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COVID-19 cases, vaccination, and SARS-CoV-2 in wastewater: insights from a Brazilian municipality

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ABSTRACT

Vaccines combatting COVID-19 demonstrate the ability to protect against disease and hospitalization, and reduce the likelihood of death caused by SARS-CoV-2. In addition, monitoring viral loads in sewage emerges as another crucial strategy in the epidemiological context, enabling early and collective detection of outbreaks. The study aimed to monitor the viral concentration of SARS-CoV-2 in untreated sewage in a Brazilian municipality. Also, it attempted to correlate these measurements with the number of clinical cases and deaths resulting from COVID-19 between July 2021 and July 2022. SARS-CoV-2 viral RNA was quantified by RT-qPCR. Pearson's correlation was performed to analyze the variables' relationship using the number of cases, deaths, vaccinated individuals, and viral concentration of SARS-CoV-2. The results revealed a significant negative correlation ($p < 0.05$) between the number of vaccinated individuals and the viral concentration of SARS-CoV-2, suggesting that after vaccination, the RNA viral load concentration was reduced in the sample population by the circulating concentration of wastewater. Consequently, wastewater monitoring, in addition to functioning as an early warning system for the circulation of SARS-CoV-2 and other pathogens, can offer a novel perspective that enhances decision-making, strengthens vaccination campaigns, and contributes to authorities establishing systematic networks for monitoring SARS-CoV-2.

Key words: epidemiological bulletins, SARS-CoV-2, statistical correlation vaccine, wastewater-based surveillance, wastewater treatment plants

HIGHLIGHTS

- The presence of SARS-CoV-2 was detected in 100% of the wastewater samples.
- Wastewater SARS-CoV-2 concentration negatively correlated with vaccinated individuals.
- Wastewater-based epidemiology is a tool for the monitoring of virus during vaccination campaigns.

1. INTRODUCTION

The COVID-19 pandemic has resulted in a significant public health crisis, impacting healthcare and socioeconomic systems (De Oliveira *et al.* 2021; Du *et al.* 2022; Rahimi & Abadi 2022). Consequently, several surveillance mitigation measures have been recommended, such as isolation, personal protection behaviors, maintaining a distance of at least 1 m from other people, properly wearing a mask that covers the mouth and nose, avoiding poorly ventilated environments, staying at home if sick, covering coughs and sneezes, washing hands frequently, getting vaccinated, and completing the vaccination cycle. Together, these measures remain essential to breaking the transmission chain of SARS-CoV-2 (Cohn *et al.* 2022; WHO 2023a) and can minimize the spread of COVID-19 caused by this etiological agent.

The different reported variants of SARS-CoV-2 (Alpha (B.1.1.7), Beta (B.1.351), Gamma (P.1), Delta (B.1.617.2), and Omicron (B.1.1.529)) can contribute to increased transmission, elevated infection and reinfection, or even reduce the immunity provided by antibodies and vaccines (Dubey *et al.* 2022; Zahmatkesh *et al.* 2022).

Between 2020 and 2023, the highest number of SARS-CoV-2 sequences in wastewater, comprising 5,681 genomic sequences across Austria, France, Italy, Liechtenstein, Ukraine, and the United States, was recorded in 2022. In this year, the Alpha and Delta variants were supplanted by the Omicron lineages (BA* + BE* + BF* + BM* + BN* + BQ* + BR* + C* + D* + XBB*), constituting 5,504 sequences (96.88%) (Pilapil *et al.* 2023). The Omicron variant displayed multiple lineages, each undergoing numerous mutations. The primary Omicron variants identified throughout the study until July 2022 were from the BA.1, BA.2, BA.3, BA.4, and BA.5 lineages (Callaway 2022).

Vaccines protect immunity based on immunological memory, in which the adaptive immune system recognizes a new pathogen and also remembers it (Sette & Crotty 2022). Thus, an individual's body can develop immune memory to the SARS-CoV-2 virus through direct infection with the coronavirus (natural immunity), COVID-19 vaccination, or hybrid immunity, which occurs through a combination of infection-induced immunity with the virus (SARS-CoV-2) and vaccine-induced immunity (Crotty 2021; Sette & Crotty 2022).

Brazil is one of the few countries that has included inactivated virus vaccines, vector-based vaccines, and mRNA-based vaccines in its vaccination schedule. During the studied period in Lavras municipality, individuals were totally immunized (primary and booster dose) with 45.1% Pfizer (mRNA-based bivalent), 29.3% AstraZeneca (adenoviral vector-based), 7.9% Janssen (adenoviral vector-based), 14.04% CoronaVac (inactivated virus whole cell), 0.7% pediatric Pfizer, and 2.7% unidentified (BRASIL 2023a). Heterologous vaccination schedules were applied, with AstraZeneca (17.5%), Pfizer (48.3%), and CoronaVac (27.7%) being administered during the primary dose. The booster dose was characterized by AstraZeneca (33%), Pfizer (44%), Janssen (9.5%), and CoronaVac (9.7%). Several countries have used booster doses with other vaccines after complete vaccination, as several studies have revealed efficacy gain (Das *et al.* 2023; Marra *et al.* 2023). From the available data, it was not possible to identify which type of dose was subsequently applied to each individual after the initial dose administration. However, the Brazilian Ministry of Health recommended that the booster dose should preferably be from the messenger RNA platform (Pfizer) or a viral vector vaccine (Janssen or AstraZeneca) (BRASIL 2023b). However, the evaluation of the effectiveness of any of the vaccines administered to the population in the study area was not the objective of the present study.

According to the World Health Organization (WHO), authorized vaccines against COVID-19 protect against the disease, reducing the rates of hospitalization and death (Cohn *et al.* 2022; Torres *et al.* 2022; WHO 2023b). However, since the start of COVID-19 vaccination, cases have been reported of vaccinated individuals who later became infected with SARS-CoV-2. It is considered possible for a vaccinated person to transmit COVID-19 to others upon infection (Cohn *et al.* 2022; WHO 2023a). However, it is supposed that a vaccinated individual, when infected with SARS-CoV-2, will experience milder symptoms than unvaccinated individuals.

Some countries have reported high COVID-19 infection cases during the second wave of SARS-CoV-2. The United Kingdom, India, Brazil, and France stood out during the ongoing pandemic period (Malik *et al.* 2022). By the first quarter of 2023, the six countries with the highest cumulative number of confirmed cases and deaths from SARS-CoV-2 infection were the United States of America (>102 million confirmed cases and >1 million deaths), China (>99 million confirmed cases), India (>44 million confirmed cases and >530,000 deaths), France (>38 million confirmed cases and >160,000 deaths), Germany (>38 million confirmed cases and >170,000 deaths), and Brazil (>37 million confirmed cases and >699,000 deaths). Globally, over 760 million confirmed cases of COVID-19 have been recorded, including more than 6 million deaths (WHO 2023c).

The vaccines developed to combat SARS-CoV-2 constitute a significant scientific accomplishment in addressing the pandemic. However, it is crucial to implement additional mitigation measures as recommended by each country (WHO 2023b). Asymptomatic individuals often do not undergo clinical testing for COVID-19; nevertheless, they can transmit the SARS-CoV-2 virus, potentially infecting others (Long *et al.* 2020; Cao *et al.* 2022; Chowdhury *et al.* 2022). Approximately 80% of asymptomatic cases for SARS-CoV-2 resolve without medical intervention, making it challenging to monitor the spread of the virus within a specific community, region, or location (Chowdhury *et al.* 2022; Robinson *et al.* 2022; Sisay *et al.* 2022). However, symptomatic and asymptomatic individuals share the release of the coronavirus genetic material in their excreta and bodily fluids, making wastewater-based epidemiology an effective surveillance tool for COVID-19 (Haramoto *et al.* 2020; Bonanno Ferraro *et al.* 2021; De Araújo *et al.* 2022; Lu *et al.* 2022; Manuel *et al.* 2022).

Based on the understanding of the presence of SARS-CoV-2 viral concentration in wastewater and considering that this study encompassed the period of advancing vaccine coverage in the Lavras municipality, this research raised the following

questions: Is it possible for the viral concentration of SARS-CoV-2 to decrease or increase after vaccination campaigns, in a particular population? How does the viral load of SARS-CoV-2 compare to the pre-vaccination period?

Therefore, this research aimed to monitor the viral load of SARS-CoV-2 in wastewater treatment plants (WWTPs) in a municipality with approximately 105,000 inhabitants located in the southern region of Minas Gerais State, Brazil. The viral concentration SARS-CoV-2 data were subjected to comparative analysis with the records of the total number of individuals vaccinated against COVID-19, clinical test results, and reports of individual deaths who tested positive for COVID-19 during the study period.

2. MATERIALS AND METHODS

2.1. Sampling

The study was conducted in Lavras, Southern Minas Gerais State, Brazil. The environmental matrices used were raw effluents collected at the inlets of three WWTPs: '*Ribeirão Vermelho* WWTP' (21°12′55.73″S 45°1′5.97″W), '*Água Limpa* WWTP' (21°14′20.78″S 45°2′23.41″W), and Federal University of Lavras WWTP – '*UFLA* WWTP', (21°13′48.98″S 44°59′22.86″W).

Sample collection consisted of aliquots taken every 15–30 min at each sampling point for eight continuous hours at *Ribeirão Vermelho* WWTP and *Água Limpa* WWTP and 40 min at *UFLA* WWTP. The sampling period starts during the 27th epidemiological week of 2021 and 2022 (SINAN), totaling 52 weeks. After excluding weeks without sampling, this investigation selected 41 out of 52 epidemiological weeks.

Samples were sent to the Applied Microbiology Laboratory at the Environmental Engineering Department of UFLA for molecular analysis processing or stored at −20 or −80 °C for future proceedings.

2.2. Samples concentration

The electronegative membrane adsorption method (HAWG047S6, cellulose ester, MILLIPORE, Darmstadt, Germany) was adopted for the wastewater concentration with the addition of $MgCl_2$ (25 mM) (Ahmed *et al.* 2020). Approximately 50 mL was filtered until membrane saturation, and subsequently, the membrane was transferred to a sterile RNase-free microtube and preserved at -20 ± 0.2 °C until RNA extraction.

2.3. Viral RNA extraction, purity, and integrity

The RNA extraction procedure followed the guidelines provided by the AllPrep PowerViral DNA/RNA Kit (Qiagen®, Hilden, Germany). In the final step of the extraction process, the RNA was eluted by adding 100 μL of RNAse-free ultra-pure water.

The integrity and purity of the extracted RNA were evaluated using a NanoDrop™ Lite Spectrophotometer (ThermoFisher Scientific, CA, USA) with a 1 μL sample. Subsequently, the RNA samples were stored at −80 °C until quantification using RT-qPCR.

2.4. Detection and quantification of the concentration of SARS-CoV-2 RNA in wastewater samples

The positive control for SARS-CoV-2 consisted of the 2019-nCoV_N_Positive Control plasmid kit (IDT). A six-point standard curve was generated by serial dilution, ranging from 200,000 to 2 gene copies per μL. The primer set CDC_N1, published by the CDC, USA, was utilized with the following sequences: 2019-nCoV_N1-Forward (5′-3′) - GACCCCAAAATCAGCGAAAT and 2019-nCoV_N1-Reverse (3′-5′) - TCTGGTTACTGCCAGTTGAATCTG (ThermoFisher Scientific). Quantitative RT-qPCR analysis was performed using the 'Power Sybr Green RNA to CT1' MasterMix from ThermoFisher Scientific. Each reaction was conducted with a total volume of 20 μL, undergoing a reaction cycle at 48 °C for 30 min, followed by 9 °C for 10 min, and 45 cycles of 95 °C for 15 s and 60 °C for 60 s.

2.5. COVID-19 clinical trials

The study considered the numbers of vaccinated individuals, daily cases, and deaths caused by COVID-19 during the epidemiological weeks (27th/2021 to 27th/2022). The daily extracted data from epidemiological bulletins are available on the website of the Municipal Government of Lavras (https://www.lavras.mg.gov.br/covid-all/5). These bulletins included records from both public and private laboratories for individuals who tested positive for COVID-19.

2.6. Population and sanitation characteristics

Lavras municipality population is approximately 105,756 inhabitants, with 100,773 corresponding to the urban population. Approximately 75.91% of the municipality's sewage is collected and treated (SNIS 2021).

2.7. Statistical analysis

Descriptive analyses and the Shapiro–Wilk test were performed to assess the normality of the data (Shapiro & Francia 1972). Subsequently, correlations between the number of cases, deaths, vaccinated individuals, and the viral load of SARS-CoV-2 in raw sewage samples were evaluated using the Pearson test, and simple linear regressions were adjusted. The R Core Team *programming language* (2022) was utilized for the described analyses.

3. RESULTS AND DISCUSSION

According to the health authority's information, the Lavras municipality administered the CoronaVac vaccine's initial doses on 20 January 2021 (LAVRAS 2021). In the first quarter of 2023, Lavras vaccination coverage reached 89.58% for the first dose and 86.56% for the second or single dose (Minas Gerais 2023). The available vaccines for the residents of the municipality included CoronaVac (Butantan), Comirnaty COVID-19 mRNA Vaccine (Pfizer/Wyeth), Oxford/AstraZeneca COVID-19 recombinant vaccine (Fiocruz and AstraZeneca), and Janssen COVID-19 recombinant vaccine (Janssen-Cilag).

Regarding the analyzed data, a descriptive statistical analysis was performed, as presented in Table 1. Throughout the 41 epidemiological weeks, the mean SARS-CoV-2 viral concentration quantification in raw sewage samples was 890.7 (gene copies/L N1). The average number of individuals testing positive for COVID-19 per week was 388.8, and the average number of deaths was 0.92. In addition, the average number of vaccinated individuals during the 41-week registration period was 405,900.

A positive correlation between the number of positive cases of COVID-19 and the number of deaths resulting from this disease ($p < 0.05$) was observed in the municipality (Table 2 and Figure 1). As also observed by Pujadas *et al.* (2020), it was possible to confirm that the greater the number of diseases, the greater the number of deaths. Therefore, it corroborates that precautionary and protective measures are extremely important in the fight against COVID-19, particularly vaccination.

Specifically, in the relationship between positive COVID-19 cases and deaths, the linear regression was statistically significant ($p < 0.05$; $R^2 = 0.15$) (Figure 1(a)), explaining the causal effect among them. However, other factors may influence the correlation, e.g., available health infrastructure, access to an appropriate treatment protocol, economic conditions of the patient, comorbidities, patient age, vaccination coverage, etc. Regarding the relationship between vaccination coverage and viral load in wastewater, the linear regression was also significant ($p < 0.05$; $R^2 = 0.13$) (Figure 1(b)), indicating an inversely proportional relationship between those variables.

According to data from the Ministry of Health, Brazil's first dose of the vaccine took place on 17 January 2021. However, in the first quarter of 2023, only 80.56% of the Brazilian population can be considered fully vaccinated (BRASIL 2023c). These data demonstrate considerable resistance to vaccination by some of the Brazilian population.

The presence of SARS-CoV-2 RNA has been detected by different researchers in studies involving the analysis of domestic wastewater. This detection indicates the presence of viral genetic material associated with excretions, such as vomit, sputum, saliva, urine, and, mainly, feces, from both symptomatic and asymptomatic patients (Alhama *et al.* 2022; Yanaç *et al.* 2022; Zahmatkesh *et al.* 2022; Arts *et al.* 2023; Nakgul *et al.* 2023). However, to date and to the best of our knowledge, the relationship between the presence of SARS-CoV-2 viral load in wastewater and vaccination records reported cases and COVID-19 deaths in the study area has not been evaluated.

The concentration of the SARS-CoV-2 virus in wastewater indicates the presence of genetic material released in feces, urine, or respiratory secretions (Koirala *et al.* 2023; Kumblathan *et al.* 2023; Maryam *et al.* 2023), and the incorporation

Table 1 | Data from the descriptive statistical analysis of the number of cases, deaths, vaccinations, and viral concentration of SARS-CoV-2 in sewage samples from the municipality of Lavras (MG) carried out in the 41 weeks between the 27th and 52nd epidemiological week of 2021 and between the 1st and 27th epidemiological week of 2022

	Cases	Deaths	Vaccinated	SARS-CoV-2 concentration in wastewater (gene copies/L N1)
Mean	388.8	0.92	4,059.0	890.7
Minimum	6.0	0.0	1,427.0	221.0
Maximum	2,568.0	6.0	8,646.0	2,359.0
Shapiro–Wilk W	0.62	0.70	0.91	0.92
Shapiro–Wilk p	<0.001	<0.001	0.003	0.006

Table 2 | Pearson correlation matrix of the variables – number of cases, deaths, vaccinated individuals, and viral load of SARS-CoV-2 in wastewater samples – from the municipality of Lavras (MG) between the 27th and 52nd epidemiological weeks of 2021 and between the 1st and 27th epidemiological weeks of 2022

		Cases	Deaths	Vaccinated	SARS-CoV-2 concentration in wastewater (gene copies/L N1)
Cases	Pearson's r	–			
	p-value	–			
	R^2	–			
	Adjusted R^2	–			
Deaths	Pearson's r	0.38*			
	p-value	0.01	–		
	R^2	0.15	–		
	Adjusted R^2	0.13	–		
Vaccinated	Pearson's r	−0.06	0.20	–	
	p-value	0.69	0.02	–	
	R^2	0.004	0.04	–	
	Adjusted R^2	−0.02	−0.01	–	
SARS-CoV − 2 concentration in wastewater (gene copies/L N1)	Pearson's r	−0.03	−0.07	−0.35*	–
	p-value	0.85	0.66		–
	R^2	0.0009	0.005	0.12	–
	Adjusted R^2	−0.02	−0.02	0.10	–

*$p < 0.05$.

of wastewater analysis represents the grouping of excretions and fluids from infected individuals, including pre-symptomatic, symptomatic, asymptomatic, and post-asymptomatic carriers. Consequently, sanitary environments, sewage systems, or wastewater may represent a sampling site that integrates surveillance efforts and its role in detecting and tracking new variants.

Thus, this study confirmed, employing wastewater analysis, that vaccines are effective in protecting against SARS-CoV-2, both for symptomatic and asymptomatic patients. The data showed a negative correlation between the number of vaccinated individuals and the concentration of SARS-CoV-2 in wastewater in the municipality (Table 2 and Figure 1). In other words, the present study obtained in the RT-qPCR analysis the minimum and maximum values of 221.0 and 2.359 copy numbers/L of sample/N1, respectively. Therefore, with the increased vaccination coverage, the study municipality recorded fewer records of infected individuals and deaths caused by COVID-19. Moreover, Armas *et al.* (2023) claim that vaccinated individuals do not contribute to SARS-CoV-2 RNA levels in wastewater.

Thus, wastewater surveillance can be an important form of early warning of the circulation of the SARS-CoV-2 virus and may even help in vaccination campaigns if properly adopted. Besides contributing to the scientific literature, these results also help the local authorities establish systematic surveillance networks for the circulation of the SARS-CoV-2 virus in raw effluents. Circulating viral load tends to occur earlier than the symptoms of COVID-19 and may even help in vaccination campaigns if properly adopted. Therefore, monitoring the presence of the SARS-CoV-2 viral load in wastewater in a pandemic scenario is of paramount importance, given the notorious decline in clinical trials and the number of individuals who are not vaccinated in different communities (Malik *et al.* 2022; Vo *et al.* 2022).

In Lavras, Brazil, the concentration of SARS-CoV-2 particles in raw sewage samples collected at the entrance of a WWTP showed a correlation with the number of cases of the disease registered in the evaluation period from May to June 2021 (Abreu *et al.* 2022). Increases in the viral load of the coronavirus in wastewater on some US college campuses have coincided with new clinical cases in the city (Reeves *et al.* 2021; Wright *et al.* 2022). This trend was also observed in other studies, such as the one by Weidhaas *et al.* (2021), in which a COVID-19 outbreak in two communities in the state of Utah, USA, was positively correlated with an increase in SARS-CoV-2 RNA from wastewater, while a decline in COVID-19 cases preceded a decrease in the viral RNA.

However, researchers noted that the effectiveness of vaccination against the SARS-CoV-2 virus would only change with the development of a robust, efficient, and proactive monitoring approach for the emergence of current and future variants. Zahmatkesh *et al.* (2022) demonstrated that, with the vaccination campaigns, there was a reduction in the quantified viral

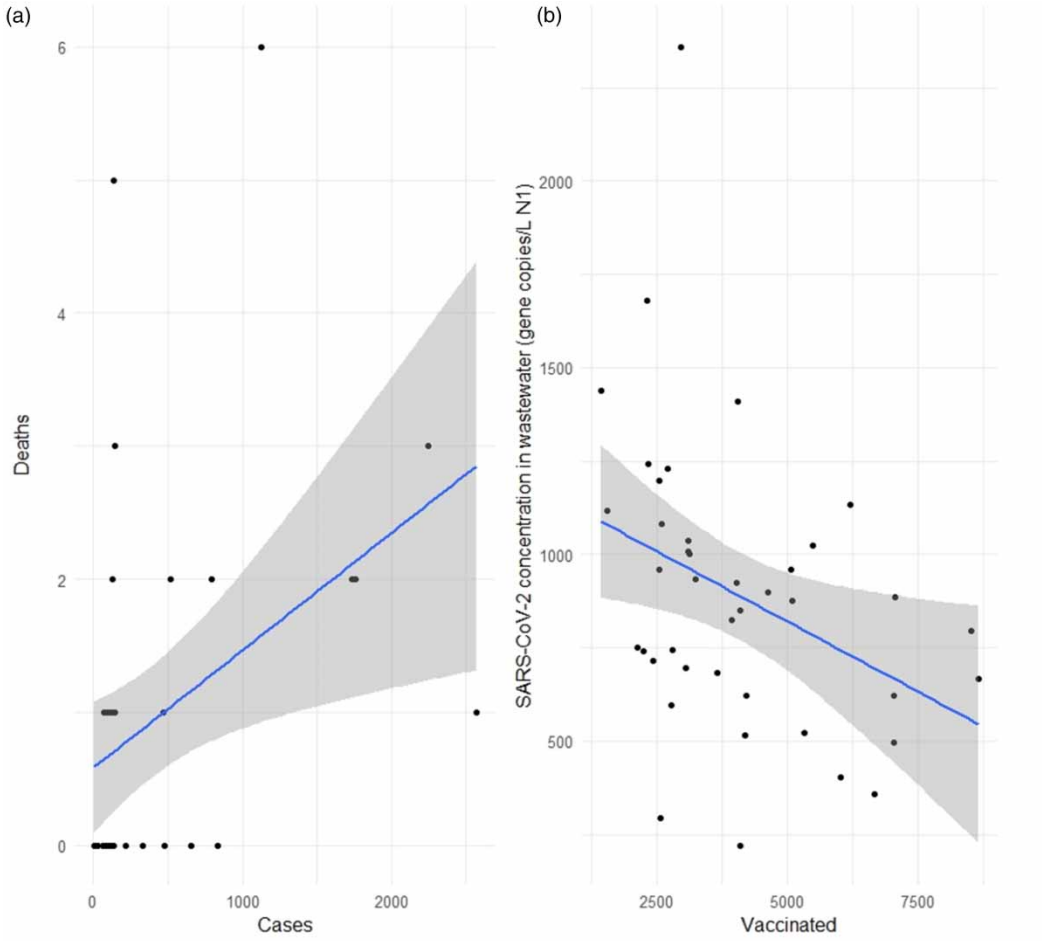

Figure 1 | Simple linear regression. (a) The number of infected individuals, which is represented by confirmed cases and deaths in the municipality of Lavras, Brazil. (b) The quantification of the viral load of SARS-CoV-2 is represented concerning the quantification of vaccinated individuals in the municipality of Lavras, Minas Gerais, Brazil, between the 27th and 52nd epidemiological weeks of 2021 and between the 1st and 27th epidemiological weeks of 2022.

load of SARS-CoV-2 in wastewater samples. Notably, the virus activation in the collected samples was not evaluated, but the presence of its viral concentration was evaluated.

The mutations of SARS-CoV-2 contribute to the emergence of new variants, leading to increased transmissibility, morbidity, and mortality (Abulsoud *et al.* 2023). Despite recognizing wastewater surveillance as a valuable source of precise information on the behavior and transmission rate of SARS-CoV-2 (Boehm *et al.* 2023), significant limitations arise in detecting the virus variant in wastewater. This can be evidenced by a lack of research evaluating the presence of these variants in raw effluent samples.

In a pioneering study, Pilapil *et al.* (2023) analyzed 8,511 genome sequences of SARS-CoV-2 variants present in wastewater from nine countries from March 2020 to May 2023. This study revealed that in effluent samples collected between January and February 2021, approximately 23.53% of the sequence composition corresponded to the B.1.1.7 variant (Alpha).

In the same study, the authors demonstrated that Delta variants, specifically the B.1.617.2 and AY.4 lineages, were detected as predominant in wastewater and clinical samples from early July 2021 to mid-December 2021. In November 2021, BA.1

(Omicron) lineages were detected in wastewater, with a noticeable increase starting in the first week of December. However, the dominance of Omicron variants was only recorded in clinical samples in mid-January 2022, with over 90% of samples being BA.2 lineages. The prevalence of Omicron variants was consistently observed in both wastewater and clinical samples throughout 2022, which later shifted in the second half of January 2023 (Pilapil *et al.* 2023).

In the present study, it was not possible to analyze the variants in raw effluent samples. Thus, the correlation between the type of vaccine and the SARS-CoV-2 variants was not investigated. However, further research on the types of vaccines individuals receive in a given sample region should be the target of future studies. Information such as the type of vaccine and the viral concentration of the SARS-CoV-2 variant present in the raw effluent sample can suggest validations on which kind of vaccine can provide greater efficacy and interfere with viral propagation. Although the study did not evaluate the lineages of SARS-CoV-2 variants, it is pioneering in comparing vaccine coverage and the concentration of the SARS-CoV-2 virus in raw effluent samples.

4. CONCLUSION

The data presented here do not challenge the notion that current vaccines are highly effective in preventing infection and reducing patient hospitalization or death but seek to present the efficiency of monitoring the viral load of SARS-CoV-2 in wastewater and its importance in demonstrating the presence or absence of the coronavirus in a Brazilian municipality. This information may be relevant in the context of Unique Health since it was possible to demonstrate the reduction in the circulation of the concentration of the SARS-CoV-2 virus after the period of local vaccination coverage.

AUTHOR CONTRIBUTIONS

MA: Conceptualization, Methodology, Validation, Formal analysis, Investigation, Data curation, Writing – original draft, Writing – review & editing, Visualization. BL: Conceptualization, Methodology, Validation, Investigation, Resources, Data Curation, Writing – review & editing, Visualization. PA: Conceptualization, Methodology, Validation, Investigation, Resources, Writing – review & editing, Visualization, Supervision, Project administration, Funding acquisition. AS: Conceptualization, Methodology, Writing – Review & Editing. LS: Conceptualization, Methodology, Validation, Formal analysis, Investigation, Resources, Data Curation, Writing – Original Draft, Writing – review & editing, Visualization, Supervision, Project administration, Funding acquisition.

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DATA AVAILABILITY STATEMENT

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

CONFLICT OF INTEREST

The authors declare there is no conflict.

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