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Unveiling the silent information of wastewater-based epidemiology of SARS-CoV-2 at community and sanitary zone levels: experience in Córdoba City, Argentina

Gisela Masachessi[a,b,*], Gonzalo Manuel Castro[a,c], María de los Angeles Marinzalda[d,e], Ariana Mariela Cachi[d,e], Paola Sicilia[c], Veronica Emilce Prez[a,b], Laura Cecilia Martínez[a], Miguel Oscar Giordano[a], María Belen Pisano[a,b], Viviana Elizabeth Ré[a,b], Carlos Martin Del Bianco[f], Sofia Parisato[f], Micaela Fernandez[f], Gustavo Ibarra[f], Laura Lopez[g], Gabriela Barbás[g] and Silvia Viviana Nates[a]

[a] Instituto de Virología Dr J. M. Vanella, Facultad de Ciencias Médicas, Universidad Nacional de Córdoba, Enfermera Gordillo Gómez s/n, Ciudad Universitaria, Córdoba X5000, Argentina
[b] Consejo Nacional de Investigaciones Científicas y Técnicas (CONICET), Godoy Cruz 2290, CABA C1425FQB, Argentina
[c] Departamento Laboratorio Central, Ministerio de Salud de la Provincia de Córdoba, T. Cáceres de Allende 421, Córdoba X5000HVE, Argentina
[d] Instituto Nacional de Medicina Aeronáutica y Espacial, FAA, Av. Fuerza Aérea Argentina Km 6 1/2 S/N B.0 Cívico, Córdoba X5010, Argentina
[e] Facultad de la Fuerza Aérea, Universidad de la Defensa Nacional, Av. Fuerza Aerea Argentina 5011, Córdoba X5000, Argentina
[f] Planta Municipal de tratamiento de efluente cloacales Bajo Grande-Laboratorio de análisis fisicoquímicos, bacteriológicos EDAR Bajo Grande, Cam. Chacra de la Merced 901, Córdoba X5000, Argentina
[g] Ministerio de Salud de la Provincia de Córdoba, Av. Vélez Sarsfield 2311 Ciudad Universitaria, Córdoba X5016 GCH, Argentina
*Corresponding author. E-mail: gmasachessi@fcm.unc.edu.ar

GM, 0000-0003-3002-4372

ABSTRACT

The emergence of COVID-19 in 2020 significantly enhanced the application of wastewater monitoring for detecting SARS-CoV-2 circulation within communities. From October 2021 to October 2022, we collected 406 wastewater samples weekly from the Córdoba Central Pipeline Network (BG-WWTP) and six specific sewer manholes from sanitary zones (SZs). Following WHO guidelines, we processed samples and detected SARS-CoV-2 RNA and variants using real-time PCR. Monitoring at the SZ level allowed for the development of a viral activity flow map, pinpointing key areas of SARS-CoV-2 circulation and tracking its temporal spread and variant evolution. Our findings demonstrate that wastewater-based surveillance acts as a sensitive indicator of viral activity, detecting imminent increases in COVID-19 cases before they become evident in clinical data. This study highlights the effectiveness of targeted wastewater monitoring at both municipal and SZ levels in identifying viral hotspots and assessing community-wide circulation. Importantly, the data shows that environmental wastewater studies provide valuable insights into virus presence, independent of clinical COVID-19 case records, and offer a robust tool for adapting to future public health challenges.

Key words: COVID-19, public health policies, SARS-CoV-2, wastewater-based epidemiology

HIGHLIGHTS

- Wastewater surveillance detects SARS-CoV-2 dynamics in real-time insights into viral circulation.
- Mapping viral activity identifies key transmission points and monitors infection trends in specific areas.
- The reemergence of SARS-CoV-2 genomes in wastewater serves as an early warning for potential COVID-19 case increases.
- Variant detection in wastewater tracks the emergence and spread of SARS-CoV-2 strains in the community.

INTRODUCTION

For decades, wastewater testing has proven to be a valuable tool in fighting infectious diseases (Hovi *et al.* 2005; Mueller *et al.* 2009; Lodder & de Roda Husman 2020; Lizasoain *et al.* 2021; Masachessi *et al.* 2022; Wani *et al.* 2023; D'Arpino *et al.* 2024). Particularly, during global pandemics like COVID-19, studies have demonstrated its effectiveness in detecting Severe Acute Respiratory Syndrome Coronavirus 2 (SARS-CoV-2), the virus responsible for COVID-19, in communities even before symptomatic cases are reported (Asghar *et al.* 2014; O'Reilly *et al.* 2020; Giraud-Billoud *et al.* 2021; Masachessi *et al.* 2022; Prado *et al.* 2023; D'Arpino *et al.* 2024). This early detection capability enables public health officials to swiftly identify and respond

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to outbreaks, potentially saving lives. A European Union (EU) report underscores the significance of wastewater surveillance for SARS-CoV-2 and its variants as a rapid and reliable method to track the virus's spread in the population, complementing genomic and epidemiological surveillance efforts (EUR Lex 2021). Since the EU's recommendation, many countries, including Argentina (Giraud-Billoud *et al*. 2021; Iglesias *et al*. 2021; Ahmed *et al*. 2021a, 2021b; Masachessi *et al*. 2022; Wade *et al*. 2022; Wu *et al*. 2022; Gupta *et al*. 2023; Prado *et al*. 2023; Giron-Guzman *et al*. 2024; Shafer *et al*. 2024), have implemented wastewater surveillance for SARS-CoV-2. However, while large sewersheds offer valuable insights, they may not provide detailed information on local areas with specific social determinants of COVID-19 risk, such as demographics and socioeconomic status, which are crucial for region-specific public health measures. To address this, recently, neighborhood-level monitoring for COVID-19 has been undertaken in some parts of the world (Spurbeck *et al*. 2021; Oh *et al*. 2022). This approach allows for more targeted public health interventions (Rusinol *et al*. 2021; Bitter *et al*. 2022; Sangsanont *et al*. 2022).

This research deepens the use of SARS-CoV-2 wastewater-based epidemiology (WBE) at the municipal or community level (Córdoba City) and its small geographical areas defined as sanitary zones (SZs). The partitioning behavior of SARS-CoV-2 in the SZs allowed for the identification of hotspots of SARS-CoV-2 circulation and tracking the spread of the virus and its variants in the community. Meanwhile, the data obtained at the municipal level allows wastewater surveillance to serve as a silent sentinel for early warning of COVID-19 outbreaks in the community and summarizes the results obtained in the SZs, reflecting the circulation of SARS-CoV-2 and its viral variants in the city's population.

MATERIALS AND METHODS

Study area

The city of Córdoba is the capital of the province of Córdoba, located in the central region of Argentina (31°25′ 00″ S 64°11′ 00″W), and has 1,505,250 inhabitants with a population density of 2,613.3 inhabitants/km^2 (INDEC 2022) (Figure 1). The city is divided into seven SZs (Figure 1), delimited to serve a specific population, accessible from all points, and capable of providing continuous, comprehensive, and permanent health care. Each SZ is represented by a set of interconnected neighborhoods. For this study, the sewer manholes of six SZs were selected. Each sewer manhole receives nearly 85% of the raw water from each selected SZ, and the raw sewage is taken 'downstream' to the city's wastewater treatment plant (WWTP) called 'Bajo Grande' (BG-WWTP) which receives approximately 51% discharge from the coverage of the city's sewerage

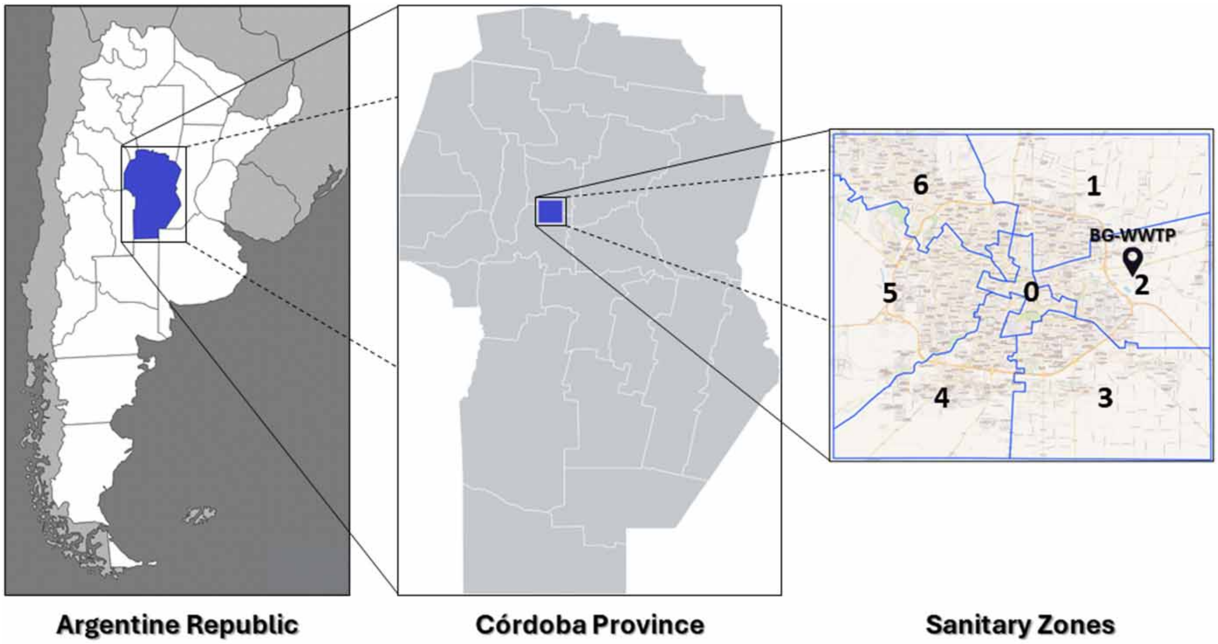

Figure 1 | Geographical representation of the area under study: Argentina and Córdoba province; Córdoba City and SZs of Córdoba City. Numbers indicate each SZ. WWTP-BG, wastewater treatment plant- Bajo Grande.

system (Figure 1). There was an SZ (SZ 4) that was not considered because the sewer percentage is below 20% and the results to be obtained would not be representative.

Wastewater sampling

A total of 406 raw sewage specimens were collected weekly: 58 were obtained from the central pipe network of the BG-WWTP and 348 were obtained from the six sewer manholes specific to each sewershed catchment from the SZs (SZs numbered: 0, 1, 2, 3, 5, and 6). Samples were collected from the beginning of October 2021 to the end of October 2022. At the BG-WWTP, 0.5 L grab samples of wastewater were taken, and at the SZs, 0.25 L samples were taken, both on weekday mornings using the method described in the WHO Guidelines for Environmental Surveillance of Poliovirus Circulation (WHO 2003). Grab samples were collected at a designated site at a given time and taken from the midstream of a sewer collector using a bucket (WHO 2003 annex 3). Each sample was transported within 6 h at 4–8 °C to the Institute of Virology, National University of Córdoba, Córdoba City, for further processing and analysis. Upon arrival at the laboratory, the samples were concentrated 100-fold using a precipitation and elution method with PEG-6000 (Lewis & Metcalf 1988).

Concentration of SARS-CoV-2 in wastewater samples

SARS-CoV-2 was concentrated in sewage specimens using the polyethylene glycol-6000 (PEG-6000) precipitation method (Lewis & Metcalf 1988), and in accordance with the WHO guidelines for environmental surveillance of poliovirus circulation (WHO 2003). This method was previously described by Masachessi *et al.* (2022). In summary, sewage was centrifuged at 4,750 × g for 20 min at 4 °C. The supernatant (S1) was kept at 4 °C for later use, while the sediment was mixed with a 3% beef extract (Lab-Lemco' Poweder, OXOID LTD, Basingstoke, Hammpshire, England)/2 M $NaNO_3$ Titolo min 99.5% (acidimetrico) (Analyticals, Farmitalia Carlo Erba S.p.A, Milano, Italy) eluant (pH 5.5) and stirred for 1 h at 4 °C. Solids were then removed by centrifugation at 10,000 × g for 20 min, and the eluate was combined with the first supernatant (S1) and adjusted to pH 7.2. PEG-6000 (Parafarm, Saporiti S.A.C.I.F.I.A, Buenos Aires, Argentina) was added to a final concentration of 10% (w/v) and NaCl pro-analysis A.C.S (Cicarelli laboratories, reagents S.A. Santa Fe, Argentina) to 2% (w/v). The resulting suspension was stirred overnight at 4 °C and centrifuged at 10,000 × g for 25 min. The supernatant containing PEG was discarded, and the pellet was suspended in 5 and 2.5 mL (from BG-WWTP and each SZ, respectively) PBS (Gibco, Invitrogen Corporation, New York, USA) (pH 7.2), adjusted to pH 8.0, incubated for 1 hour with occasional vortexing, and centrifuged at 10,000 × g for 20 min. The S1 was then stored at −70 °C. The recovery rate of the method and the controls used have been previously described by Masachessi *et al.* (2022).

Genomic RNA extraction from viral concentrates

Each concentrated sample (1 mL) was subjected to RNA automated extraction using MagNA Pure 96 DNA and Viral NA Large Volume Kit in the MagNA Pure 96 system Roche (Roche Diagnostics GmbH – Mannheim, Germany), according to the manufacturer's instructions. The extracted RNA was eluted in a final volume of 50 μL of a 60 mM Tris-HCl buffer. Blank control was included during the RNA extraction to monitor any possible cross-contamination during sample processing.

SARS-CoV-2 genome detection

Amplification reactions

Molecular detection of SARS-CoV-2 RNA was performed by real-time reverse transcription and polymerase chain reaction (RT-PCR) using the DisCoVery SARS-CoV-2 RT-PCR Detection Kit (Safecare Biotech Hangzhou Co. Ltd, China). In this study, only results derived from N gene detection, from the DisCoVery SARS-CoV-2 RT-PCR Detection Kit, were analyzed since it has a higher analytical sensitivity for detection than the Orf1ab gene (Masachessi *et al.* 2022).

In all cases, assays were run according to the manufacturer's instructions. RT-PCR reactions were carried out using a Cobas Z 480 equipment (Roche, Germany). The cycle conditions and the temperature ramps used for each of the amplification kits used were described previously by Masachessi *et al.* (2022).

SARS-CoV-2 variant of concerns (VOCs) detection

VOCs identification was performed only in N gene-positive samples using consecutive RT-PCRs to detect relevant mutations/deletions present in the Spike protein. This was achieved using the TaqMan™ SARS-CoV-2 Mutation Panel reagent (Life Technologies Corporation, Pleasanton, CA) in SARS-CoV-2-positive concentrated wastewater samples.

Through this methodology, combined mutation analysis allowed for the detection of a mutation profile compatible with VOCs Delta and Omicron.

SARS-CoV-2 clinical epidemiology in Córdoba City

The Ministry of Health of the Province of Córdoba (MinSal Córdoba) provided daily COVID-19 cases with spatiotemporal information for the city and for each SZ (MinSal Córdoba 2022).

RESULTS

Fluctuating patterns of SARS-CoV-2 circulation at SZs and municipal levels in wastewater of Córdoba City

Based on the detection of the N gene of SARS-CoV-2, the spatial and temporal spread of SARS-CoV-2 circulation in the SZs of Córdoba City can be divided into 12 main moments, as shown in Figure 2. Each of these moments reflects the viral activity in a specific timeframe in the six SZs of Córdoba City. Additionally, extensive weekly monitoring was conducted at BG-WWTP to provide data on SARS-CoV-2 circulation at the population level, as depicted in Figure 3.

During the first 7 weeks of sampling (from 1 October to 24 November 2021), all samples analyzed from the six SZs tested negative for the SARS-CoV-2 N gene (Moment 1; Figure 2). This result corresponds with the absence of viral genome detection in wastewater at the municipal level (Figure 3). Throughout this period, the total positive COVID-19 cases reported in the city of Córdoba amounted to 841, with daily cases ranging from a minimum of 2 to a maximum of 12 (Figure 3). The first sewage sample testing positive was collected from SZ 0 on 1 December 2021 (Moment 2, Figure 2). This first positive sample preceded a progressive increase in the number of COVID-19 clinical cases reported in this zone, rising from a total of 22 cases to 96 cases between 1 and 16 December 2021 (data not shown). Meanwhile, the first positive sewage sample at the municipal level was obtained 5 days after the first positive sample detected in SZ 0 (6 December 2021; Ct

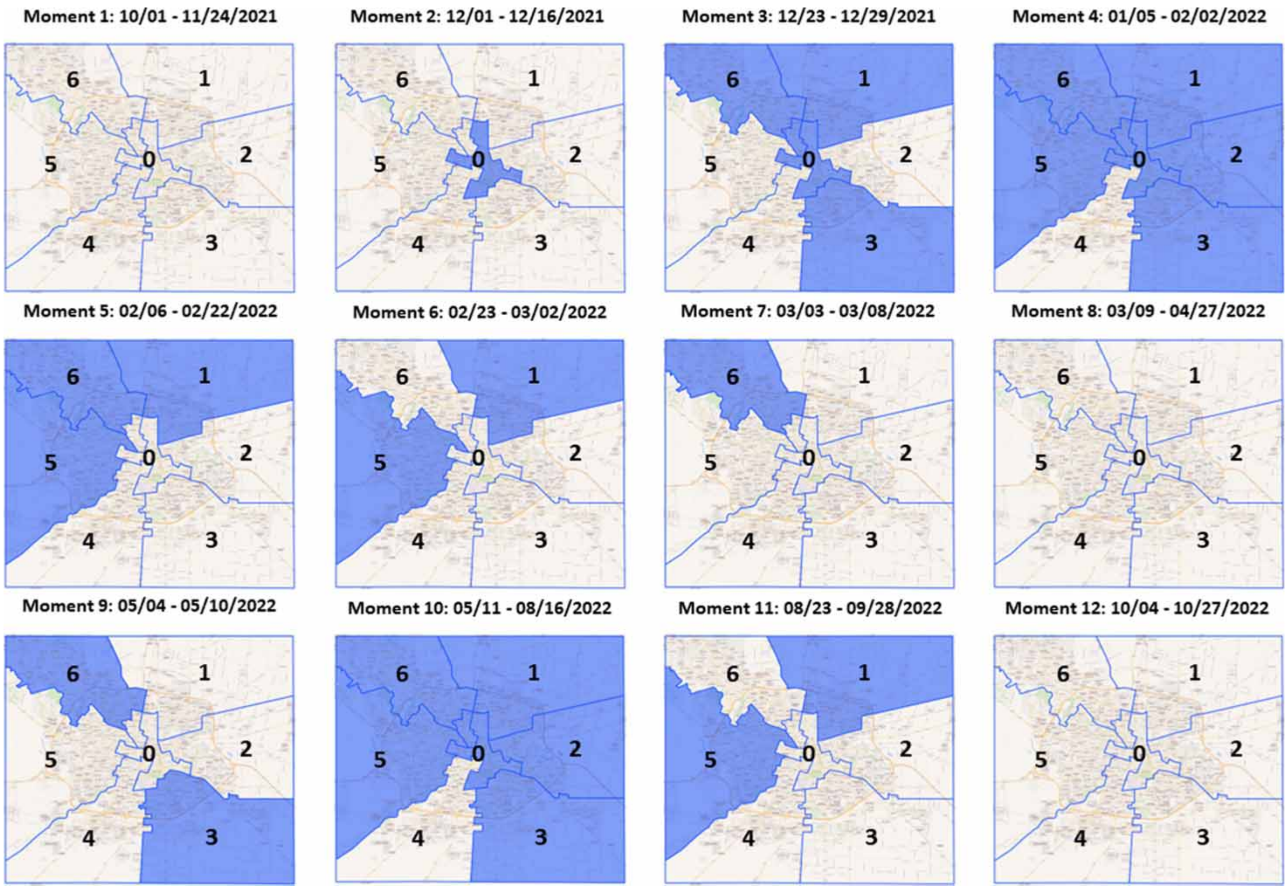
Moment 1: 10/01 - 11/24/2021
Moment 2: 12/01 - 12/16/2021
Moment 3: 12/23 - 12/29/2021
Moment 4: 01/05 - 02/02/2022
Moment 5: 02/06 - 02/22/2022
Moment 6: 02/23 - 03/02/2022
Moment 7: 03/03 - 03/08/2022
Moment 8: 03/09 - 04/27/2022
Moment 9: 05/04 - 05/10/2022
Moment 10: 05/11 - 08/16/2022
Moment 11: 08/23 - 09/28/2022
Moment 12: 10/04 - 10/27/2022

Figure 2 | SARS-CoV-2 dispersion flow map in SZs. Geographic areas in blue indicate viral activity in a particular SZ.

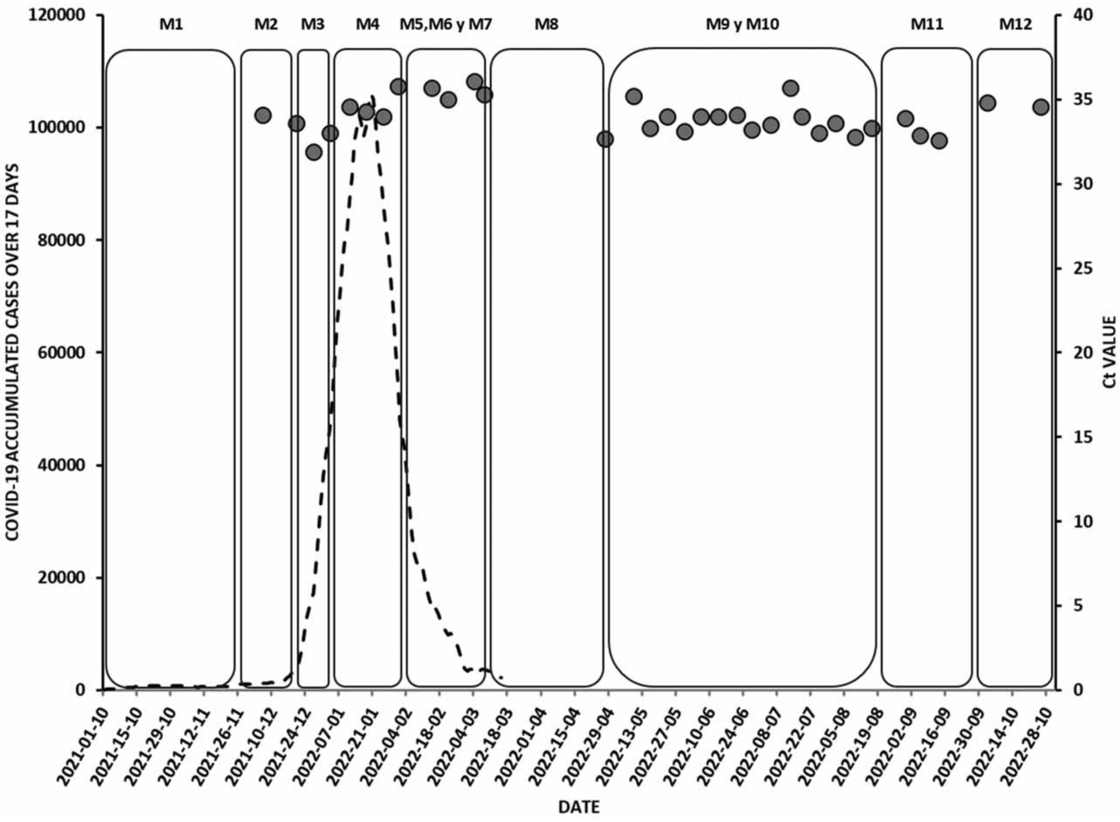

Figure 3 | Detection of SARS-CoV-2 in wastewater from Córdoba City. The curve with the dotted line shows the number COVID-19 reported cases accumulated in Córdoba city (data from Ministry of Health of Cordoba Province). Bullets represent the detection and Ct values of the SARS-CoV-2 genome by identifying the N gene in wastewater. The Moments analyzed in the SZs are delineated within boxes.

34.1). This first detection of the viral genome in BG-WWTP sewage served as an early warning sign of the subsequent increase in COVID-19 cases in Córdoba City (Figure 3). The total number of positive cases accumulated in the city of Córdoba during this period (Moment 2) was 2,317, with daily reported cases increasing from 34 to 529. This trend was evident in the SZs during Moment 3, on 23–29 December 2021 (Figure 2). At Moment 3, SZ 0 experienced an increase in COVID-19 cases, and cases of COVID-19 began to be recorded in SZ 1, 3, and 6 (data not shown). Accompanying positive cases of COVID-19 tested in SZs 1, 3, and 6, the wastewater in these ZSs began to show positive results for the N gene of SARS-CoV-2. During Moment 3, the viral genome was continuously detected in the BG-WWTP, coinciding with an increase in the reported COVID-19 cases (Ct values between 39.9 and 30.8) (Figure 3, Table 1). The total number of positive cases

Table 1 | SARS-CoV-2 Ct values and reported COVID-19 cases in wastewater samples from BG-WWTP (municipal level) at each moment studied

Moment	Period	Cumulative number of COVID-19 cases	Ct values (maximum – minimum values)
2	1/12/2021–16/12/2021	2,371	34.1[a]
3	23/12/2021–29/12/2021	27,547	34.0 (39.9–30.8)
4	**5/1/2022–2/2/2022**	**100,200**	**33.9 (35.8–34.0)**
5	16/2/2022–22/2/2022	14,511	34.5 (35.0–34.1)
6	23/2/2022	9,699	36.1[a]
7	3/3/2022	3,453	35.1[a]

[a] Just one sample tested positive for the SARS-CoV-2 N gene.

The text in bold highlights the Ct values of SARS-CoV-2 from Moment 4, which corresponds to the period with the highest number of reported COVID-19 cases.

accumulated in the city of Córdoba during this period was 27,547, with daily reported cases increasing from 1,400 to 5,506 (Figure 3). This upward trend continued into Moment 4 (period from 5 January to 2 February 2022), during which all studied SZs (SZs 0, 1, 2, 3, 5, and 6) tested positive for SARS-CoV-2 genome detection. Throughout Moment 4, all weekly wastewater samples from BG-WWTP consistently tested positive for SARS-CoV-2 (Ct values between 35.8 and 34.0; Table 1). During this period, the total number of reported COVID-19 cases in Córdoba City increased to 100,200, aligning with the third epidemic wave in Argentina. Since 2 February 2022, a gradual decline in the number of SZs testing positive for SARS-CoV-2 genomic detection has been observed, spanning Moments 5, 6, and 7, covering the period from 6 February to 8 March 2022.

Since 1 February, 2022, COVID-19 case data has not been reported at the individual SZ level but rather at the city level of Córdoba. At the municipal level, a notable decrease in reported clinical cases was evident, with 14,511 cases at Moment 5 (Ct values between 35.0 and 34.1); 9,699 cases at Moment 6 (Ct 3.1), and 3,453 cases at Moment 7 (Ct 35.1) (Figure 3 and Table 1). On 15 March 2022, the Ministry of Health of the Province of Córdoba ceased publishing daily COVID-19 case numbers on the official website. The latest data at the municipal level indicated a low and stable number of COVID-19 cases, fluctuating between 44 and 211 per day. This corresponds to Moment 8 (spanning from 9 March to 27 April 2022), during which the analysis of wastewater at the SZs and municipal level resulted in negative detection of SARS-CoV-2 N gene (Figures 2 and 3).

After a period without detecting SARS-CoV-2 in wastewater (Moment 8), the viral genome was unexpectedly found in wastewater of SZs 3 and 6 on 4 May 2022 (Moment 9) (Figure 2). A week later, the viral genome was detected in all studied SZs (Moment 10), marking 15 weeks of continuous detection from 11 May to 16 August 2022. At the municipal level, SARS-CoV-2 detection became evident on 9 May 2022, 5 days after its detection on SZs (Ct 32.8). This marked the beginning of an uninterrupted increase in the frequency of SARS-CoV-2 detection at the municipal level (Moment 10, Ct values between 36.2 and 31.0), extending from 9 May to 16 August 2022. Despite the lack of local data on clinical cases of COVID-19, a national report confirmed an increase in clinical cases from epidemiological weeks 20–32 (MinSal Argentina 2022).

After continuous detection of the SARS-CoV-2 genome in wastewater of all SZs, Moment 11 begins, with viral circulation restricted to SZs 1 and 5, where wastewater remained positive for 5 weeks, from 23 August to 28 September 2022. BG-WWTP wastewater, during Moment 11, reflected viral circulation at the municipal level (Ct values between 35.3 and 34.4).

This progression of decreasing positive SZs (Moment 10 to Moment 11) led to the final period of the study corresponding to Moment 12 (Figure 2). During Moment 12, the SARS-CoV-2 genome was not detected in wastewater samples from any of the SZs studied, indicating a significant decrease in viral activity in Córdoba City. It is important to highlight that, while there were no positive samples for the SARS-CoV-2 at the SZ level, in Moment 12, sporadic detection of SARS-CoV-2 viral genome was observed at the municipal level (Ct 36). In the period covering Moment 12 (from 4 October to 31 October), a sample that tested positive for SARS-CoV-2 at the BG-WWTP was collected on 25th October, 2022.

All Ct values obtained for each SZ and at each sampling Moment are presented in Table 1 of the supplementary material.

Ct value in wastewater samples to predict the evolution of SARS-CoV-2 outbreaks in the community

Ct values of SARS-CoV-2 N gene detection in wastewater and reported COVID-19 cases at the municipal level for Moments 2, 3, 4, 5, 6, and 7 are detailed in Table 1. Notably, the average Ct values at Moment 4, which coincided with the peak in reported cases in Córdoba, did not significantly differ ($p < 0.05$) from Ct values obtained during periods with significantly fewer reported cases of COVID-19 (Moments 2, 3, 5, 6, and 7).

Detection of SARS-CoV-2 viral mutations to monitor changes in the circulation dynamics of VOCs

The analysis of SARS-CoV-2 viral variants was conducted on samples exhibiting viral activity, as indicated by the detection of the SARS-CoV-2 N gene. By the first week of Moment 2 (1–8 December 2021), the Delta variant was identified in SZ 0 and at the municipal level (Table 2, Moment 2a). Subsequently, on 13 December 13 2021 (Table 2, Moment 2b), alongside the Delta variant, the Omicron variant was introduced, observed both in SZ 0 and at the municipal level. In Moment 3, the rise in COVID-19 cases began, with the Omicron and Delta variants co-circulating at both the municipal level and in SZs 0, 1, 3, and 6. From Moment 4 until the conclusion of the study, the Omicron variant became the sole circulating variant at the municipal level. In the SZs, the Omicron variant was consistently detected, while the presence of the Delta variant was sporadically observed (SZ 6 at Moments 4 and 7, and SZ 5 at Moment 5).

Table 2 | Detection of SARS-CoV-2 viral variants in each moment (M) at all SZs and the BG-WWTP

Year	**2021**				**2022**								
Date	**10/01-11/24**	**12/01–12/16**		**12/23–12/29**	**01/05–02/02**	**02/06–02/22**	**02/23–03/02**	**03/03–03/08**	**03/09–04/27**	**05/04–05/10**	**05/11–08/16**	**08/23–09/28**	**10/04–27/10**
Sanitary zone	**M1**	**M2a**[a]	**M2b**[b]	**M3**	**M4**	**M5**	**M6**	**M7**	**M8**	**M9**	**M10**	**M11**	**Mt12**
0	–	Delta	Delta Omicron	Delta Omicron	Omicron	–	–	–	–	–	Omicron	–	–
1	–	–	–	Delta Omicron	Omicron	Omicron	Omicron	–	–	–	Omicron	Omicron	–
2	–	–	–	–	Omicron	–	–	–	–	–	Omicron	–	–
3	–	–	–	Delta Omicron	Omicron	–	–	–	–	Omicron	Omicron	–	–
5	–	–	–	–	Omicron	Delta Omicron	Omicron	–	–	–	Omicron	Omicron	–
6	–	–	–	Delta Omicron	Delta Omicron	Omicron	–	Delta Omicron	–	Omicron	Omicron	–	–
WWTP-BG	–	Delta	–	Delta Omicron	Omicron	Omicron	Omicron	Omicron	–	Omicron	Omicron	Omicron	Omicron

[a] 5 December 2021.

[b] 13 December 2021.

DISCUSSION

Environmental surveillance in wastewater samples has been implemented since 1989 and has played a fundamental role in monitoring polio eradication (Manor *et al.* 1999; Asghar *et al.* 2014; Hamisu *et al.* 2022). However, the emergence of COVID-19 in 2020 heightened attention toward monitoring wastewater as a tool for detecting infectious diseases, transforming it into an increasingly widespread public health practice that could facilitate effective surveillance of various pathogens at the community level (Shaw *et al.* 2020; Farkas *et al.* 2024; Giron-Guzman *et al.* 2024; Smith *et al.* 2024). Numerous studies on a global scale have compiled valuable information provided by the WBE of SARS-CoV-2 to characterize large sewersheds and to approach community-wide COVID-19 surveillance. However, there has been limited research adopting a more focused approach to WBE, investigating different catchment levels with flow rates varying from medium flow (neighborhood or SZ level) to low flow (city block level) to ultralow flow (building level) (Thompson *et al.* 2020; Rouchka *et al.* 2021; George *et al.* 2022; Oh *et al.* 2022). Over the past 4 years, our research team has been actively involved in wastewater surveillance to monitor the presence of SARS-CoV-2 at the municipal level. This surveillance displayed that in geographical areas with a stable population, the first detection of the SARS-CoV-2 viral genome in wastewater after a period of non-detection is an early warning sign of the subsequent increase in COVID-19 cases (Masachessi *et al.* 2022). To deepen our studies, since October 2021, we have simultaneously conducted wastewater monitoring at the municipal level and in small geographical areas defined as SZs in Córdoba City. Of the seven SZs into which the city of Córdoba is divided, one zone was excluded because its waste disposal system is not based on a convergent sewage network but rather on septic tanks. Therefore, the sewer conditions in this SZ do not allow for informative sampling of virus circulation in that geographical area (Manor *et al.* 1999; O'Reilly *et al.* 2020).

In summary, monitoring wastewater at SZs level allowed us to identify critical points of SARS-CoV-2 circulation in the city of Córdoba and to create a flow map of viral activity. This flow map pinpointed geographical areas where outbreaks started (Figure 2; Moment 2 SZ 0, Moment 9 SZ 3 and 6), and subsequently visualized the expansion of viral circulation in the city (Figure 2; Moments 4 and 10, SZ 0, 1, 2, 3, 5, and 6), as well as the moments of viral activity retraction (Moments 5 SZ 1, 5, and 6; Moment 6 SZ 1 and 5; and Moment 7 SZ 6) and non-viral detection (Moments 1, 8, and 12). Identifying this viral circulation flow allowed targeted public health actions by SZ, independent of the COVID-19 notification actions carried out by the Córdoba province Health Ministry. Specifically, these actions were aimed at surrounding the SZ to limit the spread of the virus within the city. Additionally, efforts were made to increase COVID-19 testing to ensure early detection and monitoring of infected individuals. By identifying cases promptly, it became possible to provide appropriate home-based care, reducing the need for hospital visits. Furthermore, strict criteria were implemented for referring patients to healthcare facilities, which helped alleviate the burden on hospitals by ensuring that only those who required intensive care were admitted. These measures collectively aimed to control the virus's spread and optimize the use of healthcare resources.

In recent years, studies conducted in various parts of the world have addressed WBE at the levels of medium- and low-flow sewershed capture. These studies have demonstrated that the sensitivity of environmental surveillance to detect SARS-CoV-2 circulation in a given population depends on the nature of the sewer network, the suitability of the sampling site, and the quality of sample handling and processing in the laboratory. Furthermore, highly sensitive pre-analysis and analysis techniques are crucial for timely outbreak detection, ensuring that the absence of detection truly indicates low virus circulation in the community (O'Reilly *et al.* 2020; Rouchka *et al.* 2021; George *et al.* 2022; Morvan *et al.* 2022; Oh *et al.* 2022; Giron-Guzman *et al.* 2024). In our study, the ability to obtain a wastewater sample that captures about 85% of excretory from each health zone, combined with highly sensitive pre-analytical and analytical techniques, allowed for the detection of the viral genome even when the recorded COVID-19 cases ranged between 3 and 5 (data not shown). This data should be interpreted with caution, as it is not possible to accurately estimate the number of asymptomatic individuals contributing SARS-CoV-2 genetic material to the sewage system. The variability in viral shedding among individuals and the potential presence of asymptomatic carriers make it challenging to determine the exact contribution of these individuals to the overall viral load detected in wastewater. Therefore, conclusions drawn from this data must consider these uncertainties to avoid overestimating or underestimating the true prevalence of the virus in the population.

In addition, the municipal-level study summarized the moments identified by SZs, generating a population-level pattern of viral circulation. This level of population sampling did not enable the identification of viral circulation hotspots within the city of Córdoba. However, the detection of a positive sample after a period of non-detection (Moment 2) was predictive of the COVID-19 third wave 2 weeks before it was recorded through clinical case notifications in the city of Córdoba. Similarly, the detection of the SARS-CoV-2 genome at Moment 9, following a period of undetectable genomes, was also predictive of an increase in COVID-19 cases reported in the city of Córdoba, despite the absence of daily COVID-19 case numbers published on the official website. In both instances (Moments 2 and 9), the initial positive sample was followed by the detection of several positive samples over time. Therefore, the study of the SARS-CoV-2 genome at the municipal level could silently indicate an imminent increase in COVID-19 cases. Finally, for a comprehensive and accurate interpretation of the sporadic positivity observed at Moment 12, maintaining regular and sustained sampling at the municipal level is essential. This approach will enable the collection of data necessary to better understand trends and patterns of positivity in the population over time. According to our results, different research conducted in other parts of the world and under different epidemiological conditions has highlighted that wastewater-based environmental surveillance provides valuable predictive insights into the increase of COVID-19 cases in the population (Bonanno Ferraro *et al.* 2022; Bowes *et al.* 2023; Ciannella Gonzalez-Fernandez & Gomez-Pastora 2023; Wani *et al.* 2023; D'Arpino *et al.* 2024). Therefore, SARS-CoV-2 WBE allows for the early detection of the virus's presence in communities before COVID-19 manifests in individuals, thus offering a crucial tool for the rapid and efficient intervention of public health authorities (Chavarria-Miro *et al.* 2021; Prado *et al.* 2021; Fondriest *et al.* 2024; Islam *et al.* 2024).

In the present study, the quantification of the SARS-CoV-2 genome was not performed. This decision was based on previous results obtained in our laboratory and by other authors, which have shown that changes in the SARS-CoV-2 genome concentration in sewage samples collected from large or small communities do not correlate with the number of COVID-19 cases in the same time period (Barrios *et al.* 2021; Giraud-Billoud *et al.* 2021; Masachessi *et al.* 2022). It is known that the Ct value is indirectly linked to the genomic load. In the present study, comparable Ct values were obtained during periods with the highest number of COVID-19 cases in the community (Moment 4, Ct between 35.8 and 34.0) compared to periods with the lowest number of clinical cases (Moment 2, Ct value 34). This reinforces that the viral load is not always linked to the magnitude of circulation in the community. This could be due to the fact that the genomic load and consequently the Ct value detected in wastewater depend on multiple factors such as: (a) the percentage of individuals connected to the sewage system in a community, (b) the percentage of the population vaccinated against COVID-19 (which means that reinfections generally do not translate into clinical COVID-19 cases requiring medical attention, but they could be SARS-CoV-2 genome sewersheds), (c) the methodology used for sample concentration and genome detection in wastewater, (d) virus dilution events in wastewater due to weather conditions (i.e., storm water runoff), and (e) discharge of industrial effluents or from animal farms or slaughterhouses, among others (Hamouda *et al.* 2021; Masachessi *et al.* 2022). This underscores the limitation of interpreting results based on processing a single wastewater sample, as the Ct value obtained may not correlate with the extent of virus activity in the community. Therefore, the value of SARS-CoV-2 genome studies in wastewater lies in systematic and longitudinal sampling. The interpretation of results hinges on consistently detecting the viral genome qualitatively, reflecting the virus's circulation in the community. Conversely, the absence of virus detection indicates either its absence or very low circulation. Detecting the viral genome after a period of non-detection predicts an increase in community cases.

As the SARS-CoV-2 pandemic evolved, new variants of the virus emerged, prompting questions about potential differences in transmissibility, severity, or other clinical characteristics compared to the original strain. Concerns also arose regarding the effectiveness of existing vaccines developed based on the original Wuhan strain against these new variants. Given this uncertain landscape, there was a critical need for early detection and identification of emerging viral variants in laboratory settings. Our study covered the last period of Delta variant circulation and the entry and spread of the Omicron variant in the population. Municipal level wastewater monitoring described the progressive decrease in Delta variant circulation until its extinction and the emergence and establishment of the Omicron variant in the community. SZ-level monitoring enabled geographical localization of the residual circulation of the Delta variant, the entry of Omicron, the co-circulation of both variants, and finally, the exclusive circulation of Omicron. Therefore, detecting SARS-CoV-2 variants through wastewater monitoring is a valid approach for early understanding of the emergence and population circulation of variants in a community, providing valuable information for public health and decision-making. The entry of Omicron circulation in the community occurred when a large percentage of the population (89.5%) had received the first dose of the COVID-19 vaccine and 78.6% had received two doses, along with a high record of previous natural infections. Omicron triggered the third epidemic wave of COVID-19 in the Córdoba population, characterized by a high number of mild to moderate clinical cases and a low rate of severe cases, hospitalizations, and deaths (JIC XXIII 2022). Other authors have described the utility of methodologies such as next-generation sequencing to study the polymorphism of variants of interest or concern within a community and in smaller areas to detect the introduction and emergence of these variants (Rouchka *et al.* 2021; Ishige 2024; Vigil *et al.* 2024; Wang *et al.* 2024).

The insights gained from this study offer municipalities and other institutions a new approach to implementing a wastewater-based surveillance system at both municipal and smaller geographic scales, capable of adapting to future public health challenges.

CONCLUSIONS

Wastewater-based surveillance undoubtedly functions as a silent sentinel of viral activity within a population. This approach has proven to be an invaluable tool for monitoring the presence of viruses such as SARS-CoV-2, even when other indicators are not readily available.

The success of the targeted surveillance conducted by SZs is evident in its ability to identify specific geographic areas with active viral transmission. This method allows for a precise assessment of the extent of virus circulation within the population, thus facilitating timely public health interventions.

Furthermore, studying wastewater at the population level provides a reflective measure of viral circulation within the community. It serves as an early warning system, alerting to potential increases in COVID-19 cases before they become apparent through clinical case records.

Importantly, the interpretation of data from environmental wastewater studies does not rely solely on the availability of clinical COVID-19 case records. Instead, it offers a complementary perspective that can enhance our understanding of virus dynamics and improve public health response strategies.

DATA AVAILABILITY STATEMENT

All relevant data are included in the paper or its Supplementary Information.

CONFLICT OF INTEREST

The authors declare there is no conflict.

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