRED 21.

Center for Disease Control, 2019. Research Use Only 2019-Novel Coronavirus (2019-nCoV) Real-time RT-PCR Primers and Probes. CDC's Diagnostic Test COVID-19 Only Supplies 2019–2020.

Claro, I.C.M., Cabral, A.D., Augusto, M.R., Duran, A.F.A., Graciosa, M.C.P., Fonseca, F.L.A., Speranca, M.A., Bueno, R. de F., 2021. Long-term monitoring of SARS-COV-2 RNA in wastewater in Brazil: A more responsive and economical approach. Water Res. 203, 117534. https://doi.org/10.1016/j.watres.2021.117534

Daughton, C.G., 2020. Wastewater surveillance for population-wide Covid-19: The present and future. Sci. Total Environ. 736, 139631. https://doi.org/10.1016/j.scitotenv.2020.139631

Dey, R.S., Ghosh, S., Chawla-Sarkar, M., Panchalingam, S., Nataro, J.P., Sur, D., Manna, B., Ramamurthy, T., 2011. Circulation of a novel pattern of infections by enteric adenovirus serotype 41 among children below 5 years of age in Kolkata, India. J. Clin. Microbiol. 49, 500–505. https://doi.org/10.1128/JCM.01834-10

Elmahdy, E.M., Shaheen, M.N.F., Rizk, N.M., Saad-Hussein, A., 2020. Quantitative Detection of Human Adenovirus and Human Rotavirus Group A in Wastewater and El-Rahawy Drainage Canal Influencing River Nile in the North of Giza, Egypt. Food Environ. Virol. 12, 218–225. https://doi.org/10.1007/s12560-020-09429-x

Espinosa, M.F., Verbyla, M.E., Vassalle, L., Leal, C., Leroy-Freitas, D., Machado, E., Fernandes, L., Rosa-Machado, A.T., Calábria, J., Chernicharo, C., Mota Filho, C.R., 2022. Reduction and liquid-solid partitioning of SARS-CoV-2 and adenovirus throughout the different stages of a pilot-scale wastewater treatment plant. Water Res. 212, 118069. https://doi.org/10.1016/j.watres.2022.118069

Fongaro, G., Stoco, P.H., Souza, D.S.M., Grisard, E.C., Magri, M.E., Rogovski, P., Schörner, M.A., Barazzetti, F.H., Christoff, A.P., de Oliveira, L.F.V., Bazzo, M.L., Wagner, G., Hernández, M., Rodríguez-Lázaro, D., 2021. The presence of SARS-CoV-2 RNA in human sewage in Santa Catarina, Brazil, November 2019. Sci. Total Environ. 778, 146198. https://doi.org/10.1016/j.scitotenv.2021.146198

Forés, E., Bofill-Mas, S., Itarte, M., Martínez-Puchol, S., Hundesa, A., Calvo, M., Borrego, C.M., Corominas, L.L., Girones, R., Rusiñol, M., 2021. Evaluation of two rapid ultrafiltration-based methods for SARS-CoV-2 concentration from wastewater. Sci. Total Environ. 768, 144786. https://doi.org/10.1016/j.scitotenv.2020.144786

Fumian, T.M., Fioretti, J.M., Lun, J.H., Dos Santos, I.A.L., White, P.A., Miagostovich, M.P., 2019. Detection of norovirus epidemic genotypes in raw sewage using next generation sequencing. Environ. Int. 123, 282–291. https://doi.org/10.1016/j.envint.2018.11.054

Gonçalves, J., Gutiérrez-Aguirre, I., Balasubramanian, M.N., Zagorščak, M., Ravnikar, M., Turk, V., 2018. Surveillance of human enteric viruses in coastal waters using concentration with methacrylate monolithic supports prior to detection by RT-qPCR. Mar. Pollut. Bull. 128, 307–317. https://doi.org/10.1016/j.marpolbul.2018.01.040

Gonçalves, J., Koritnik, T., Mioč, V., Trkov, M., Bolješič, M., Berginc, N., Prosenc, K., Kotar, T., Paragi, M., 2021. Detection of SARS-CoV-2 RNA in hospital wastewater from a low COVID-19 disease prevalence area. Sci. Total Environ. 755, 143226. https://doi.org/10.1016/j.scitotenv.2020.143226

Gonzales-Gustavson, E., Cárdenas-Youngs, Y., Calvo, M., da Silva, M.F.M., Hundesa, A., Amorós, I., Moreno, Y., Moreno-Mesonero, L., Rosell, R., Ganges, L., Araujo, R., Girones, R., 2017. Characterization of the efficiency and uncertainty of skimmed milk flocculation for the simultaneous concentration and quantification of water-borne viruses, bacteria and protozoa. J. Microbiol. Methods 134, 46–53. https://doi.org/10.1016/j.mimet.2017.01.006

Gonzales-Gustavson, E., Rusiñol, M., Medema, G., Calvo, M., Girones, R., 2019. Quantitative risk assessment of norovirus and adenovirus for the use of reclaimed water to irrigate lettuce in Catalonia. Water Res. 153, 91–99. https://doi.org/https://doi.org/10.1016/j.watres.2018.12.070

Guerrero-Latorre, L., Collado, N., Abasolo, N., Anzaldi, G., Bofill-Mas, S., Bosch, A., Bosch, L., Busquets, S., Caimari, A., Canela, N., Carcereny, A., Chacón, C., Ciruela, P., Corbella, I., Domingo, X., Escoté, X., Espiñeira, Y., Forés, E., Gandullo-Sarró, I., Garcia-Pedemonte, D., Girones, R., Guix, S., Hundesa, A., Itarte, M., Mariné-Casadó, R., Martínez, A., Martínez-Puchol, S., Mas-Capdevila, A., Mejías-Molina, C., Rafa, M.M.I., Munné, A., Pintó, R.M., Pueyo-Ros, J., Robusté-Cartró, J., Rusiñol, M., Sanfeliu, R., Teichenné, J., Torrell, H., Corominas, L., Borrego, C.M., 2022. The Catalan Surveillance Network of SARS-CoV-2 in Sewage: design, implementation, and performance. Sci. Rep. 12, 16704. https://doi.org/10.1038/s41598-022-20957-3

Guerrero-Latorre, L., Romero, B., Bonifaz, E., Timoneda, N., Rusiñol, M., Girones, R., Rios-Touma, B., 2018. Quito's virome: Metagenomic analysis of viral diversity in urban streams of Ecuador's capital city. Sci. Total Environ. 645, 1334–1343. https://doi.org/10.1016/j.scitotenv.2018.07.213

Hart, O.E., Halden, R.U., 2020. Computational analysis of SARS-CoV-2/COVID-19 surveillance by wastewater-based epidemiology locally and globally: Feasibility, economy, opportunities and challenges. Sci. Total Environ. 730, 138875. https://doi.org/10.1016/j.scitotenv.2020.138875

Hellmér, M., Paxéus, N., Magnius, L., Enache, L., Arnholm, B., Johansson, A., Bergström, T., Norder, H., 2014. Detection of pathogenic viruses in sewage provided early warnings of hepatitis A virus and norovirus outbreaks. Appl. Environ. Microbiol. 80, 6771–6781. https://doi.org/10.1128/AEM.01981-14

Hernroth, B.E., Conden-Hansson, A.-C., Rehnstam-Holm, A.-S., Girones, R., Allard, A.K., 2002. Environmental factors influencing human viral pathogens and their potential indicator organisms in the blue mussel, Mytilus edulis: the first Scandinavian report. Appl. Environ. Microbiol. 68, 4523–4533. https://doi.org/10.1128/AEM.68.9.4523-4533.2002

Kazama, S., Miura, T., Masago, Y., Konta, Y., Tohma, K., Manaka, T., Liu, X., Nakayama, D., Tanno, T., Saito, M., Oshitani, H., Omura, T., 2017. Environmental Surveillance of Norovirus Genogroups I and II for Sensitive Detection of Epidemic Variants. Appl. Environ. Microbiol. 83. https://doi.org/10.1128/AEM.03406-16

Kitajima, M., Ahmed, W., Bibby, K., Carducci, A., Gerba, C.P., Hamilton, K.A., Haramoto, E., Rose, J.B., 2020. SARS-CoV-2 in wastewater: State of the knowledge and research needs. Sci. Total Environ. 739, 139076. https://doi.org/10.1016/j.scitotenv.2020.139076

Kiulia, N.M., Gonzalez, R., Thompson, H., Aw, T.G., Rose, J.B., 2021. Quantification and Trends of Rotavirus and Enterovirus in Untreated Sewage Using Reverse Transcription Droplet Digital PCR. Food Environ. Virol. 13, 154–169. https://doi.org/10.1007/s12560-020-09455-9

Kobayashi, N., Oshiki, M., Ito, T., Segawa, T., Hatamoto, M., Kato, T., Yamaguchi, T., Kubota, K., Takahashi, M., Iguchi, A., Tagawa, T., Okubo, T., Uemura, S., Harada, H., Motoyama, T., Araki, N., Sano, D., 2017. Removal of human pathogenic viruses in a down-flow hanging sponge (DHS) reactor treating municipal wastewater and health risks associated with utilization of the effluent for agricultural irrigation. Water Res. 110, 389–398. https://doi.org/10.1016/j.watres.2016.10.054

Lizasoain, A., Tort, L.F.L., García, M., Gillman, L., Alberti, A., Leite, J.P.G., Miagostovich, M.P., Pou, S.A., Cagiao, A., Razsap, A., Huertas, J., Berois, M., Victoria, M., Colina, R., 2018. Human enteric viruses in a wastewater treatment plant: evaluation of activated sludge combined with UV disinfection process reveals different removal performances for viruses with different features. Lett. Appl. Microbiol. 66, 215–221. https://doi.org/10.1111/lam.12839

Loisy, F., Atmar, R.L., Guillon, P., Le Cann, P., Pommepuy, M., Le Guyader, F.S., 2005. Real-time RT-PCR for norovirus screening in shellfish. J. Virol. Methods 123, 1–7. https://doi.org/10.1016/j.jviromet.2004.08.023

Mabasa, V. V, Meno, K.D., Taylor, M.B., Mans, J., 2018. Environmental Surveillance for Noroviruses in Selected South African Wastewaters 2015-2016: Emergence of the Novel GII.17. Food Environ. Virol. 10, 16–28. https://doi.org/10.1007/s12560-017-9316-2

Mans, J., 2019. Norovirus Infections and Disease in Lower-MiddleandLow-Income Countries, 1997¯2018. Viruses 11. https://doi.org/10.3390/v11040341

McCall, C., Wu, H., Miyani, B., Xagoraraki, I., 2020. Identification of multiple potential viral diseases in a large urban center using wastewater surveillance. Water Res. 184, 116160. https://doi.org/10.1016/j.watres.2020.116160

Medema, G., Heijnen, L., Elsinga, G., Italiaander, R., Brouwer, A., 2020. Presence of SARS-Coronavirus-2 RNA in Sewage and Correlation with Reported COVID-19 Prevalence in the Early Stage of the Epidemic in the Netherlands. Environ. Sci. Technol. Lett. 7, 511–516. https://doi.org/10.1021/acs.estlett.0c00357

MINSA, 2022. National Center for Epidemiology, Disease Prevention and Control: Epidemiological Bulletin (Centro Nacional de Epidemiología, Prevención y Control de Enfermadades: Boletín Epidemiológico).

PAHO, 2021. Guide for the analysis and quantification of SARS-CoV-2 in wastewater (Guía para el análisis y la cuantificación del SARS-CoV-2 en aguas residuales).

PAHO, 2007. Epidemiological surveillance of diarrhea caused by rotavirus: practical guide (Vigilancia epidemiológica de diarreas causadas por rotavirus: guía práctica). Publicación Científica y Técnica No. 623 ISBN 92 75, 43.

Philo, S.E., Keim, E.K., Swanstrom, R., Ong, A.Q.W., Burnor, E.A., Kossik, A.L., Harrison, J.C., Demeke, B.A., Zhou, N.A., Beck, N.K., Shirai, J.H., Meschke, J.S., 2021. A comparison of SARS-CoV-2 wastewater concentration methods for environmental surveillance. Sci. Total Environ. 760, 144215. https://doi.org/10.1016/j.scitotenv.2020.144215

Plaza-Garrido, A., Ampuero, M., Gaggero, A., Villamar-Ayala, C.A., 2023. Norovirus, Hepatitis A and SARS-CoV-2 surveillance within Chilean rural wastewater treatment plants based on different biological treatment typologies. Sci. Total Environ. 863, 160685. https://doi.org/10.1016/j.scitotenv.2022.160685

Polo, D., Quintela-Baluja, M., Corbishley, A., Jones, D.L., Singer, A.C., Graham, D.W., Romalde, J.L., 2020. Making waves: Wastewater-based epidemiology for COVID-19 - approaches and challenges for surveillance and prediction. Water Res. 186, 116404. https://doi.org/10.1016/j.watres.2020.116404

Prado, T., de Castro Bruni, A., Barbosa, M.R.F., Garcia, S.C., de Jesus Melo, A.M., Sato, M.I.Z., 2019. Performance of wastewater reclamation systems in enteric virus removal. Sci. Total Environ. 678, 33–42. https://doi.org/10.1016/j.scitotenv.2019.04.435

Prado, T., Fumian, T.M., Mannarino, C.F., Maranhão, A.G., Siqueira, M.M., Miagostovich, M.P., 2020. Preliminary results of SARS-CoV-2 detection in sewerage system in Niterói municipality, Rio de Janeiro, Brazil. Mem. Inst. Oswaldo Cruz 115, e200196. https://doi.org/10.1590/0074-02760200196

Prado, T., Fumian, T.M., Mannarino, C.F., Resende, P.C., Motta, F.C., Eppinghaus, A.L.F., Chagas do Vale, V.H., Braz, R.M.S., de Andrade, J. da S.R., Maranhão, A.G., Miagostovich, M.P., 2021. Wastewater-based epidemiology as a useful tool to track SARS-CoV-2 and support public health policies at municipal level in Brazil. Water Res. 191, 116810. https://doi.org/10.1016/j.watres.2021.116810

R Core Team (2023). R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. URL https://www.R-project.org/.

Randazzo, W., Truchado, P., Cuevas-Ferrando, E., Simón, P., Allende, A., Sánchez, G., 2020. SARS-CoV-2 RNA in wastewater anticipated COVID-19 occurrence in a low prevalence area. Water Res. 181, 115942. https://doi.org/10.1016/j.watres.2020.115942

Rodriguez, D., Serrano, H., Delgado, A., Nolasco, D., Saltiel, G., 2018. De residuo a recurso: Cambiando paradigmas para intervenciones más inteligentes para la gestión de aguas residuales en América Latina y el Caribe. Bancomundial.org 63.

Rusiñol, M., Girones, R., 2017. Summary of Excreted and Waterborne Viruses. Water Sanit. 21st Century Heal. Microbiol. Asp. Excreta Wastewater Manag. (Global Water Pathog. Proj. https://doi.org/10.14321/waterpathogens.19

Rusiñol, M., Zammit, I., Itarte, M., Forés, E., Martínez-Puchol, S., Girones, R., Borrego, C., Corominas, L., Bofill-Mas, S., 2021. Monitoring waves of the COVID-19 pandemic: Inferences from WWTPs of different sizes. Sci. Total Environ. 787, 147463. https://doi.org/10.1016/j.scitotenv.2021.147463

Silva-Sales, M., Martínez-Puchol, S., Gonzales-Gustavson, E., Hundesa, A., Gironès, R., 2020. High Prevalence of Rotavirus A in Raw Sewage Samples from Northeast Spain. Viruses 12. https://doi.org/10.3390/v12030318

Stehlík, M., Kiseľák, J., Dinamarca, M.A., Li, Y., Ying, Y., 2021. On COVID-19 outbreaks predictions: Issues on stability, parameter sensitivity, and precision. Stoch. Anal. Appl. 39, 383–404. https://doi.org/10.1080/07362994.2020.1802291

Tandukar, S., Sthapit, N., Thakali, O., Malla, B., Sherchan, S.P., Shakya, B.M., Shrestha, L.P., Sherchand, J.B., Joshi, D.R., Lama, B., Haramoto, E., 2022. Detection of SARS-CoV-2 RNA in wastewater, river water, and hospital wastewater of Nepal. Sci. Total Environ. 824, 153816. https://doi.org/10.1016/j.scitotenv.2022.153816

Tang, A., Tong, Z.-D., Wang, H.-L., Dai, Y.-X., Li, K.-F., Liu, J.-N., Wu, W.-J., Yuan, C., Yu, M.-L., Li, P., Yan, J.-B., 2020. Detection of Novel Coronavirus by RT-PCR in Stool Specimen from Asymptomatic Child, China. Emerg. Infect. Dis. 26, 1337–1339. https://doi.org/10.3201/eid2606.200301

Whitney, O.N., Kennedy, L.C., Fan, V., Hinkle, A., Kantor, R., Greenwald, H., Crits-Christoph, A., Al-Shayeb, B., Chaplin, M., Maurer, A.C., Tjian, R., Nelson, K.L., 2020. Sewage, Salt, Silica and SARS-CoV-2 (4S): An economical kit-free method for direct capture of SARS-CoV-2 RNA from wastewater. medRxiv Prepr. Serv. Heal. Sci. https://doi.org/10.1101/2020.12.01.20242131

Wölfel, R., Corman, V.M., Guggemos, W., Seilmaier, M., Zange, S., Müller, M.A., Niemeyer, D., Jones, T.C., Vollmar, P., Rothe, C., Hoelscher, M., Bleicker, T., Brünink, S., Schneider, J., Ehmann, R., Zwirglmaier, K., Drosten, C., Wendtner, C., 2020. Virological assessment of hospitalized patients with COVID-2019. Nature 581, 465–469. https://doi.org/10.1038/s41586-020-2196-x

Ye, Y., Ellenberg, R.M., Graham, K.E., Wigginton, K.R., 2016. Survivability, Partitioning, and Recovery of Enveloped Viruses in Untreated Municipal Wastewater. Environ. Sci. Technol. 50, 5077–5085. https://doi.org/10.1021/acs.est.6b00876

Zeng, S.Q., Halkosalo, A., Salminen, M., Szakal, E.D., Puustinen, L., Vesikari, T., 2008. One-step quantitative RT-PCR for the detection of rotavirus in acute gastroenteritis. J. Virol. Methods 153, 238–240. https://doi.org/10.1016/j.jviromet.2008.08.004

Zhou, N., Lin, X., Wang, S., Tao, Z., Xiong, P., Wang, H., Liu, Y., Song, Y., Xu, A., 2016. Molecular epidemiology of GI and GII noroviruses in sewage: 1-year surveillance in eastern China. J. Appl. Microbiol. 121, 1172–1179. https://doi.org/10.1111/jam.13218

Table 1. Detections and quantifications in genomic copies (GC) per 100 mL of wastewater sample for SARS-CoV-2 by each processing method in both districts.

District	Direct analysis		Ultrafiltration		Skimmed milk flocculation	
	Positives / Total (%)	Max. quantification (GC/100 mL)	Positives / Total (%)	Max. quantification (GC/100 mL)	Positives / Total (%)	Max. quantification (GC/100 mL)
Huancayo	16 / 25 (64.0)	9.32E+06	14 / 23 (60.8)	3.53E+05	3 / 25 (12.0)	2.54E+04
El Tambo	16 / 25 (64.0)	8.42E+06	11 / 23 (47.8)	5.55E+05	2 / 25 (8.0)	3.53E+03

Table 2. Median, minimum, and maximum values in genomic copies (GC) per 100 mL of wastewater sample for rotavirus (RV), norovirus genogroup II (NoV GGII), and human adenovirus (HAdV) concentrated by skimmed milk flocculation in both district.

District	Virus	Median (GC/100 mL)	Min. quantification (GC/100 mL)	Max. quantification (GC/100 mL)
Huancayo	RV	3.10E+06	5.17E+04	2.38E+08
	NoV GGII	1.47E+06	1.78E+04	1.02E+07
	HAdV	1.68E+06	7.47E+04	5.03E+07
El Tambo	RV	9.13E+06	2.08E+05	2.75E+08
	NoV GGII	1.27E+06	2.31E+04	1.22E+07
	HAdV	3.69E+06	1.26E+05	1.92E+07

Table 3. Mean, minimum, and maximum values of each physicochemical parameters in wastewater from both districts: pH, temperature, electrical conductivity (EC), and total dissolved solids (TDS).

	Huancayo District			El Tambo District		
	Mean ± SD[a]	Min. value	Max. value	Mean ± SD	Min. value	Max. value
pH	7.85 ± 0.23	7.30	8.24	8.13 ± 0.21	7.64	8.80
Temperature (°C[b])	16.82 ± 1.33	13.60	20.10	17.82 ± 1.51	14.30	21.40
EC (mS/cm[c])	1.36 ± 0.26	1.00	2.24	1.46 ± 0.21	1.05	1.94
TDS (ppt[d])	0.68 ± 0.12	0.50	1.09	0.73 ± 0.10	0.53	0.97

[a] Standard deviation; [b] degree Celsius; [c] milliSiemens per centimeter; [d] parts per trillion.

Fig. 1. Monitoring of SARS-CoV-2 concentrations in wastewater and weekly registration of COVID-19 cases. The blue bars represent the reported cases of COVID-19 by the health authority of Peru, while the red line represents the concentration of genomic copies (GC) per 100 mL of wastewater sample evaluated in this study, across the epidemiologic weeks, for the districts of Huancayo (Fig. A) and El Tambo (Fig. B).

Fig. 2. Monitoring of rotavirus (RV) and human adenovirus (HAdV) concentrations in wastewater and weekly registration of acute gastroenteritis cases. The blue bars represent the reported cases of acute gastroenteritis by the health authority of Peru, while the red line represents the concentration of genomic copies (GC) per 100 mL of wastewater sample evaluated in this study. Fig. A and B shown the concentrations of RV and the number of cases in children under 1 year of age for El Tambo District (Fig. A) and the number of cases in children between 1 to 4 years of age for Huancayo District (Fig. B). Fig. C shows the concentrations of HAdV and the number of cases in children between 1 to 4 years for Huancayo District.

Table 1. Detections and quantifications in genomic copies (GC) per 100 mL of wastewater sample for SARS-CoV-2 by each processing method in both districts.

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Table 2. Median, minimum, and maximum values in genomic copies (GC) per 100 mL of wastewater sample for rotavirus (RV), norovirus genogroup II (NoV GGII), and human adenovirus (HAdV) concentrated by skimmed milk flocculation in both district.

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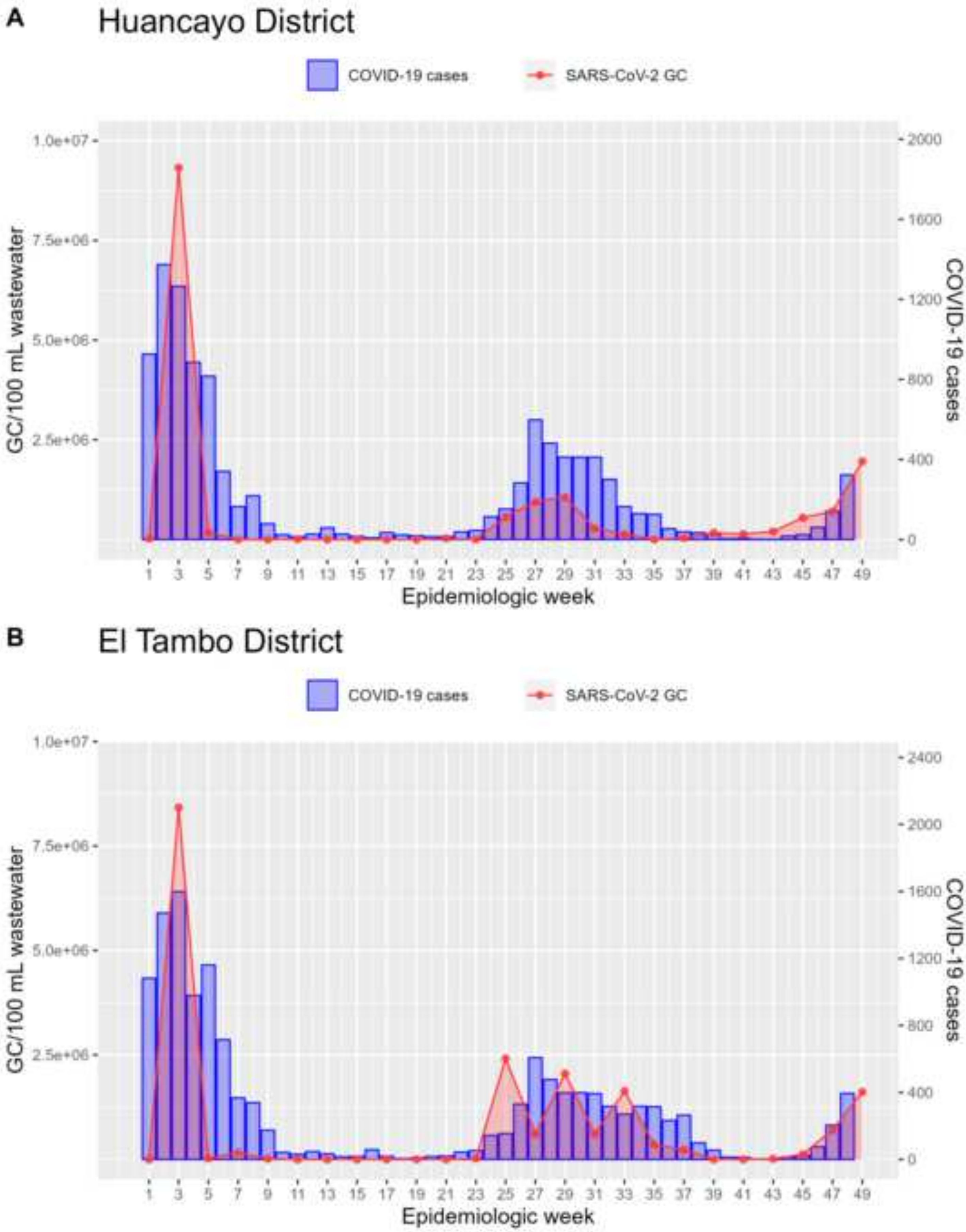
Figure


A
Huancayo District
COVID-19 cases
SARS-CoV-2 GC
GC/100 mL wastewater
COVID-19 cases
1.0e+07
7.5e+06
5.0e+06
2.5e+06
2000
1600
1200
800
400
0
1 3 5 7 9 11 13 15 17 19 21 23 25 27 29 31 33 35 37 39 41 43 45 47 49
Epidemiologic week
B
El Tambo District
COVID-19 cases
SARS-CoV-2 GC
GC/100 mL wastewater
COVID-19 cases
1.0e+07
7.5e+06
5.0e+06
2.5e+06
2400
2000
1600
1200
800
400
0
1 3 5 7 9 11 13 15 17 19 21 23 25 27 29 31 33 35 37 39 41 43 45 47 49
Epidemiologic week

Figure

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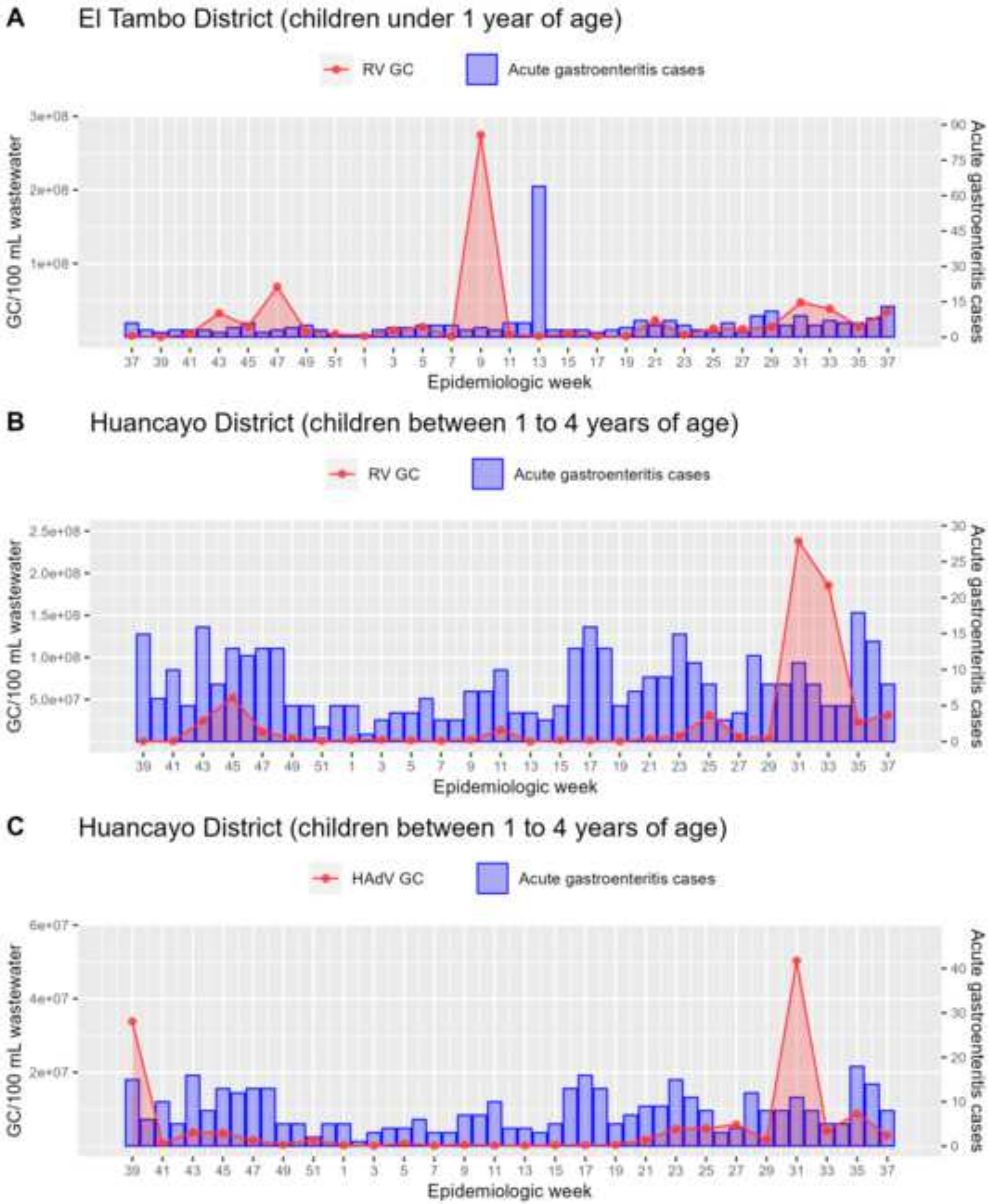

Declaration of interests

☒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.

☐The authors declare the following financial interests/personal relationships which may be considered as potential competing interests:

CRediT authorship contribution statement

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