WASTEWATER-BASED EPIDEMIOLOGY FOR SARS-COV-2 SURVEILLANCE IN SOUTH AFRICA

NATIONAL INSTITUTE FOR COMMUNICABLE DISEASES
Division of the National Health Laboratory Service

WEEK 39

OVERVIEW

This report summarises and interprets findings from detection and quantification of SARS-CoV-2 levels by the NICD Centre for Vaccines and Immunology in influent (untreated) wastewater in 18 wastewater treatment plants across five provinces. Levels of SARS-CoV-2 in wastewater correlate with population levels of SARS-CoV-2 over time and indicate the geographic distribution of disease. SARS-CoV-2 is shed from symptomatic and asymptomatic persons in stool, but is not transmitted by faeco-oral route nor in wastewater. This report is based on data collected from June 2020 until the end of September 2021.

HIGHLIGHTS

- In Gauteng, levels of SARS-CoV-2 in wastewater treatment plants from City of Tshwane and City of Johannesburg show consistent decreases, correlating with the ongoing downward curve of the third wave. Levels of SARS-CoV-2 in Ekurhuleni at two plants increased in week 38 after a low at week 37.
- In eThekwini, KwaZulu-Natal Province, levels of SARS-CoV-2 in wastewater continue to drop. Levels at week 38 correlate with those seen at week 23 (2nd week June 2021).
- In Mangaung, SARS-CoV-2 levels show an increase in south-eastern Mangaung (Bloemspruit WWTF) from week 33. **Health authorities should strengthen surveillance in this area.** Levels are stable and/or decreasing in central Mangaung (Sterkspruit WWTF).
- In the Eastern Cape Province, Nelson Mandela Metro, SARS-CoV-2 levels in wastewater have steadily increased since week 23, and have paralleled the increase in clinical cases. In Buffalo City Metro, SARS-CoV-2 levels are low and stable in eastern coastal region (East Bank WWTF), but increasing in the north western part of the metro, suggesting increased transmission in this area (Mdantsane).
- In the Western Cape Province, at two facilities in the City of Cape Town (Borcherds Quarry and Zandvleit WWTFs), SARS-CoV-2 levels appear to be decreasing, suggesting reduced SARS-CoV-2 transmission.

DETECTION AND QUANTITATION OF SARS-COV-2 AT SENTINEL WASTEWATER TREATMENT SITES IN SOUTH AFRICAN URBAN AREAS, MARCH-AUGUST 2021

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BACKGROUND

The detection and monitoring of SARS-CoV-2 epidemiology through wastewater was first proposed in April 2020. Initial reports describing the feasibility and practical usefulness of this approach emerged simultaneously from several countries during August 2020. Recent evidence has shown that SARS-CoV-2 can be detected in wastewater prior to the appearance of clinical cases, and longitudinal tracking of SARS-CoV-2 viral load in wastewater correlates with the burden of clinically diagnosed cases. Sequencing of SARS-CoV-2 RNA fragments in wastewater has identified variants of concern as well as mutations not detected in clinical cases.

In South Africa, SARS-CoV-2 epidemiology is monitored through laboratory testing of clinical cases using reverse-transcriptase polymerase chain reaction (RT-PCR) tests and rapid antigen tests, COVID-19 hospital admissions and COVID-19 - related deaths. Laboratory testing data is relayed by testing laboratories to the National Institute for Communicable Diseases (NICD) via the DATCOV system. From these data sources, epidemiological indicators including incidence rates of testing and case detection, hospitalisation and death rates are made available to key stakeholders and the general public.

Clinical epidemiology based on reporting of laboratory-confirmed cases of SARS-CoV-2 has limitations. Household transmission studies in South African urban and rural settings have demonstrated that a large proportion of cases are asymptomatic, and a high proportion of cases so mild as not to elicit health seeking, and that laboratory-confirmed cases likely represent less than 10% of SARS-CoV-2 cases prevalent in a community at any given time Secondly, there is increasing use of rapid antigen detection tests in clinical settings. Results of these tests may not be reported to surveillance networks. Consequently, laboratory-diagnosis is increasingly less representative of the burden of disease.

In November 2020, a network of testing laboratories, which became known as the South African Collaborative COVID-19 Environmental Surveillance System (SACCESS) network, was established in order to support the development of a common testing methodology, identify and address challenges, and share best practices related to qualitative, quantitative and RNA sequencing of SARS-CoV-2 in waste water. Treatment of wastewater in South Africa is the responsibility of local government. Approximately 1050 waste water treatment works (WWTPs) are administered by metropolitan councils and local

government, and treat industrial and domestic waste. SACCESS partners and the NICD have engaged with local government to support sample collection, interpretation and utilisation of the results for public health purposes.
The SACCESS network aims to detect and quantify SARS-CoV-2 in wastewater in urban settings in South Africa, to compare trends temporal and geographic distribution of in wastewater levels with trends in clinical epidemiology so as to support the use of wastewater based epidemiology for COVID-19 outbreak prevention and response activities.

METHODS

Outbreak context and clinical case epidemiology
Since the first case of SARS-CoV-2 in South Africa was detected on 3 March 2020, laboratories in the country have conducted over 13 million RT-PCR and antigen tests. Three distinct waves of SARS-CoV-2 infection occurred, with the first two peaking in June and December 2020 respectively, whilst the third is still unfolding as of 2 September 2021. The current de-duplicated and geospatially allocated national line list of laboratory-confirmed cases of SARS-CoV-2 (identified by RT-PCR or antigen test) is provided by the NICD for comparison with results from SARS-CoV-2 testing of wastewater.

Establishment of the laboratory testing network
Commencing in 2018, the NICD had been conducting testing of wastewater for poliovirus as part of the National Department of Health's polio surveillance programme. In 2020, the NICD commenced testing of influent wastewater samples from these 18 sites, including eight in Gauteng Province, two in the City of Cape Town (Western Cape Province), two in Mangaung (Free State Province), two in eThekwini (KwaZulu-Natal Province) and four in Eastern Cape Province (two in Buffalo City Metro and two in Nelson Mandela Metro). Quantitative testing results for these sites are available from week 8 of 2021, onwards.

SARS-CoV-2 detection, quantitation methodology and interpretation of results
At identified wastewater treatment facilities, one litre grab samples of influent are collected and transported at <5°C to the testing facility. Samples are concentrated using Centricon® Plus-70 centrifuge. RNA is extracted using the QIAamp® viral RNA mini kit. SARS-CoV-2 RNA is detected using the Allplex™ 2019-nCoV Assay. A positive PCR test result is defined as detection of any SARS-CoV-2 gene target (amongst the N, E or RdRP genes). A negative PCR test is defined as a positive internal control without a positive N, E or RdRP gene target. An invalid test result is defined as failure to detect the N, E or RdRP genes along with a negative internal control. Quantitative PCR results in genome copies/ml were log transformed when graphed. All RT-PCR detection methodologies use in-built positive and negative controls to eliminate processing errors or contamination. Quantitative testing (in copies/ml of wastewater) is conducted by the NICD using a fourplex RT-qPCR assay. The Allplex 2019-nCoV assay (Seegene, catalogue number RP10243X) includes proprietary primers and probes that amplify the E, N and RdRP genes. The assay also amplifies an internal control that helps monitor for PCR inhibition. Standard curves, from which SARS-CoV-2 copy numbers are calculated, are constructed using the EDX SARS-CoV-2 Standard (Exact Diagnostic, catalogue number COV019) consisting of synthetic RNA transcripts containing the E, N and RdRP genes. Table 1 provides interpretive principles to support public health preparedness and response activities.

Table 1. Principles of SARS-CoV-2 detection and quantification on influent samples from wastewater treatment plants and interpretive principles to guide application of test results to support COVID-19 public health responses, South Africa.

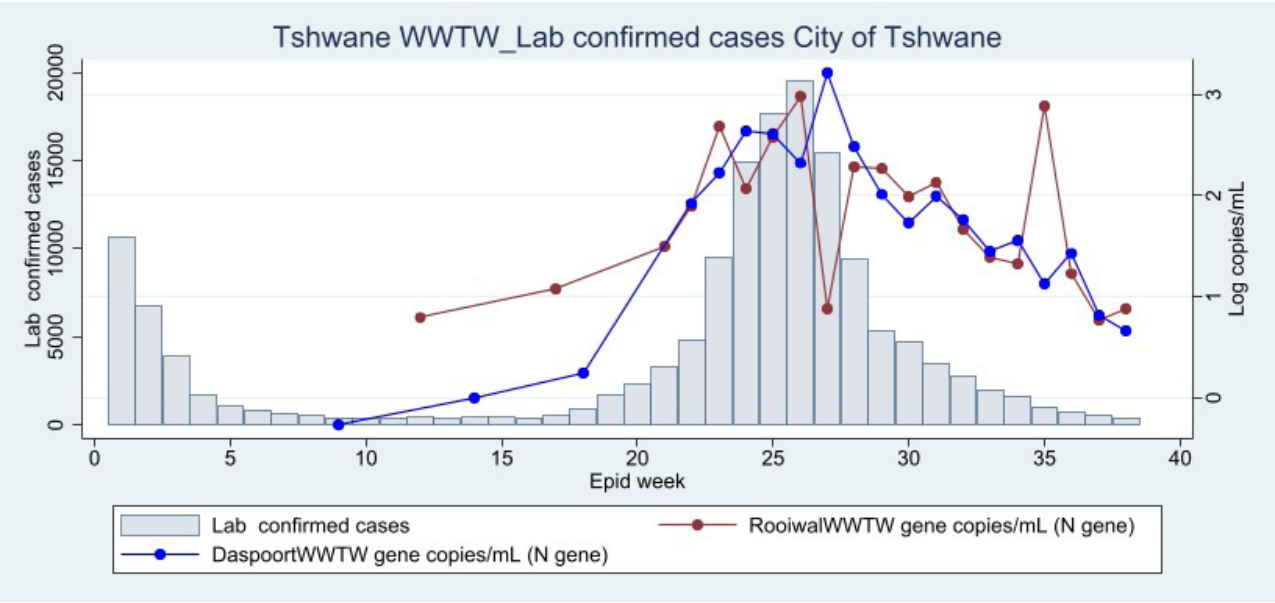
Testing modality	Test modalities	Interpretive principles to support public health responses
Detection of SARS-CoV-2	Concentration of viruses from influent wastewater samples followed by RT-PCR* testing using commercial kits with primers specific for SARS-CoV-2 virus. Interpretive criteria for PCR results are specific to the test kit used for detection. Ct values are recorded for each of the genes detected by the PCR.	When a test result changes from • positive to negative, this signifies fewer/ no cases in population • negative to positive, this indicates the need for increased population awareness and action Changes in Ct values with time may indicate changing concentrations of virus in the influent (low Ct value equates to high viral load)
Quantification of SARS-CoV-2	Concentration and RT-PCR as above, with comparison to a standard curve drawn from RT-PCR with a known concentration of plasmid containing one/more genes of SARS-CoV-2. The PCR Ct value results are compared to a standard curve to determine quantity of SARS-CoV-2 in the influent sample.	The concentration of SARS-CoV-2 at a particular facility may be used to infer the burden of SARS-CoV-2 in the population served by the wastewater treatment facility. Trends in the rate of change of concentration give an indication of whether the burden of disease is increasing or decreasing

*RT-PCR=reverse transcriptase polymerase chain reaction; Ct=cycle threshold

RESULTS

Gauteng Province

A



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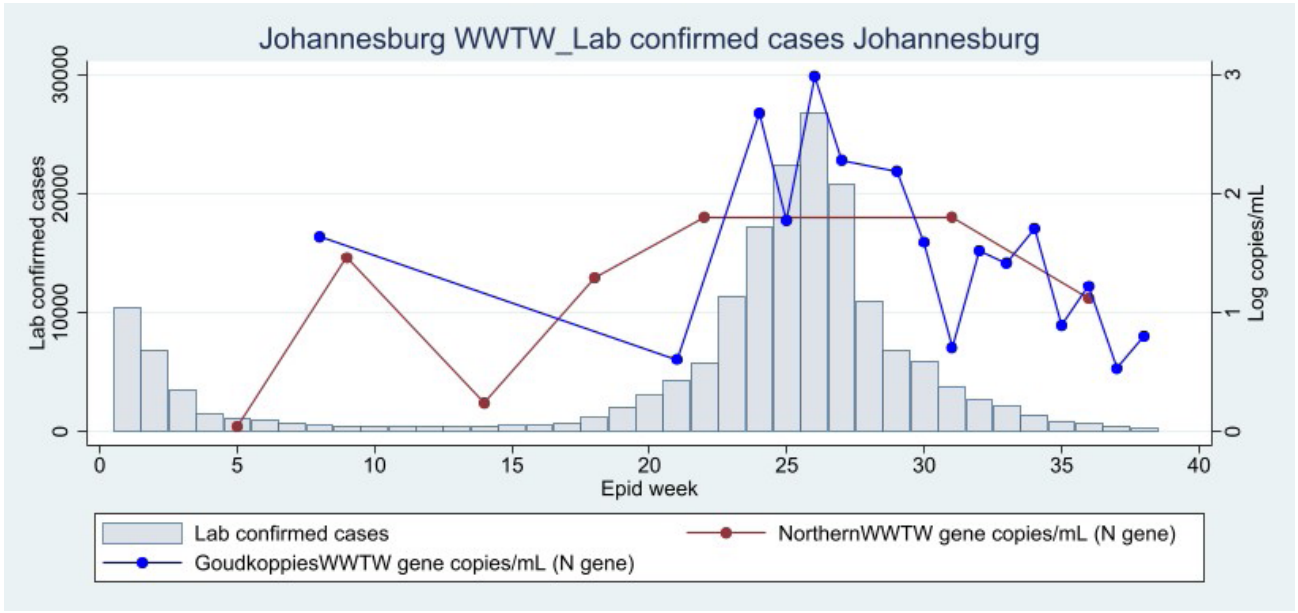

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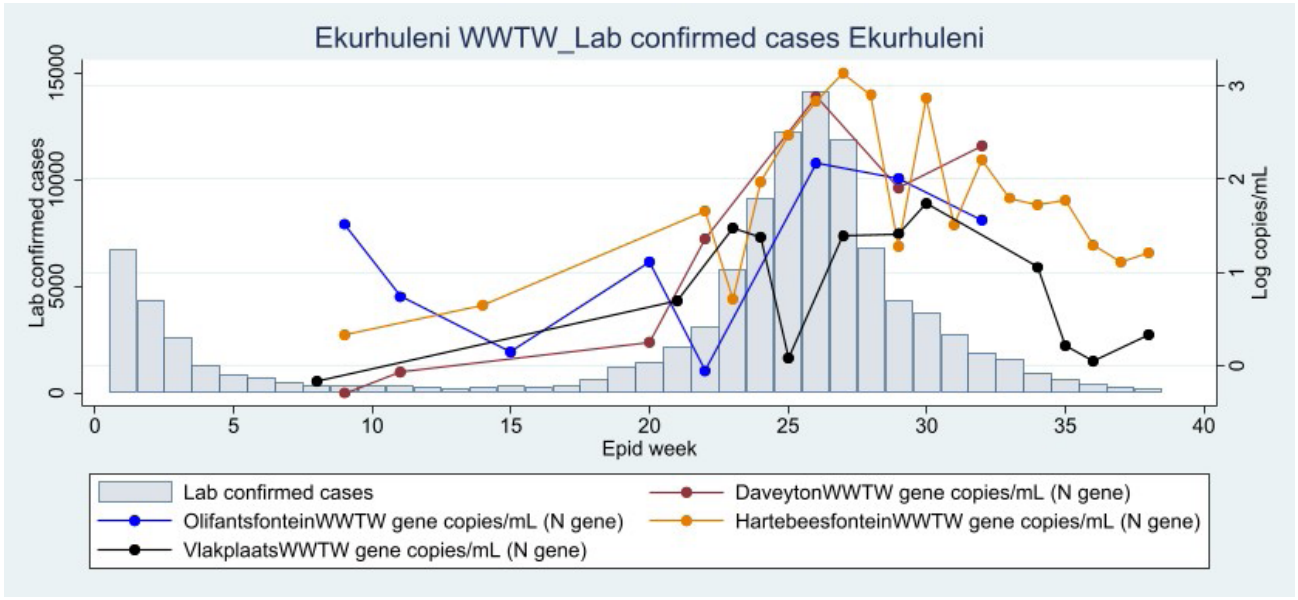


Figure 1. A-C Laboratory confirmed cases of SARS-CoV-2 (bars) and levels of SARS-CoV-2 in log copies/ml of wastewater (coloured lines) for selected metropolitan areas in Gauteng Province during epidemiological weeks 1-38, 2021.

In Gauteng province (Figure 1A-C), quantitative testing commenced in epidemiological week 8 in City of Tshwane (two treatment plants Figure 1A), epidemiological week 5 in City of Johannesburg (two treatment plants, Figure 1B), epidemiological week 8 in Ekurhuleni Metro (four treatment plants, Figure 1C). In all metros and all treatment plants, the peak of SARS-CoV-2 in wastewater levels has corresponded with the peak in clinical cases at week 25. SARS-CoV-2 levels in all plants are decreasing. However levels in wastewater from two WWTF in Ekuruhleni have increased from low in week 36. Health authorities should strengthen surveillance in these areas, namely south eastern Johannesburg including Vosloorus, Katlehong and Thokoza (Vlakplaats WWTF) and north eastern Ekurhuleni including Tembisa (Hartebeesfontein WWTF). This may suggest increasing transmission of SARS-CoV-2 in this catchment area.

KwaZulu-Natal Province

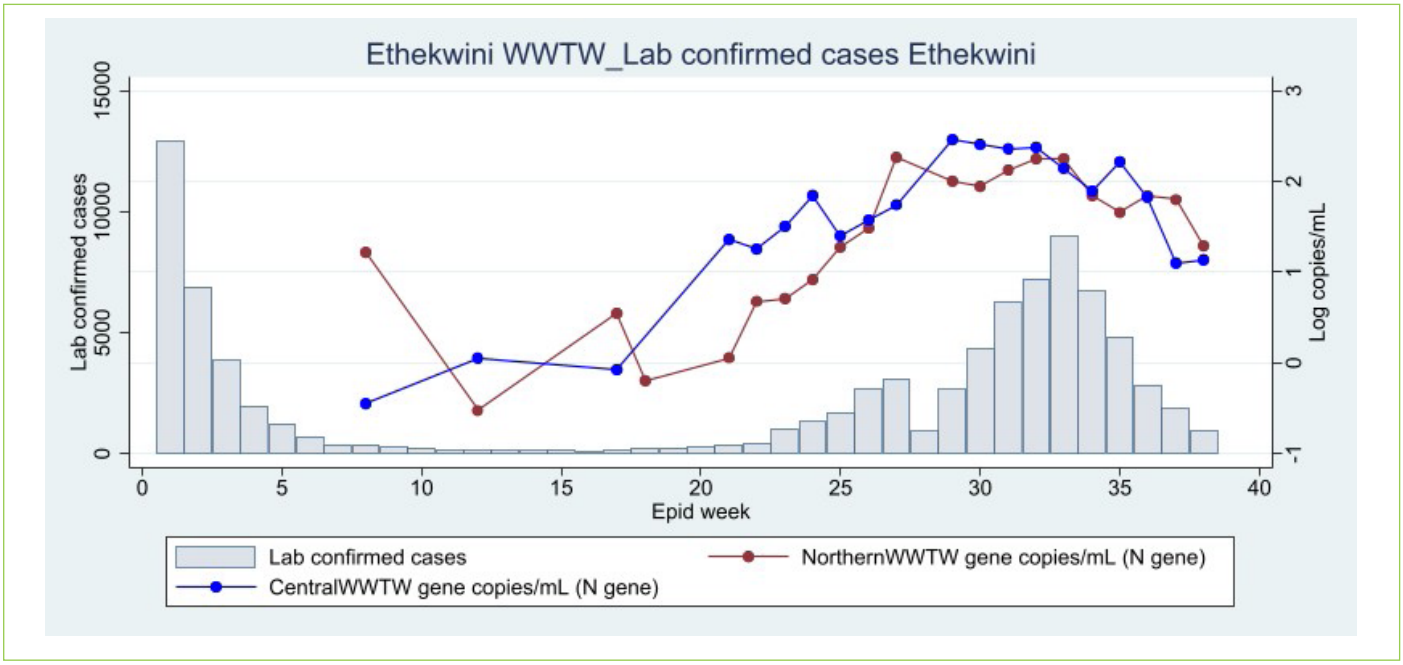


Figure 2. Laboratory confirmed cases of SARS-CoV-2 (bars) and levels of SARS-CoV-2 in log copies/ml of wastewater (coloured lines) in Ethekwini, KwaZulu-Natal Province during epidemiological weeks 1-38, 2021.

In eThekwini, quantitative testing by the NICD commenced in week 8, 2021 at two wastewater plants at the tail end of the second wave (Figure 3). Levels of SARS-CoV-2 reached a low between weeks 12-18, and rose steadily in parallel with clinical cases until week 29. From week 29 until week 33, levels remained more-or-less constant whilst clinical cases continued to increase. Week 34 -38 have shown a decrease in wastewater levels which may reflect a decreased clinical burden of SARS-CoV-2 in the catchment areas of these wastewater treatment plants.

Free State Province

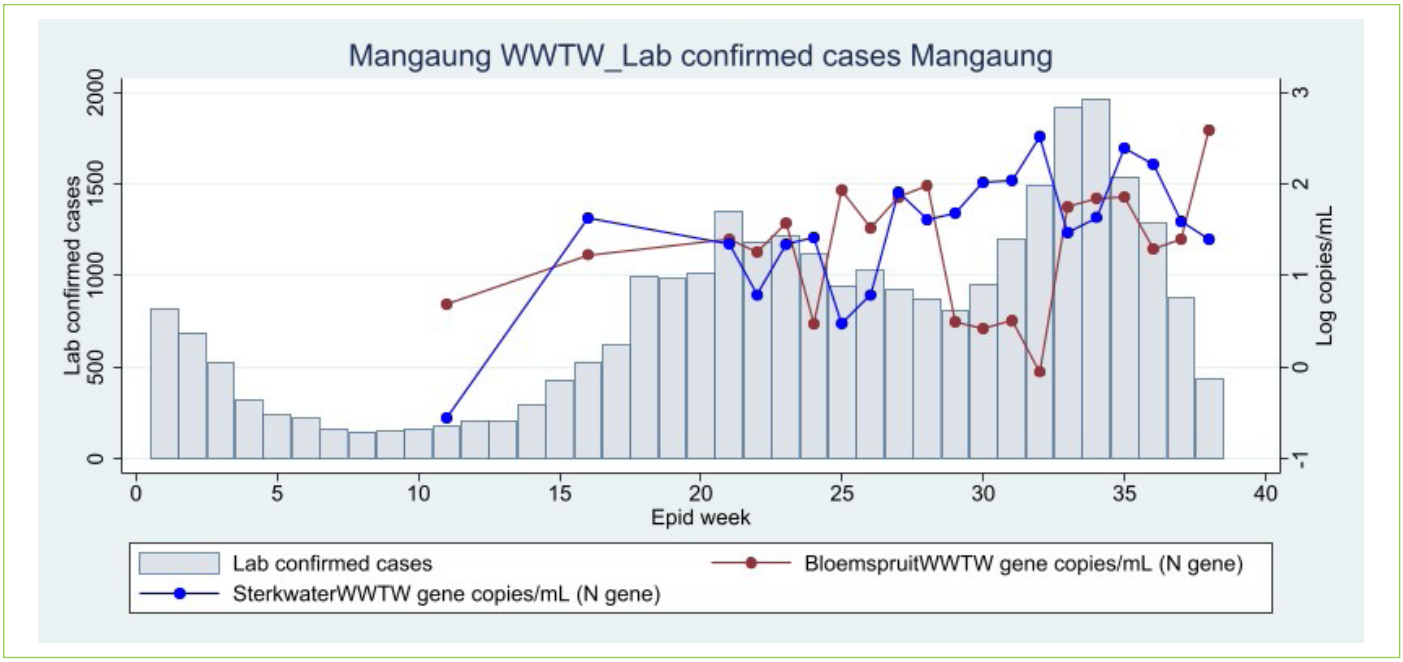
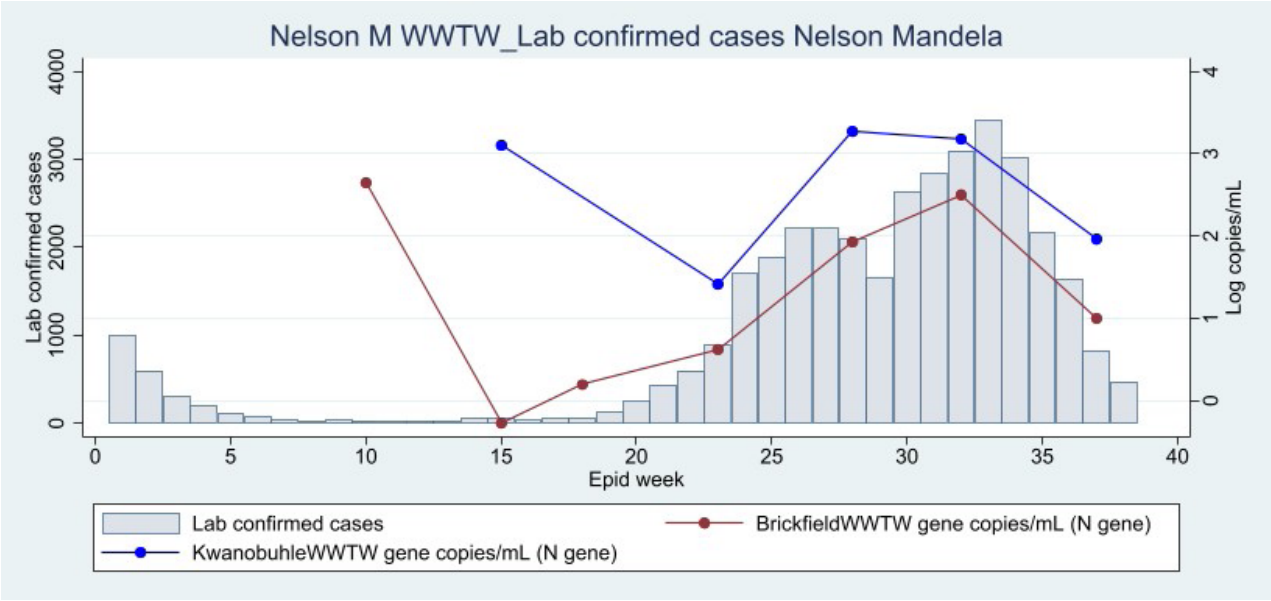


Figure 3. Laboratory confirmed cases of SARS-CoV-2 (bars) and levels of SARS-CoV-2 in log copies/ml of wastewater (coloured lines) in Mangaung, Free State Province during epidemiological weeks 1-38, 2021.

In Mangaung, quantitative testing by the NICD commenced in week 11, 2021 at two wastewater treatment plants. SARS-CoV-2 levels rose from week 11 and then plateaued from week 15 until week 23 (Figure 3). A transient decrease from weeks 23-25 at both plants paralleled a moderate decrease in clinical cases. An increase in wastewater levels from week 25 (Sterkwater) and week 33 (Bloemspruit) cases was observed, and has reflected an increase in clinical cases from week 29. •In south-eastern Mangaung (Bloemspruit WWTF), SARS-CoV-2 levels show an increase from from week 33. Health authorities should strengthen surveillance in this area. .Presently levels are stable and/or decreasing in central Mangaung (Sterkspruit WWTF).

Eastern Cape Province

A



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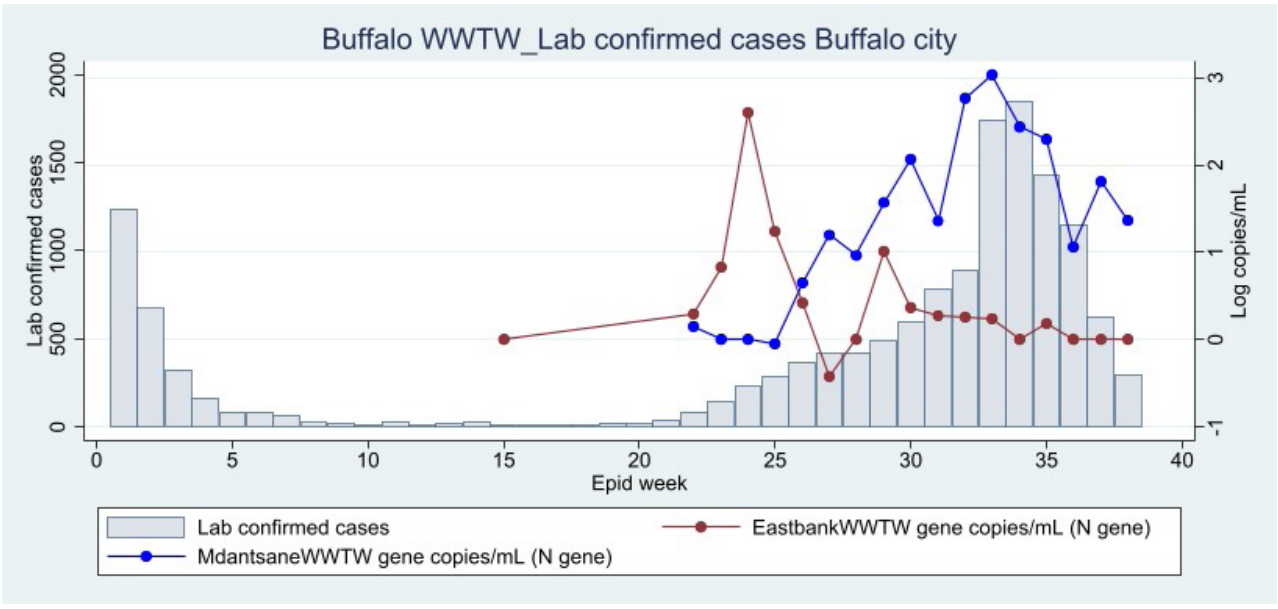

Figure 4. Laboratory confirmed cases of SARS-CoV-2 (bars) and levels of SARS-CoV-2 in log copies/ml of wastewater (coloured lines) in Nelson Mandela Metro (A) and Buffalo City Metro (B), Eastern Cape Province during epidemiological weeks 1-38, 2021.

In the Eastern Cape Province, the NICD commenced quantitative testing in week 10 (Nelson Mandela Metro) and week 15 (Buffalo City) (Figure 4, A-B). In Nelson Mandela Metro, SARS-CoV-2 levels in wastewater have steadily increased since week 23, and have paralleled the increase in clinical cases. A marked decrease in levels in week 38 has paralleled the reduction in clinical cases. In Buffalo City Metro, SARS-CoV-2 levels increased transiently in a single treatment plant (East Bank) and have continued at low levels, whilst at a second plant (Mdantsane), levels show a slow decline in parallel with clinical cases.

Western Cape Province

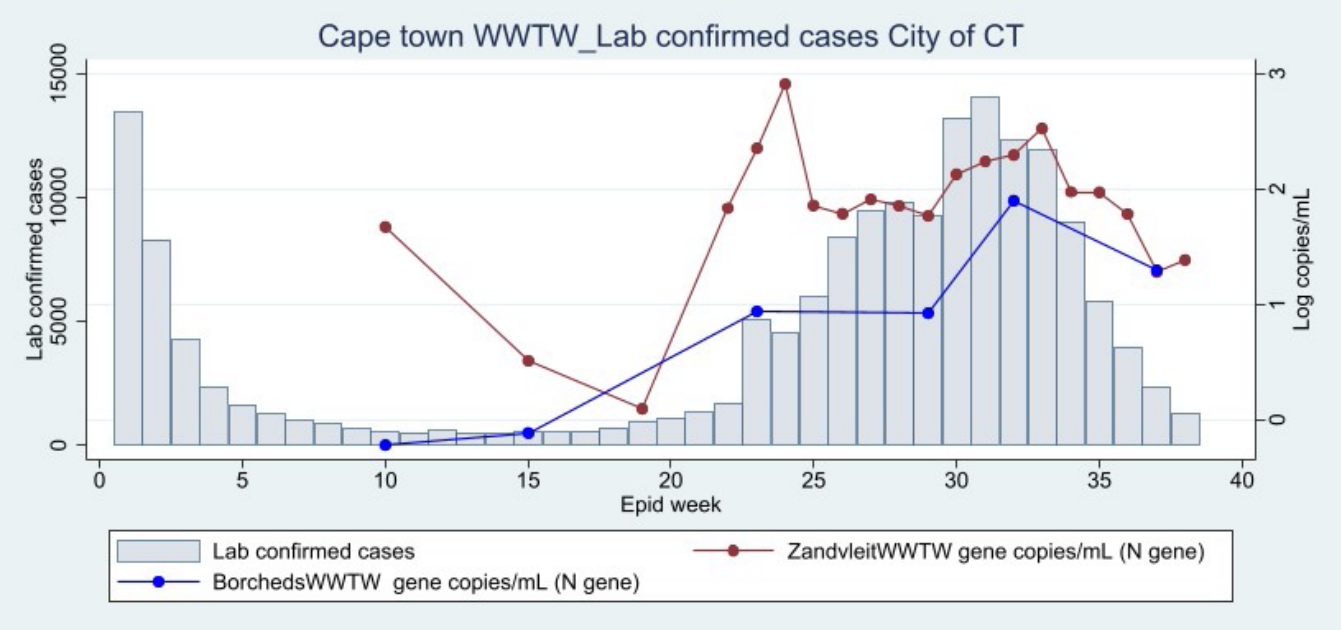

Figure 5. Laboratory confirmed cases of SARS-CoV-2 (bars) and levels of SARS-CoV-2 in log copies/ml of wastewater (coloured lines) in City of Cape Town, Western Cape Province during epidemiological weeks 1-38, 2021.

In the Western Cape Province, the NICD commenced quantitative testing in week 10, 2021. SARS-CoV-2 levels at a single facility (Borcherds quarry) continue to rise and in parallel with the increase in clinical cases until week 33 but presently are at lower levels. At a second facility (Zandvliet), levels reached a nadir at week 19, and havedecreased since then. These results should be interpreted with reference to SARS-CoV-2 epidemiology in areas draining into these treatment plants. The MRC website provides data from additional wastewater treatment plants in City of Cape Town and other Western Cape districts (https://www.samrc.ac.za/wbe/).

LIMITATIONS

It is not possible to estimate population burden of disease using wastewater testing of SARS-CoV-2 as sources of variability are multiple, including variation in length and concentration of SARS-CoV-2 excretion by infected persons, variation in degradation rate of viral RNA in wastewater and sampling error. Interpretation of results from quantitative testing of SARS-CoV-2 in wastewater is enhanced when the population served by the wastewater treatment plants are well characterised in terms of SARS-CoV-2 testing rates, health seeking behaviour, hospital admissions and deaths due to SARS-CoV-2, as well as other general indicators of health. Further exploration of the relationship between quantitative SARS-CoV-2 results, local trends in clinical case burden, environmental factors and test methodology will support interpretation of observed fluctuations in RNA levels.

CONCLUSION

SARS-CoV-2 data from wastewater at South African sentinel sites show concordance with clinical epidemiologic curves in the respective locations, illustrating the potential of the SACCESS network to provide descriptive epidemiological data pertaining to geographic variation and burden of SARS-CoV-2.

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