

### Presence of SARS-CoV-2 in urban effluents in southeast Buenos Aires, Argentina, May 2020 to March 2022

Carlos Cimmino<sup>1</sup>, Leandro Rodrigues Capítulo<sup>2</sup>, Andrea Lerman<sup>1</sup>, Andrea Silva<sup>1</sup>, Gabriela Von Haften<sup>3</sup>, Ana P. Comino<sup>3</sup>, Luciana Cigoy<sup>3</sup>, Marcelo Scagliola<sup>3</sup>, Verónica Poncet<sup>1</sup>, Gonzalo Caló<sup>4</sup>, Osvaldo Uez<sup>1</sup>, and Corina M. Berón4

Suggested citation Cimmino C; Rodrigues Capítulo L; Lerman A; Silva A; Von Haften G; Comino AP, et al. Presence of SARS-CoV-2 in urban effluents in south-east Buenos Aires, Argentina, May 2020 to March 2022. Rev Panam Salud Publica. 2023;47:e94. https://doi.org/10.26633/RPSP.2023.94

#### **ABSTRACT**

**Objectives.** To implement and evaluate the use of wastewater sampling for detection of severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) in two coastal districts of Buenos Aires Province, Argentina. Methods. In General Pueyrredon district, 400 mL of wastewater samples were taken with an automatic sampler for 24 hours, while in Pinamar district, 20 L in total (2.2 L at 20-minute intervals) were taken. Samples were collected once a week. The samples were concentrated based on flocculation using polyaluminum chloride. RNA purification and target gene amplification and detection were performed using reverse transcription polymerase chain reaction for clinical diagnosis of human nasopharyngeal swabs.

Results. In both districts, the presence of SARS-CoV-2 was detected in wastewater. In General Pueyrredon, SARS-CoV-2 was detected in epidemiological week 28, 2020, which was 20 days before the start of an increase in coronavirus virus disease 2019 (COVID-19) cases in the first wave (epidemiological week 31) and 9 weeks before the maximum number of laboratory-confirmed COVID-19 cases was recorded. In Pinamar district, the virus genome was detected in epidemiological week 51, 2020 but it was not possible to carry out the sampling again until epidemiological week 4, 2022, when viral circulation was again detected.

Conclusions. It was possible to detect SARS-CoV-2 virus genome in wastewater, demonstrating the usefulness of the application of wastewater epidemiology for long-term SARS-CoV-2 detection and monitoring.

#### **Keywords**

SARS-CoV-2; wastewater; disease outbreaks; environmental monitoring; Argentina.

The etiological agent of coronavirus disease 2019 (COVID-19) is severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), which belongs to a large family of single-strand RNA viruses that can infect animals including humans. SARS-CoV-2 infection in humans mainly presents as respiratory, gastrointestinal, hepatic, and neurological diseases. The virus can spread from an infected person in the form of small liquid particles (1). SARS-CoV-2 RNA has been detected in upper and lower respiratory tract fluids and also in the stool, blood, and urine of infected people. The presence of the virus in feces seems to be independent of the presence of gastrointestinal symptoms (2).

the extent of infection circulating in the human population. The number of positive cases of COVID-19 gives some measure of disease prevalence in the population; however, it only provides information about people who have been tested and have shown symptoms of the disease. Asymptomatic carriers who do not have any symptoms of COVID-19 may still be infected and can spread the infection to other people without knowing. Therefore, relying solely on the number of positive cases can underestimate the true prevalence of the virus in the population. Other measures, such as

Effective surveillance of SARS-CoV-2 is vital to estimate



This is an open access article distributed under the terms of the Creative Commons Attribution-NonCommercial-NoDerivs 3.0 IGO License, which permits use, distribution, and reproduction in any medium, provided the original work is properly cited. No modifications or commercial use of this article are permitted. In any reproduction of this article there should not be any suggestion that PAHO or this article endorse any specific organization or products. The use of the PAHO logo is not permitted. This notice should be preserved along with the article's original URL. Open access logo and text by PLoS, under the Creative Commons Attribution-Share Alike 3.0



Instituto Nacional de Epidemiología "Dr. Juan H. Jara", Mar del Plata, Argentina.

Centro de Estudios Integrales de la Dinámica Exógena, Universidad Nacional de La Plata, La Plata, Argentina.

Obras Sanitarias Sociedad de Estado, Mar del Plata, Argentina.

Instituto de Investigaciones en Biodiversidad y Biotecnología and FIBA, Mar del Plata, Argentina. Argentina. Corina M Berón, corina.beron@inbiotec-conicet.gob.ar

testing, contact tracing, and monitoring of the spread of the disease, are necessary to obtain a more accurate picture of its prevalence (3).

The use of wastewater to detect human enteric viruses began with the detection of polyomavirus; since then, different DNA and RNA viruses have been found in such samples (4, 5). Viral nucleic acid particles can be protected by organic components in untreated wastewater, and can be used to monitor and characterize the prevalence of the human virome (collection of viruses in and on the human body) through polymerase chain reaction (PCR) amplification of virus-specific sequences (5, 6), especially in populations where health systems do not allow more specific and expensive monitoring (7).

As SARS-CoV-2 is shed in feces in relatively high amounts (8), wastewater analysis can provide a useful indicator of disease incidence at any point in time, particularly as most urban centers are served by only one or two centralized wastewater treatment plants. This provides a single integrated signal for up to millions of people in just a few samples (9). As such, laboratories in many countries have evaluated the detection of SARS-CoV-2 in wastewater, envisaging the possibility of using this measure as an early warning of virus circulation in the community (9–11).

During the pandemic, the most critical moment was at the beginning of 2020, when it was essential to know the number of active cases of COVID-19 so as to take the appropriate public health measures to limit its spread, for example, whether to enforce mandatory isolation or to allow people to travel. However, at this time, the hospital and laboratory supplies required for diagnosis of SARS-CoV-2 infection were insufficient in almost all countries. Given this situation, the possibility of detecting viral circulation in a few wastewater samples was proposed as an effective epidemiological tool. Such a tool could be a quick, sensitive, and reasonable option to monitor the spread of the virus in the population. However, wastewater samples are heterogeneous, and the first important step is wastewater concentration. Therefore, many of the proposed protocols included the use of costly high-tech equipment and supplies, such ultrafiltration or the use of electronegative membranes (9, 12–15), which are not always available in all areas. RNA extraction and specific gene amplification steps also have methodological challenges, especially the presence of inhibitors, sensitivity of the technique, and its reproducibility (16–18). All the steps have to be carried out in laboratories with a high biological safety level, which are not available in all countries or regions.

In this context, the objective of this study was to implement and evaluate appropriate low-cost protocols for the sampling and concentration of wastewater from different locations on the south-east coast of the province of Buenos Aires, Argentina, in order to detect SARS-CoV-2.

#### **METHODS**

## Sampling sites and collection of wastewater samples

In this study, untreated wastewater samples were collected from effluent treatment plants in two coastal districts in the south-east of the Buenos Aires Province, Argentina. The first plant, run by the Obras Sanitarias Sociedad de

Estado company, was in Mar del Plata, the largest coastal city in General Pueyrredon district (Figure 1), with a population of 682 605 inhabitants. The district covers a total surface area of 1 453.44 km², of which Mar del Plata urban area covers 79.48 km². Mar del Plata is an important tourist center in Argentina, and receives more than 8 000 000 tourists each year, mainly from December to March (the summer season). General Pueyrredon district is also an important fishing port and has food, textile, and metalwork industries. The area is also one of the main production centers for horticultural soils in the country.

The wastewater effluent network of General Pueyrredon is shown in Figure 2A. The pumping stations, located in low points of the city, receive the wastewater liquids that arrive by gravity from different watersheds. The liquids are then pumped out to the maximum collectors. The sampling zone was a point before the effluent treatment stage (Figure 2A), where 97% of the urban effluent from homes and industries connected to the system is concentrated. Given the variation in the population size (depending on the tourist season) and industrial activities, the volume and heterogeneity of the effluent varies considerably by time.

The second study site was in the district of Pinamar (Figure 1), which is a seaside area 345 km from Buenos Aires city. It has a total area of 66 km<sup>2</sup>, and a stable population of 40 259 inhabitants. The business of Pinamar is tourism and about 2 900 000 tourists a year visit the area during the summer season. In spite of its extensive beach front and clean sand, the general infrastructure is quite limited and only the 18% of the population is connected to the wastewater network. This network dates back to the 1940s and has not been updated since then (Figure 2B). The town with the greatest sanitation coverage is Pinamar, followed by Ostende, Valeria del Mar, and the coastal area of Cariló. The wastewater system consists of fiber cement pipelines that transport the effluent by gravity to a series of coastal enclosures (19). These enclosures are interconnected to a central chamber from which the effluent is conveyed to the wastewater treatment plant. The effluent access consists of seven pipes that discharge, simultaneously, into a central storage chamber, and from there to a series of aeration lagoons. The wastewater treatment plant is run by the Cooperativa de Agua y Luz Pinamar and treats around 4 000 m<sup>3</sup> and 14 000 m<sup>3</sup> of effluent daily during the winter and summer seasons, respectively. Finally, the treated effluent permeates to infiltration ponds located in the south, which occupy a surface area of 26 hectares and represent 40% of the annual recharge of the aquifer (20). The sewage effluent samples were taken at the sewage treatment plant inlet pipe where the seven pipes mentioned above are mixed.

Samples were collected using two types of sampling techniques. In the General Pueyrredon wastewater treatment plant, samples were collected weekly for 24 hours from May 2020 to March 2022 using an automatic sampler located at the entrance of the plant (Figure 3, panels A and B). A sample of 400 mL of wastewater water was collected between Saturday mornings and Sunday mornings to avoid industrial effluents. At Pinamar, samples were taken manually from the open mixing pipe by drawing a total equivalent volume of 20 L at 20-minute intervals (2.2 L per sample) between 09:00 and 12:00 on Fridays during the summers of 2020–2021 and 2021–2022 (Figure 3, panel C).

**Buenos Aire** city Rio de La Plata **Estuary** Study Area Samborombon bay 36° 18.504' S -**Buenos Aires Province** Pinamar city Atlantic Ocean Mar del Plata City Kilometers

FIGURE 1. Sampling areas in the province of Buenos Aires, Argentina

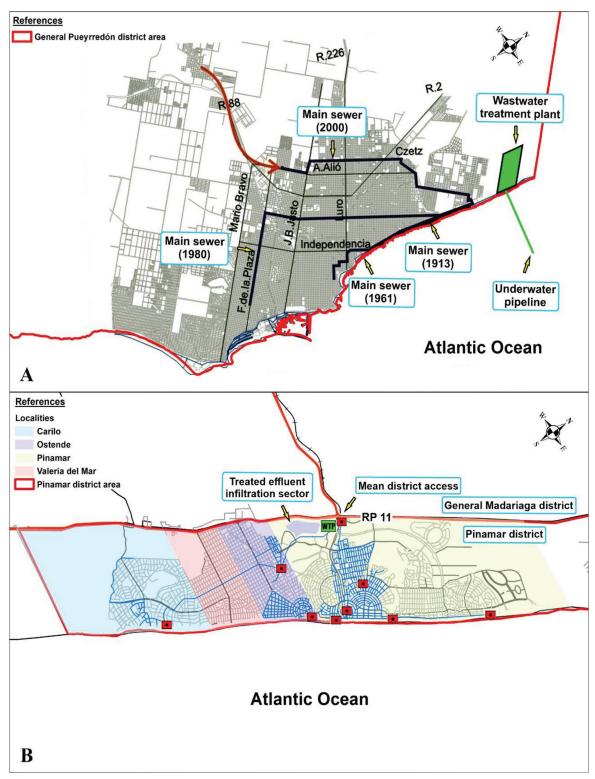
Source: prepared by authors

### Sample concentration and RNA extraction

Concentration of SARS-CoV-2 genetic material from the wastewater collected at the General Pueyrredon plant was carried out by aluminum-driven flocculation according to the Standard Methods for the Examination of Water and Wastewater (21) and Randazzo et al. (22). For this, 400 mL water samples were adjusted to pH 6.0 and precipitated with aluminum hydroxide. The final pellet was resuspended in 1 mL of phosphate-buffered saline. Wastewater concentration was performed six times. Samples from the wastewater treatment plant in Pinamar could not be concentrated there because of the lack of adequate equipment. Therefore, at the beginning of the sampling, an attempt to concentrate the solids in the wastewater was made using a commercially available flocculant (Figure 3, panels C-H). For this, 5 mL of 15% aluminum chloride solution (Clorotec Clarificador®, Escobar, Buenos Aires, Argentina) was dissolved in 20 L of untreated water effluent. Then, the samples were left to decant for 2 h at room temperature and out of sunlight to avoid re-suspension of the flocs. The supernatant was discarded and the precipitated sludge (360 mL) was collected in three sterile 120 mL containers each. Wastewater concentration was performed twice. The samples were labeled and transported to the Administración Nacional de Laboratorios e Institutos de Salud-Instituto Nacional de Epidemiología J.H. Jara in Mar del Plata in portable coolers, according to the WHO biosafety recommendations. In order to compare protocols, some of the General Pueyrredon wastewater water samples were concentrated using the polyaluminum chloride flocculation protocol described by Wehrendt et al. (23).

The samples from Pinamar taken in 2022 were concentrated according to the Wehrendt et al. protocol (23). For this step, 250  $\mu$ L of each sample was used to perform the RNA purification using the TRIzol<sup>TM</sup> reagent (Invitrogen<sup>TM</sup>, Waltham, MA, USA) protocol, followed by RNA isolation using QIAamp R Viral RNA Mini Kit (QIAGEN, Hilden, Germany) according to the manufacturer's instructions.

FIGURE 2. Wastewater effluent networks in the two districts sampled, Argentina: A) General Pueyrredon; B) Pinamar



**Notes:** Panel B. The blue lines indicate the route of the wastewater pipelines which carry the effluent to the discharge chambers (red rectangles), and from there to the municipal treatment plant (green rectangle). Finally, the treated effluent goes to the infiltration ponds. **Source:** prepared by authors

FIGURE 3. Wastewater sampling of two districts, Argentina: A) and B) automatic sampler in General Pueyrredon wastewater treatment plant; C–H) wastewater treatment plant-Cooperativa de Agua y Luz Pinamar, Pinamar, C) extraction of sewage effluent samples, D) flocculant addition, E) effluent homogenization, F) pH and temperature determination, G) appearance of the concentrate after 3 hours, H) conditioning of the samples for transfer to the laboratory in Mar del Plata.



Source: all photographs were taken by the authors and are unpublished.

### **Reverse transcription PCR analysis**

The following reverse transcription PCR (RT-PCR) analyses were done. For SARS-CoV-2 RNA detection in the wastewater samples, the Real-Time Fluorescent RT-PCR Kit (BGI, Cambridge, MA, USA) was used to amplify SARS-CoV-2 ORF1ab gene, including amplification of human  $\beta$ -actin gene as an internal control according to the manufacturer's instructions. E and

RdRp genes were also detected, using qScript XLT One-Step RT-qPCR ToughMix 2X (Quanta BioSciences, Inc., Gaithersburg, MD, USA) which contained 0.4  $\mu$ M of each primer, 0.25  $\mu$ M of probe and 5  $\mu$ L of template RNA. AccuPower® SARS-CoV-2 Multiplex Real-Time RT-PCR Kit (Cat. No. SCVM-2112, Daejeon, Republic of Korea), which is able to detect E, RdRp and N genes, was used following manufacturer's guidelines. Additionally, DiaplexQ Novel Coronavirus (SolGent Co. Ltd,

Daejeon, Republic of Korea), with Orf1a and N target genes and an internal positive control, was used as test validation. All RT-PCR amplifications were performed in 20  $\mu L$  of reaction mixtures. These detection systems changed due to the difficulties in obtaining supplies during the project.

The RT-PCR assays were performed using a Bio-Rad CFX96 thermal cycler (Bio-Rad Laboratories, Hercules, CA, USA). For each PCR run, a series of positive and negative controls was included. To avoid contaminant effects on RT-PCR assays, 1/10 dilutions of some RNA samples were used. Primer sequences and probes are described in Table 1.

#### **Epidemiological data**

Epidemiological data from General Pueyrredon and Pinamar districts, such as number of confirmed active and suspected COVID-19 cases a day, were obtained from the national and provincial health agencies, and from data provided by the Administración Nacional de Laboratorios e Institutos de Salud-Instituto Nacional de Epidemiología Dr. J.H. Jara.

#### **RESULTS**

#### **Effluent characteristics**

Biological oxygen demand (BOD) and chemical oxygen demand (QOD) values were similar in both districts, with values ranging between 150–200 mg  $\rm O_2/L$  and 250–350 mg  $\rm O_2/L$  respectively. Although these parameters can be assigned to domestic wastewater effluents, in Mar del Plata, the service provider indicated that after the resumption of industrial activity, the BOD and QOD values increased to 240 mg  $\rm O_2/L$  and 485 mg  $\rm O_2/L$ , respectively. This did not occur in Pinamar because there is no industrial activity, only tourism.

#### Analysis of SARS-CoV-2 in wastewater

From the onset of cases in the region, protocols for wastewater sampling and concentration, and detection of SARS-CoV-2

were developed based on virus detection systems using human nasopharyngeal swab samples.

In General Pueyrredon district, during epidemiological week 28 in 2020, the presence of the virus was detected in the effluent for the first time, while 167 COVID-19 cases were confirmed by laboratory diagnosis from 508 suspected cases. This first detection was 20 days before the start of the increase in cases of the first wave of the COVID-19 epidemic in General Pueyrredon district (epidemiological week 31) and 9 weeks before the maximum number of cases. In epidemiological week 34 of 2020, the cycle threshold (Ct) value reached 34, equivalent to about 4 500 viral copies per mL of effluent, based on the target copy number gene used as the positive SARS-CoV-2 control (6). The maximum number of confirmed cases in the first wave in General Pueyrredon district was in epidemiological week 39, 2020 with 1 043 confirmed cases of 2 084 suspected cases. In epidemiological week 52, 2020, laboratory-confirmed cases reached a peak, and the virus was detected in the effluent, while between epidemiological weeks 2, 2021 and 16, 2021, the virus was not detected. In epidemiological week 17, 2021, the virus was again detected in the wastewater, which anticipated the peak of cases confirmed in nasopharyngeal swabs: 1 931 cases detected by RT-PCR in epidemiological week 20, 2021 (Figure 4A).

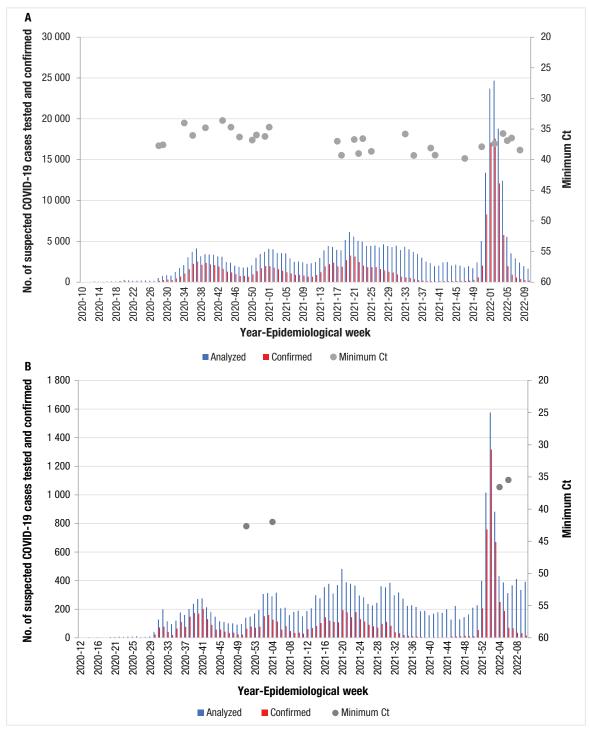
In the collection and concentration of the samples from Pinamar effluent, alternative protocols were tested, which may be useful for small towns without proper infrastructure and equipment. The first sample (in summer 2020–2021) was concentrated by a commercially available 15% aluminum chloride solution (Clorotec Clarificador®). The presence of the virus genome was seen in epidemiological week 51, 2020 when 64 confirmed cases were recorded. The summer of 2021 was rainy and when the town received the highest influx of tourists, during the Easter holidays in 2021, a big storm occurred which flooded some areas. As a result, it was not possible to carry out the sampling again in Pinamar until epidemiological week 4, 2022, when viral circulation was again detected in the wastewater and 251 cases of COVID-19 confirmed by laboratory diagnosis were recorded (Figure 4B).

TABLE 1. Primers and probes used in real-time reverse transcription polymerase chain reaction for SARS-CoV-2 RNA amplification

Target	Name	Sequence (5' $ ightarrow$ 3')	Reference
Orf1ab	ORF1ab-F	CCCTGTGGGTTTTACACTTAA	(24)
	ORF1ab-R	ACGATTGTGCATCAGCTGA	
	ORF1ab-P	CCGTCTGCGGTATGTGGAAAGGTTATGG	
E	E_Sarbeco_F1	ACAGGTACGTTAATAGTTAATAGCGT	(25)
	E_Sarbeco_R2	ATATTGCAGCAGTACGCACACA	
	E_Sarbeco_P1	ACACTAGCCATCCTTACTGCGCTTCG	
RdRp	RdRP_SARSr-F2	GTGARATGGTCATGTGTGGCGG	
	RdRP_SARSr-R1	CARATGTTAAASACACTATTAGCATA	
	RdRP_SARSr-P1	CCAGGTGGWACRTCATCMGGTGATGC	
Human β-globin	β-globin F	TGCACGTGGATCCTGAGAACT	(26)
	β-globin R	AATTCTTTGCCAAAGTGATGGG	
	β-globin P	CAGCACGTTGCCCAGGAGCCTG	
Orf1a	Orf1a	Not available	DiaPlexQ™ novel coronavirus (2019-nCoV)
N	N	Not available	detection kit (SolGent Co., Ltd, Daejeon, Korea)

SARS-CoV-2, severe acute respiratory syndrome coronavirus 2. **Source:** prepared by authors

FIGURE 4. Presence of the SARS-CoV-2 viral genome in wastewater and number of COVID-19 cases (assessed and confirmed), by epidemiological week: A) Mar del Plata; B) Pinamar



SARS-CoV-2, severe acute respiratory syndrome coronavirus 2; COVID-19, coronavirus disease 2019; Ct, cycle threshold; RT-PCR, real-time reverse transcription polymerase chain reaction. **Note:** Data are available on request from the corresponding author. **Source:** prepared by the authors from the results.

#### **DISCUSSION**

Wastewater-based epidemiology has great potential as a complementary solution to clinical testing. This is because wastewater testing can cover an entire community with only a few samples, the cost is relatively low, and it can detect asymptomatic or unreported cases. This strategy was not only successfully applied for the detection of SARS-CoV-2 but was previously used for other human viruses (6, 8). This monitoring method has been implemented in many countries around the world in surface wastewater and treatment plants (27, 28).

In this study, to assess wastewater testing as a tool to evaluate the circulation of SARS-CoV-2 in the community and to establish the relationship between confirmed cases of SARS-CoV-2 infection and SARS-CoV-2 titer in the effluent, a systematic follow-up was carried out by epidemiological week. The two districts chosen had different population sizes and infrastructure. The concentration and detection protocols used allowed the early detection of SARS-CoV-2 in the effluent when the number of laboratory-confirmed cases was low in both districts, especially during lockdowns. Based on these results, we hoped to find an association between the decline in the Ct value (number of viral copies) and the rise of the number of cases. However, the Ct values detected throughout the monitoring, did not vary much.

The difficulties imposed by the COVID-19 pandemic did not give enough time to standardize the protocols for wastewater testing, so each region had to adapt the monitoring method based on its health infrastructure and economy. Among the different concentration methods for wastewater effluent for the detection of pathogens, the most used methods were based on ultrafiltration (29), precipitation (30), and flocculation (31).

At the wastewater treatment plant in General Pueyrredon, two aluminum-driven flocculation concentration methods were tested (22, 23) and the viral presence in the effluent was similar in both cases based on Ct values (data not shown). On the other hand, using a commercial flocculant in Pinamar, SARS-CoV-2 could only be detected during the lockdown period, and no viral presence was recorded after tourism restarted. This finding may be because of the dilution of the samples due to the increase in water consumption in the town. For this reason, another concentration protocol was used (23) and viral presence in the samples from the Pinamar wastewater treatment plant was again recorded (Figure 4B).

To understand our results, it is important to highlight how people traveled during the time of our study. To limit the spread of SARS-CoV-2 in the population, the national government of Argentina issued a preventive and mandatory social isolation order throughout the country from March 20, 2020 (decree 297/2020). This compulsory measure banned people from traveling within cities and across the country, with the exception of personnel considered essential. On April 27, 2020, in some areas of the country, more flexible measures were introduced through decree 520/20, based on the health situation. This decree allowed greater movement of people but with social distancing and observation of health measures, such as the use of personal protection and hygiene. General Pueyrredon introduced this measure on November 29, 2020 and Pinamar on June 28, 2020. Since December 2020 both regions have been open to tourism, and the vaccination campaign began throughout the country.

The highest number of laboratory-confirmed cases in General Pueyrredon was in epidemiological week 39, 2020. However, the first RT-PCR signal from the wastewater samples was detected in epidemiological week 28, 2020, when industries were closed and the mandatory social isolation order was in place. This signal anticipated the increase in cases about 20 days before the start of the first wave of the pandemic (in epidemiological week 31), and 9 weeks before the maximum peak of cases. In epidemiological week 51, 2021, when the lockdown finished and tourism restarted, the virus titer increased because of family gatherings during the Christmas and New Year

holidays. As a result, COVID-19 cases increased from 5 038 to 24 673 in epidemiological week 2, 2022. Despite this increase, the Ct of the effluents did not change in the same way, and only a small decrease in the Ct value was observed, perhaps because of the dilution effects as a result of the seasonal increase in the population.

In the case of Pinamar, SARS-CoV-2 was monitored only during the summers of 2020–2021 and 2021–2022. The virus was detected in epidemiological week 51, 2020 for the first time, when 64 laboratory-confirmed cases of SARS-CoV-2 infection were also recorded. Because false negatives in the Ct of the effluent were suspected, the concentration method was modified and a viral titer was again detected in the summer of 2021–2022 (Figure 4B).

Although the two districts analyzed have their own characteristics and different wastewater management systems, they both have variable populations during the summer season. The difficulty in obtaining prediction models that relate Ct values to the total number of cases, reported and not reported, has already been raised by other researchers (31, 32).

Among the challenges we had to deal with in this study were: the characteristics of the wastewater network; the relationship of the residual water flow with rainfall; the time and method of sampling; the concentration protocols; the detection of the virus; and interpretation of results. However, early detection of the viral genome in urban wastewater was successful. Our findings indicate that, even with the particularities of this region, wastewater epidemiology is a reliable tool that provides an objective analysis of the progress of the pandemic in the community. It overcomes the limitations of other epidemiological control tools that are expensive, such as lockdown and mass testing, and it is a valid alternative for the detection of asymptomatic people. This tool should be useful for many low- and middle-income countries with insufficient resources to conduct clinical trials in their communities. Indeed, for such countries, wastewater epidemiology may be the only feasible means of effective population surveillance (9). Similar successful experiences have been reported in the Americas regions, such as in other areas of Argentina (23, 28, 30, 33, 34), Brazil (35), and Peru (16).

#### **Conclusions**

During 2 years of monitoring, we were able to detect SARS-CoV-2 virus genome in wastewater early on, demonstrating once again the usefulness of the application of wastewater epidemiology for SARS-CoV-2 detection and monitoring.

**Author contributions.** CC, AS, VP, and OU undertook preliminary assays of sensitivity and RT-PCR. LRC undertook the Pinamar sample collection, concentration precipitation, and logistics coordination. AL and CC did the RT-PCR assays with different diagnostic kits and viral load testing. AS and VP did the concentration and purification of viral RNA. GVH, APC, LC, and MS undertook data collection, concentration, and logistics coordination of Mar del Plata city samples. GC assessed the different methods of concentration and validation of RNA extraction. CMB undertook investigations, Pinamar sample concentration, and obtaining funding. CC, LRC, and CMB did the graphics design, and wrote, reviewed, and edited the paper. All authors approved the final version for submission.

Acknowledgements. We thank the Administración Nacional de Laboratorios e Institutos de Salud-Instituto Nacional de Epidemiología Dr. Juan H. Jara and Dra. Irene Pagano for allowing institutional interaction between Obras Sanitarias Sociedad de Estado, Administración Nacional de Laboratorios e Institutos de Salud-Instituto Nacional de Epidemiología Dr. J.H. Jara, and the Instituto de Investigaciones en Biodiversidad y Biotecnología. We also thank the following people: Mrs Anguiano Samanta (Secretary of Landscape and Environment of Pinamar) for carrying out sampling; Dr D'Agostino Eduardo (Secretary of Health of Pinamar) for providing the epidemiological data and the logistics of transporting the samples to the INE-J.H. Jara laboratory; Dr Henk Braig for inspiring this research; and Professor Ana Tassi (former Professor of English Grammar,

Universidad Nacional de Mar del Plata) for proofreading the draft manuscript.

Conflicts of interest. None declared.

**Funding.** Ministry of Science, Technology, and Innovation, Grant ID BUE 188: Program for federal articulation and strengthening of capacities in science and technology for COVID-19.

**Disclaimer.** The authors hold sole responsibility for the views expressed in the manuscript, which may not necessarily reflect the opinion or policy of the *Revista Panamericana de Salud Pública / Pan American Journal of Public Health* and/or those of the Pan American Health Organization.

#### **REFERENCES**

- Ortiz-Prado E, Simbaña-Rivera K, Gómez-Barreno L, Rubio-Neira M, Guaman LP, Kyriakidis NC, et al. Clinical, molecular, and epidemiological characterization of the SARS-CoV-2 virus and the coronavirus disease 2019 (COVID-19): a comprehensive literature review. Diagn Microbiol Infect Dis. 2020;98:115094. doi: 10.1016/j. diagmicrobio.2020.115094
- Yan Y, Chang L, Wang L. Laboratory testing of SARS-CoV, MERS-CoV, and SARS-CoV-2 (2019-nCoV): current status, challenges, and countermeasures. Rev Med Virol. 2020;30:e2106. doi: 10.1002/rmv.2106
- 3. Wu SL, Mertens AN, Crider YS, Nguyen A, Pokpongkiat NN, Djajadi, et al. Substantial underestimation of SARS-CoV-2 infection in the United States. Nat Commun. 2020;11: 4507. doi: 10.1038/s41467-020-18272-4
- 4. Bosch A, Guix S, Sano D, Pinto RM. New tools for the study and direct surveillance of viral pathogens in water. Curr Opin Biotechnol. 2008;19:295–301. doi: 10.1016/j.copbio.2008.04.006
- 5. Blinkova O, Rosario K, Li L, Kapoor Â, Slikas B, Bernardin F, et al. Frequent detection of highly diverse variants of cardiovirus, cosavirus, bocavirus, and circovirus in wastewater samples collected in the United States. J Clin Microbiol. 2009;47:3507–13. doi: 10.1128/JCM.01062-09
- 6. Bosch A. Human enteric viruses in the water environment: a minireview. Int Microbiol. 1998;1:191–6.
- 7. Nemudryi A, Nemudraia A, Wiegand T, Surya K, Buyukyoruk M, Cicha C, et al. Temporal detection and phylogenetic assessment of SARS-CoV-2 in municipal wastewater. Cell Rep Med. 2020;1:100098. doi: 10.1016/j.xcrm.2020.100098
- Rampelli S, Biagi E, Turroni S, Candela M. Retrospective search for SARS-CoV-2 in human faecal metagenomes. 2020. doi: 10.2139/ ssrn.3557962.
- Ahmed W, Angel N, Edson J, Bibby K, Bivins A, O'Brien JW, et al. 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. 2020;728:138764. doi: 10.1016/j.scitotenv.2020.138764
- Chavarria-Miró G, Anfruns-Estrada E, Guix S, Paraira M, Galofré B, Sánchez G, et al. Sentinel surveillance of SARS-CoV-2 in wastewater anticipates the occurrence of COVID-19 cases. MedRxiv. 2020. doi: 10.1101/2020.06.13.20129627 [preprint].
- 11. Maryam S, Ul Haq I, Yahya G, Ul Haq M, Algammal AM, Saber S, et al. COVID-19 surveillance in wastewater: an epidemiological tool for the monitoring of SARS-CoV-2. Front Cell Infect Microbiol. 2023;12:1743. doi: 10.3389/fcimb.2022.978643
- 12. Medema G, Been F, Heijnen L, Petterson S. Implementation of environmental surveillance for SARS-CoV-2 virus to support public health decisions: opportunities and challenges. Curr Opin Environ Sci Health. 2020;17:49–71. doi: 10.1016/j.coesh.2020.09.006
- 13. Sherchan SP, Shahin S, Ward LM, Tandukar S, Aw TG, Schmitz B, et al. First detection of SARS-CoV-2 RNA in wastewater in North America: a study in Louisiana, USA. Sci Total Environ. 2020;743:140621. doi: 10.1016/j.scitotenv.2020.140621

- Hillary, LS, Malham SK, McDonald, JE, Jones, DL. Wastewater and public health: the potential of wastewater surveillance for monitoring COVID-19. Curr Opin Environ Sci Health. 2020;17,14–20. doi: 10.1016/j.coesh.2020.06.001
- 15. Mousazadeh M, Ashoori R, Paital B, Kabdaşlı I, Frontistis Z, Hashemi M, et al. Wastewater based epidemiology perspective as a faster protocol for detecting coronavirus RNA in human populations: a review with specific reference to SARS-CoV-2 virus. Pathogens. 2021;10:1008. doi: 10.3390/pathogens10081008
- 16. Pardo-Figueroa B, Mindreau-Ganoza E, Reyes-Calderon A, Yufra SP, Solorzano-Ortiz IM, Donayre-Torres, et al. Spatiotemporal surveillance of SARS-CoV-2 in the sewage of three major urban areas in Peru: generating valuable data where clinical testing is extremely limited. ACS ES T Water. 2022;2:2144–57. doi: 10.1021/acsestwater.2c00065
- 17. Martins RM, Carvalho T, Bittar C, Quevedo DM, Miceli RN, Nogueira ML, et al. Long-term wastewater surveillance for SARS-CoV-2: one-year study in Brazil. Viruses. 2022;14:2333. doi: 10.3390/v14112333
- 18. Pérez-Cataluña A, Cuevas-Ferrando E, Randazzo W, Falcó, Allende A, Sánchez G. Comparing analytical methods to detect SARS-CoV-2 in wastewater. Sci Total Environ. 2021;758:143870. doi: 10.1016/j. scitotenv.2020.143870
- 19. Rodrigues Capitulo L, Kruse EE. Relationship between geohydrology and Upper Pleistocene–Holocene evolution of the eastern region of the province of Buenos Aires, Argentina. J South Am Earth Sci. 2017;76:276–89. doi: 10.1016/j.jsames.2017.03.011
- 20. Rodrigues Capitulo L. Evaluación geohidrológica en la región costera oriental de la Provincia de Buenos Aires [Geohydrological evaluation in the eastern coastal region of the Province of Buenos Aires]. [Thesis]. La Plata, Universidad Nacional de La Plata; 2015.
- 21. Standard methods for the examination of water and wastewater. Standard Methods Committee. Washington, DC: American Public Health Association, American Water Works Association, Water Environment Federation; 2011.
- Randazzo W, Piqueras J, Evtoski Z, Sastre G, Sancho R, Gonzalez C, et al. Interlaboratory comparative study to detect potentially infectious human enteric viruses in influent and effluent waters. Food Environ Virol. 2019;11:350–63. doi: 10.3389/fmicb.2020.01911
- 23. Wehrendt DP, Massó MG, Machuca AG, Vargas CV, Barrios ME, Campos J, et al. A rapid and simple protocol for concentration of SARS-CoV-2 from wastewater. J Virol Methods. 2021;297:114272. doi: 10.1016/j.jviromet.2021.114272
- 24. Chinese Center for Disease Control and Prevention; National Institute for Viral Disease Control and Prevention. Specific primers and probes for detection 2019 novel coronavirus [internet]. Beijing: Chinese Center for Disease Control and Prevention 2020; 2:332-336. Available from: https://weekly.chinacdc.cn/en/article/doi/10.46234/ccdcw2020.085
- Corman VM, Landt O, Kaiser M, Molenkamp R, Meijer A, Chu DK, et al. Detection of 2019 novel coronavirus (2019-nCoV) by

- real-time RT-PCR. Euro Surveill. 2020;25:2000045. doi: 10.1016/j.pathol.2020.08.002
- 26. Îrenge LM, Robert A, Gala JL. Quantitative assessment of human β-globin gene expression in vitro by TaqMan real-time reverse transcription-PCR: comparison with competitive reverse transcription-PCR and application to mutations or deletions in noncoding regions. Clin Chem. 2005;51:2395–6. doi: 10.1373/ clinchem.2005.056630
- 27. Iglesias NG, Gebhard LG, Carballeda JM, Aiello I, Recalde E, Terny G, et al. SARS-CoV-2 surveillance in untreated wastewater: detection of viral RNA in a low-resource community in Buenos Aires, Argentina. Rev Panam Salud Publica. 2021;45:e137. doi: 10.26633/RPSP.2021.137
- 28. Naughton CC, Roman FA, Alvarado AGF, Tariqi AQ, Deeming MA, Kadonsky KF, et al. Show us the data: global COVID-19 wastewater monitoring efforts, equity, and gaps. FEMS Microbes. 2023,4:xtad003. doi: 10.1093/femsmc/xtad003
- 29. Forés E, Bofill-Mas S, Itarte M, Martínez-Puchol S, Hundesa S, Calvo M, et al. Evaluation of two rapid ultrafiltration-based methods for SARS-CoV-2 concentration from wastewater. Sci Total Environ. 2021;768:144786. doi: 10.1016/j.scitotenv.2020.144786
- 30. Masachessi G, Castro G, Cachi AM, Marinzalda DLA, Liendo M, Pisano MB, et al. Wastewater based epidemiology as a silent sentinel of the trend of SARS-CoV-2 circulation in the community in central Argentina. Water Research. 2022;219:18541. doi: 10.1016/j. watres.2022.118541
- 31. Giraud-Billoud M, Cuervo P, Altamirano JC, Pizarro M, Aranibar JN, Catapano A, et al. Monitoring of SARS-CoV-2 RNA in wastewater as an epidemiological surveillance tool in Mendoza, Argentina.

- Sci Total Environ. 2021;796:148887. doi: 10.1016/j.scitotenv.2021. 148887
- 32. Ma Y, Liu X, Tao W, Tian Y, Duan Y, Xiang M, et al. Estimation of the outbreak severity and evaluation of epidemic prevention ability of COVID-19 by province in China. Am J Public Health. 2020;110:1837–43. doi: 10.2105/AJPH.2020.305893
- 33. Barril PA, Pianciola LA, Mazzeo M, Ousset MJ, Jaureguiberry MV, Alessandrello M, et al. Evaluation of viral concentration methods for SARS-CoV-2 recovery from wastewaters. Sci Total Environ. 2021;756:144105. doi: 10.1016/j.scitotenv.2020.144105.
- 34. Cruz CM, Sanguino-Jorquera D, González AM, Irazusta PV, Poma RH, Cristóbal HA, et al. Sewershed surveillance as a tool for smart management of a pandemic in threshold countries. Case study: tracking SARS-CoV-2 during COVID-19 pandemic in a major urban metropolis in northwestern Argentina. Sci Total Environ. 2023; 862:160573. doi: 10.1016/j.scitotenv.2022.160573
- 35. Calábria de Araújo J, Madeira CL, Bressani T, Leal C, Leroy D, Machado EC, et al. Quantification of SARS-CoV-2 in wastewater samples from hospitals treating COVID-19 patients during the first wave of the pandemic in Brazil. Sci Total Environ. 2023;860:160498. doi: 10.1016/j.scitotenv.2022.160498

Manuscript received on 6 February 2023. Revised version accepted for publication on 14 March 2023.

## Presencia del SARS-CoV-2 en aguas residuales urbanas del sudeste de Buenos Aires, Argentina, de mayo del 2020 a marzo del 2022

#### **RESUMEN**

**Objetivos.** Aplicar y evaluar la utilización de muestreos de aguas residuales como método para la detección del coronavirus del síndrome respiratorio agudo severo de tipo 2 (SARS-CoV-2) en dos distritos costeros de la Provincia de Buenos Aires, Argentina.

**Métodos.** Se utilizó un dispositivo de muestreo automático para tomar muestras de 400 mL de las aguas residuales de 24 horas en el distrito de General Pueyrredon, mientras que en el distrito de Pinamar se tomaron muestras de 2,2 L a intervalos de 20 minutos hasta un volumen total de 20 L. Los muestreos se realizaron una vez por semana. Las muestras se concentraron mediante floculación con policloruro de aluminio. La purificación del ARN y la amplificación y detección del gen diana se llevaron a cabo mediante la prueba de reacción en cadena de la polimerasa con retrotranscripción para el diagnóstico clínico a partir de hisopados nasofaríngeos.

**Resultados.** Se observó la presencia de SARS-CoV-2 en las aguas residuales de ambos distritos. En General Pueyrredon, el SARS-CoV-2 se halló en la semana epidemiológica 28 del 2020, es decir, 20 días antes del inicio del aumento de casos de enfermedad por coronavirus 2019 (COVID-19) registrado en la primera ola (semana epidemiológica 31) y nueve semanas antes de que se alcanzara el número máximo de casos de COVID-19 con confirmación de laboratorio. En el distrito de Pinamar se detectó el genoma viral en la semana epidemiológica 51 del 2020, pero solo se pudo volver a realizar el muestreo en la semana epidemiológica 4 del 2022, en la que se volvió a detectar la circulación del virus.

**Conclusiones.** Se pudo detectar el genoma del virus SARS-CoV-2 en aguas residuales, lo que muestra la utilidad de la aplicación de la epidemiología de aguas residuales como método para la detección y el seguimiento del SARS-CoV-2 a largo plazo.

Palabras clave

SARS-CoV-2; aguas residuales; brotes de enfermedades; monitoreo del ambiente; Argentina.

# Presença de SARS-CoV-2 em águas residuais urbanas no sudeste de Buenos Aires, Argentina, de maio de 2020 a março de 2022

#### **RESUMO**

**Objetivos.** Implementar e avaliar o uso de amostragem de águas residuais na detecção do coronavírus da síndrome respiratória aguda grave 2 (SARS-CoV-2) em dois distritos costeiros da Província de Buenos Aires, Argentina.

**Métodos.** No distrito de General Pueyrredon, amostras de 400 mL de águas residuais foram coletadas ao longo de 24 horas com um amostrador automático; já no distrito de Pinamar, foram coletados 20 L no total (2,2 L a intervalos de 20 minutos). As amostras foram coletadas uma vez por semana e concentradas por floculação com cloreto de polialumínio. A purificação do RNA e a amplificação e detecção de genes-alvo foram realizadas por meio de reação em cadeia da polimerase com transcrição reversa para diagnóstico clínico de esfregaços nasofaríngeos humanos.

**Resultados.** Detectou-se presença de SARS-CoV-2 em águas residuais dos dois distritos. Em General Pueyrredon, o SARS-CoV-2 foi detectado na semana epidemiológica 28 de 2020, ou seja, 20 dias antes do início de um aumento no número de casos da doença provocada pelo coronavírus de 2019 (COVID-19) na primeira onda (semana epidemiológica 31) e 9 semanas antes de se registrar o número máximo de casos de COVID-19 confirmados em laboratório. No distrito de Pinamar, o genoma viral foi detectado na semana epidemiológica 51 de 2020, mas não foi possível realizar a amostragem novamente até a semana epidemiológica 4 de 2022, quando a circulação do vírus foi novamente constatada.

**Conclusões.** Foi possível detectar o genoma do vírus SARS-CoV-2 em águas residuais, demonstrando a utilidade da aplicação da epidemiologia baseada em águas residuais para detectar e monitorar o SARS-CoV-2 em longo prazo.

#### Palavras-chave

SARS-CoV-2; águas residuárias; surtos de doenças; monitoramento ambiental; Argentina.