Science of the Total Environment 800 (2021) 149578

Contents lists available at ScienceDirect

Science of the Total Environment

journal homepage: www.elsevier.com/locate/scitotenv

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

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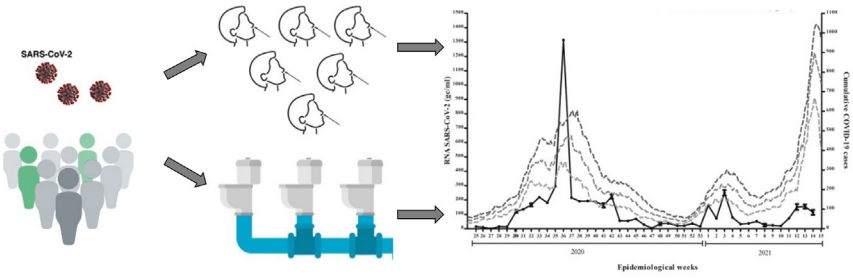
HIGHLIGHTS

- SARS-CoV-2 loads in wastewater from Buenos Aires region ranged from 10^{-1} to 10^{3} gc/ml.
- The concentration of SARS-CoV-2 in wastewater correlated with COVID-19 cases.
- Bovine coronavirus is proposed as SARS-CoV-2 surrogate for concentration from wastewater.

GRAPHICAL ABSTRACT



ARTICLE INFO

Article history:
Received 14 June 2021
Received in revised form 6 August 2021
Accepted 7 August 2021
Available online 12 August 2021

Editor: Damia Barcelo

Keywords:
SARS-CoV-2
Wastewater-based epidemiology
Virus recovery
Bovine coronavirus

ABSTRACT

In the current pandemic of COVID-19, sewage surveillance of SARS-CoV-2 genome has been used to complement viral epidemiology in different countries. The aim of this work was to introduce and evaluate this wastewater-based tool in the metropolitan region of Buenos Aires, Argentina. As a pilot study, surveillance of SARS-CoV-2 in wastewater from three districts of this area was performed for more than nine months from June 2020 to April 2021. Viruses present in the samples were concentrated using polyethylene glycol precipitation and quantified using RT-qPCR CDC N1 assay. Virus recovery for SARS-CoV-2 and a potential surrogate, bovine coronavirus Mebus strain, that shares the *Betacoronavirus* genus and structural characteristics with SARS-CoV-2, were evaluated after concentration and detection procedures. Recovery of both viruses did not differ significantly, with a median for SARS-CoV-2 and BCoV of 0.085 (95% CI: 0.021-0.179) and 0.262 (95% CI: 1.18×10^{-5}-0.564) respectively. The concentration of SARS-CoV-2 genome in wastewater ranged from 10^{-1} to 10^{3} cg/ml, depending on the wastewater treatment plant, type of collection site, viral recovery of the concentration method and the epidemiological situation of the outbreaks. Significant correlations were observed between SARS-CoV-2 concentration in wastewater and reported clinical cases, reinforcing the utility of this approach to monitor the epidemiological status of populations.

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https://doi.org/10.1016/j.scitotenv.2021.149578
0048-9697/

1. Introduction

Infection of a population caused by microbial agents that are shed via stool and urine can be tracked by wastewater analysis. In the current pandemic of COVID-19, sewage surveillance of SARS-CoV-2 genome has been used to complement viral epidemiology in different countries, which was reviewed recently (Medema et al., 2020a).

Viral detection in wastewater is possible due to the prolonged viral shedding of SARS-CoV-2 in stools of patients even after respiratory samples became negative (Parasa et al., 2020; van Doorn et al., 2020; Wölfel et al., 2020). The prevalence of SARS-CoV-2 in feces of patients ranges between 15.3 and 83.3% according to different studies and metanalyses (Cheung et al., 2020; Foladori et al., 2020). Respiratory fluids excreted to the sewer system also might contribute to the viral genome detection in these samples. Wastewater-based surveillance has been applied mostly to non-enveloped viruses (Chen et al., 2020; WHO, 2003; Wyn-Jones et al., 2011) and so it is necessary to adapt concentration methods for enveloped viruses such as SARS-CoV-2. Also, the performance of detection procedures designed for clinical samples must be evaluated when applied to environmental samples.

Epidemiological data based on nasopharyngeal sample tests only gives information about a fraction of the infected persons, either due to the limited testing capacity or the unrecognized asymptomatic infections. However, the analysis of the wastewater could render information about the whole population. Depending on the point of the sewer system selected for sampling, the results can inform about the viral circulation in larger or restricted populations (Ahmed et al., 2020a; Betancourt et al., 2021; Wurtzer et al., 2020).

The brief of the WHO about the status of environmental surveillance for SARS-CoV-2 virus sustain that among the main uses of this epidemiological strategy, the following can be listed: early warning and monitoring of viral circulation in a community; detection of the virus in locations with limited clinical surveillance, and research purposes (Global Infectious Hazard Preparedness WHO Headquarters (HQ) WHO Worldwide, 2020).

In Argentina, the first imported case of SARS-CoV-2 was detected on March 3rd, 2020. The main affected area in the country was the Buenos Aires Metropolitan Region (referred to as BAMR), where the communitarian circulation of the virus began on March 23rd. A lockdown was implemented on March 20th, which delayed the exponential increase in the number of cases. In September, the BAMR reached a temporary maximum of reported cases and started a stabilization trend while the virus increased its spreading through all the country. By March of 2021, the second wave of the pandemic was beginning, and during April 11-15% of the population had received at least one dose of the vaccination scheme.

The aim of this work was to conduct a pilot wastewater-based epidemiological study of SARS-CoV-2 of three districts of the BAMR between June 2020 and April 2021. The evaluation of the possible correlation between SARS-CoV-2 RNA levels in wastewater and COVID-19 cases could show how useful this tool can be to assist public health responses.

2. Materials and methods

2.1. Sampling

The Buenos Aires Metropolitan Region (BAMR) is a wide territory with a surface of 13,285 km^2 that includes Buenos Aires city and 40 districts that represent approximately 14.8 million inhabitants. The districts of the Region possess different population densities and different kinds of sewer systems. A pilot study to evaluate the usefulness of the wastewater-based epidemiological tool in our region was planned. Raw sewage was weekly collected for more than nine months (from mid-June 2020 to early April 2021) from four sewer systems. Sampling sites, named Alejandro Korn (AK), San Vicente (SV), San Pablo (SP) and San Fernando (SF), represented different sized catchments of Buenos Aires Metropolitan Region, in the jurisdiction of the Province of Buenos Aires, Argentina.

AK and SV represented very crowded communities, close connected, placed in the South of the BAMR both belonging to a superior administrative-territorial division, called *Partido* San Vicente, from here on, named district San Vicente (Fig. 1). These sewer networks were selected because they serve well-defined geographic regions and are not impacted by storm discharges. The population registered according to the last census in 2010, was estimated as 35,407 inhabitants in AK location, 21,411 inhabitants in SV location and 59,478 inhabitants in the whole district San Vicente (Instituto Nacional de Estadística y Censos (INDEC), 2012). A new population survey that was meant to be conducted in 2020 was postponed due to the pandemic. Wastewater samples were taken from the inlet pipe of the WWTPs of AK and SV, which serve population equivalents of 20,000 and 15,000, respectively. Then, samples collected at this point of the sewer networks, represent the excretion from 56.47% of the population of AK, 70.06% of the population of SV and 58.85% of the whole district San Vicente.

The other two sampling points in the north of the BAMR, SP and SF, belong to other two different districts and represented sewer systems that serve smaller populations of lower-income neighborhoods (3500 and 5000 population equivalents, respectively). SP samples were taken from the inlet pipe of the WWTP while the samples from SF were collected from a manhole of the sewer system.

Grab samples of 250 ml were collected weekly, at the same hour in the morning, in these four locations. Samples were refrigerated at 10 °C and transported to the laboratory, to be immediately processed.

2.2. Viral concentration and nucleic acids extraction

Samples were pasteurized in a 60 °C water bath for 90 min, with periodic rotation. Then, 200 ml of each sample were concentrated by precipitation with polyethylene glycol 8000 (10% [wt/vol]; Sigma-Aldrich, Inc.) and NaCl (0.385 M; Sigma-Aldrich, Inc.), as previously described (Wu et al., 2020) with minor modifications.

Samples with PEG/NaCl were incubated on iced water for 30 min with rotation and centrifuged at 12,000 ×*g* for 1 h, at 4 °C. The pellet was resuspended in 1.0 ml of TRIzol reagent (ThermoFisher Scientific) for nucleic acids extraction; 0.2 ml of chloroform was added and mixed by shaking. After a 3 min incubation, samples were centrifuged for 15 min at 12,000 ×*g* at 4 °C and the aqueous phase was retained. Then, 4 μl de polyA (High Pure Viral Nucleic Acid Kit, Roche Diagnostics) and 0.5 ml of isopropanol were added to the aqueous phase, incubated for 30 min at -20 °C and centrifuged for 10 min at 12,000 ×*g* at 4 °C. The pellet was suspended in 0.2 ml of Binding Buffer (High Pure Viral Nucleic Acid Kit, Roche Diagnostics) and nucleic acid extraction was performed with the kit according to the manufacturer's instructions. The nucleic acids were collected in a final volume of 50 μl of elution buffer and stored at -80 °C.

2.3. SARS-CoV-2 RT-qPCR

The RT-qPCR directed to the N1 genetic region of SARS-CoV-2 was implemented, using the CDC 2019-Novel Coronavirus (2019-nCoV) primers and probes (Lu et al., 2020) with modifications (0.4 μM of 2019-nCoV_N1 Forward Primer and 2019-nCoV_N1 Reverse Primer, 0.25 μM of 2019-nCoV_N1 Probe), 0.3 μg/μl of BSA, and TaqMan™ Fast Virus 1-Step Master Mix (ThermoFisher Scientific). Each reaction contained 5 μl of the 50 μl eluted nucleic acids and the reaction volume was adjusted to a final volume of 20 μl with MilliQ water. Thermal cycling (15 min at 50 °C, 20 s at 95 °C followed by 45 cycles of 15 s at 95 °C, 60 s at 59 °C) was carried out on a Step One Plus (Applied Biosystems) thermocycler. A sample was considered positive if the N1 2019-nCoV marker cycle threshold growth curve crossed the threshold line within 40.00 cycles (< 40.00 Ct).

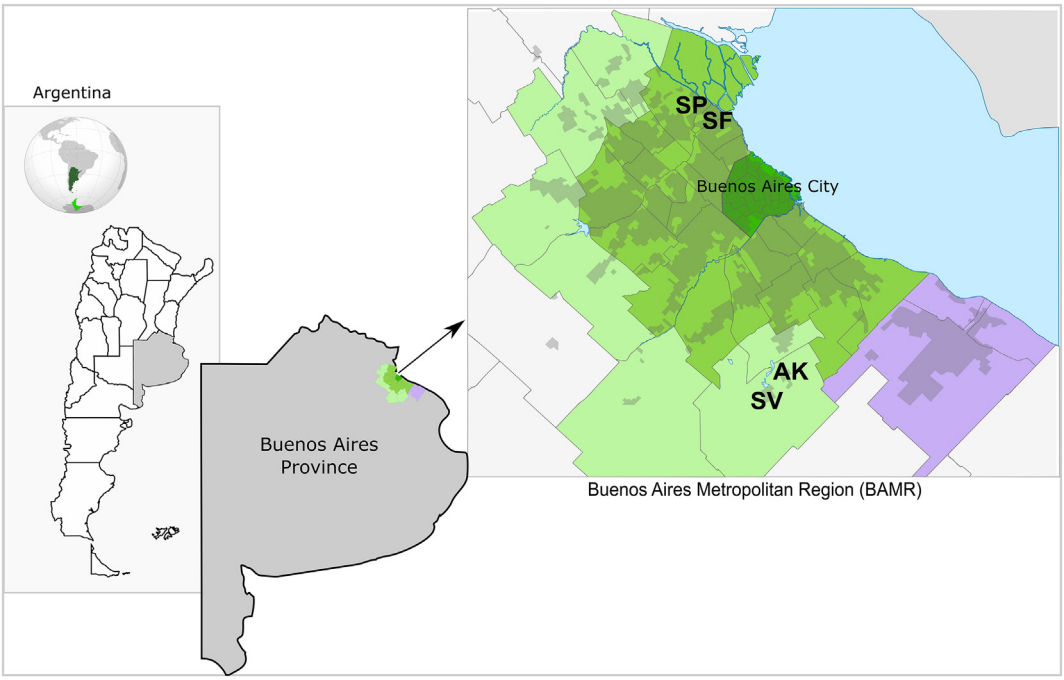

Fig. 1. Location of sampled points, Alejandro Korn (AK), San Fernando (SF), San Pablo (SP), and San Vicente (SV) in the Buenos Aires Metropolitan Region (BAMR) in the Buenos Aires province, Argentina.

SARS-CoV-2 concentrations were estimated using a standard curve per run based on 10-fold serial dilutions (from 1 to 10^5 gc per reaction) of the plasmid DNA control nCoV-ALL+001 (Eurofins) quantified by fluorometric measure (Qubit® 3.0 Fluorometer). NTCs were included in each run. The absolute limit of detection (the lowest viral load that gives 95% of positive results (ALOD95%) was estimated by the Probability of Detection (POD) model (Wilrich and Wilrich, 2009) implemented in the PODLOD calculation program v.9. The absolute limit of quantification (ALOQ) was estimated as the lowest quantity measured with a coefficient of variation (CV) lower than 0.35.

2.4. Human polyomavirus qPCR

As a human viral indicator, we quantified human polyomavirus (HPyV) in sewage samples to rule out failures in the viral concentration, detection, or other sampling issues such as sewage dilution. Quantification was performed in a subset of the samples including those with non-detectable results for SARS-CoV-2 ($n = 62$, Table S1 in Supplementary Material). A qPCR assay, previously developed by our workgroup, that simultaneously quantifies JCPyV and BKPyV targeting the VP2 genetic region was used (Barrios et al., 2018). Briefly, real-time TaqMan PCR assays were performed using the FastStart Universal Probe Master (ROX) mix (Roche Diagnostics), 0.5 of μM S1-JC-BK primer, 0.5 μM of AS2-JC-BK primer, 0.1 μM of probe-JC-BK and 0.3 μg/μl of BSA. Each reaction contained 5 μl of the 50 μl eluted nucleic acids and the reaction volume was adjusted to a final volume of 20 μl with MilliQ water. Thermal cycling (10 min at 95 °C followed by 40 cycles of 15 s at 95 °C, 60 s at 60 °C) was carried out on a Step One Plus (Applied Biosystems) thermocycler. Despite the procedure we applied using TRIzol reagent is indicated for RNA isolation, we were able to co-isolate DNA in the same nucleic acid extracts.

To estimate HPyV concentrations, a standard curve per run was used. It was based on 10-fold serial dilutions (from 1 to 10^5 gc per reaction) of a plasmid DNA containing a JCPyV VP2-VP1 sequence (subtype 2 A), quantified by fluorometric measure (Qubit® 3.0 Fluorometer).

2.5. Bovine coronavirus Mebus strain RT-qPCR

The concentration of the bovine coronavirus (BCoV), Mebus strain, was determined by RT-qPCR as described previously (Cho et al., 2010) in the concentrated samples as well as in the initial sample. Briefly, real-time TaqMan PCR assays were performed using the TaqMan™ Fast Virus 1-Step Master Mix (ThermoFisher Scientific), 0.4 μM of BCoVF primer, 0.4 μM of BCoVR primer, 0.2 μM BCoV-probe and 0.3 μg/μl of BSA. Each reaction contained 5 μl of the 50 μl eluted nucleic acids and the reaction volume was adjusted to a final volume of 20 μl with MilliQ water. Thermal cycling (15 min at 50 °C, 20 s at 95 °C followed by 45 cycles of 15 s at 95 °C, 60 s at 60 °C) was carried out on a Step One Plus (Applied Biosystems) thermocycler. A standard curve was build using 10-fold serial dilutions (from 10^3 to 10^8 equivalent genome copies per reaction) of a DNA copy of a nucleic acid extract of BCoV propagated in cell culture, quantified for total RNA by fluorometric measure (Qubit® 3.0 Fluorometer). Real-time TaqMan PCR assays were performed using the TaqMan™ Fast Virus 1-Step Master Mix (ThermoFisher Scientific) in a Step One Plus thermocycler.

2.6. Viral recovery (VR)

To characterize the concentration process, we estimated the recovery of SARS-CoV-2 and a bovine coronavirus, Mebus strain, that could behave as a potential surrogate. Viral recovery (VR) of both viruses was calculated as the ratio of the total equivalent genome copies (eq gc) in the viral concentrate and the total eq gc in the wastewater sample:

$$VR = \frac{eq\ gc\ in\ viral\ concentrate}{eq\ gc\ in\ wastewater\ sample} \quad (1)$$

In a preliminary assay, BCoV Mebus recovery and its associated variability were calculated in quadruplicate from one sample. Briefly, 800 ml of a wastewater sample belonging to the SP location were spiked with the BCoV Mebus strain before virus concentration, achieving an initial concentration of $2{,}5 \times 10^2$ UFF/ml. Then, the sample was homogenized and processed in four parallel subsamples of 200 ml. Also, to

assess VR variability across different samples, BCoV was used to spike additional wastewater samples ($n = 15$) and viral recovery was also calculated. VR of SARS-CoV-2 was evaluated using non-spiked wastewater samples from point SP. Selected samples ($n = 7$) were those in which the virus was in a concentration high enough, quantifiable in the wastewater sample before the concentration process.

Statistical analyses were performed using RStudio v1.4.1106. Mean, median, percentiles, range, variance, standard deviation, and coefficient of variation of viral recovery were calculated for each virus using the stats package. F test (stats) was used to compare variances and a non-parametric Wilcoxon rank-sum test (stats) was used to compare viral recovery for SARS-CoV-2 and BCoV Mebus. The coefficient of variation test (Zar5) was used to compare the CV of both viruses' recoveries.

Also, to describe viral recovery distributions of SARS-CoV-2 and BCoV, data that could be used in future prevalence studies, the shape parameters of a beta distribution were estimated using the ebeta function from the EnvStats package. Monte Carlo simulations were performed to obtain a random sample ($n = 10{,}000$) of the estimated distributions of both viruses. Beta distributions were then compared using the Two-sample Kolmogorov-Smirnov test (stats package).

2.7. Sample limit of detection (SLOD) and sample limit of quantification (SLOQ)

Sample limit of detection and quantification was calculated as described previously (Rajal et al., 2007) with minor modifications:

$$SLOD = \frac{ALOD}{V_{na,qPCR}} \times \frac{V_{f,\,ext}}{V_{i,\,ext}} \times \frac{V_{conc}}{V_{sample}} \times \frac{1}{VR}$$

$$SLOQ = \frac{ALOQ}{V_{na,qPCR}} \times \frac{V_{f,\,ext}}{V_{i,\,ext}} \times \frac{V_{conc}}{V_{sample}} \times \frac{1}{VR}$$

where ALOD and ALOQ are the absolute limits of detection and quantification of the qPCR, $V_{na,qPCR}$ is the volume of nucleic acids in the qPCR reaction, $V_{f,ext}$ is the final volume of the nucleic acids extraction, $V_{i,ext}$ is the concentrated sample volume used for nucleic acids extraction, V_{conc} is the volume of the viral concentrate, V_{sample} is the volume of the wastewater sample and VR is the viral recovery based on the SARS-CoV-2. All volumes were expressed in ml.

2.8. Calculation of SARS-CoV-2 and human polyomavirus concentration in the original wastewater samples

The concentration of SARS-CoV-2 and HPyV determined in the concentrated samples was used to calculate the concentration of the viruses in the original wastewater samples, following equations, based on that described by Rajal et al. (2007):

$$C_{sample} = \frac{gc_{qPCR}}{V_{na,qPCR}} \times \frac{V_{f,\,ext}}{V_{i,\,ext}} \times \frac{V_{conc}}{V_{sample}} \times \frac{1}{VR}$$

where C_{sample} is the genomic copies of the qPCR reaction, $V_{na,qPCR}$ is the volume of nucleic acids in the qPCR reaction, $V_{f,ext}$ is the final volume of the nucleic acids extraction, $V_{i,ext}$ is the concentrated sample volume used for nucleic acids extraction, V_{conc} is the volume of the viral concentrate, V_{sample} is the volume of the wastewater sample and VR is the mean of the viral recovery based on the SARS-CoV-2 estimation. All volumes were expressed in ml.

A non-parametric Wilcoxon rank-sum test (stats) was used to compare SARS-CoV-2 and HPyV concentrations in wastewater and correlation analyses were performed using the Spearman test package using RStudio v1.4.1106.

2.9. SARS-CoV-2 genome concentration in wastewaters and its relationship with COVID-19 cases

The cumulative number of COVID-19 cases was calculated for the district San Vicente (AK + SV) and for the individual sampling points for the different time spans: 10, 15 and 20 days. Information regarding the number of COVID-19 cases was provided by the Sanitary Authorities of Buenos Aires Province. The correlation between COVID-19 cumulative cases and SARS-CoV-2 viral titers in wastewater was evaluated with the Spearman correlation test (Spearman's rank correlation coefficient rho, ρ) using the GraphPad Prism V5 software. The correlation coefficient ranges from −1 (perfect negative correlation) to +1 (perfect positive correlation). The criterion used in the comparisons for intermediate values was: $\rho \geq 0.7$, strong correlation; $0.7 < \rho \geq 0.5$, moderate correlation and $0.5 < \rho \geq 0.3$, weak correlation. However, this correlation analysis could not be carried out for the neighborhoods where SP and SF are located, given the lack of epidemiological data.

In the correlation analyses samples where SARS-CoV-2 was detected below the ALOQ, a value of half of the SLOQ (median) was used. Instead, for those samples where SARS-CoV-2 could not be detected or was detected below the ALOD95%, half of the SLOD (median) was used.

3. Results

3.1. Viral concentration

Viruses present in wastewater samples were concentrated by precipitation with polyethylene glycol and SARS-CoV-2 and human polyomavirus JCPyV and BKPyV genomes were quantified by RT-qPCR and qPCR, respectively. Several authors demonstrated the association of viruses with solids in sewage (Arraj et al., 2005; Ye et al., 2016), particularly for enveloped viruses such as murine coronavirus. Therefore, we concentrated the entire matrix of the wastewater samples without prior clarification and extracted the viral nucleic acids from the pellet with several purification steps to remove the qPCR inhibitors.

To characterize the whole procedure from viral concentration to quantification, we determined viral recovery for SARS-CoV-2 and a potential surrogate virus: a bovine coronavirus, Mebus strain (Table 1). Since viral recovery is highly variable, we compared its performance within and between samples using BCoV Mebus. In a preliminary assay, viral recovery in a single sample tested in quadruplicate was evaluated. The median of BCoV Mebus VR in this assay was 0.155 (95% CI = 0.124-0.338) with a coefficient of variation of 0.573.

Then viral recovery was estimated between different wastewater samples for SARS-CoV-2 ($n = 7$) and BCoV Mebus ($n = 15$). VR median for SARS-CoV-2 and BCoV were 0.085 (95% CI = 0.021-0.179) and 0.262 (95% CI: 1.18×10^{-5}-0.564) respectively. When comparing process performance for SARS-CoV-2 and BCoV Mebus, we found that viral recovery and the coefficient of variation were not significantly different among both viruses (p-value of 0.078 and 0.647, respectively). Also, recovery and coefficient of variation of BCoV Mebus recovery within one sample and between samples (n = 15) were not significantly different (p-value of 0.736 and 0.655 respectively). Recovery of human polyomaviruses (JC and BK) could not be calculated since their viral load was not quantifiable prior to concentration in 22 assayed samples (data not shown).

Because of its high variability, viral recovery is often better represented by beta distributions, data that can also be used in future prevalence studies. Empiric data was then used to estimate shape parameters of a beta distribution to represent viral recovery. Distributions were then simulated (10,000 mc simulations) and their corresponding statistics were obtained (Table 1). Although empiric recovery data were not significantly different between SARS-CoV-2 and BCoV Mebus, the corresponding estimated beta distributions did differ (p-value $= 2.2 \times 10^{-16}$).

Table 1
Virus recovery of SARS-CoV-2 and its potential surrogate BCoV Mebus.

Virus recovery – empirical data									
Virus	n	Min	5% percentile	Median	Mean	95% percentile	Max	Standard deviation	Coefficient of variance
SARS-CoV-2	7	0.004	0.021	0.085	0.098	0.179	0.194	0.061	0.625
BCoV Mebus	4[a]	0.120	0.124	0.155	0.200	0.338	0.370	0.115	0.573
	15	1.18×10^{-5}	2.29×10^{-5}	0.262	0.271	0.564	0.823	0.215	0.795
Virus recovery – simulated data									
	n	Min	5% percentile	Median	Mean	95% percentile	Max	Shape 1 (α)	Shape 2 (β)
SARS-CoV-2	10,000	3.18×10^{-5}	0.010	0.077	0.096	0.244	0.594	1.352	12.78
BCoV Mebus	10,000	1×10^{-6}	4.76×10^{-4}	0.131	0.232	0.777	0.999	0.426	1.355

[a] Single sample tested in quadruplicate.

3.2. Viral detection and quantification

SARS-CoV-2 genome was detected and quantified by RT-qPCR using CDC N1 primers and probe set (Lu et al., 2020) which was originally designed for clinical samples. In the wastewater samples studied in this work, the ALOD95% of the implemented technique was estimated as 2.6 gc per reaction, (95% CI = 1.3–5.2). The limit of quantification (ALOQ) has been estimated as 10.0 gc per reaction (CV = 28.4%).

The sample limit of detection (SLOD) is the minimum viral concentration in the original sample that could be detected after the concentration procedure. It depends not only on the sensitivity of the RT-qPCR but also on the variables of the whole molecular detection and the characteristics of the samples that affect the concentration process. Considering an RT-qPCR ALOD of 2.6 gc and a median viral recovery for SARS-CoV-2 of 0.085, the sample limit of detection of this procedure was 1.53 gc/ml with a 95% CI of 0.73-6.19 gc/ml which reflects dispersion in the viral recovery factor.

A total of 172 samples (43 per sample site) were analyzed and SARS-CoV-2 was detected in 92.44% of them (159/172 samples) (Table S1 in Supplementary Material). SARS-CoV-2 genome was detected in all sewage samples from AK and SV, with concentrations between 0.18 and 792.54 gc/ml in AK and between 0.17 and 521.91 gc/ml in SV (Table 2). Meanwhile, viral genome was detected in 95.35% of SP samples (41/43 samples with concentrations between 0.60 and 1784.97 gc/ml) and 74.42% of SF samples (32/43 samples with concentrations between 0.2 and 472.31 gc/ml) (Table 2).

Samples were collected during established outbreaks in the BAMR region, the dynamics of SARS-CoV-2 concentration in sewage are shown in Fig. 2.

We also analyzed the presence of human polyomavirus in 62 samples, especially in those where SARS-CoV-2 RNA was non-detectable. These viruses are excreted by a high proportion of the population, persistently infected. Due to their high concentration and constant presence in wastewater, they are used to track human fecal/urine contamination. In most of the samples (11/12) where SARS-CoV-2 RNA was non-detectable, we were still able to detect human polyomavirus. Levels of HPyV ranged between 0.15 and 4.72×10^5 gc/ml and did not correlate with SARS-CoV-2 load (p-value = 0.398). HPyV concentration in these samples could be higher than reported since its recovery could be lower than SARS-CoV-2 recovery used to correct viral loads due to the extraction procedure directed to RNA viruses. The concentration of HPyV genomes was not significantly different between samples of AK, SV, and SF, but the concentration in SP samples was significantly higher than in the other sample points (Table 2). From 62 samples analyzed for HPyV, only two samples belonging to SF were negative, showing the human fecal/urinary source of the samples and that HPyV detection performed well despite the nucleic acid extraction method was primarily intended to recover RNA. The lack of viral detection in those two samples could be due to wastewater dilution since samples were collected from a manhole liable to receive runoff water.

3.3. Correlations between viral concentration in wastewater and clinical cases of COVID-19

Correlations between the concentration of SARS-CoV-2 RNA in wastewater and the cumulative number of COVID-19 cases were evaluated for the whole district San Vicente and for the individual locations AK and SV. We analyzed three different span times of cumulative COVID-19 cases: 10, 15, and 20 days, since the mean time of fecal shedding is highly variable (van Doorn et al., 2020) and the viral RNA decay in wastewater was described to cover a wide range, from 8.04 to 27.8 days for a 1-log reduction in viral concentration (Ahmed et al., 2020b). Correlations were compared using Spearman's rank correlation coefficient rho (ρ) following the criteria described in the Material and Methods section. Correlations were observed for all assessed span times and locations, with different strengths (Table 3, and scatterplots of the correlations are shown in Fig. S1 in Supplementary Material). AK sampling location showed a weak correlation with cumulative

Table 2
Statistical analysis of viral loads in wastewater samples.

Virus	HPyV (gc/ml)				SARS-CoV-2 (gc/ml)			
Sampling site	AK	SF	SP	SV	AK	SF	SP	SV
Minimum	0.96	0.15	0.2	0.25	0.18	0.10	0.59	0.17
1st quartil	3.52	1.96	111.70	0.62	8.99	0.41	4.46	12.89
Median	65.58	27.50	248.0	15.45	21.23	1.74	30.71	35.49
Mean	142.05	182.72	32,249.20	115.04	59.75	35.22	266.05	63.17
3rd quartil	280.75	156.03	1640.10	65.91	56.21	11.46	428.69	86.55
Maximum	414.65	1279.95	471,735.3	1032.06	792.54	472.31	1784.97	521.91
Wilcox test	vs SF: p-value: 0.486 vs SP: p-value: 0.073 vs SV: p-value: 0.193	vs SP: p-value: 0.009 vs SV: p-value: 0.518	vs SV: p-value: 0.007		vs SF: p-value: 5.26×10^{-5} vs SP: p-value: 0.452 vs SV: p-value: 0.414	vs SP: p-value: 3.55×10^{-5} vs SV: p-value: 8.54×10^{-6}	vs SV: p-value: 0.735	

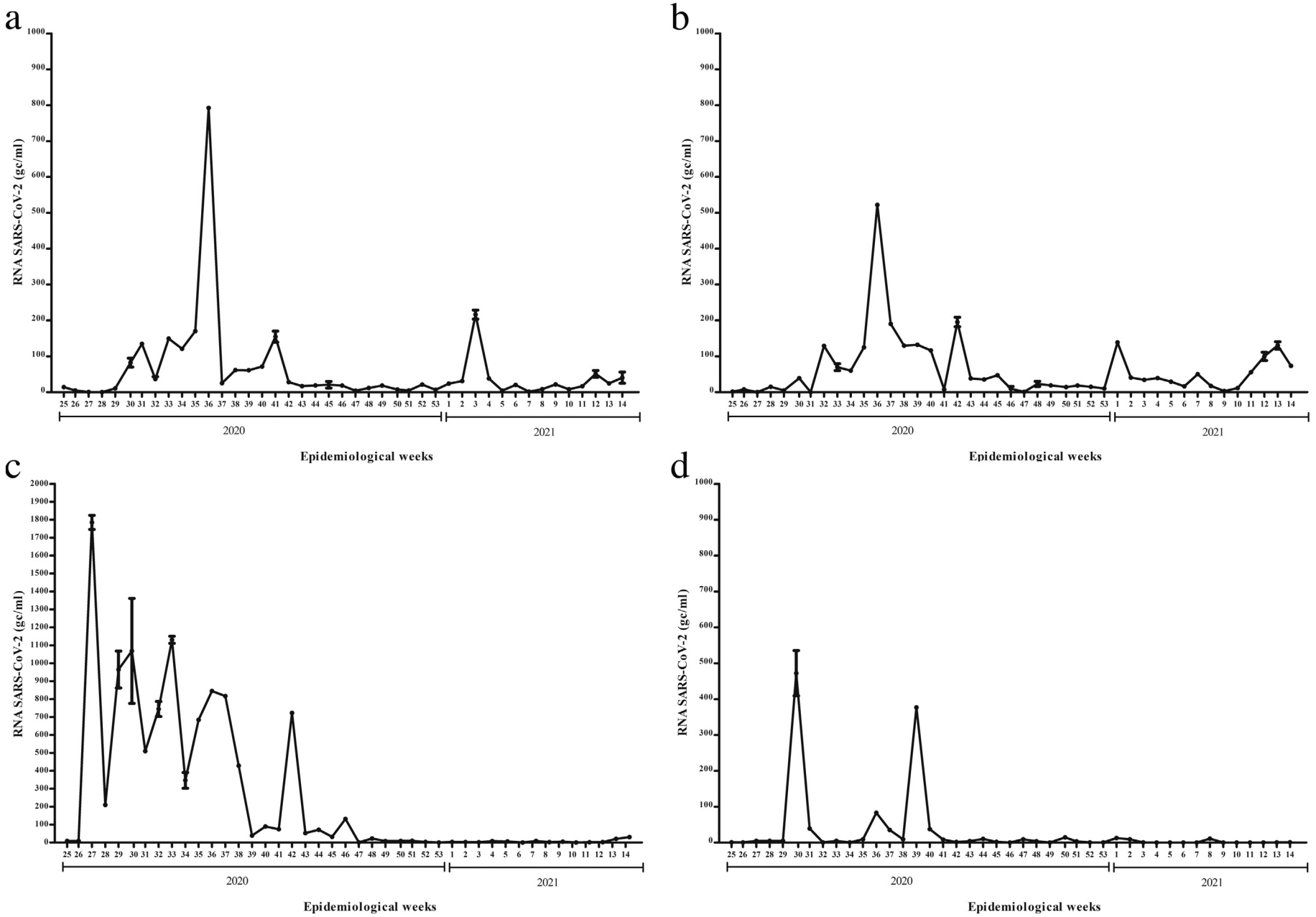

Fig. 2. Dynamics of SARS-CoV-2 RNA concentration along the studied period.
Line graph showing SARS-CoV-2 RNA concentration (virus RNA gene copies per ml of wastewater) in the different locations: a) AK b) SV c) SF d) SP in the epidemiological weeks.

Table 3
Correlation between the viral RNA concentrations in wastewater and the cumulative number of cases for different span times.

	AK		SV		AK + SV	
	ρ	*p* Value	ρ	p Value	ρ	p Value
10 days	0.476	0.001	0.693	2.55×10^{-7}	0.795	1.98×10^{-10}
15 days	0.443	0.003	0.727	3.44×10^{-8}	0.807	6.23×10^{-11}
20 days	0.499	0.001	0.716	6.73×10^{-8}	0.812	4.085×10^{-11}

COVID-19 cases ($\rho = 0.443$-0.499), but SV and AK + SV showed strong correlations ($\rho = 0.7$-0.8). The strongest correlation was observed for the whole district (AK + SV) and the 20-day cumulative COVID-19 cases ($\rho = 0.812$).

The time course of SARS-CoV-2 RNA concentration in wastewater and the cumulative COVID-19 cases are shown in Fig. 3. Viral concentrations in wastewater reflected the dynamics of clinical information, with increases and decreases preceding those of COVID-19 cases. Information about COVID-19 cases for SF and SP neighborhoods was not available so correlation analysis with SARS-CoV-2 concentration in wastewater was not performed.

4. Discussion

The management of water and sanitation in Latin America is a matter of concern since there are regions where households lack a sewer connection, furthermore, less than 50% of the collected wastewater is adequately treated. However, during the last years, improvements have been made (Economic Commission for Latin America and the Caribbean, (ECLAC), 2019). Successful public interventions will benefit from the interactions with academic and research institutions. The pandemic of COVID-19 provided an opportunity for this cooperation in many countries where the researchers offered the results of the wastewater-based epidemiological study of SARS-CoV-2. The first regional wide monitoring was reported by Prado et al., which rendered a tool as a complementary indicator in the surveillance of COVID-19 cases in Niterói city, Brazil (Prado et al., 2020, 2021).

The present study reported a pilot wastewater-based epidemiological survey of SARS-CoV-2 in three districts of the Buenos Aires Metropolitan Region, the main affected area in Argentina, in a nine-month longitudinal analysis to track SARS-CoV-2 dynamics. The general strategy included the weekly collection of grab samples taken at the entry of the WWTPs and manholes, viral concentration by PEG/NaCl precipitation and genome quantification by RT-qPCR. The correlation with the epidemiological information of the catchment area of the WWTPs was analyzed.

Some checkpoints of the entire process were considered. First, SARS-CoV-2 recovery of the concentration-detection procedure was characterized without spiking samples. This approach, also used by other authors (Bertrand et al., 2021), was possible during the exponential phase of transmission when virus concentration was high enough to be quantifiable before concentration. We used the median recovery of SARS-CoV-2 to calculate the viral concentration in the original samples, which avoids an underestimation of the viral concentrations. However, virus recovery from environmental samples shows a wide range and therefore the goal would be to calculate the recovery for each sample. This could be performed from now on using an appropriate surrogate virus such as BCoV Mebus. BCoV is a member of the *Betacoronavirus* genus, as well as SARS-CoV-2. Both viruses share structural characteristics, but they are genetically distant enough to be differentiated by species-specific qPCR assays, which allows simultaneous quantification of both viruses in the same sample without cross-reactions. Structural similarities contribute to their similar performance during viral concentration procedures, making BCoV a good surrogate virus for SARS-CoV-2. Since the virus can be grown and titrated in tissue culture, the production of laboratory working stocks is feasible. Another study recently published also proposes the use of a bovine coronavirus as a surrogate for SARS-CoV-2 (LaTurner et al., 2021). In the present study, the recovery values of BCoV were higher than those of SARS-CoV-2, although not statistically significant. This could be explained either by the concentration levels or by the ratio of RNA/viral particles, which could be higher for BCoV.

As another checkpoint, we quantified human polyomaviruses JCPyV and BKPyV, as viral indicators, associated with human fecal/urine contamination, especially in those samples where SARS-CoV-2 was not detected. Detection and quantification of these viruses' load (JC and BK in this work) can account for sewage dilutions due to runoffs, rain and pipe infiltrations that can occur along the sewage system. These variations can also cause fluctuation in SARS-CoV-2 loads in sewage besides COVID-19 cases in the community. Also, they can account for failures in the viral concentration or detection inhibitions. In this study, these viral controls performed well for the warning of such events.

As the main goal of this survey, the concentration of SARS-CoV-2 genome in wastewater was measured and it ranged from 10^{-1} to 10^{3} gc/ml, values within the range reported by other authors (Ahmed et al., 2020a; Randazzo et al., 2020; Wu et al., 2020; Wurtzer et al., 2020). This broad range is comprehensible since it depends not only on the epidemiological situation of the outbreak but also on the sampled sewer system, type of collection site, and viral recovery of the concentration method.

Dynamics of SARS-CoV-2 in the larger WWTP studied (AK and SV) correlated with the number of COVID-19 cases. However, changes in the concentration of SARS-CoV-2 in samples from the smaller communities (SF and SP) did not exhibit clear tendencies. SP wastewater presented the highest concentrations of SARS-CoV-2 and HPyV despite been a small catchment area; it showed to be less susceptible to stormwater runoff and other dilution events. It must be considered that the SP WWTP collects mainly household wastes; meanwhile, AK

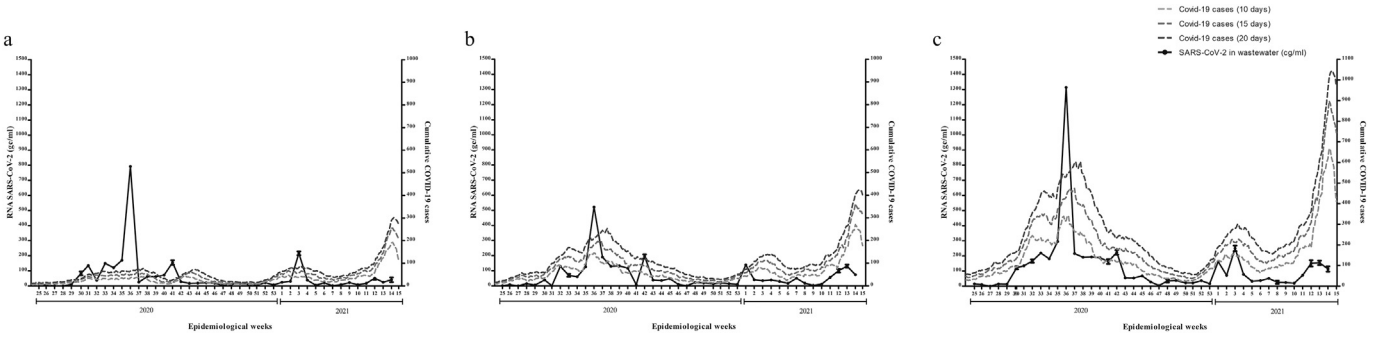

Fig. 3. Time course of SARS-CoV-2 RNA concentration in wastewater and the cumulative COVID-19 cases.
Line graph showing SARS-CoV-2 RNA concentration (virus RNA gene copies per ml of wastewater, y-axis on the left) in the different locations: a) AK, b) SV c) District SV (AK + SV). The COVID-19 cases in these locations (y-axis on the right), accumulated during periods of 10, 15 and 20 days are shown in dashed lines from light to dark gray, respectively, along the epidemiological weeks.

and SV also collect wastes from stores and small industries. Samples collected from a manhole, in SF, showed reduced viral concentrations, with some undetectable results. The scarce resident time and lack of the mixing of the sewage flows at this point of the sewer systems, added to the contribution of runoff water from rainfall and other urban fluids may be responsible for this random concentration of SARS-CoV-2 RNA.

Then, in addition to a real difference in viral excretion among the populations, dilution effects on the wastewaters due to the commercial activities or run-off water, or a non-characterized negative effect on the viral concentration/detection processes cannot be ruled out.

In this study, more consistent results were obtained when samples were collected from the inlet pipes of the WWTP than to the manhole. The waste homogenization obtained in these downstream collection points could have compensated for the lack of a composite sample, which may not occur when moving upstream to collect samples from the manholes. Unfortunately, epidemiological information representing the small communities of SP and SF neighborhoods was not available for this study.

When comparing levels of SARS-CoV-2 in wastewater to the cumulative number of cases, correlation strength may also be affected by eventual changes in testing policy throughout the study. Despite these limitations, a clear and significant correlation was observed between the SARS-CoV-2 RNA concentration in wastewater samples and the cumulative number of COVID-19 cases in the district San Vicente in the Southern Buenos Aires Metropolitan Region. Given that the two sampling points in this district were closely located, the movement of individuals between them for commercial and industrial purposes and medical assistance can justify that the region behaves epidemiologically better as a unit, which is supported by the correlations that were stronger for the entire district than for the individual localities ($\rho = 0.795$ to 0.812 vs $\rho = 0.443$ to 0.727).

In this study, the best correlation between the viral RNA concentrations in wastewater and the clinical data was obtained for a 20-day span time of reported COVID-19 cases. A long duration of viral shedding in feces has been informed, even for several weeks, however, the integrity and thus, the detection of viral genomes in wastewater matrices can be altered over time. The decay of the viral RNA excreted in wastewater was shown to vary in a wide range (Ahmed et al., 2020b). Then, the appropriate period of accumulated cases to evaluate in these studies should be a balance between the duration of fecal viral excretion and the decay of viral RNA in these environments. According to our results, the cumulative number of cases in periods of 20 days correlates strongly with the viral RNA quantified in wastewaters ($\rho = 0.812$), as other authors also found (Medema et al., 2020b). This was also observed for shorter periods of 10 and 15 days ($\rho = 0.795$-0.807). Correlation between daily or weekly COVID-19 cases and SARS-CoV-2 concentration in wastewater per capita per day has also been described recently (Weidhaas et al., 2021).

Viral concentrations in wastewater reflected the exponential increase in the number of reported COVID-19 cases between July and mid-September. The increment of SARS-CoV-2 genome concentration in wastewater preceded the increases in the COVID-19 cases as it was also reported in other studies (Ahmed et al., 2021; Panchal et al., 2021; Randazzo et al., 2020). The earlier trends in the viral dynamics observed in wastewaters can be explained by the early excretion of viral genomes in feces of infected persons, even in pre-symptomatic stages, the eventual delay in the clinical testing and reports, and the number of asymptomatic cases in every population. The reduction in the cases was also evidenced by the decline in viral concentration in the effluents after mid-September, preannounced by the viral genome dynamics in the effluents. Wastewater viral concentration and the number of cases showed a clear increase since the third week of December, probably due to social gatherings around Christmas and New Year festivities. The beginning of the second wave of COVID-19 cases was evident by March of 2021, along with the rise in viral RNA concentration in wastewater. The dynamics in this district are representative of the epidemiological behavior of the entire Buenos Aires Metropolitan Region.

5. Conclusion

Sewage surveillance of SARS-CoV-2 reflected the population excretion dynamics into the wastewater system. It will be very useful when the number of COVID-19 cases decreases and mass vaccination can be achieved, as it will early warn about an increase in viral circulation in the population. However, differences in the dynamics in different sewer systems can be the results not only of the spread of the virus in the community but also of the intrinsic variables of each sewer. These results can assist local authorities in taking actions of public health, enabling the cooperation between research and management institutions.

CRediT authorship contribution statement

Melina Elizabeth Barrios: Methodology, Formal analysis, Investigation, Writing – original draft, Writing – review & editing. **Sofía Micaela Díaz:** Methodology, Investigation, Formal analysis. **Carolina Torres:** Methodology, Investigation, Formal analysis. **Damián Matías Costamagna:** Resources. **María Dolores Blanco Fernández:** Formal analysis, Writing – original draft, Writing – review & editing. **Viviana Andrea Mbayed:** Conceptualization, Investigation, Resources, Writing – original draft, Writing – review & editing, 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.

Acknowledgments

This work was possible thanks to the collaboration with several organizations of the Buenos Aires Province Government in charge of public and environmental health, the wastewater treatment company, and the Ministry of Science, Technology, and Innovation of Argentina. Then, we acknowledge the contribution of Aguas Bonaerenses S.A. (ABSA) and in particular Eng. Luis Bruzzone, Autoridad del Agua (ADA), Organismo Provincial para el Desarrollo Sostenible (OPDS) and the Dirección de Vigilancia epidemiológica y control de brotes, Ministerio de Salud de la Provincia de Buenos Aires, for the provision of samples and epidemiological information. We also thank the Ministerio de Ciencia, Tecnología e Innovación de Argentina (MinCyT) for its collaboration in coordinating institutional interactions. We express our gratitude to Dr. V. Parreño and M. Bok, from the Instituto Nacional de Tecnología Agropecuaria (CICV y A, INTA Castelar) for the provision of the bovine coronavirus stock.

Funding

This work was supported by the Agencia Nacional de Promoción de la Investigación, el Desarrollo Tecnológico y la Innovación, MINCyT (IP-COVID-19 233 and 785), Argentina.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.scitotenv.2021.149578.

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