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Wastewater-based SARS-CoV-2 airport surveillance: key trends at the Cape Town International Airport

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ABSTRACT

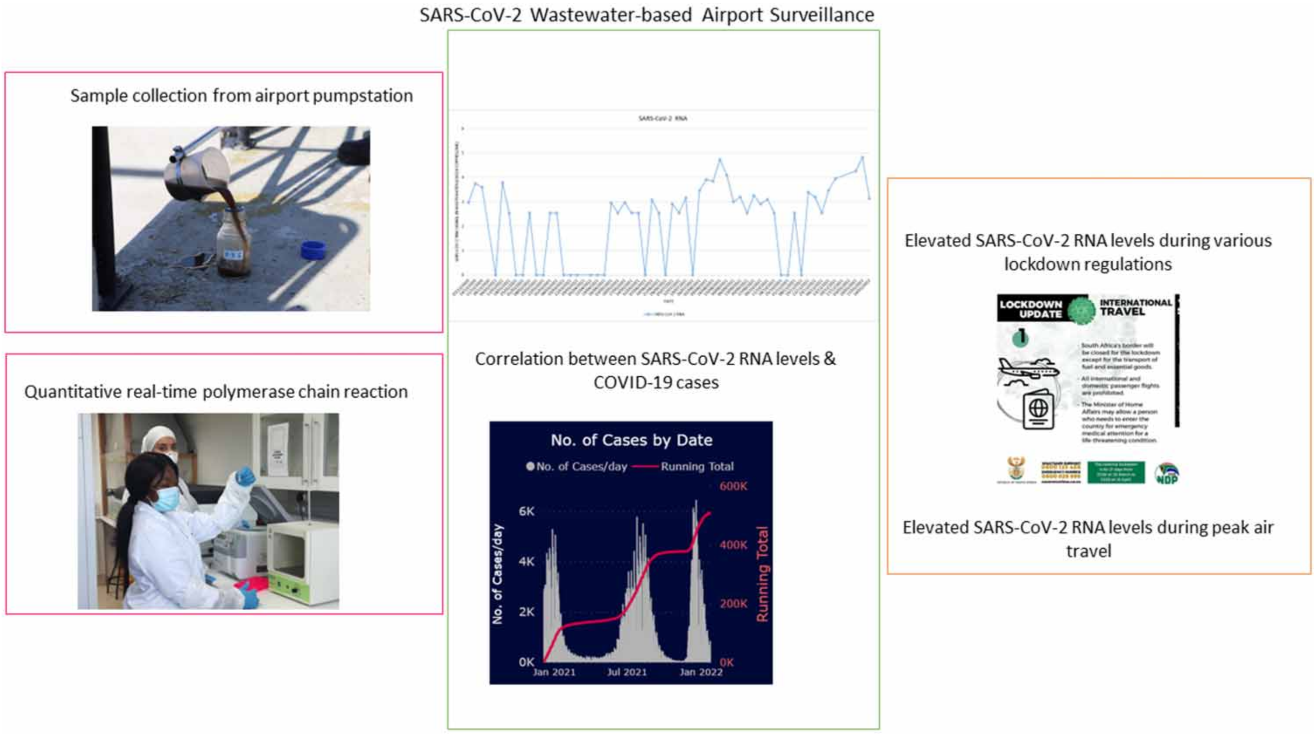
Monitoring of SARS-CoV-2 RNA in wastewater has revealed the role of mobility in the transmission of coronavirus disease (COVID-19), and the surveillance of airport wastewater in cities across the world has demonstrated how travel entry points can give an indication of trends in transmission. This study undertook wastewater surveillance at the Cape Town International Airport (CTIA) to assess the use of a WBE approach to provide supplementary information on the presence of COVID-19 at a key air travel entry point in South Africa. Grab wastewater samples ($n = 55$) were collected from the CTIA wastewater pump station and analysed using quantitative real-time polymerase chain reaction (qRT-PCR) method. The study found a correlation between the wastewater data and clinical cases reported in the City of Cape Town during various time periods and during the peak of a COVID-19 wave. Highly elevated viral loads in the wastewater were observed at times there was increased mobility through the airport. The study also revealed elevated viral load levels at the airport despite the stricter restrictions and through the lower restrictions. The study findings indicate wastewater surveillance and airports can provide supplementary information to airport authorities to assess the impacts of imposed travel restrictions.

Key words: airport surveillance, COVID-19, SARS-CoV-2, wastewater-based epidemiology

HIGHLIGHTS

- This is the first published work to report on airport wastewater surveillance in Africa.
- There was a strong correlation between wastewater data and clinical case data.
- This study demonstrates the utility of wastewater monitoring at air travel entry points in an African setting.

GRAPHICAL ABSTRACT



INTRODUCTION

In the current coronavirus disease (COVID-19) pandemic, wastewater-based epidemiology (WBE) has been utilized as a novel approach to track the presence of severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) in wastewater and use the findings as an early indicator of COVID-19 infections within communities. The WBE approach has been adopted in various settings, predominantly the surveillance of Wastewater Treatment Plants (WWTPs), and more recently in more defined locations, for example, by sewage systems within residential buildings (Zdenkova *et al.* 2021; Ahmed *et al.* 2022). More recently, WBE has been touted as an effective public health tool to not only determine the presence or absence of SARS-CoV-2 RNA but also to quantify the viral loads and provide aggregate information for a community, particularly in the absence of frequent individual testing and asymptomatic cases (Foladori *et al.* 2020). In addition, next-generation sequencing has also become a cost-effective tool for the early identification of new variants. The surveillance of travel industries such as aviation has demonstrated its impact on the transmission of SARS-CoV-2 across and within borders. For example, 62.8% of cases in Australia were found to be individuals who were infected with COVID-19 while travelling internationally (Ahmed *et al.* 2020). Research by Lodder & de Roda Husman (2020) was among the first to detect SARS-CoV-2 in wastewater that was collected at Amsterdam Airport Schiphol, with these wastewater samples testing positive 4 days after the first COVID-19 case was identified in the Netherlands. Based on these findings, research has expanded to conduct wastewater surveillance at international airports in countries/cities including the Netherlands, Australia, Prague, and Dubai (Ahmed *et al.* 2020; Medema *et al.* 2020; Albastaki *et al.* 2021; Zdenkova *et al.* 2021).

As South Africa developed COVID-19 response strategies to limit the transmission of SARS-CoV-2 in the country, a series of lockdowns were implemented, varying in their restrictive measures. For air travel, these measures included restricting international flights to and from the country, limiting domestic air travel for business purposes only subject to authorization, screening all passengers entering terminal buildings, and requiring a negative COVID-19 test 72 h prior to permitted travel. With mobility within and between populations among the key contributors to the spread of COVID-19, there is an imperative to investigate the impact the transportation industry has on the transmission of SARS-CoV-2. As restrictions lifted and airports increasingly returned to full operations, the Cape Town International Airport (CTIA) has offered important opportunities to provide insights into the impact of restrictive measures. The CTIA is the third largest airport in Africa, processing over 10 million passengers annually. The CTIA terminal buildings consist of domestic and international passengers travelling in and out of a city, retail stores, and restaurants. Therefore, this study undertook wastewater surveillance at the CTIA to determine the utility of using such an approach to provide supplementary information to public health and airport authorities on the presence of COVID-19 at a key air travel entry point in an African setting.

MATERIALS AND METHODS

Wastewater sampling

Between December 2020 and January 2022, 500 mL of raw influent wastewater grab samples were collected from the pump station at the CTIA in South Africa (Figure 1). According to the 2011 census, the City of Cape Town municipality has a population of 3,740,026. The most recent available data indicate that the CTIA handled over 10 million passengers over the 2019/2020 financial year (Mokhele & Mokhele 2022). This pump station receives the wastewater from the arrivals and departures terminal buildings, restaurants, and airport staff offices. A total of 55 samples were collected during this sampling period between 07 December 2020 and 24 January 2022. The samples were collected weekly on Mondays and at a similar time over the 14-month period. Samples were collected on Mondays to allow laboratory analysis to be completed, provide results in a timeous manner, and afford municipal stakeholders the opportunity to use the information on a weekly basis for their response strategies. Samples were transported to the laboratory on ice and processed on the same day for the extraction of RNA. They were later stored at −80 °C after which quantitative real-time polymerase chain reaction (qRT-PCR) was conducted.

Sample concentration and RNA extraction

A modified method described by Peccia *et al.* (2020) and optimized by Johnson *et al.* (2021) was used to extract RNA (Johnson *et al.* 2021). In this method, 100 mL of influent wastewater was spun down at 2500 × g for 20 min, whereafter 2–5 mL of the resulting pellet was added to a 15 mL PowerBead® Tube containing lysis buffer to inactivate the virus and stabilize the viral RNA (Johnson *et al.* 2021). Subsequently, the sample was homogenized and phase-separated, with the quantity and quality of the total RNA measured by spectrophotometry using the NanoDrop ® ND-1000 instrument (Johnson *et al.* 2021). Due to the absence of a surrogate, a clinical SARS-CoV-2 positive nasal swab sample with a known viral load was used to spike a wastewater sample to examine the efficacy of the extraction method (Johnson *et al.* 2021). A 10-fold serial dilution was made using the 2019-nCoV-N-positive plasmid control as a standard, which was supplied at 200,000 copies/μL (Johnson *et al.* 2021). The recovery for extraction efficiency was previously tested and reported by Johnson *et al.* (2021).

Quantitative real-time polymerase chain reaction analysis

In order to detect SARS-CoV-2 viral RNA in the wastewater samples, the study used the Centers for Disease Control and Prevention (CDC)-approved qRT-PCR nucleocapsid gene (N1 and N2) primer/probe (CDC 2020). The wastewater samples were reverse transcribed, amplified, and quantified using the Bio-Rad iTaq Universal Probes One-Step Kit (Bio-Rad) according to

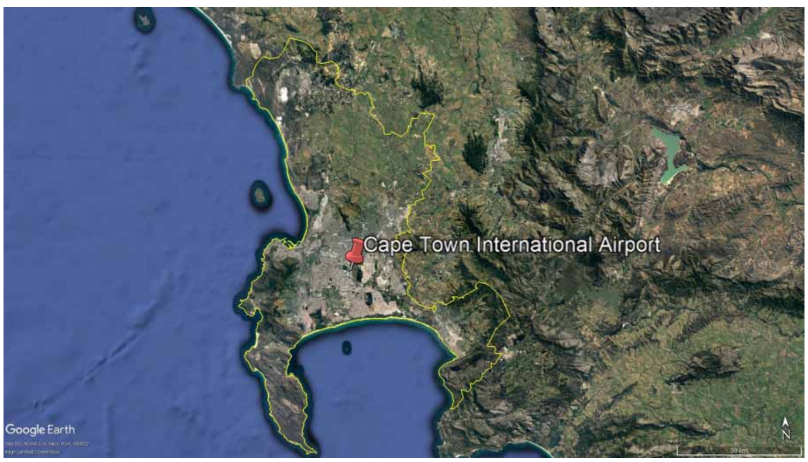


Figure 1 | Location of the CTIA in Cape Town, South Africa.

the manufacturer's instructions. There was a 100% alignment between the N1 and N2 primer and probe sets with the N protein of the SARS-CoV-2 strain (Johnson *et al.* 2021). To detect the presence of and quantify SARS-CoV-2 viral RNA in wastewater, a one-step qRT-PCR was performed in duplicate for all samples. A positive control was included for all experimental runs and SARS-CoV-2 was quantified using the standard curve (Johnson *et al.* 2021). Finally, to reduce possible contamination, RNA extraction and qRT-PCR were performed in separate laboratories (Johnson *et al.* 2021).

COVID-19 alert levels

When sampling commenced at the CTIA in December 2020, the country was on alert level 1, international travel had resumed and domestic passenger flights were permitted. Following a rise in clinical case numbers, there was a change in restrictions to alert level 3 from 29 December 2020 to 28 February 2021, which prohibited all international passenger flights, and only passengers were allowed inside the terminal building. Between 16 June and 25 July 2021, in response to increasing clinical cases, the alert level was adjusted from levels 2 to 4, signifying a moderate to high COVID-19 spread in the country. During this period, there were multiple lockdown regulations implemented in the country that affected the operation of commercial passenger flights at the CTIA. Among the restrictions were the prohibitions of international flights, limiting domestic air travel for business purposes only, and only allowing passengers inside the terminal building. The COVID-19 alert level had eased with adjustments made to alert level 1 as of 1 October 2021 and international airports increasingly returned their operations to full capacity.

Statistical analysis

Summary statistics were used to describe the SARS-CoV-2 RNA copies per millilitre of wastewater. To determine the correlation between the N1 and N2 primers, Spearman's rank correlation was performed at a significance level of <0.05. The N1 and N2 values were combined and the average value was used as a single measurement. Additionally, Spearman's rho correlation analysis was performed between the SARS-CoV-2 in wastewater collected on Mondays and clinical cases in the City of Cape Town a week prior. For analysis, the RNA values were log-transformed. The SARS-CoV-2 RNA values are presented as virus concentrations without normalization due to the lack of information and the certainty of flow rate and population size. Due to an absence of clinical case reporting at the airport, Spearman's rank correlation was computed to assess the relationship between the wastewater data and reported clinical cases in the City of Cape Town municipality. The clinical case data on the number of cases in the City of Cape Town municipality were obtained from the Western Cape Provincial COVID-19 Cases Dashboard (https://coronavirus.westerncape.gov.za/vaccine/covid-19-cases-dashboard). Cases were defined as the number of cases diagnosed with COVID-19 by the date of diagnosis. An analysis was conducted to observe trends in wastewater and clinical cases during three distinctive time periods. SARS-CoV-2 RNA viral load below the limit of detection (LOD) (700 genome copies/mL) was replaced by half of the LOD. Statistical analysis was conducted using STATA, version 17.

RESULTS AND DISCUSSION

Spearman's correlation indicated a strong and positive correlation between the N1 and N2 values ($rho = 0.82$, $p = 0.0001$). During the study period (Figure 2), 69% of wastewater samples ($n = 55$) collected from the pump station at the CTIA tested positive for SARS-CoV-2 RNA with the highest viral load equalling 66,483 copies/mL (17 January 2022) and a median viral load of 639 copies/mL. The non-detection of SARS-CoV-2 RNA in the wastewater could be attributed to inhibitions during the PCR analysis caused by the wastewater chemistry. Similar to Zdenkova *et al.* (2021) results, this study has successfully demonstrated the detection of SARS-CoV-2 from grab samples collected from airport wastewater. There was a moderate and positive correlation between the wastewater data and clinical case data, which was statistically significant ($rho = 0.52$, $p = 0.0001$). These findings are in contrast to those of Zdenkova *et al.* (2021), who found a weak correlation at locations that were not predominantly large residential areas, such as the airport WWTP in Prague. However, this is to be expected as airport wastewater surveillance represents a sample of those working at the airport and travellers arriving that have not yet contributed to the COVID-19-positive cases. In instances when the RNA levels in wastewater were high but clinical cases were low, this could be attributed to changes in testing criteria limiting testing to only high-risk groups or to changes in people's health seeking behaviour and their opting not to test despite presenting COVID-19 symptoms, and asymptomatic individuals shedding the virus in the wastewater.

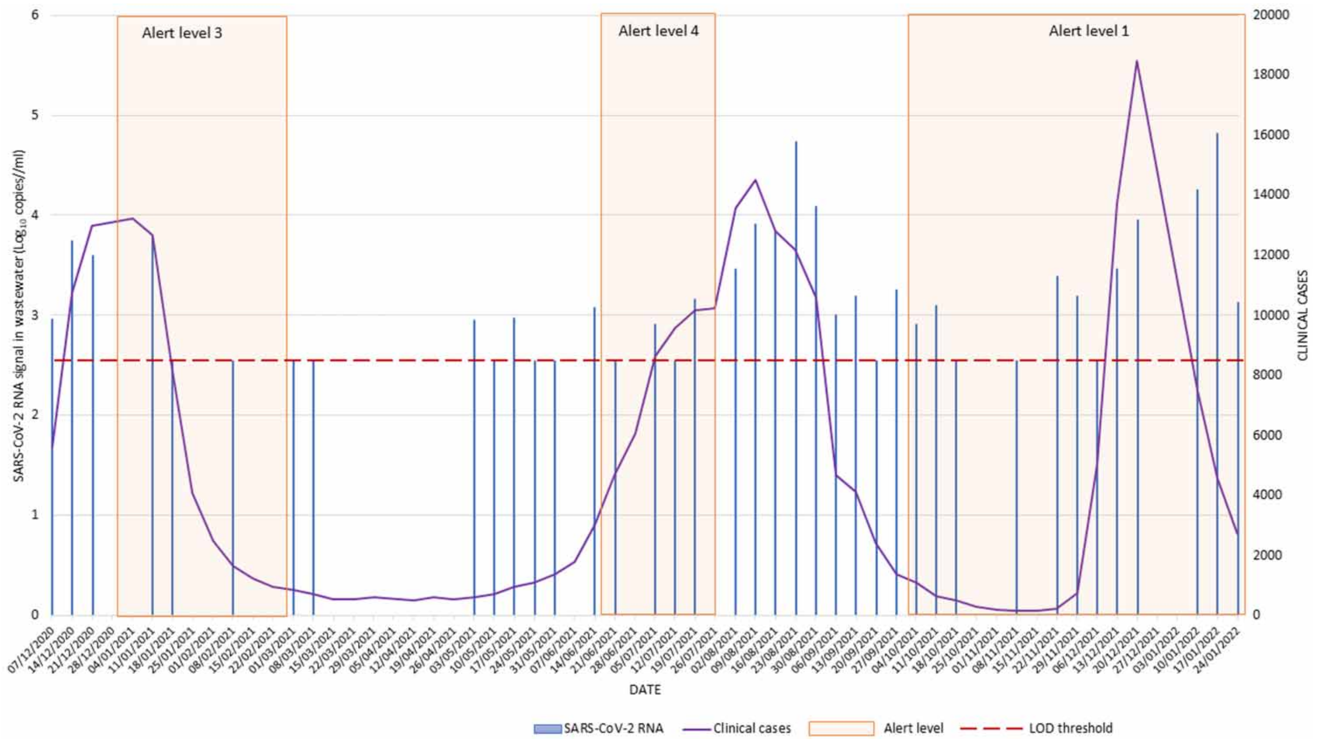


Figure 2 | Trends in wastewater data (SARS-CoV-2) from the CTIA and clinical cases reported in the City of Cape Town during adjusted alert levels 3 (29 December 2020 to 28 February 2021), 4 (16 June 2021 to 25 July 2021) and 1 (1 October 2021 onwards).

During the period of alert level 3, the median viral RNA load detected was 581 copies/mL with the highest viral load equalling 6,076 copies/mL. Although a moderate correlation was observed between the wastewater data and clinical cases, this relationship was not statistically significant ($rho = 0.54$, $p = 0.084$). Highly elevated viral loads in the wastewater were observed over the 3 weeks in December 2020 when there would typically be increased mobility through the airport due to the end-of-year holiday season in the country, the associated easing of travel restrictions during the pandemic, and the city being a tourism destination. In this same time period in December 2020, there was an increase in case of data with reported cases remaining above 10,000 a week in the city. Subsequently, there were decreases in the viral load detected in the wastewater and from the reported clinical cases during the early parts of February 2021 when air travel was not dominated by passengers travelling for tourism purposes.

During alert level 4 restrictions, the highest SARS-CoV-2 viral load was detected in the wastewater (53,683 copies/mL), with a median viral load of 1,098 copies/mL. Although the country experienced increased restrictions in various sectors and industries, regulations still permitted domestic flights. International air travel was also permitted, with passengers required to provide proof of a negative PCR COVID-19 test 72 h before their departure date. At this stage, proof of COVID-19 vaccination and/or recovery was not requested. In this 14-week period, SARS-CoV-2 was detected in 86% ($n = 14$) of the wastewater samples. Analysis between the wastewater data and clinical cases indicated a moderately strong and significant correlation ($rho = 0.54$, $p = 0.05$). Through media reports, there was an increase in commuters through the CTIA with volumes recovering to 66% of domestic arrivals and recovering to 29% of international arrivals when compared to pre-pandemic levels. In total, 869,119 air passengers travelled through the CTIA between October and December 2021. When examining the wastewater trends over this 15-week period, the highest viral load was 66,483 copies/mL, with a median of 2,010 copies/mL. Moreover, Spearman's correlation indicated a strong positive and statistically significant relationship between the wastewater data and reported cases in the City of Cape Town ($rho = 0.69$, $p = 0.0046$).

Air passenger data were unavailable throughout the other periods of this study, specifically during alert levels 3 and 4, thus limiting the interpretation of RNA in wastewater. This study also found a moderate and statistically significant relationship between the RNA levels in wastewater and RNA levels from the City of Cape Town municipality ($rho = 0.55$, $p = 0.0001$). This result indicates that the wastewater samples collected at the CTIA pump station may be used as a convenient sampling location to provide supplementary data on the spread of COVID-19. Given that various restrictive measures were meant to prevent the presence and transmission of COVID-19 in and out of the CTIA, this study revealed elevated RNA levels at the airport during the stricter restrictions (alert level 4) and through the lower restrictions (alert level 1). This indicates that airport authorities can benefit from wastewater surveillance, which can guide their assessments of how effective restrictions have been at international airports such as the CTIA.

CONCLUSION

This study reports on the detection of SARS-CoV-2 in wastewater samples from the CTIA wastewater pump station and demonstrates the utility of monitoring air travel entry points during various stages of travel restrictions in an African setting. Although there are limitations to using grab wastewater samples, which reflect one-time point of the week, the routine sampling strategy did allow trends to be observed. Other limitations to the study were the lack of available air passenger data during alert levels 3 and 4 to make comparisons with the wastewater data throughout all restriction levels. The results emanating from this study indicated that the viral load detected in airport wastewater coincided with trends in clinical case data in the City of Cape Town Wastewater surveillance at the CTIA can provide supplementary information on trends of COVID-19 cases in the CTIA, by indicating potential surges in transmission. In comparing the wastewater data and clinical case data during the three time periods, the correlation was stronger when the median SARS-CoV-2 viral load was higher in periods during alert levels 4 and 1. This indicates that wastewater surveillance of the CTIA would be most beneficial in periods of an increased prevalence of COVID-19.

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ETHICAL APPROVAL

Approval was obtained from the SAMRC Human Research Ethics Committee.

DATA AVAILABILITY STATEMENT

Data cannot be made publicly available; readers should contact the corresponding author for details.

CONFLICT OF INTEREST

The authors declare there is no conflict.

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