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RESEARCH ARTICLE

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The potential of long-term wastewater-based surveillance to predict COVID-19 waves peak in Mexico

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Abstract

Wastewater-based surveillance (WBS) is valuable method for monitoring the dispersion of pathogens at a low cost. However, their impact on public health decision-making is limited because there is a lack of long-term analyses, especially in low- and middle-income countries. This study aimed to assess the effectiveness of using WBS to predict the occurrence of COVID-19 waves and estimate the prevalence of infection, emphasizing the impact of SARS-CoV-2 variants. During 17 months of influent monitoring of two wastewater treatment plants in Queretaro City, Mexico, wave prediction time was influenced by variant dispersion. Waves dominated by the Delta and Omicron variants circulation showed lead days values from 5 to 14 and 1 to 4 days, respectively. According to the Monte Carlo model, disease prevalence prediction by WBS aligned with clinically reported cases at wave onsets, but the variant's transmissibility explained the overestimation during peaks. This work provides new insights into the potential and limitations of using WBS as an epidemiological tool for detecting pathogens and predicting their occurrence.

Practitioner Points

- Long-term wastewater monitoring allowed early prediction of COVID-19 case waves.
- The prediction capability is related to the variant presence and their infectivity.
- The prevalence estimated by wastewater surveillance was higher in all case waves.
- The prevalence estimation has limitations regarding variations in data input.

KEYWORDS

Monte Carlo simulation, SARS-CoV-2 variants, viral transmissibility, wastewater-based surveillance

INTRODUCTION

The COVID-19 pandemic had a devastating global impact, with more than 770 million cases and 6.9 million deaths; despite the declaration of the end of the emergency phase, the SARS-CoV-2 dispersion is still a concern (WHO, 2024). Controlling pandemics relies on clinical surveillance, which is limited by public health infrastructure. This leads to delays in case detection and monitoring system implementation, especially in low- and middle-income countries (LMICs), where the process can be expensive and politicized. Moreover, it has limitations regarding early identification of outbreaks and monitoring asymptomatic cases (Chan et al., 2010; Nieuwenhuijse & Koopmans, 2017; Wannigama et al., 2023).

Due to the limitations and rapid spread of SARS-CoV-2 in the population, monitoring its presence in wastewater emerged as an alternative to tracking the pandemic globally (Ahmed et al., 2020; Ampuero et al., 2020). Wastewater-based surveillance (WBS) is a tool that can predict disease outbreaks before clinical diagnosis. It analyzes a population's risk factors and health status, helping with timely decision-making and public health interventions (Calabria De Araujo et al., 2021; Parkins et al., 2024). This tool is based on extracting, detecting, and interpreting chemical or biological compounds, often called biomarkers in wastewater, referring to the concentration of pathogens, drugs, pharmaceuticals, or pollutants (Escolà Casas et al., 2021; Vitale et al., 2021). According to the World Bank Group, wastewater testing is a more cost-effective surveillance method than clinical testing for LMICs (Manuel et al., 2022). WBS studies in LMIC are limited, focusing for instance, on the spread of human polyomavirus (JCPyV), as one of the main enteric viruses present in wastewater in Brazil, Argentina, Chile, and Thailand (Fumian et al., 2010; Levican et al., 2019; Sangsanont et al., 2022; Torres et al., 2016).

Recently, SARS-CoV-2 detection has been evaluated in LMIC such as India, Brazil, and Mexico by correlating the SARS-CoV-2 viral load in wastewater with the number of COVID-19 cases in each country (Carrillo-Reyes et al., 2021; Hemalatha et al., 2020; Kumar et al., 2020; Mota et al., 2021). To better understand the capacity and potential applications of WBS for SARS-CoV-2, a few cases have analyzed temporal variations in genetic material through long-term. Quantitative model development has also been applied to predict the number of infected people or viral prevalence under different conditions compared to clinical prevalence from a Bayesian framework to the implementation of the Monte Carlo model (Dai et al., 2024; De Araújo et al., 2022; Fu et al., 2023; Mota et al., 2021). Also, temperature variation throughout the year due to seasons is a critical factor influencing RNA degradation. Failing to consider this can lead to underestimating or overestimating pathogen dissemination changes within the population (Hart & Halden, 2020a, 2020b). SARS-CoV-2 infections have become evident through symptomatic people attending medical facilities; however, the detection may occur sometime after a new wave has started (Ngwira et al., 2022; Pincombe et al., 2021).

The world struggled with the spread of SARS-CoV-2 infections as new variants emerged caused by mutations in the virus's genome that have spread globally, with a natural occurrence. The WHO has identified five “variants of concern”: Alpha (B.1.1.7) and Beta (which were detected in December 2020), Gamma (detected in January 2021), Delta (in May 2021), and Omicron (in November 2021). These variants are more concerning due to their greater epidemic potential and ability to evade the immune system, which can lead to faster spread and replacement of previous variants (Cherian et al., 2021; Loza et al., 2023; Taboada et al., 2023). There is a potential benefit of monitoring the population for SARS-CoV-2 by analyzing wastewater over an extended period, particularly when there is a high risk of transmission. However, very few studies have been conducted in the long term to evaluate the predictive capacity of WBS with the presence of different SARS-CoV-2 variants. Studies have shown that the prediction time for the next wave of cases ranges from 1 to 2 weeks (Hopkins et al., 2023; Kumar et al., 2023). In Latin America, there are long-term monitoring WBS studies of 15, 17, 20, and 26 months for COVID-19; however, their prediction has not been evaluated based on the intensities of different disease prevalence rates in different waves (Belmonte-Lopes et al., 2023; De Araújo et al., 2022; Marques Dos Santos et al., 2023; Masachessi et al., 2022), respectively. In Mexico, the monitoring of SARS-CoV-2 in wastewater has been evaluated in some regions (Carrillo-Reyes et al., 2021; González-Reyes et al., 2021; Hernández-Terrones et al., 2023; Padilla-Reyes et al., 2022; Schilmann et al., 2023; Zarza et al., 2022), but these investigations have been carried out in periods not exceeding 6 months. In this sense, Latin American countries lack knowledge about how various COVID variants affect the prevalence and predictive capacity of WBS, creating a gap in comparative studies.

This study aimed to evaluate the potential of using WBS to predict the emergence of COVID-19 waves and estimate the prevalence of infection with respect to the impact of SARS-CoV-2 variants. To achieve this, a long-term case study was conducted in Queretaro, Mexico, monitoring the influent of two wastewater treatment plants (WWTPs) during three waves of COVID-19 cases between March 2020 and December 2021. This study provides evidence of WBS's potential to complement pathogen and disease monitoring in the population.

MATERIAL AND METHODS

Sampling

Two WWTPs were evaluated in this study, located in the municipalities of Corregidora (population 212,567) and Santiago de Queretaro (population 1,049,777) both within the metropolitan area of Queretaro, Mexico (Table S1). The wastewater monitoring was divided into two periods. In Period 1, 24-point samples every 15 days were collected in the influent of two WWTPs denoted PS and SRJ. Both WWTPs operate with a conventional activated sludge process. During Period 1, 1 L of grab samples were collected using a manual sampler during the morning (at 8:00 h). Considering that the volume of wastewater a WWTP receives varies throughout the day and can influence the maximum virus load in the sewage system, (Farkas et al., 2018; Gerba et al., 2017) suggest using 1-day composite samples to improve concentration estimates. Therefore, for Period 2 (September 2021–July 2022) 24-h composite samples were collected using an autosampler, taking a 100-mL sample every 30 min and accumulated in a cold chamber (4°C) every 15 days in the influent of the WWTP-PS (obtaining a total of 12 samples). For both periods, once samples were collected, they were transported to the laboratory and kept at 4°C until they were processed.

RNA concentration and extraction

On the same day of sampling collection, ribonucleic acid concentration was performed in duplicate using the filtration method with electronegatively charged membranes for both periods. Sample volumes ranging from 20 to 50 mL were taken up and adjusted to a pH of 3.5 with a 2-N HCl solution. The samples were then filtered through a negatively charged nitrocellulose membrane with a pore size of 0.45 μm (Millipore, The Netherlands) using a glass funnel and a base (Millipore, The Netherlands) (Carrillo-Reyes et al., 2021). The membranes were kept at −20°C no more than 2 days until the RNA extraction procedure was performed. According to the manufacturer's instructions, RNA was extracted from the membranes using the RNeasy Power Microbiome kit (QIAGEN, The Netherlands); 50 μL of RNA extract was recovered from each sample and was kept at −80°C until its quantification, no more than 2 days.

SARS-CoV-2 quantification by RT-qPCR

From the RNA extracted in Period 1, quantification of the RdRP gene specific for SARS-CoV-2 was performed, following the methodology proposed by (Oyervides-Muñoz et al., 2024). The primers and probe were synthesized by Sigma-Aldrich (USA). The Wuhan coronavirus 2019 RdRP gene control (European Virus Archive Global, Germany) was used as calibration suspension using four tenfold dilutions. All RT-qPCR assays were duplicated using a Real-Time PCR system (Applied Biosystems StepOne™, USA). A Cq of 22.87 ± 0.67 was obtained for the calibration curve, the details of the calibration curve are shown in Table S2.

During and after the first sampling period of this study, different SARS-CoV-2 genes were evaluated in the literature as targets in wastewater, finding a higher detection sensitivity for N1 and N2 genes for the RdRP gene (Kaya et al., 2022; Pérez-Cataluña et al., 2021). Considering the latter findings, for Period 2, the N gene of SARS-CoV-2 was quantified by RT-qPCR. Quantification was carried out for all the samples using the Water SARS-CoV-2-RT-PCR Test, as previously reported (Oyervides-Muñoz et al., 2024). The calibration curve details are reported in Table S2. A positive and negative control was included for every RT-qPCR assay. An internal control was evaluated to detect inhibition in amplification during Period 1, implementing the RnaseP gene quantification method (Carrillo-Reyes et al., 2021). For the N gene, all the RT-qPCR reactions included an internal control based on the IDEXX Internal Water Control (IDEXX Part Number: 99-57010-00).

SARS-CoV-2 quantification in the influent of WWTPs

RNA quantification data were adjusted to a degradation factor determined from WWTP data, and the wastewater temperature was measured on-site (Equation 1), as previously reported (Schilmann et al., 2023).

$$Degradation\ Factor = 0.5\left\{\frac{Mean\ travel}{Half\ life\ in\ sampling\ conditions}\right\} \tag{1}$$

where mean travel (h) is the travel time of the wastewater in the sewer until it reaches the WWTPs, and half-life in sampling conditions (h) is the lifespan of the virus at water temperature (Table S3, Equation S1 and Equation S2). The RNA of SARS-CoV-2 genes in wastewater between sampling days was estimated by implementing the locally estimated smoothed scatterplot (LOESS) model to model the weekly viral load as a function of time, to produce an estimate of the potential curve as close as possible to the observed data, subject to the smoothness of the adjustment (Cleveland, 1979; Shokrzadeh et al., 2014), as has been proposed for WBS of SARS-CoV-2 (Sakarovitch et al., 2022).

Clinical COVID-19 cases

The dynamic of genes in wastewater was compared to active COVID-19 cases reported by health authorities to assess the potential of WBS as an early warning system and estimate prevalence in each wave of cases for both WWTPs. Clinical data were obtained from the Official Mexican government dashboard (https://datos.covid-19.conacyt.mx). Active cases were calculated as the sum of confirmed cases that began symptoms within 14 days before the wastewater sampling date, considering that viral shedding can start 5–6 days before symptoms appear (He et al., 2020). This time range for active cases determination and comparison for WBS has been previously reported (Schilmann et al., 2023). Three wave periods were considered in this work, corresponding to the reported waves and COVID-19 cases determined by the National Epidemiological Surveillance System (https://www.sinave.gob.mx) from October 5, 2020, to April 16, 2021, June 7 to October 29, 2021, and December 27, 2021, to March 4, 2022, for the second, third, and fourth COVID-19 waves in Mexico, respectively. Clinical prevalence is considered as the number of COVID-19 infected in the population, estimated for each municipality using the equation (Equation 2) proposed by (De Freitas Bueno et al., 2022). This study reported prevalence results as the number of cases per 1000 inhabitants (National Institute of Mental Health, 2024).

$$
\begin{aligned}
&\mathit{Clinical\ prevalence}\left(\mathit{number\ of\ cases}\right)\\
&=\left(\frac{\mathit{People\ who\ have\ the\ condition\ at\ point\ in\ time}}{\mathit{People\ living\ in\ the\ population\ at\ point\ in\ time}}\right)\\
&\quad\times 1000
\end{aligned}
\tag{2}
$$

where people with the condition at the point in time refers to the estimated active cases to the sampling date, people living in the population to the total number of inhabitants in each municipality. The Mexican Consortium for Genomic Surveillance (CoViGen-Mex by its Spanish acronym, http://mexcov2.ibt.unam.mx) obtained data on variant dispersion in Mexico.

Lead time of WBE surveillance

The WBS implementation was evaluated as an early warning system prior to clinical case reporting; thus, the early warning time was statistically determined with a specific lag in days using Pearson's R to compare adjusted RNA concentrations in wastewater and active cases. The procedure to locate the maximum R was implemented, as previously reported using Stata software (v 16.1, StataCorp LLC, TX, USA) (Schilmann et al., 2023).

Infection prevalence of SARS-CoV-2 estimation

The WBS prevalence estimate of SARS-CoV-2 infection was compared with the prevalence estimated with clinical cases. The prevalence estimated by WBS followed Equation 3 proposed by (Schilmann et al., 2023).

$$
\begin{aligned}
&\mathit{WBS\ prevalence}\left(\mathit{numer\ of\ cases}\right)\\
&=\left\{\frac{\left\{\dfrac{\mathit{SARS{-}CoV2\ quantification}\times \mathit{WWTP\ flow}}{\mathit{Degradation\ Factor}\times \mathit{Recovery\ percentage}}\right\}}{\dfrac{\mathit{normal\ fecal\ load}\times \mathit{fecal\ excretion\ rate}}{\%\mathit{of\ infected\ who\ shed\ SARS{-}CoV{-}2\ fecally}}}\right\}
\end{aligned}
\tag{3}
$$

where SARS-CoV-2 quantification (copies/mL) is the virus quantification from the wastewater sample (Section 2.3), WWTP flow (m^3/s) is the mean daily inflow at the WWTPs obtained in situ, degradation factor (Section 2.4). The amount of viral genetic material lost during sample processing was evaluated, where a bacteriophage MS2 (ATCC 15597-B1) recovery assay was performed. The assay consisted of inoculating wastewater samples at a concentration of 2.3*x*1011pfu/mL; the inoculated samples were treated with the concentration and isolation procedures described above, then a double agar layer assay was performed to measure the active bacteriophages retained as plaque-forming units per milliliter (PFU/mL), as reported by (Cormier & Janes, 2014), recovery percentage is the mean recovery virus (0.292). Some parameters were obtained from the literature as normal fecal load (g/day*person) is the amount of feces excreted per person per day (Rose et al., 2015), fecal excretion rate (copies/mL) is the viral load excreted in feces (Brown et al., 1996), fecal density (g/mL) (Weiss et al., 2020), and % of infected who shed SARS-CoV-2 fecally (Cheung et al., 2020) (Table S4). Due to the wide range of variation in these parameters, interpreting the results of the infected population and the predicted prevalence is difficult. To address this, a Monte Carlo statistical approach was used to calculate the prevalence estimate by generating 1000 estimates for each sample using different ranges of values for each parameter (Schilmann et al., 2023).

RESULTS AND DISCUSSION

Dynamics of SARS-CoV-2 concentration in wastewater

The behavior of SARS-CoV-2 RNA adjusted loads showed a similar trend to the three waves of cases present during the study (Figure 1). Concerning the PS WWTP (Figure 1a, Period 1), during the second wave, the cases decreased when the sampling period began. The RdRP gene's quantification values in wastewater ranged from 1.7 to 3.3 Log_{10}/mL. For the SRJ WWTP (Figure 1b), quantification values of the RdRP gene in wastewater were obtained in a range of 0.9 to 3.3 Log_{10}/mL in the second wave of cases. In the PS WWTP, N gene concentrations ranged from 2.4 to 3.2 Log_{10}/mL during the peak of the third wave and subsequently decreased to 0 to 0.6 Log_{10}/mL until the onset of the fourth wave and then increased to 2.6 Log_{10} copies/mL. During the third wave, the viral concentrations of the N gene at the SRJ WWTP remained consistent at around 2.4 to 3.1 Log_{10}/mL until the end of the sampling period, indicating that SARS-CoV-2 concentration peaks align with other studies during the monitoring period. Similar ranges of SARS-CoV-2 genes concentrations were quantified in wastewater, using different RNA concentration methods, in São José do Rio Preto, Brazil, four different cities from Argentina, and in Ahmedabad, India, reported concentrations ranging from 0 to 2 Log_{10}/mL for N1 and N2 genes using centrifugal ultrafiltration, 0 to 3.2 Log_{10}/mL for N1 gene, and around 1 Log_{10}/mL for of the SARS-CoV-2 genome, both using polyethylene glycol-NaCl precipitation (Barrios et al., 2021; Kumar et al., 2023; Martins et al., 2022).

The similar and, in some cases, higher range of viral concentrations from this study compared to others, corroborates that the WBS is a reliable tool for peak concentration detection in the case of SARS-CoV-2 prevalence under different waves of infections.

In Mexico, wastewater from Tapachula, northeast of Mexico City, Monterrey and Querétaro cities, a minimum concentration of about 1 Log_{10}/mL was obtained in all cases while the maximum concentrations were 2.8, 2.3, 3, and 3.7 Log_{10}/mL of the N gene, respectively (Carrillo-Reyes et al., 2021; González-Reyes et al., 2021; Padilla-Reyes et al., 2022; Zarza et al., 2022), and a minimum and maximum concentrations in a range of 1.5 to 5 Log_{10}/mL for the RdRP gene in Queretaro and Cancun (Carrillo-Reyes et al., 2021; Hernández-Terrones et al., 2023). However, these previous works were limited by sampling time to predict subsequent waves of cases but are consistent with the concentrations observed in our study, which was conducted a month before the third

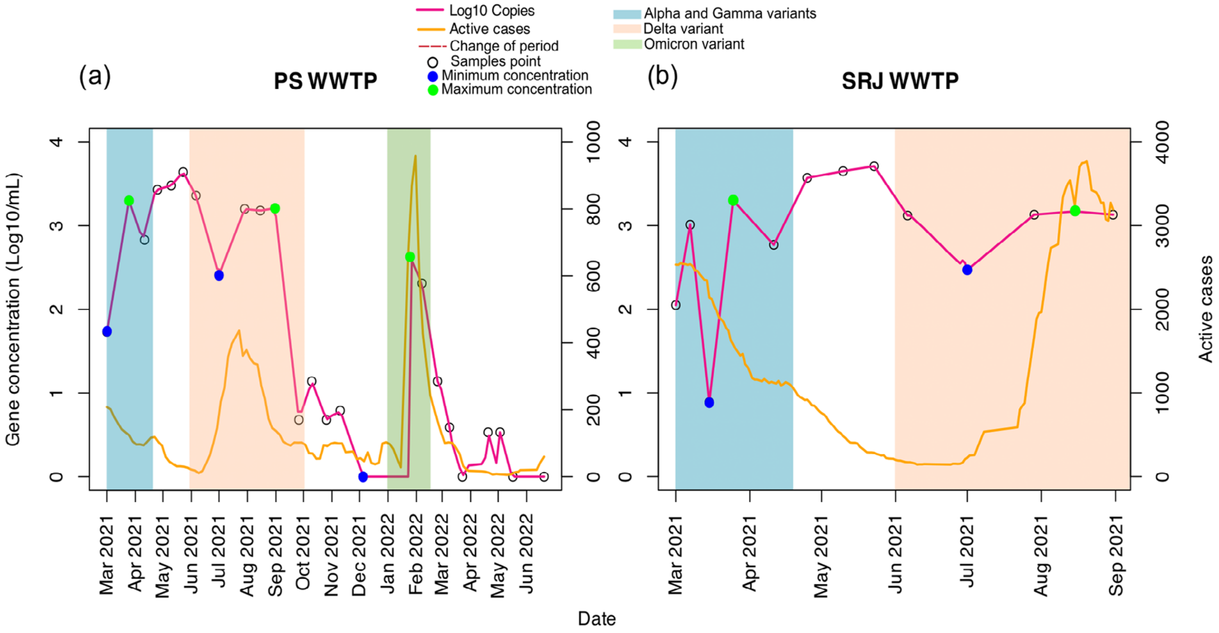

FIGURE 1 Logarithmic genomic concentration of SARS-CoV-2 genes in wastewater evaluated in Periods 1 (a) and 2 (b) for WWTP-PS and only Period 1 for WWTP SRJ, for RdRP (Period 1) and N genes (Period 2), respectively. The pink line represents the locally estimated smoothed scatterplot (LOESS) model applied to gene concentration. Minimum and maximum genomic concentrations corresponding to different COVID-19 waves are pointed out. Active cases represent the number of confirmed COVID-19 cases reported in each municipality.

wave of cases started. Due to this study began at the end of the second wave (March 2021), it was impossible to predict the variation in active COVID-19 cases considering viral genes in wastewater. However, in the third wave, there was a variation between 445 and 4000 cases in Corregidora and Santiago de Queretaro, respectively, and 922 cases in Corregidora during the fourth wave. The difference in population explains the contrast between municipalities; Santiago de Querétaro has five times more inhabitants than Corregidora, and it is necessary to highlight that clinical data are limited at the municipal level and the WWTP SRJ serves only 1.6% of the population, so clinical data may be overestimated. Virus concentrations in wastewater increased from 1.7 to 3.3 and from 0.9 to 3.3 $\mathrm{Log_{10}/mL}$ during the end of the second wave and from 2.4 to 3.2 and from 2.4 to 3.1 $\mathrm{Log_{10}/mL}$ in the third wave, for PS WWTP and SRJ WWTP, respectively. The increases found in this study are like the range of viral concentrations found by (Pang et al., 2022) in wastewater in 10 communities in Alberta Canada; from 0 to a range of 1.8 to 2.2 $\mathrm{Log_{10}/mL}$ for the first, second, third, and fourth waves of cases, respectively.

Lead time of WBE surveillance

To evaluate the WBS as an early warning system for case peaks during monitoring, the Pearson correlation coefficients (Rho) were calculated (Figure 2), and the lead days concerning the active cases corresponded. For PS WWTP stronger correlations were obtained with Rho values of 0.95 ranging from 11 to 14 and from 1 to 4 lead days for third and fourth waves, respectively (Figure 2a). For SRJ WWTP in the third wave (Figure 2b), the Rho values were 0.9 and 0.79, ranging from 5 to 7 and 50 lead days (this is not reliable for pre-wave detection), respectively. At the end of the second wave, negative Rho values were obtained for both WWTPs, and therefore a lead time could not be determined. Rho values worldwide have remained between 0.8 and 0.9 and lead times between 2 to 4 days (Hillary et al., 2021; Nemudryi et al., 2020). While in Monterrey, Mexico, correlation values around 0.6 were found with 2 to 7 days of lead times before clinical cases were reported. In Guadalajara, Mexico City, and Leon, Rho values of 0.71, 0.82, and 0.75 were reported with lead times of 2.1 and 40 days, respectively, with lower Rho values in smaller WWTPs (Padilla-Reyes et al., 2022; Schilmann et al., 2023). However, these works have only been evaluated in a short period (first wave of cases). WBS has been implemented in other countries, with lead times determined as 10 days, 7 to 14 days, 5 to 9 and 8.8 days during different monitoring periods, 26 months in New York (USA), 17 months in Xàtiva (Spain), 7 months in Athens (Greece) and 6 months in Catalonia region (Spain), respectively (Galani et al., 2022; Hill et al., 2023; López-Peñalver et al., 2023; Mattei et al., 2023); but previous studies report on predicting different waves without evaluating the nature of the waves against different SARS-CoV-2 variants. Variants' transmissibility can influence the speed of spread of the disease (Castelán-Sánchez et al., 2023). In Mexico, based on the monitoring of clinical cases, the CoViGen-Mex (Loza et al., 2023) determined the spread of the Alpha, Gamma, and Delta variants from May 2021 to November 2021 and the Omicron variant from the last week of December 2021

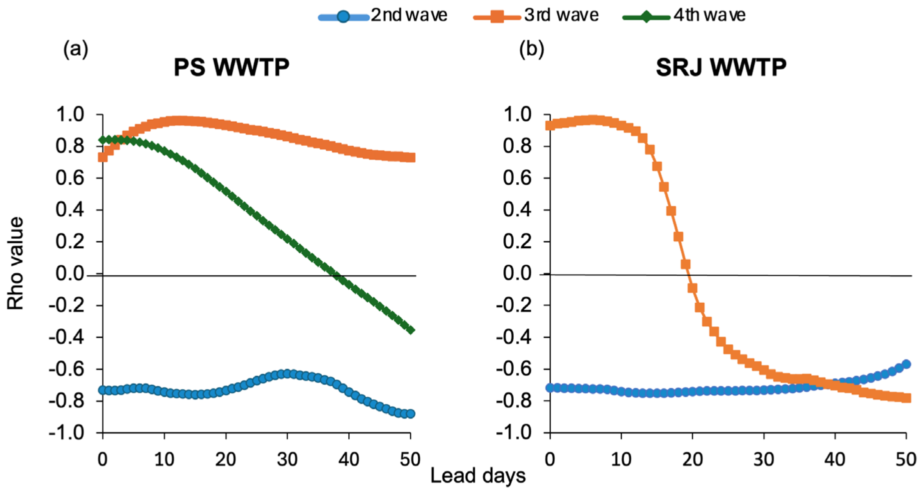

FIGURE 2 Pearson correlation coefficient (Pearson's Rho) between SARS-CoV-2 RNA concentration and COVID-19 active cases at different waves of cases and lag days estimated, (a) WWTP-PS, (b) WWTP SRJ. The blue line represents the second COVID-19 wave when Alpha and Gamma variants were circulating; the orange line represents the third wave when the Delta variant was circulating, and the green line represents the fourth wave when the Omicron variant was circulating.

(Figure 1). This information can be correlated with reported cases and waves detected in wastewater. In the third wave, 11 to 14 and 5 to 7 days of lead were obtained in the PS WWTP and SRJ WWTP, respectively, where the Delta variant appeared in June 2021 and quickly became the dominant variant until the first weeks of December 2021 (Loza et al., 2023; Zárate et al., 2023). On the other hand, in the fourth wave, a range of 1 to 4 lead days was obtained only in PS WWTP; this wave was caused by the Omicron BA.1.x variant that entered Mexico during the last week of December 2021, spreading rapidly throughout the country (Taboada et al., 2023; Zárate et al., 2023).

The Omicron variant contains many mutations that enable it to evade neutralizing antibodies effectively and is highly transmissible, even in vaccinated individuals, and symptoms develop faster than Delta variant (Bouzid et al., 2022; Chu & Yuen, 2022). As a result, the dispersion of Omicron variant may have affected the high transmission rates observed in Mexico's fourth wave of COVID-19, leading to a lower predictive capacity of the WBS. Similar to the present study, (Hopkins et al., 2023) evaluated wastewater from Houston (USA), where they obtained ranges of lead times to predict hospitalizations of 10 to 12, 13 to 16, and 7 to 10 days for Alpha, Delta, and Omicron waves, respectively. They obtained 6 6-day lead time for test positivity only for the Alpha wave, with no lead time for the Delta and Omicron waves, this attributed to differences in elimination of feces and appearance symptoms due to the different virus variants present in each wave. In Yamanashi, Japan, Shrestha et al. (2023) assessed wastewater for 23 months, applying models to estimate the average of COVID-19 cases. They obtained a 3-day lead time during the spread period of the Omicron variant. This study indicates that WBS prediction capability is affected by illness infectivity, determined by SARS-CoV-2 variants, which requires long-term studies. Considering that the lead time calculation requires a correlation of the viral load in wastewater and active cases, its reliability depends on the accuracy of collecting clinical case data. Other studies have analyzed WBS's predictive capability through modeling and identifying inflection points, but their applications are constrained (Galani et al., 2022; Shrestha et al., 2023). One limitation of WBS is the lack of standardization for lead time prediction in disease case waves.

COVID-19 prevalence estimation by WBS

Approaches to estimating true prevalence based on wastewater surveillance data have varied mainly in estimating infected cases in the population by applying Monte Carlo simulation due to the variety of value ranges of some variables (Claro et al., 2021; De Freitas Bueno et al., 2022; Perez-Zabaleta et al., 2023; Prandi et al., 2022). In this study, a Monte Carlo simulation estimated prevalent infection cases based on wastewater and clinics per 1000 inhabitants for each wave of cases in both WWTPs (Figure 3). According to the simulation, the estimated prevalence of WBS consistently exceeded the clinical prevalence reported. Specifically, after the third and fourth waves the prevalence was 90 to 150 and six times higher, respectively. Previous works have estimated wastewater prevalence using data models, reporting higher values either as cases or percentages

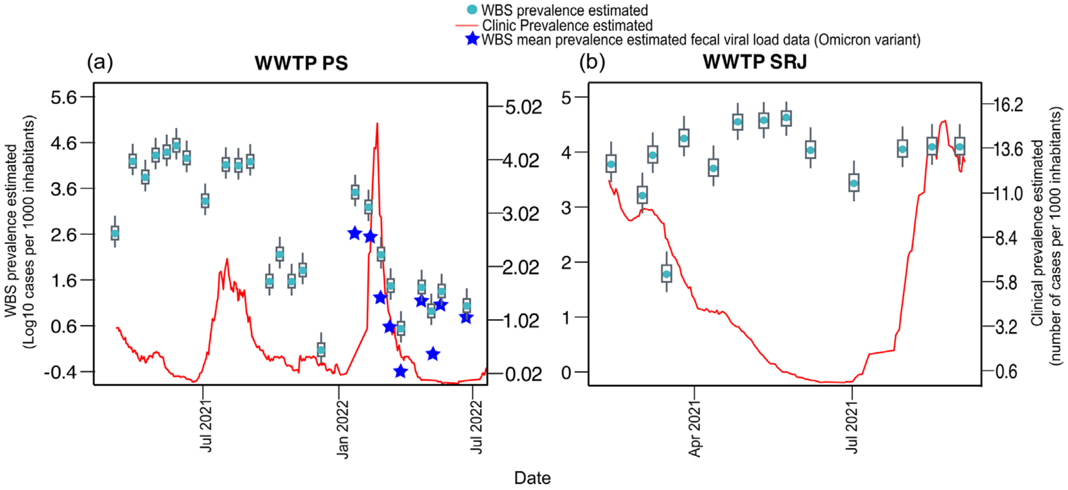

FIGURE 3 Boxplots show the estimated prevalence of SARS-CoV-2 infection in the population by wastewater-based surveillance (WBS). The boxes represent the simulations performed for each wastewater sample, and the circles represent the median in Log_{10} number of cases for each wastewater sample. Stars symbols represent the WBS estimation using average excretion parameter for Omicron variant.

(Table S5). Schilmann et al. (2023) evaluated wastewater in WWTPs located in 10 cities in Mexico during the second wave of cases, where they estimated that the WBS prevalence was 2 to 20 times higher than the clinical prevalence. In Brazil, (Castelán-Sánchez et al., 2023) and (Claro et al., 2021) during 1 year and 10 months of surveillance, respectively, reported estimated prevalences 5 to 10 times higher and 20 times higher than the clinical prevalence, in Porto Alegre and in the ABC region, respectively; and in Foz do Iguaçu, a similar trend was reported during 1-year following-up, being from 10 to 200 higher than clinical data (De Freitas Bueno et al., 2022). Furthermore, in Tehran (Iran), a study found a WBS prevalence up to 29 times higher than confirmed clinical cases (Amereh et al., 2022). This modeling suggests that the WBS consistently exhibits a predictive pattern globally in specific cases, specifically, when the disease prevalence is moderate, but not for the peaks of cases, where the trend behaves differently, as explained below.

In this study, at the end of the second, during the third, and fourth waves, the WBS prevalence was at least 219 times higher than the clinical prevalence (Table S5). A few factors can explain this inconsistency between WBS-calculated data and clinical test results. Firstly, the WWTP could have an excess viral load due to an unmeasured flow of people in the study area, such as tourism or other economic activities. Secondly, the actual number of cases may be underestimated due to limited access to PCR tests (registered in the government's surveillance system) and a preference for rapid tests (not recorded in the surveillance) during the study period. Lastly, there is uncertainty in the modeling due to different infectivity parameters and viral load dispersion caused by variables like different virus variants. Previous studies have also shown variability in WBS prevalence data estimated with several orders of magnitude. Pang et al. (2022) correlated RNA concentrations in Alberta, Canada, wastewater with new COVID-19 cases, suggesting that low and moderate correlations may be due to RNA degradation from freeze–thaw cycles and high tourist numbers (67 times more than permanent residents), where their viral load contribution to wastewater is difficult to estimate, even so, it did not affect the correlations obtained. Also, Fernandez-Cassi et al. (2021) evaluated wastewater from WWTPs in Switzerland and found higher SARS-CoV-2 RNA loads in the Lugano WWTP than at the Zurich WWTP despite similar numbers of confirmed cases in the catchment. They attributed this difference to lower virus recovery in Zurich and reduced precision in RNA copies quantification due to greater decay during sample storage. Wang et al. (2021) conducted a study on wastewater from five WWTPs in Los Angeles County, USA. Where the estimated number of infected individuals based WBS is nearly 2 million higher than the number of clinical cases reported within a 20 days, the estimated infection rate in the wastewater from one of the WWTPs was 440,000 cases per 100,000 individuals, attributed to uncertainties about the infected population, laboratory processes, sample handling, and SARS-CoV-2 variants that could alter input and output parameters. The high prevalence estimation by WBS in this study can be partially explained by the fact that Corregidora and Santiago de Querétaro are highly industrial and touristic areas that experience sporadic influxes of people during the day. In 2023, the Tourism authority in the State of Queretaro indicated that the entity had a tourist flow of 7.6 million, three times the population size; however, this information is at the state level, and there is no specific information at the municipal level to evaluate the contribution of tourism to each WWTP. Therefore, increased fecal matter and genetic material in wastewater could lead to overestimating the prevalence estimated by WBS. Recently, Gawlik et al. (2024) recommended using human-specific biomarkers, which can be biological or chemical parameters, to measure the level of human fecal content in wastewater and to normalize the measured concentration of SARS-CoV-2.

An uncertainty parameter is the ability of each country to accurately determine COVID-19 infectivity. Despite the prevalence estimated by WBS, considering the % of infected people who excrete the SARS-CoV-2 virus (Equation 3), parameters such as the symptomatic/asymptomatic ratio may partially explain its inconsistency with clinical data. Throughout the pandemic in Mexico, the average number of total accumulated COVID-19 cases dependent on the availability of diagnostic tests was approximately 2.2, 2.9, and 5.3 millions during the second, third, and fourth waves, corresponding to infectivity rates of 1.7%, 2.3%, and 4.1%, respectively. For instance, Brazil had a low infectivity rate of 0.02% to 0.07% (Claro et al., 2021; De Freitas Bueno et al., 2022), while Los Angeles (USA) had a high rate of 37.5% (Wang et al., 2021). The limited availability of PCR diagnostic tests during the pandemic can explain the low percentage of reported people infected in Mexico, similar to other LMICs (Manuel et al., 2022). Previously, Li et al. (2021) found that higher wastewater and air temperatures led to increased RNA recovery efficiency, suggesting that the virus is more prevalent in warmer sewers. Recent research about factors such as fecal viral load has also been evaluated for the Omicron variant (Li et al., 2024). This could affect the prevalence estimation in different waves of cases through data modeling. Therefore, in this study, the mean prevalence by WBS was also estimated for the fourth wave, using the fecal viral load data reported for the Omicron variant. Prevalence results ranged from 0.5 to 1 $\log_{10}$ less compared to using fecal viral load data when parameters when the predominant COVID-19 variants were B.1, B.1.1, and B.1.1.222 (Figure 3); however, as expected, the trend was similar. This study was conducted during 2021–2022 when the Alpha, Gamma, Delta, and Omicron variants were present, leading to changes in the value ranges of the variables due to the variants' transmissibility and lower severity of disease symptoms, these changes can lead to uncertain prevalence estimates, either overestimating or underestimating the true prevalence. To overcome such limitations, prevalence modeling must be followed by periodically updated viral load and excretion information.

Limitations and perspectives

Several factors can influence wastewater monitoring results. These include the type and size of the sewer basin, population size, and environmental conditions such as rainfall and temperature (Gawlik et al., 2024; Weidhaas et al., 2021). Additionally, because many cities in Latin and Central America still lack adequate sanitation services (Madrigal et al., 2020; Prado et al., 2023), it is essential to expand monitoring efforts beyond WWTPs to include sewer networks and natural water bodies such as rivers and streams to improve our understanding of virus circulation (Prado et al., 2023). Thus, the implementation of the WBS carried out in this work can be extrapolated mainly in places without a centralized sewer system, and same climate and temperature conditions like LMICs countries.

The persistence and decay of SARS-CoV-2 in wastewater is temperature-dependent. In this study, the RNA half-life in sampling conditions (*t*1/2) ranged from 8.59 to 15.44 and 9.95 to 15.44 h at 17.1 to 23.5 and 15 to 22°C for the PS and SRJ WWTPs, respectively (Table S3). Previously, similar *t*1/2values for SARS-CoV-2 RNA in wastewater have been estimated to range from 12.8 to 14 h at 4°C and 16 h at 10°C (Weidhaas et al., 2021). Considering RNA lifetime, the adjusted SARS-CoV-2 loads in wastewater can lead to up to 40% and 55% higher concentrations in cold months (8 to12°C) compared to warmer months (20 to 25°C), as have been reported in Wisconsin, USA (Schussman & McLellan, 2022). In this study, the influence of *t*1/2 (Figure 4 and Table S4) was evaluated by season of the year, where no statistically significant difference was obtained between the seasons of the year for each WWTP. This is attributed to the fact that there was no extreme difference in the average ambient temperatures, which remained at 15 to 23°C in both municipalities throughout the sampling time. In this sense, the adjusted SARS-CoV-2 loads in wastewater increased 2% and 25%, at 17.1 and 23.5°C, respectively; this study's lowest and highest water temperatures.

Other important factors that may influence monitoring results are the sampling method (grab or composite sampling) and sampling duration. These factors may affect the integrity of the viral RNA, as well as the consistency and comparability of the results. Some studies have suggested using 24-h composite samples to improve

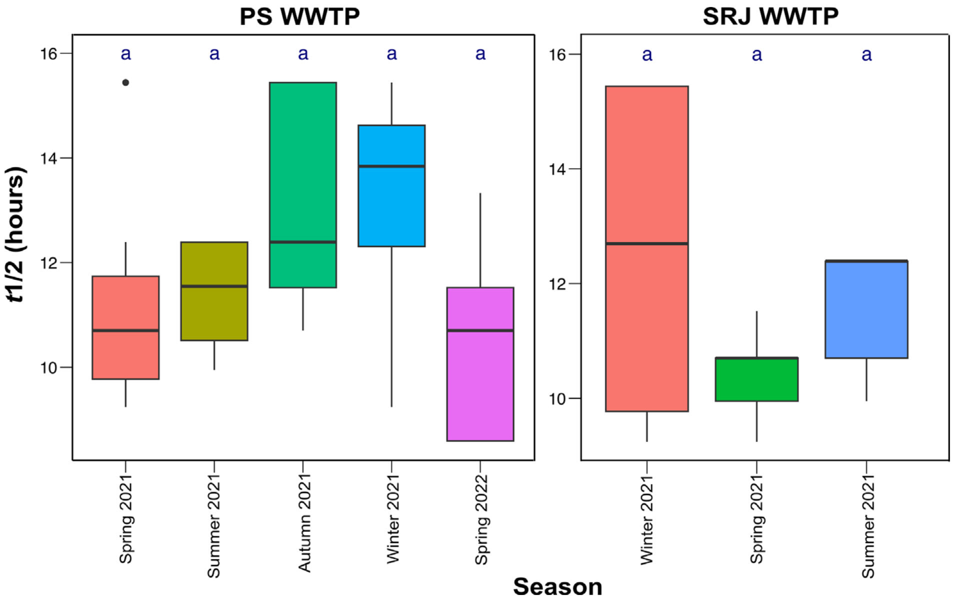

FIGURE 4 Boxplots show the effect of SARS_CoV_2 RNA t1/2 during different seasons of the year at different wastewater treatment plants (WWTP).

sample representativeness and enhance concentration estimates (Farkas et al., 2018; Feng et al., 2021; Gerba et al., 2017; Gerrity et al., 2021). George et al. (2022) indicated that grab sampling at sites with high inflow is equivalent to 24-h composite sampling, and at sites with low inflow, composite sampling is recommended. Although sampling twice a week has been reported to be sufficient for infection trend analysis compared to sampling once a week (Feng et al., 2021), other studies have been performed every 15-day sampling, similar to this study, obtaining sufficient data to correlate SARS-CoV-2 RNA concentration in wastewater to clinical cases (Sakarovitch et al., 2022; Wang et al., 2021).

The limitation of clinical data at the municipal level means that when evaluating a small WWTP serving <20,000 inhabitants (WWTP SRJ), it is associated with obtaining low concentrations of the virus in wastewater compared to the reported clinical cases. Previously, Feng et al. (2021) suggested that the relationship between the incidence of COVID-19 and SARS-Cov-2 RNA in wastewater may be specific to the WWTP. Therefore, the trends obtained by WBS in the SRJ WWTP would reflect the circulation of the virus in the population served, even when clinical cases are not reported. This condition highlights the WBS's potential as a complementary tool to the clinical surveillance system, especially when cases requiring medical attention are scarce, which limits the visibility of infection trends.

Furthermore, it is important to establish standardized reporting units for WBS prevalence estimates. While the use of WBS can be helpful in estimating the prevalence of COVID-19, it is still limited due to the complexity and uncertainties involved in the process. Considering the WBS limitations is crucial to understanding the possible bias and interpreting their results, even though it is a valuable complementary epidemiological tool. In this sense, WBS provides relevant information from early detection, where the virus was not present, quantitative trends showing peaks and declines, and surveillance of the emergence of variants to identify hotspots, assess the spread of the disease, and make informed public health and policy decisions; the monitoring of other pathogens of epidemiological interest, as well as the development of monitoring networks and dashboards (De Araújo et al., 2022; de Calabria Araujo et al., 2024; Gawlik et al., 2024; Trigo-Tasende et al., 2023).

CONCLUSIONS

The long-term surveillance of SARS-CoV-2 genetic material in wastewater allowed for validation of its utility for wave occurrence prediction and showed a different grade of effectiveness according to the estimation of the prevalence of infection. The intensity of the waves was strongly associated with SARS-CoV-2 variants present in each one, reflecting the severity of their symptoms in the population and determining their predictive capability using WBS during the Delta variant circulation; the lead time was longer compared to the Omicron variant. Most Omicron cases did not require hospitalization, leading to underestimated clinical data. The study emphasizes the significance of continuous monitoring of SARS-CoV-2 in wastewater to early detect potential surges. However, there are limitations in estimating the disease prevalence based on wastewater. The uncertainty arises from various factors such as infectivity, viral load affected by virus mutations, viral shedding in feces, and the absence of standardized methods for modeling. This study demonstrated the potential of WBS as a complementary tool to clinical surveillance for the early detection of COVID-19 cases. Additionally, it thoughtfully examines the limitations of this approach.

AUTHOR CONTRIBUTIONS

Marcela Zavala-Méndez: Conceptualization; methodology; data curation; formal analysis; validation; investigation; writing—original draft. **Andrés Sánchez-Pájaro:** Conceptualization; methodology; writing—original draft; data curation; formal analysis. **Astrid Schilmann:** Conceptualization; methodology; data curation; writing—review and editing. **Juliana Calábria de Araújo:** Conceptualization; writing—review and editing; supervision. **Germán Buitrón:** Supervision; writing—review and editing; conceptualization. **Julián Carrillo-Reyes:** Conceptualization; methodology; data curation; supervision; formal analysis; validation; investigation; funding acquisition; project administration; writing—original draft; writing—review and editing.

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CONFLICT OF INTEREST STATEMENT

The authors declare no conflicts of interest.

DATA AVAILABILITY STATEMENT

The data that support the findings of this study are available from the corresponding author upon reasonable request.

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SUPPORTING INFORMATION

Additional supporting information can be found online in the Supporting Information section at the end of this article.

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