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Wastewater-based monitoring of SARS-CoV-2: Insights from small and low-income Brazilian cities

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Abstract

Severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), the etiological agent of COVID-19, has affected millions of people worldwide and significantly impacted public health. The detection of viral RNA in the feces of infected individuals prompted several countries to adopt wastewater-based epidemiology (WBE) as a tool to monitor SARS-CoV-2 circulation within communities. However, most surveillance studies have focused on large urban centers. This study aimed to analyze the presence and persistence of SARS-CoV-2 in wastewater from two small Brazilian towns with low socioeconomic indicators, limited sewage coverage, and low population density, located in northern Minas Gerais. Wastewater samples were collected biweekly over 12 months, between 2023 and 2024, from three sampling points in Salinas and one in Rubelita. Viral RNA was concentrated using electronegative membranes, and SARS-CoV-2 detection was performed using real-time quantitative polymerase chain reaction. SARS-CoV-2 RNA was detected at all sampling points, with a high frequency of positive samples observed in May, June, October, November 2023, and January 2024. Notably, viral RNA was detected even after the World Health Organization declared the end of the global health emergency on May 5, 2023, and in the absence of officially reported human cases. These findings underscore the value of wastewater surveillance as a tool for monitoring SARS-CoV-2, particularly in areas with limited clinical testing capacity. The study highlights the potential of WBE to detect ongoing viral transmission across diverse urban and socioeconomic contexts, including regions with case underreporting and challenges in mass testing, thereby supporting public health preparedness and response.

Keywords: Coronavirus; Wastewater-based epidemiology; Severe acute respiratory syndrome coronavirus 2; COVID-19 pandemic

1. Introduction

The severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), a member of the *Coronaviridae* family and the *Betacoronavirus* genus, is the etiological agent of COVID-19. Transmission occurs primarily through person-to-person contact through respiratory secretions; transmission through contaminated surfaces may also occur. Clinical manifestations include fever, cough, dyspnea, headache, anosmia, ageusia, diarrhea, rhinorrhea, and muscle pain (Ebrille *et al*., 2020).

First identified in Wuhan, China, in December 2019, SARS-CoV-2 triggered a pandemic declared by the World Health Organization (WHO) on March 11, 2020. In Brazil, the first case of COVID-19 was reported on February 26, 2020, in the state of São Paulo (Oliveira *et al*., 2020), and started to spread rapidly across all states in the country.

As of February 18, 2025, a total of 715,026 deaths and 39,168,245 cases of the disease had been reported in Brazil (Ministry of Health, 2024). The rapid progression of COVID-19 prompted authorities to implement intervention measures to control the disease, including social distancing, border closures, and an urgent search for vaccines. Numerous tests were developed to detect SARS-CoV-2, with clinical testing serving as the primary surveillance method. These tests provided essential data needed to detect, assess, notify, and report public health events, in accordance with the 2005 International Health Regulations.

However, traditional surveillance based on clinical testing continued to face structural challenges, including unequal access, infrastructure limitations, resource scarcity, and underreporting of cases. These factors disproportionately affect peripheral regions or areas with limited testing capacity, such as territories with low population density and reduced healthcare coverage. The northern region of Minas Gerais, which, together with the Jequitinhonha Valley, presents the lowest socioeconomic indicators in the state (Luiz *et al.,* 2012), has been particularly affected. These regions were strongly impacted, reporting more than 319,000 cases between January 2020 and February 2025 (State Health Department of Minas Gerais, 2024).

In this context, the need to overcome the limitations of clinical surveillance has stimulated the use of alternative monitoring sources, including both environmental and digital sources. Environmental surveillance through wastewater-based epidemiology (WBE) has become a valuable strategy for identifying the circulation of SARS-CoV-2, as it enables the detection of viral RNA even in asymptomatic individuals (Ferraro *et al*., 2022). In parallel, social media-based approaches have emerged as tools capable of identifying epidemiological trends before any intervention by health authorities. Lopreite *et al*. (2021) demonstrated that Twitter data could anticipate potential COVID-19 outbreaks in Europe by detecting unusual increases in the use of terms directly associated with respiratory symptoms. Thus, both WBE and social media platforms represent important methodologies that can support the monitoring of viral circulation, particularly in contexts where underreporting is prevalent. In areas with poor access to public health services due to logistical or financial limitations, the lack of clinical testing has led to many underreported cases (C. L. F. Silva *et al*., 2020). In this context, wastewater monitoring can be especially useful in low- and middle-income countries, where resources such as viral and antibody testing capacity, hospital infrastructure, qualified personnel, and personal protective equipment may be limited (de Freitas Bueno *et al*., 2022). However, several characteristics of the sewage network and the environment are fundamental to the effectiveness of WBE, including wastewater composition (industrial or domestic), associated microbiota, in-sewer travel time, and temperature, which can accelerate the decay of SARS-CoV-2 RNA (Ahmed *et al*., 2020; Bivins *et al*., 2020; Hart & Halden, 2020; Korajkic *et al*., 2024). Population coverage and representativeness of the contributing sewage network are also crucial factors for interpreting WBE data in socially heterogeneous settings.

The monitoring of SARS-CoV-2 in wastewater was extensively explored during the pandemic, both in Brazil and globally. However, most studies were conducted in developed countries with temperate climates, suggesting that this tool can be used to analyze viral dynamics in the environment and predict potential outbreaks through increased viral loads in samples (de Freitas Bueno *et al*., 2022; Medema *et al*., 2020; Prado *et al*., 2021). Most of these investigations focused on large metropolitan areas and high-density populations. Furthermore, research has primarily focused on large urban centers or their surroundings, and was predominantly carried out before the WHO declared the end of the COVID-19 pandemic on May 5, 2023 (Pan American Health Organization, 2023). In addition, limited information is available about the potential of WBE for SARS-CoV-2 surveillance in small towns, particularly those located far from major urban centers and characterized by low socioeconomic and demographic indices, where limited access to sewage collection systems may further constrain monitoring coverage and representativeness. In such contexts, socioeconomic vulnerability and limited sanitation infrastructure may reduce the population representativeness captured by wastewater surveillance, thereby challenging the interpretation of viral circulation patterns. Moreover, little is known about the performance of WBE in post-pandemic scenarios, when vaccination, repeated exposure, and reduced testing availability may further weaken the association between wastewater viral signals and reported clinical cases.

Therefore, this study aims to monitor the dynamics of SARS-CoV-2 in wastewater from the cities of Salinas and Rubelita, two low-income municipalities located in a tropical region, characterized by low population density and remote from major urban centers. We also seek to contrast the frequency of viral detection in wastewater with the officially reported COVID-19 cases in the region. By addressing this underexplored public-health and sanitation setting, our study provides new insights into the applicability, strengths, and limitations of WBE as a complementary surveillance tool under conditions of underreporting and low sanitation coverage.

2. Materials and methods

2.1. Study area and wastewater sampling

The study was conducted between February 7, 2023, and January 1, 2024, at strategic locations within the raw sewage collection network in Salinas and Rubelita, municipalities in the state of Minas Gerais (Figure 1), located in the northern mesoregion of Minas Gerais. Salinas has an estimated population of 40,178 inhabitants, with 66.15% of the population having access to sanitation services. The municipality of Rubelita has 5,679 inhabitants, and 25.7% of them have access to adequate sewage systems (IBGE, 2022). The region where the cities are located is classified as tropical-semi-arid (Aw type) according to the Köppen climate classification, with an average annual temperature ranging from 22 to 24°C (Alvares *et al.*, 2013).

Four sampling points were selected based on accessibility and population vulnerability, capturing both commercial and domestic effluents. Three points were located in the municipality of Salinas. The first was the Raquel neighborhood, which receives wastewater from a local long-term care institution for older adults, making it a point of particular interest due to the increased vulnerability of this population to COVID-19. During the pandemic, older adults, particularly those residing in long-term care facilities, were identified as sentinel groups for early detection of SARS-CoV-2 outbreaks. This classification is attributed to their heightened vulnerability resulting from immunosenescence and the high prevalence of chronic illnesses and multiple comorbidities (Benksim *et al.*, 2020). In addition, long-term care facilities are closed environments where frequent interactions among residents and between residents and external community members, such as the staff, facilitate the rapid transmission of viruses. Studies have shown that increases in COVID-19 incidence within long-term care often precede regional peaks in confirmed cases by 1–3 weeks (Arons *et al.*, 2020). The second point was the Federal Institute of Northern Minas Gerais (IFNMG), which receives sewage from the academic and school community, representing a strategic monitoring site due to the high population turnover and intense daily interpersonal contact. The third point corresponded to the wastewater treatment plant (WWTP), which is responsible for collecting all sewage generated in the municipality, predominantly of domestic origin, with a

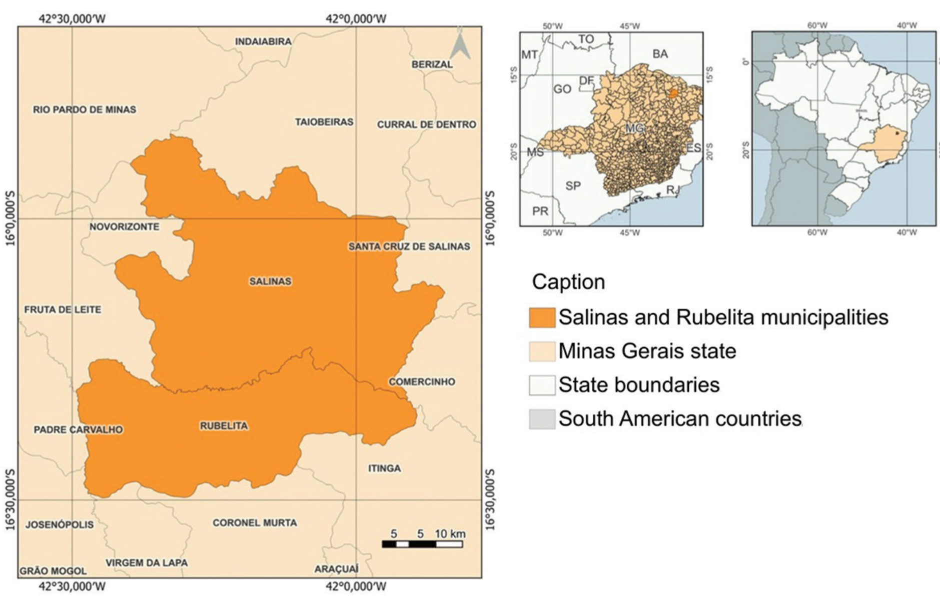

Figure 1. Location map of the municipalities of Salinas and Rubelita, Minas Gerais, Brazil

limited contribution from small commercial establishments and no significant industrial effluent. The fourth sampling point was located in Rubelita, also at the municipality's WWTP, which similarly receives predominantly domestic wastewater with minor commercial contributions and no industrial discharge.

Samples were collected biweekly using the composite sampling technique, which involves collecting three subsamples of approximately 1 L of sewage at 10-min intervals. The collected sewage was then homogenized, and 500 mL was transferred to properly labeled plastic containers. Samples were stored in a cooler with ice and transported to the Molecular Biology Laboratory at IFNMG, Salinas Campus, within 2 h for SARS-CoV-2 RNA analysis. To prevent contamination, researchers wore appropriate personal protective equipment, including lab coats, masks, and gloves.

2.2. Sample concentration and RNA extraction

The viral content from the sewage samples was concentrated using electronegative membrane filtration, a widely used method for enteric viruses that requires minimal specialized laboratory infrastructure (Ahmed *et al*., 2020; Ahmed *et al*., 2015). Initially, 1 mL of magnesium chloride was added to each 100 mL aliquot of the sample. The samples were then acidified by adding acetic acid until the pH reached between 3.0 and 3.5 (Ahmed *et al*., 2020).

The samples were then filtered through 0.45 μm cellulose ester electronegative membranes (47 mm in diameter; HAWP09000; Merck Millipore, Ltd., Australia), using a Büchner funnel connected to a vacuum pump. After filtration, the membranes were placed in tubes containing RNAlater™ (Invitrogen, ThermoFisher, United States) and silica beads for subsequent membrane disruption, and stored in a −80°C freezer until RNA extraction, which was performed within 15 days of sample collection.

After membrane disruption using a Loccus (Brazil) L-Beader 24® tissue homogenizer, 600 μL of lysis solution (PureLink RNA Mini Kit®, ThermoFisher, United States) was added to the tube and mixed with 6 μL of 2-mercaptoethanol. The samples were centrifuged (14,000 g, 15 min, at 8°C), and 800 μL of supernatant was used for the extraction process, following the manufacturer's protocol.

2.3. Viral detection

For viral RNA detection, real-time quantitative polymerase chain reaction was performed, targeting the *SARS-CoV-2 E* gene (envelope) and the *ribonuclease P* gene as a reaction control, both of which are included in the commercial SARS-CoV-2 (EDx) Bio-Manguinhos (Brazil) molecular kit, following the manufacturer's recommendations. Reactions were conducted on a QuantStudio 3 thermal cycler (ThermoFisher, United States) under the following cycling conditions: 50°C for 15 min, 95°C for 2 min, followed by 40 cycles of 95°C for 15 s and 58°C for 30 s. Specific primers were used for the envelope gene (E_Sarbeco_F1: ACAGGTACGTTAATAGTTAATAGCGT; E_Sarbeco_R2: ATATTGCAGCAGTACGCACACA; and E_Sarbeco_P1: ACACTAGCCATCCTTACTGCGCTTCG-NFQ) and for the *ribonuclease P* gene (RdRP_SARSr-F2: GTGARATGGTCATGTGTGGCGG; RdRP_SARSr-R1: CARATGTTAAASACACTATTAGCATA; and RdRP_SARSr-P2: VIC-CAGGTGGAACCTCATCAGGAGATGC-NFQ) (Corman *et al*., 2020). Each sample was tested in duplicate, and samples were considered negative when the cycle threshold value was >40 (Ahmed *et al*., 2022).

2.4. Data analysis

The overall positivity rate was calculated by dividing the number of samples with detected viral RNA by the total number of samples tested. This calculation was also performed separately for each sampled municipality and each sampling point to obtain the positivity rates for each location. Data were compiled using Microsoft Excel to monitor viral circulation throughout the sampling period.

The number of confirmed human COVID-19 cases reported biweekly was obtained from the official sources of the Minas Gerais State Health Department (State Health Department of Minas Gerais, 2024). These data were compiled and compared with the results of viral RNA detection in wastewater samples.

2.5. Scientific dissemination

To generate information for controlling the spread of the virus and provide the community with feedback on the research, we prepared epidemiological bulletins, disseminated to the authorities through official IFNMG channels, as well as in the form of informative posts on social media. This allowed for the visualization of SARS-CoV-2 presence at sampling points over time. Social media was also utilized to facilitate clear and accessible communication for the general public.

3. Results

3.1. Sample collection and SARS-CoV-2 RNA detection in wastewater samples

Between February 2023 and January 2024, we conducted 26 biweekly collections at each of the three sampling points in the municipality of Salinas and one sampling point in Rubelita. A total of 104 wastewater samples were collected, with 78 samples from Salinas and 26 from Rubelita. In total, SARS-CoV-2 RNA was detected in 55 (52.88%) of the samples (Table 1). In Salinas, 46 (58.9%) samples tested positive, and in Rubelita, 9 (34.6%) samples tested positive. The highest positivity rate was observed at the WWTP in Salinas, with 21 positive samples, whereas the lowest rates were found at IFNMG and at the Rubelita WWTP, with nine positive samples each (Table 1). The mean cycle threshold values of the positive samples ranged from 31.0 to 39.0 (Table S1).

Importantly, the virus was detected in at least one of the sampling points during every collection, except on February 7, 2023, February 24, 2023, and March 8, 2023 (Figure 2). Despite the recurrent detection of SARS-CoV-2 RNA in wastewater, only 15 human COVID-19 cases were confirmed in the same 1-year period (State Health Department of Minas Gerais, 2024). Throughout the year, the distribution of positive samples fluctuated, revealing clear periods of increased viral circulation. Notably, detection peaks—during which viral RNA was identified simultaneously at all sampling points—were observed between sampling rounds 8 and 11 (May 11 to June 22, 2023), a period when only a single human case was reported in Salinas and none in Rubelita. Similar peaks occurred in sampling rounds 20 and 21 (October 25 to November 6, 2023), again coinciding with minimal reported cases: One in Salinas and none in Rubelita (Secretaria Estadual de Saúde de Minas Gerais, 2024). In addition, rounds 25 and 26 (January 4 to January 15, 2024) exhibited widespread positivity across nearly all sampling points, with IFNMG being the only exception, reinforcing the value of wastewater surveillance as a sensitive tool for detecting viral circulation independently of clinical notifications, as illustrated in Figure 2.

Table 1. Detection of SARS-CoV-2 RNA in wastewater collected in Salinas and Rubelita

Municipality	Sampling point	No. positive samples/No. total samples (%)
Salinas	WWTP Salinas	21/26 (80.7)
	IFNMG	9/26 (34.6)
	Raquel neighborhood	16/26 (61.53)
Rubelita	WWTP Rubelita	9/26 (34.6)
Total		55/104 (52.88)

Abbreviations: IFNMG: Federal institute of northern minas gerais; WWTP: Wastewater treatment plant.

3.2. Scientific dissemination

The periodic results of the collections were communicated firsthand to the health authorities of the involved municipalities to support local epidemiological surveillance and facilitate decision-making. In addition, during the collection period, digital content was created for the Instagram account of the Laboratory of Insect Behavior, @lacoi_ifnmg, including 8 feed posts and 24 story posts as a means of scientific dissemination (Table 2). Bulletins were also produced to share the results on social media and the institutional website of IFNMG, as shown in Table 2.

4. Discussion

The SARS-CoV-2 virus has a high transmission capacity and was responsible for a pandemic with profound social, economic, and public health impacts (Gunawardana *et al.*, 2021). Although the WHO declared the end of the COVID-19 global health emergency public health emergency of international concern (PHEIC) in May 2023 (World Health Organization, 2023), continuous surveillance of the virus is essential for outbreak detection and the implementation of control measures. In this context, wastewater monitoring emerges as an important

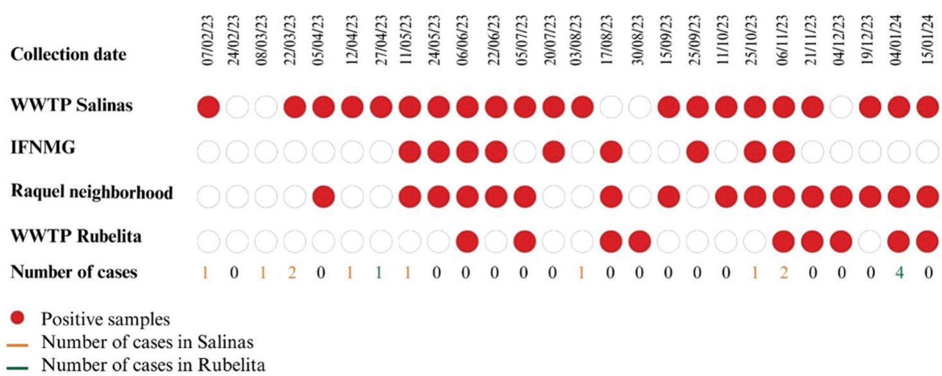

Figure 2. Results of sewage samples and numbers of human cases in Salinas and Rubelita
Abbreviations: IFNMG: Federal institute of northern minas gerais; WWTP: Wastewater treatment plant.

Table 2. Links to access digital content used for scientific dissemination

Feed of the laboratory of Insect behavior	Website of the federal institute of northern minas gerais
Link 1	Link 1
Link 2	Link 2
Link 3	
Link 4	
Link 5	
Link 6	
Link 7	
Link 8	

surveillance tool, and WBE has proven to be a valuable approach for monitoring health-related aspects in different parts of the world (de Freitas Bueno *et al*., 2022).

SARS-CoV-2 detection rates in wastewater are typically directly proportional to the size of the contributing population, with larger populations facilitating more consistent detection of viral RNA (Wu *et al*., 2020). However, this study underscores the applicability of wastewater surveillance as an epidemiological tool, even in small municipalities with low Human Development Index, such as Salinas and Rubelita (41,178 and 5,679 inhabitants, respectively). Gudra *et al.* (2022) similarly identified SARS-CoV-2 RNA in wastewater from two small towns in Latvia, Jelgava (~55,000 inhabitants) and Kuldiga (~11,000 inhabitants), further validating the sensitivity and adaptability of this method. Furthermore, other cities in Minas Gerais, such as Belo Horizonte and Contagem (Chernicharo *et al*., 2021), have implemented wastewater-based surveillance within large urban centers characterized by a high Human Development Index, substantial population densities, and significant commuter inflows. These contrasting scenarios highlight the versatility of wastewater surveillance in providing actionable epidemiological insights across diverse demographic and socioeconomic contexts.

Notably, viral RNA was detected in 24 out of 26 sampling rounds, including all samples collected after the WHO announced that COVID-19 no longer constituted a PHEIC on May 5, 2023 (Figure 2). Similarly, Tateishi *et al.* (2025) and C. C. M. Silva *et al.* (2024) reported SARS-CoV-2–positive wastewater samples after the PHEIC was lifted, supporting wastewater surveillance as an effective early warning system for ongoing viral transmission. Thus, the declaration of the pandemic's end does not equate to the eradication of COVID-19, and preventive measures must continue, particularly the reinforcement of vaccine doses for at-risk groups such as the elderly and individuals with comorbidities. Although WBE is a powerful tool for monitoring viral circulation, it does not directly indicate the occurrence of an outbreak, but rather reflects the presence of the virus in the contributing population. In this context, wastewater surveillance can serve as a powerful and low-cost indicator of viral circulation in the population, especially in remote or underserved areas where access to individual testing is limited, thereby enabling public health authorities to make informed decisions, such as reinforcing vaccination or prevention campaigns (Claro *et al*., 2021; Gudra *et al*., 2022; Prado *et al*., 2021). Monitoring viral variants also remains crucial for the detection of more pathogenic lineages or those with vaccine escape potential (Eales *et al*., 2024).

Although it was not our objective to statistically correlate the detection of viral RNA in wastewater with clinical cases, it is worth noting that, despite the almost constant detection of the virus in wastewater, only a few cases were reported during the entire sampling period. The State Health Department of Minas Gerais issued weekly epidemiological bulletins (https://info.saude.mg.gov.br/1/paineis/2) to analyze and disseminate information on COVID-19 cases across the state. During the study period, the bulletins reported only 10 human cases in Salinas and five in Rubelita (Figure 2) (Minas Gerais State Health Department, 2024). Several factors may explain this underreporting, such as limited access to clinical testing, the ongoing vaccine rollout since 2021, and prior infections, all of which may contribute to milder symptoms (Rodda *et al*., 2021), thereby reducing the demand for testing and visits to primary healthcare units.

Throughout the year, viral detection peaks were observed when viral RNA was found at all collection sites (Figure 2). These peaks coincided with significant climatic and behavioral factors, such as the winter period, which is conducive to the transmission of respiratory viruses, as well as national holidays in October/November and the year-end recess, which increases the movement of people between different states/municipalities. This trend mirrors the pattern observed in Brazil since 2020 (Berra *et al*., 2024; Minas Gerais State Health Department, 2024). Nevertheless, it must be emphasized that, due to the absence of viral load quantification, these peaks represent qualitative detection events rather than true quantitative surges in viral circulation. Although wastewater analysis in these two small towns proved useful for tracking the spread of the SARS-CoV-2 virus and assessing case control and reporting efforts, the quantification of viral RNA in wastewater would be necessary for evaluating potential COVID-19 impacts (Corpuz *et al*., 2022).

The disparity in the proportion of positive samples between Salinas and Rubelita, with 58.9% and 34.6% positivity rates, respectively, can be attributed to Salinas having a population 7 times larger than Rubelita (41,000 vs. 5,600 inhabitants) and a higher population density (21 inhabitants/km^2 vs. 5 inhabitants/km^2) (IBGE, 2022). Salinas is a micro-region of significant importance for the regional economy, education, and healthcare, making it a location with a notable presence of individuals from other municipalities. The IFNMG campus in Salinas, for instance, has two boarding schools for students from neighboring municipalities, accommodating 164 residents (direct communication). Furthermore, a nephrology service is available at the local hospital, serving patients from several cities, with a capacity of up to 180 patients (Dilson Godinho Foundation, 2024), which further contributes to the movement of people in the city, increasing the likelihood of viral transmission (Wu *et al*., 2020).

Another factor that may influence the differences in positivity between the municipalities is the level of sanitation coverage available to the population. Salinas has an estimated population of 40,178 inhabitants, with 66.15% receiving wastewater services, whereas Rubelita has only 25.7% coverage for its 5,679 inhabitants. This discrepancy reduces the number of individuals contributing to the sewer network in Rubelita and, consequently, decreases the likelihood of detecting viral RNA (IBGE, 2022). Previous studies have shown that the extent of sewerage networks is important for the performance of wastewater-based surveillance, as incomplete systems result in lower population participation and greater environmental dispersion of waste (Gudra *et al*., 2022). Thus, the low sanitation coverage in Rubelita likely intensifies the dilution and loss of viral genetic material, reinforcing that sanitation infrastructure is a critical variable for the success of WBE, particularly in low-income regions. Since socially deprived populations are often disproportionately affected by health conditions, this context also highlights an important limitation of WBE in areas with low sanitation coverage, where population representativeness in wastewater surveillance may be reduced.

Furthermore, although we did not directly evaluate this variable, previous studies have shown that the fate of viruses in wastewater can be affected by inactivation, degradation, dispersion, and retardation (Kallem *et al*., 2023). Temperature is the most important factor, as it plays a decisive role in RNA stability (Hart & Halden, 2020). However, temperature did not appear to compromise the detection of SARS-CoV-2 in our samples. Considering that our study was conducted in one of the hottest regions of Brazil (Hart & Halden, 2020; Korajkic *et al*., 2024), the continuous detection of viral RNA throughout most of the analyzed period reinforces that, even under adverse environmental conditions, wastewater-based surveillance can still provide valuable epidemiological information.

This study presents some limitations, including its restricted geographical scope, which is limited to two municipalities, and the absence of viral load quantification due to technical limitations. This limitation is particularly relevant when interpreting peak detection events, as the lack of quantitative data prevents robust inferences regarding transmission intensity and the magnitude of outbreaks. In addition, the protocol adopted for sample concentration and detection, although widely used in the early stages of the COVID-19 pandemic, is currently considered to have lower sensitivity compared to more recent methodologies (Juel *et al*., 2021). Nevertheless, even under these constraints and in a region marked by socioeconomic and structural vulnerabilities, the method proved effective in detecting SARS-CoV-2 RNA in wastewater samples throughout the study period. These findings reinforce the feasibility and relevance of WBE as a complementary surveillance tool, particularly in low-resource settings where clinical testing may be scarce or underutilized. When combined with more frequent sampling and viral load quantification, WBE has the potential to serve as an early warning system for detecting viral circulation and outbreaks. In this context, scientific dissemination efforts, such as those undertaken in this study, are essential to ensure that academic research findings extend beyond the academic community. We also emphasize the importance of maintaining immunization efforts, including the expansion of booster doses for at-risk populations, such as the elderly and individuals with comorbidities. Overall, our results demonstrate that even with limited resources, WBE can offer valuable epidemiological insights and support more equitable health surveillance strategies.

5. Conclusion

From a public policy perspective, the results reinforce WBE as a strategic, low-cost, and scalable surveillance tool that can be formally incorporated into routine public health monitoring, especially in small and socioeconomically vulnerable municipalities. The continuous detection of SARS-CoV-2 after the end of the COVID-19 global health emergency highlights the need for permanent surveillance systems rather than temporary emergency actions. Our findings support the formulation of policies that integrate environmental surveillance with clinical reporting systems, strengthen sanitation infrastructure, and prioritize investments in early warning systems to guide timely vaccination campaigns.

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Conflict of interest

The authors declare they have no competing interests.

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Ethics approval and consent to participate

Not applicable.

Consent for publication

Not applicable.

Availability of data

All data analyzed have been presented in the paper.

Further disclosure

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