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Research article

Temporal assessment of SARS-CoV-2 detection in wastewater and its epidemiological implications in COVID-19 case dynamics

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A R T I C L E I N F O

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A B S T R A C T

This research evaluated the relationship between daily new Coronavirus Disease 2019 (COVID-19) cases and Severe Acute Respiratory Syndrome Coronavirus 2 (SARS-CoV-2) concentrations in wastewater, followed by effects of differential SARS-CoV-2 shedding loads across various COVID-19 outbreaks. Linear regression analyses were utilized to examine the lead time of the SARS-CoV-2 signal in wastewater relative to new COVID-19 clinical cases. During the Delta wave, no lead time was evident, highlighting limited predictive capability of wastewater monitoring during this phase. However, significant lead times were observed during the Omicron wave, potentially attributed to testing capacity overload and subsequent case reporting delays or changes in shedding patterns. During the Post-Omicron wave (Febuary 23 to May 19, 2022), no lead time was discernible, whereas following the lifting of the COVID-19 state of emergency (May 30, 2022 to May 30, 2023), the correlation coefficient increased and demonstrated the potential of wastewater surveillance as an early warning system. Subsequently, we explored the virus shedding in wastewater through feces, operationalized as the ratio of SARS-CoV-2 concentrations to daily new COVID-19 cases. This varied significantly across the Delta, Omicron, other variants and post-state-emergency phases, with the Kruskal-Wallis H test confirming a significant difference in medians across these stages ($P < 0.0001$). Despite its promise, wastewater surveillance of COVID-19 disease prevalence presents several challenges, including virus shedding variability, data interpretation complexity, the impact of environmental factors on viral degradation, and the lack of standardized testing procedures. Overall, our findings offer insights into the correlation between COVID-19 cases and wastewater viral concentrations, potential variation in SARS-CoV-2 shedding in wastewater across different pandemic phases, and underscore the promise and limitations of wastewater surveillance as an early warning system for disease prevalence trends.

1. Introduction

Since the onset of the global COVID-19 pandemic in 2020, wastewater-based epidemiology (WBE) has proven to be an effective tool for SARS-CoV-2 monitoring, widely utilized to estimate viral prevalence and disease circulation within a given sewershed [1–4]. As a data and knowledge input to decision-making processes, WBE presented significant potential as an early warning tool for identifying

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the presence of the virus within a community. In developing countries, where constrained testing resources pose a significant challenge, WBE has emerged as a vital tool for decision-making during the pandemic [5–7]. This approach capitalizes on the fact that SARS-CoV-2 RNA is shed in the feces of infected individuals whether they are symptomatic or asymptomatic, subsequently entering the wastewater system. WBE data was the only available information at community level when clinical testing data was not available due to lack of testing supplies and/or preference of individuals to not get tested upon experiencing COVID-19 symptoms [8].

As different variants of SARS-CoV-2 have emerged and spread globally, it has become imperative to understand their impact on WBE and disease transmission dynamics. Since the first emergence of COVID-19 in December 2019 until now, different variants of SARS-CoV-2 have emerged and spread throughout many countries, including Alpha variant (B.1.1.7, November 2020), Delta variant (B.1.617.2, May 2021), and Omicron variant (B.1.1.529, November 2021) [9–13]. In the summer of 2021, the Delta variant was the dominant strain in the US, accounting for more than 99 % of COVID-19 cases, with a significant increase in hospitalization at the time [10]. It was proven that Delta variants were more contagious than the original viruses and put unvaccinated people more at risk [14]. At the end of 2021, the Omicron variant emerged at a rapid pace and took over as the main strain in the US, which continued to spread more quickly than any other strain in many countries. Even though the Omicron variant tends to be more contagious to date, it is generally observed to cause less severe symptoms than the Delta variant [9,13].

Substantial research has probed into the correlations between SARS-CoV-2 marker genes present in wastewater and the incidence and prevalence of COVID-19 cases in various sewersheds, with the goal of establishing a predictive model for estimating present and future COVID-19 cases. The focus has been on exploring the wastewater surveillance as an early warning system for pandemics, underscoring its capacity to provide diverse lead times before the clinical detection of cases [15–17]. The lead time is identified as the difference in time between when an increase in viral detection is observed in wastewater and when a corresponding increase in clinical cases is reported [18]. Lead time can be different because of the sensitivity and specificity of the viral detection methods, the incubation period of the target disease, and the testing and reporting practices for clinical cases. So, it is important to take these factors into account when interpreting the results [19]. The efficacy of WBE as an early warning system has been critically evaluated by Bibby et al. [20], focusing on the onset of viral shedding and the delay between WBE results and clinical test reporting. Despite the uncertainties surrounding the clinical presentation of COVID-19 and various factors influencing WBE results, its potential in early disease detection remains undeniable. Specifically, to date, no study has explored how the dominant variants of SARS-CoV-2 might affect the lead time and the correlations between wastewater signals and clinical cases in WBE. Furthermore, temporal fluctuations have been observed in the ratio of viral load in the wastewater to clinical cases [21–23]. These variations may be driven by changes in diagnostic testing rates, alterations in fecal shedding, and the temporal discrepancy between wastewater surveillance and clinical case data. Subsequent studies could model these influential factors to derive a more accurate approximation of actual case counts.

Li et al. [4] and Haak et al. [24] previously utilized WBE to investigate the original strain of COVID-19 in Northern Nevada, USA, in October 2020. Their work established correlations between concentrations of SARS-CoV-2 gene markers and daily new clinical cases of COVID-19 in designated sewersheds and local clusters during the pandemic's first wave. This research prompted us to further question the predictive power of wastewater SARS-CoV-2 marker concentrations for forecasting COVID-19 trends in a community, especially under the influence of changing dominant viral strains. Could the variations of SARS-CoV-2 affect virus shedding in feces and disease transmission dynamics? Additionally, we explored how shifts in health policies, such as the withdrawal of face mask mandates—which could influence home testing rates and the public's willingness for undergoing testing—impacted the efficacy of reported clinical cases in monitoring the spread of COVID-19. These policy changes could, in turn, alter the correlation between SARS-CoV-2 RNA markers in wastewater and confirmed clinical cases. To investigate these issues, we extended the wastewater surveillance in Northern Nevada from the July 2021 through May 2023. Our study focused on distinct periods: the Delta wave, the Omicron wave, the Post-Omicron wave, and the Post-Emergency following the lifting of face mask mandates and state emergencies. This paper seeks to delineate the relationships between SARS-CoV-2 concentrations and COVID-19 clinical cases across these periods and evaluate how different SARS-CoV-2 variants and policy changes have influenced the predictive potential of WBE.

2. Methods and materials

2.1. Study area, sample collection and pretreatment

Wastewater samples were procured from Truckee Meadows Water Reclamation Facility (TMWRF), one of the largest water reclamation facilities in Northern Nevada, USA. TMWRF handles approximately 80 % of the wastewater across Washoe County, encompassing over 75 % of the region's population. The summary of the samples across four study periods is presented in Table 1. During the study period, the flowrate was $(109.8 \pm 3.8) \times 10^3$ m^3/day, facilitating enhanced biological phosphorus removal in the secondary treatment and nitrification/denitrification in tertiary treatment. The specific sewershed is shown in Fig. 1. We obtained 1L

Table 1
Summary of samples across four study periods.

Study Period	Dominant Variant	Policy	No. of Samples
July 1, 2021–November 24, 2021	Delta	Facemask Mandate	146
Nov 25, 2021–Feb 22, 2022	Omicron	Facemask Mandate	87
Feb 23 – May 19, 2022	B2	Facemask Mandate Lifted	86
May 20, 2022–May 30, 2023	BA.4/5, BQ, XBB	State COVID-19 Emergency Lifted	376

samples of wastewater after preliminary treatment from TMWRF. These 24-h composite samples were collected daily, seven days a week. All of the samples were transported on ice to the laboratory. We centrifuged the 200 mL of samples at 3000 g for 15 min to eliminate large particles. The supernatant was then sequentially filtered through sterile membrane filters of 1.5, 0.8, and 0.45 μm to remove debris and larger particles, including eukaryotic microorganisms. The resulting liquid sample was used to extract the virus, which was subsequently analyzed for RNA using reverse transcription and quantitative polymerase chain reaction (RT-qPCR) method.

2.2. *Virus recovery, RNA extraction, and RT-qPCR analysis*

The methodology for virus recovery was adapted from the methods described previously with minor modifications [4,25]. We utilized 100 KDa Amicon® Ultra-15 Centrifugal Filter Units (Millipore Sigma, St. Louis, MO, USA) to filter a 60 mL sample through three cycles of centrifugation at 4000*g* for 5 min each, resulting in approximately 500 μL of concentrated sample within a cartridge. Occasionally, the duration of centrifugation was extended based on lab experience judgment to address pore blockage caused by particles in the Amicon filters. The concentration factors for each sample were computed in accordance with the methods described by Li et al. [4]. Unless analyzed on the same day, the concentrated viral samples were typically stored at −80 °C for downstream analysis.

Total RNA was extracted from the concentrated samples using the AllPrep PowerViral DNA/RNA kit (QIAGEN, Inc., Germantown, MD, USA), following the instructions in the user manual. The RT-qPCR analysis was performed on the CFX96 Touch Real-Time PCR Detection System (BioRad, Hercules, CA, USA) using the recommended N1 and N2 primers and probes as per the US CDC guidelines. We employed the SARS-CoV-2 RT-qPCR Kits for Wastewater (Promega, Madison, WI, USA), adhering to the instructions provided with the kit. Each reaction incorporated ten μL of GoTaq® Wastewater Probe qPCR MasterMix (2 ×), one μL of N1 or N2 & PMMoV Primer/Probe/IAC Mix (20 ×), 0.2 μL of GoScript® Enzyme Mix (50 ×), and five μL of the total genomic RNA template in a final volume of 20

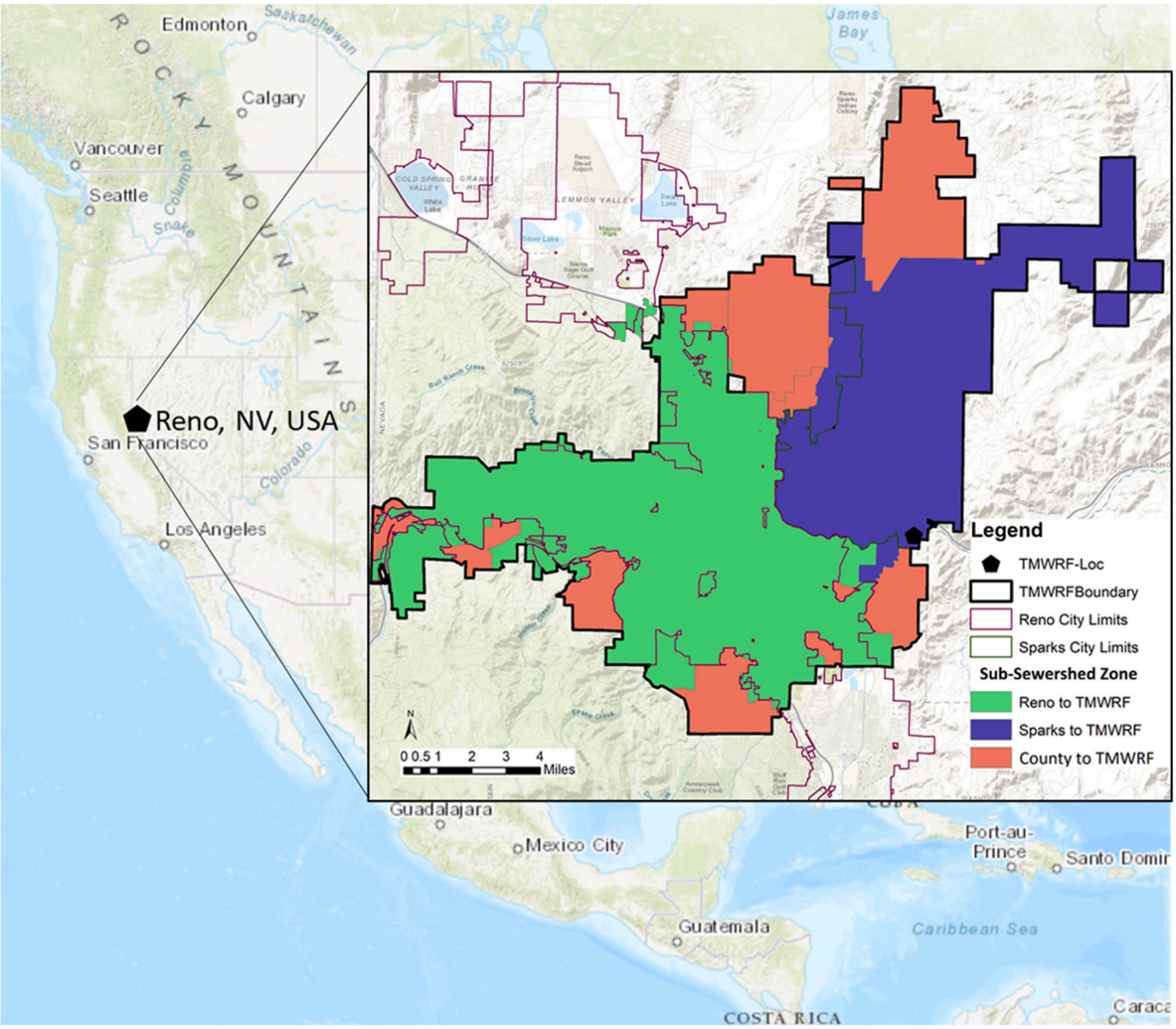

Fig. 1. Sampling location during the study period.

μL. The RT-qPCR procedure included reverse transcription at 45 °C for 15 min, and GoTaq® activation at 95 °C for 2 min, followed by 40 cycles of 15-s denaturation at 95 °C and 60-s annealing/extension at 62 °C, with a plate reading after each cycle. The threshold cycle (Ct) was determined using the default algorithm in the CFX Manager Software (BioRad, Hercules, CA, USA). Both positive and non-template controls were incorporated in each run. Calibration curves spanning a 6-log range were created by serially diluting the SARS-CoV-2 positive control (IDT, Coralville, IA, USA) 10-fold in the range from 4,000,000 to 4 gc/μL. All calibration curves exhibited correlation coefficients (R^2) greater than 0.99, with amplification efficiencies ranging from 90 % to 110 %. The limit of detection (LoD) in each qPCR assay was >4 gc/μL of RNA elute, representing the lowest concentration after serial dilution of the positive controls that still showed more than 50 % positive amplification with obtainable Ct values.

2.3. SARS-CoV-2 recovery efficiency, quality control, and PCR inhibition

SARS-CoV-2 RT-qPCR Kits for Wastewater (Promega, Madison, WI, USA) contains a process control and internal amplification control (IAC) in each amplification. Pepper Mild Mottle Virus (PMMoV) is an endogenous virus in wastewater and can validate the virus concentrating and recovery method. For the samples that did not yield a positive amplification by RT-qPCR for SARS-CoV-2 but yielded a detectable amplification of PMMoV, the concentration of SARS-CoV-2 is considered below the LoD in the wastewater. Any samples measured to be negative for PMMoV were re-analyzed from the pretreatment step within 48 h. IAC is added in each RT-qPCR, including the primers and the CAL Fluor® 560 dye-labeled probe, to amplify and detect a 435bp product from a given DNA template that includes in every amplification. This is to evaluate the inhibition of DNA polymerase and/or Reverse Transcriptase in RT-qPCR amplification. Compared to the non-template control (NTC) well, if the IAC Ct in a sample well was changed significantly (Ct ≥ 2), PCR inhibitors are considered to be present in the sample. The samples were labeled for qualitative but not quantitative analysis.

The human coronavirus OC43 strain (HCoV-OC43) was used as a surrogate to study the virus recovery rate in the concentration method because of its enveloped structure, which is similar to SARS-CoV-2. 100 μL of HCoV-OC43 strain was spiked into 180 mL wastewater, followed by the Amicon ultrafiltration method (Section 2.2). The recovery of HCoV-OC43 in untreated wastewater was 24 ± 2 %, which is the same as our previous study [4].

2.4. Data analysis

The concentrations of SARS-CoV-2 viral gene markers were determined by averaging the N1 and N2 gene concentrations from identical samples. To mitigate variability from sampling operations, molecular biological assays, and other environmental factors, we computed the 7-day moving averages for both viral gene marker concentrations and clinically reported COVID-19 cases. The data on daily new cases of COVID-19 were sourced from the Washoe County Health District COVID-19 Dashboard and COVID-19 Epidemiology Surveillance Program Annual Report [26,27]. We normalized the reported clinical COVID-19 cases by the population within wastewater treatment facility's sewershed, and these were articulated as cases per 0.1 million people. We chose not to adjust the results according to flow rate due to the minimal variation in flow ($(109.8 \pm 3.8) \times 10^3$ m^3/day) throughout the study period. Furthermore, we did not employ PMMoV results to standardize the SARS-CoV-2 data, as it did not enhance the correlations, which was in agreement with previous study [28]. Nonparametric Spearman correlation coefficients (r) and linear regression models were developed and applied using GraphPad Prism 10 Software (Graphpad Inc., San Diego, CA) for statistical analysis of the data.

3. Results and discussion

Table 1 presents a summary of samples collected across four study periods, including different dominant COVID-19 variants and associated policies. From July 1 to November 24, 2021, the Delta variant had been dominant, along with a facemask mandate. During this period, 146 samples were gathered. From November 25, 2021, and February 22, 2022, the Omicron variant was dominant, with continued facemask mandates. In this phase, 87 samples have been analyzed. In the Post-Omicron wave from February 23 to May 19, 2022, the B2 variant was dominant, with the lifting of the facemask mandate. 86 samples were collected during this period. During the Post-Emergency sampling period, from May 20, 2022, to May 30, 2023, various sublineages of the Omicron variants were dominant and 376 samples were collected. This progression highlights the dynamic nature of the pandemic response and the shifts in dominant variants alongside policy changes over time.

3.1. SARS-CoV-2 wastewater surveillance and COVID-19 prevalence with insights from different waves in study areas, along with pandemic timeline

As the number of people tested per week for COVID-19 decreased since Spring 2021 [26], SARS-CoV-2 monitoring in wastewater was proposed as a complementary data resource for public health decision-making. The presence of SARS-CoV-2 viral markers was confirmed in wastewater samples in the study region and validated using the Amicon filtration method in the study area. On July 27, 2021, the US Centers for Disease Control (CDC) issued a guideline stating that all individuals, regardless of vaccination status, should wear masks in public indoor settings in counties with substantial or high transmission rates of COVID-19. In July 2021, the Delta variant became the dominant strain and spread in Washoe County, Nevada [10]. A new mask mandate was issued on July 30, 2021 in response to the high transmission of the Delta variant, which peaked in the first week of September 2021. We observed that the concentration of SARS-CoV-2 viral markers in wastewater corresponded to the number of COVID-19 cases in Washoe County, USA, reaching its peak concentration in early September during the Delta wave (146 samples tested, Table 1, Fig. 2). On December 14, 2021, the first case of the Omicron variant was confirmed in Nevada, USA, and rapidly spread throughout the entire state. We found that the concentration of SARS-CoV-2 viral markers started to increase in mid-December 2021 and reached its peak in the third week of January 2022 (87 samples tested, Table 1, Fig. 2). Moreover, the temporal trends of SARS-CoV-2 gene concentrations in wastewater corresponded to both the Delta and Omicron waves, which is consistent with findings from other studies [21,29,30].

Starting from February 10, 2022, the Nevada state government removed the facemask mandate, meaning that masks are no longer compulsory in public in the region of interest. In April 2022, there was a notable rise in COVID-19 cases attributed to the easing of pandemic restrictions and the emergence of a new Omicron subvariant (86 samples tested, Table 1, Fig. 2). Although hospitalizations also increased, they did not reach a level that posed a significant burden on healthcare facilities. Consequently, on May 20, 2022, the state of emergency was lifted, and plans were put in place to gradually phase out related programs. 376 samples were tested in this period (Table 1, Fig. 2). Subsequently, hospitalizations began to decline from July to September 2022. During this period, the state reported a decrease in cases; however, it was cautioned that the numbers might be undercounted due to the increased use of at-home COVID-19 testing and not reported to the public health agencies. In the following sections, we examine and analyze the relationships between SARS-CoV-2 concentrations in wastewater and reported clinical COVID-19 cases during the Delta, Omicron, and other variants, like BA2, BA.4/5, BQ and XBB, waves, and following the cessation of compulsory face mask mandates and the lifting of the state emergency period.

3.2. A comparison of the correlations between daily new COVID-19 cases and the SARS-CoV-2 signal in wastewater during different waves of the pandemic

To discern the correlations and establish the lead time between SARS-CoV-2 viral RNA concentrations in wastewater and clinically reported COVID-19 cases, we performed a correlation analysis. This process involved generating the 7-day moving averages for both the SARS-CoV-2 gene concentrations in wastewater and the daily new COVID-19 cases within Washoe County. Spearman's correlation coefficient (r) was employed to evaluate the relationship between these variables. In addition, we examined the wastewater RNA marker concentrations (7-day moving average) at lead times of 3, 7, and 10 days to evaluate the potential of SARS-CoV-2 monitoring as a predictive factor for community-level diagnosis estimates.

Table 2 provides a detailed comparison of the correlation coefficients (Spearman's r) between SARS-CoV-2 viral RNA marker concentrations (in gene copies per liter, gc/L) and daily COVID-19 cases across four different phases: the Delta, Omicron, Post-Omicron wave, and Post-Emergency wave. The correlations were evaluated for both daily results and 7-day moving average case counts, under various lead times (0, 3, 7, and 10 days). During the Delta wave, the correlation coefficients ranged from 0.54 to 0.70 for daily clinical cases and from 0.66 to 0.79 for the 7-day average clinical cases. The highest correlation was observed when considering the 7-day average clinical cases with a 0-day lead time. During the Omicron wave, we observed stronger correlations, with Spearman r ranging from 0.68 to 0.84 for daily clinical cases; this correlation was improved to range from 0.75 to 0.87 when using the 7-day average clinical cases. The strongest correlation was observed with a 7-day lead time and the 7-day moving average of clinical cases. The Post-Omicron wave showed correlation coefficients between 0.19 and 0.68 for daily clinical cases and 0.19 to 0.78 for the 7-day average clinical cases. Remarkably, the strongest correlation was observed with a 10-day lead time, which is three days longer than previous wave. The Post-Emergency wave presented correlation coefficients ranging from 0.62 to 0.77 for daily clinical cases and 0.70 to 0.83 for the 7-day average clinical cases, exhibiting strongest correlation with a 10-day lead time. Overall, this data suggests that the correlation between SARS-CoV-2 signals in wastewater and reported COVID-19 cases varies across different pandemic phases. Notably, stronger correlations appear during certain waves, particularly the Omicron and Post-Omicron waves, and at specific lead times.

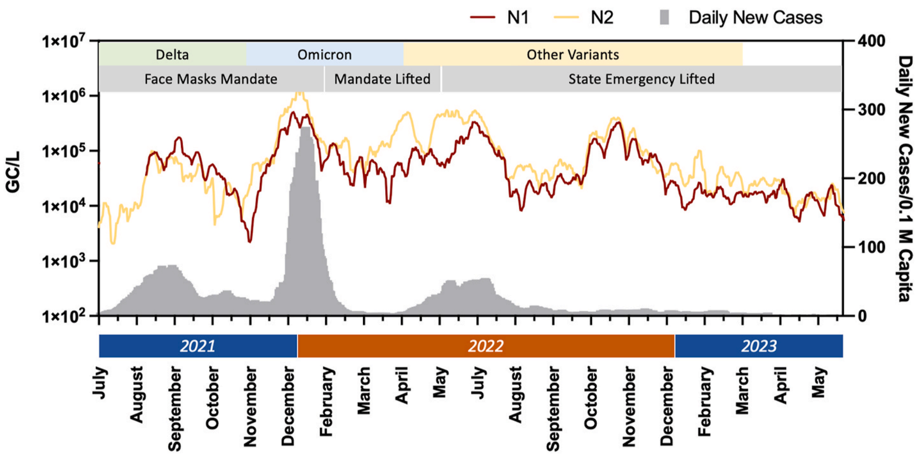

Fig. 2. Time series analysis of SARS-CoV-2 monitoring under Delta, Omicron and other variants waves in Washoe County, NV, USA. The red line shows the SARS-CoV-2 N1 gene concentrations and the yellow line shows the N2 gene concentrations of the sampling day. The grey bar shows the clinical cases per 0.1 M population during the study period. (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)

Table 2
Summary of correlations between SARS-CoV-2 viral RNA concentrations in wastewater and clinically new COVID-19 cases.

SARS-CoV-2 Wastewater Signal (gc/L)	Delta wave		Omicron wave		Post-Omicron wave		Post-emergency wave	
	Daily cases	Daily cases (7 day-moving avg)	Daily cases	Daily cases (7 day-moving avg)	Daily cases	Daily cases (7 day-moving avg)	Daily cases	Daily cases (7 day-moving avg)
0-day lead, daily result	0.54[a]	0.66[a]	0.68[a]	0.75[a]	0.19	0.19	0.62[a]	0.70[a]
0-day lead, 7-day avg	0.70[a]	0.79 [b]	0.76 [b]	0.81[b]	0.27	0.30	0.74[a]	0.80[b]
3-day lead, 7-day avg	0.65[a]	0.72[a]	0.80 [b]	0.85[b]	0.37	0.44	0.75[a]	0.81[b]
7-day lead, 7-day avg	0.59[a]	0.65[a]	0.84 [b]	0.87[b]	0.54[a]	0.61[a]	0.75[a]	0.82[b]
10-day lead, 7-day avg	0.48	0.55[a]	0.84 [b]	0.86[b]	0.68[a]	0.78[b]	0.77 [b]	0.83[b]

[a] Moderate correlation ($0.50 < r \leq 0.75$).
[b] Strong correlation ($r > 0.75$).

Employing a 7-day moving average tends to yield stronger correlations than daily clinical case counts. This approach also effectively smooths the data, helping to mitigate short-term fluctuations and provides a clearer view of longer-term trends.

One possible reason for these observed differences could be virus decay, which may result from natural degradation of the virus during in-sewer transportation or changes in wastewater temperatures [31]. These factors could subsequently impact the correlation in different periods. For instance, in the study area, temperatures are typically higher in summer (coinciding with the Delta wave) and lower in winter (during the Omicron wave). These seasonal temperature fluctuations may lead to an accelerated virus decay during the Delta wave, resulting in a weaker correlation during that period. However, our current understanding of the impact of these factors on the correlations between SARS-CoV-2 concentrations in wastewater and the number of daily new COVID-19 cases remains limited. A stronger correlation has been observed since Post-Omicron wave with a longer leading time. This could be attributed to the decrease in public COVID-19 testing capacity and the subsequent delay in test reporting after the state emergency was lifted.

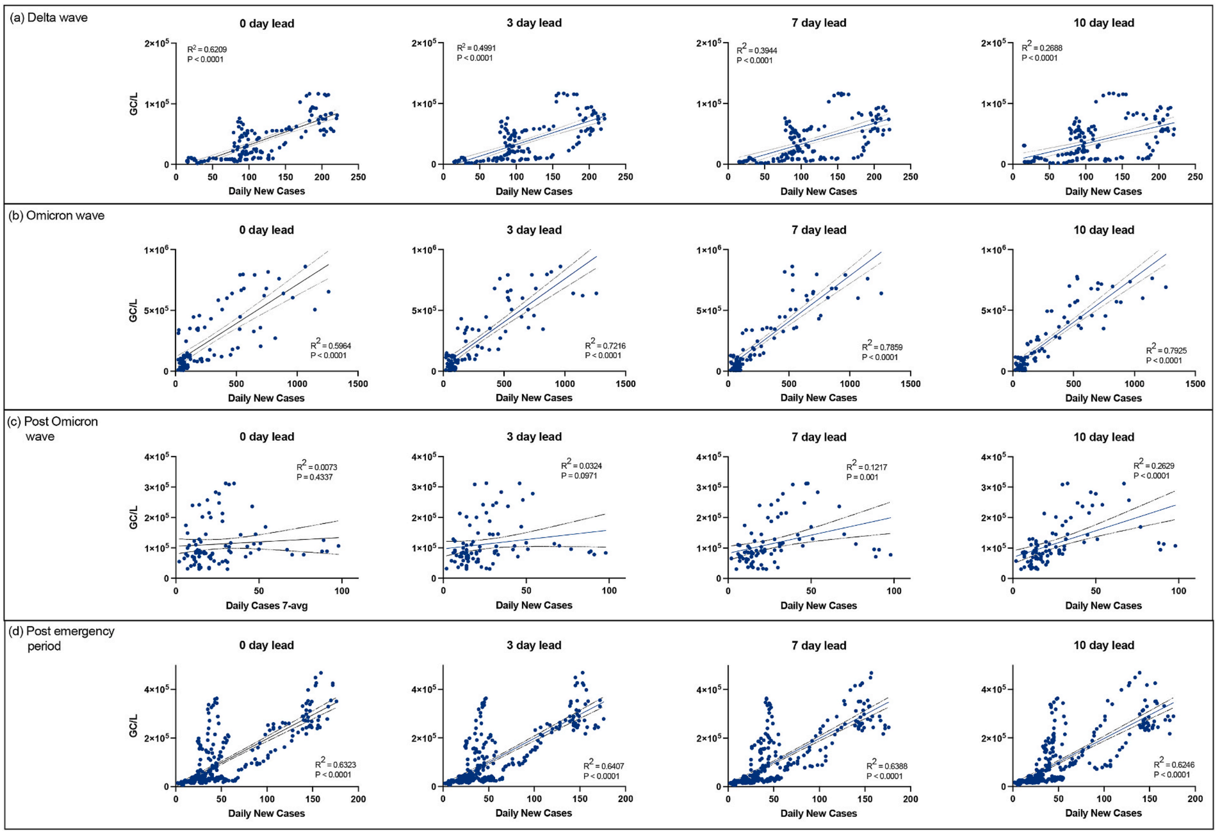

Fig. 3. Linear regression analysis to investigate the correlations between daily new COVID-19 cases and SARS-CoV-2 Viral RNA marker concentrations across four study periods: (a) Delta wave, from July 1, 2021 to Nov 24, 2021; (b) Omicron wave, from Nov 25, 2021 to Feb 21, 2022; (c) Post-Omicron wave, from Feb 22, 2022 to May 10, 2022; and (d) Post-Emergency wave, from May 10, 2022 to May 31, 2023.

3.3. Assessing the variability of SARS-CoV-2 wastewater surveillance lead times across different periods

In order to examine the variability in the lead time of the SARS-CoV-2 signal in wastewater, we conducted simple linear regression analyses over four distinct study periods. The analyses compared the SARS-CoV-2 signal with daily new COVID-19 cases, examining lead times of 0, 3, 7, and 10 days. The results of these investigations are presented in Fig. 3. During the Delta wave, we found no detectable lead time. The most robust regression results were observed when comparing same-day wastewater monitoring data with reported COVID-19 clinical cases ($R^2 = 0.62$, $P < 0.0001$). When lead times were integrated into the analysis, the regression performance deteriorated, as shown in Fig. 3(a). Consequently, the utility of wastewater monitoring for predicting SARS-CoV-2 prevalence was limited during this period. Another study applied a grid search from −6 to 6 weeks and did not confirm any lead time [32]. A study in Ireland found a significant decrease in lead time from 29 days to 6 days during the Delta wave. This could potentially be attributed to changes in the fecal shedding in wastewater and duration [23]. One study found that wastewater signal lagged behind the COVID-19 positivity rate during the Delta wave, as evidenced by the maximum cross-correlation coefficients. During the Delta wave, testing resources were more readily available, which might have led to more prompt diagnostic testing [33]. This could have resulted in a shift in the timing of wastewater signals relative to those seen in the Alpha wave.

During the Omicron wave, linear regression analysis identified significant lead times (3-day lead time: $R^2 = 0.72$; 7-day lead time: $R^2 = 0.78$ and 10-day lead time: $R^2 = 0.79$, Fig. 3(b)), demonstrating a marked increase compared to the Delta wave. This observation contradicts the findings of an another study [32], where no lead time was detected during the Omicron wave. The increase in lead time during the Omicron wave could be due to overwhelmed testing capacity, potentially causing delays in the reporting of COVID-19 cases. Another plausible explanation could be a shift in the virus shedding pattern during Omicron wave.

On February 10, the face mask mandate was lifted in Nevada, USA. In line with our previous study [13], the Post-Omicron variant was the predominant strain of SARS-CoV-2 during this study period. Linear regression analysis of this study period (Post- Omicron wave) did not identify an optimal lead time. The coefficient of determination (R^2) increased from 0.01 to 0.27 with a 0-day to a 10-day lead time (Fig. 3(c)). Concurrently during this period, free at-home test kits were widely distributed in the United States, and symptoms were generally milder, likely owing to the increased rates of full vaccination [23].

Since May 20, 2022, when the state of emergency related to COVID-19 was lifted, daily new cases have consistently remained at a low to moderate level comparing with Omicron wave with an increasing tread. Meanwhile, the linear regressions demonstrated an increase in the coefficient of determination, ranging from 0.62 to 0.64 (Fig. 3(d)). These strong coefficients underscore the effectiveness of WBE as an early warning system to reflect the level of disease in the community. However, we noted large residuals in the regression when daily new cases were fewer than 50 (Fig. 3(c)). This suggests that the regression model may become less sensitive when the virus prevalence is low in the community.

During the Alpha wave in Northern Nevada, Li et al. [4] confirmed a 7-day lead time of wastewater SARS-CoV-2 monitoring. Our study highlighted the lead time shifting across different monitoring phases in the same study area with the same WBE method. One reason for this variability could be the influence of different variants on the virus shedding in feces, and consequently, in wastewater. Another potential factor could be the implementation of vaccination. As of the 30th week of 2021, the COVID-19 vaccination rate in Washoe County was at 49.2 %. So far, to the best of our knowledge, no studies have been conducted to investigate the effect of vaccination rates on virus shedding in feces and transmission. Some studies have reported that fully vaccinated individuals are often asymptomatic or show very mild symptoms, which might result in a decreased or delay of viral load in feces [34,35].

3.4. Differences in wastewater SARS-CoV-2 viral RNA concentrations/daily new COVID-19 cases (W/C ratio) across various pandemic waves

In order to clarify the differences in virus shedding in wastewater through feces between the Delta and Omicron variants of SARS-CoV-2, we calculated the ratio of SARS-CoV-2 viral RNA marker concentrations in wastewater to daily new COVID-19 cases (W/C Ratio). In this section, the W/C ratio is taken to indicate potential changes in viral shedding patterns, but it is important to note that

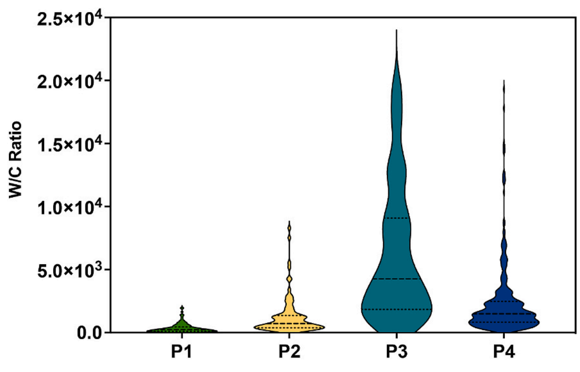

Fig. 4. Wastewater SARS-CoV-2 viral RNA concentrations/Daily new COVID-19 cases (W/C Ratio) in the study area. P1: Delta wave; P2: Omicron wave; P3: Post-Omicron wave; P4: Post-Emergency wave.

variability in this ratio may also be due to the use of clinical versus at-home testing and the overall reporting of clinical case data.

Our dataset spans four distinct phases of the pandemic and measures the ratio of SARS-CoV-2 concentration in wastewater to daily new COVID-19 cases (referred to as P1 through P4). The concentration of SARS-CoV-2 is in gc/L. In the Delta wave (P1), the mean ratio was 349, indicating a relatively lower level of viral shedding in the community. We conducted Kruskal-Wallis test to assess whether the W/C Ratio exhibited differences in central tendency across P1 to P4 phases. The test yielded a Kruskal-Wallis statistic of 292.20 and a P-value <0.0001, indicating a statistically significant difference in the W/C Ratio between at least two of the phases. During the Omicron wave (P2) we observed a significant increase in this ratio, with the mean rising to 1263, suggesting an increased level of viral shedding. The Post-Omicron wave (P3) exhibited an even more substantial rise in viral shedding, with the mean ratio spiking to 6191, marking a more than four-fold increase compared to the Omicron wave (Fig. 4).

Lastly, in the Post-Emergency wave (P4), the mean ratio decreased to 2292, nearly a third of the mean during the Post-Omicron wave. However, the spread of data was wider during this phase, indicating greater variability in W/C Ratio. These observations were statistically validated with a Kruskal-Wallis H test, which showed a highly significant result ($P < 0.0001$), confirming that the medians of the ratios differed significantly across the four phases.

The accuracy of WBE in reflecting new daily diagnosed case may be directly linked to the shedding dynamics of SARS-CoV-2. Currently, sputum and feces are thought to be the primary shedding sources of SARS-CoV-2 RNA in wastewater. This is primarily attributed to their propensity for shedding, both in terms of probability and magnitude, along with their more frequent discharge into the sewer system. These factors render sputum and feces more indicative of the viral prevalence in wastewater compared to other potential shedding sources like saliva, blood, and urine [36,37]. With the expansion of testing capacity in Nevada, USA during November and December of 2021, delayed tests of clinical cases may have been reported at the same time as new cases. Despite this, it is plausible that these delays in testing did not significantly influence the concurrent wastewater viral concentrations, potentially leading to an elevated W/C ratio compared to the result from Delta wave. Intriguingly, a contrasting observation was made in a study from the Detroit metropolitan area, USA, which reported a relatively low W/C ratio during the Omicron wave. The researchers suggested that this discrepancy could be attributed to changes in virus transmission dynamics and an observable increase in reported clinical cases that was not mirrored by a proportional increase in wastewater viral concentrations [38].

Throughout the third wave when other variants were dominant, we observed a persistent rise in the W/C Ratio, accompanied by larger fluctuations. This increase could be attributed to a reduction in clinical cases and the widespread use of home-test kits within the study area. Interestingly, after the lifting of the COVID-19 emergency on May 10, 2022, the W/C ratio began to decline. This decreasing trend likely reflects the lower disease prevalence within the study area post-emergency.

4. Conclusions

Our study has revealed significant correlations between the concentrations of SARS-CoV-2 in wastewater and the number of COVID-19 clinical cases in the community. These findings affirm the potential of WBE as an early warning system for tracking the prevalence of COVID-19. However, it is important to note that this correlation varied across different phases of the pandemic. During the Delta wave, no lead time was detected. This could be due to the increased availability of testing resources, leading to more prompt diagnostic testing and thus shifting the timing of wastewater signals. The W/C Ratio and duration also exhibited significant changes during this period, further complicating the situation. Contrastingly, during the Omicron wave, we observed significant lead times. This discrepancy from the Delta wave findings could be attributed to the overwhelmed testing capacity, leading to delayed COVID-19 test reporting. Furthermore, a potential shift in the virus shedding pattern indicated by the W/C ratio during this wave might have also contributed to the increased lead time. The study also highlighted changes in W/C Ratio across different pandemic phases. The Delta wave demonstrated a low W/C ratio, which indicated a lower level of viral shedding in the community compared to the Omicron and Post-Omicron waves. Importantly, the potential variations in virus shedding through feces that were indicated by the W/C ratio could explain the differences in lead time for wastewater monitoring across the different waves.

However, the role of vaccination in influencing virus shedding and transmission remains unclear. Fully vaccinated individuals often result in mild or no symptoms, which might cause a decrease or delay in viral load in feces. Further research is needed to investigate the effect of vaccination on virus shedding and transmission. The potential of WBE as an early warning system in public health is evident from our findings. However, there are limitations to consider. The variability in virus shedding in feces among individuals and the impact of environmental factors on virus degradation in sewage can lead to inconsistencies in the data. Additionally, the interpretation of WBE data is complex as it represents a combined signal from symptomatic, asymptomatic, and pre-symptomatic individuals.

Despite these challenges, our study highlights the value of WBE as a supplementary tool for traditional disease surveillance systems. With further research and development, it could serve as a robust resource in pandemic preparedness, enabling early interventions and more efficient use of healthcare resources.

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Data availability statement

The data that support the findings of this study are not publicly available due to privacy concerns. Details about the data and how to request access are available from the corresponding author upon reasonable request.

CRediT authorship contribution statement

Lin Li: Writing – review & editing, Writing – original draft, Validation, Methodology, Investigation, Formal analysis, Data curation, Conceptualization. **Laura Haak:** Writing – review & editing, Validation, Investigation, Formal analysis, Data curation. **Madeline Carine:** Validation, Methodology, Investigation, Data curation. **Krishna R. Pagilla:** Writing – review & editing, Resources, Project administration, Funding acquisition, Conceptualization.

Declaration of competing interest

The authors declare no conflicts of interest.

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