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

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

WEEK 40

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 up until the week ending 8th October 2021 (epidemiological week 40)

HIGHLIGHTS

- In Gauteng, levels of SARS-CoV-2 in wastewater treatment plants from City of Tshwane show a general downward trend (with a transient increase in week 39) correlating with the ongoing downward curve of the third wave. **Levels of SARS-CoV-2 in northern Ekurhuleni (Tembisa, Hartebeesfontein plant) and northern City of Johannesburg (Northern plant) are showing increases and health authorities should strengthen surveillance for cases in these areas.**
- In eThekwini, KwaZulu-Natal Province, levels of SARS-CoV-2 in wastewater continue to drop, despite a transient increase in week 39 in Mangaung, SARS-CoV-2 levels showed an increase in south-eastern Mangaung (Bloemspruit wastewater treatment facility [WWTF]) from week 33 until week 38. However, from week 38 till week 40, the levels at both plants in Mangaung continue to drop with a corresponding decrease in clinical cases.
- In the Eastern Cape Province, Nelson Mandela Metro, SARS-CoV-2 levels in wastewater have steadily declined since week 32, and have paralleled the decrease in clinical cases. In Buffalo City Metro, SARS-CoV-2 levels were low and stable in eastern coastal region (East Bank WWTF) from week 29 until week 40 when a sudden increase was observed. **Health authorities should strengthen surveillance for cases in this area.**
- In the Western Cape Province, at two facilities in the City of Cape Town (Borcherd's Quarry and Zandvleit WWTFs), SARS-CoV-2 levels appear to be steadily 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- OCTOBER 2021

CO-FUNDED BY THE WATER RESEARCH COMMISSION AND THE NICD

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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 wastewater. Treatment of wastewater in South Africa is the responsibility of local government. Approximately 1050 wastewater 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 just under 18 million RT-PCR and antigen tests. Three distinct waves of SARS-CoV-2 infection occurred, peaking in June 2020, December 2020 and July 2021, respectively. 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 <5oC 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.

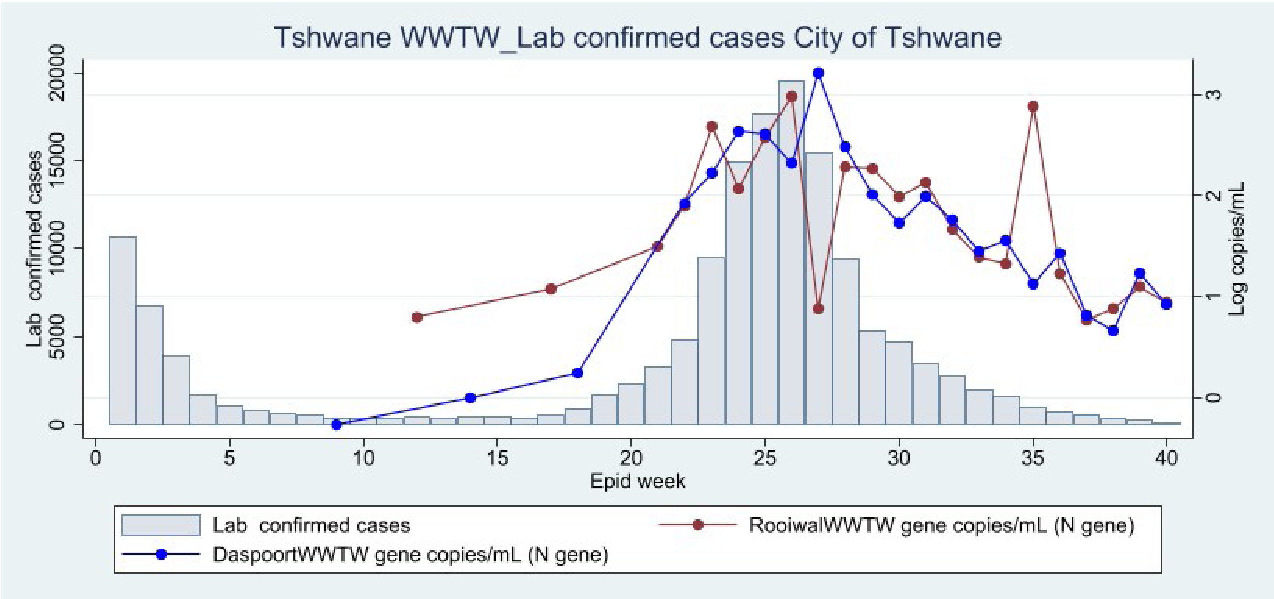
Testing modality	Test modalities	Interpretive principles to support public health responses
Detection of SARS-CoV-2	Concentration of viruses from influent wastewater samples followed by RT-PCR* testing using commercial kits with primers specific for SARS-CoV-2 virus. Interpretive criteria for PCR results 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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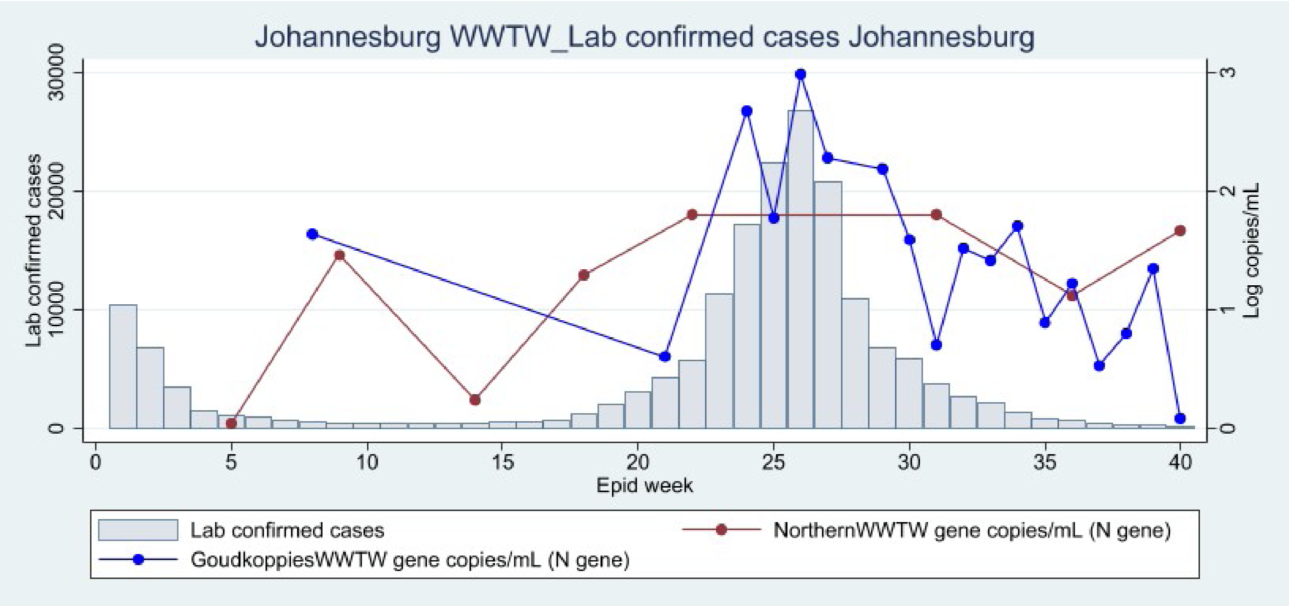

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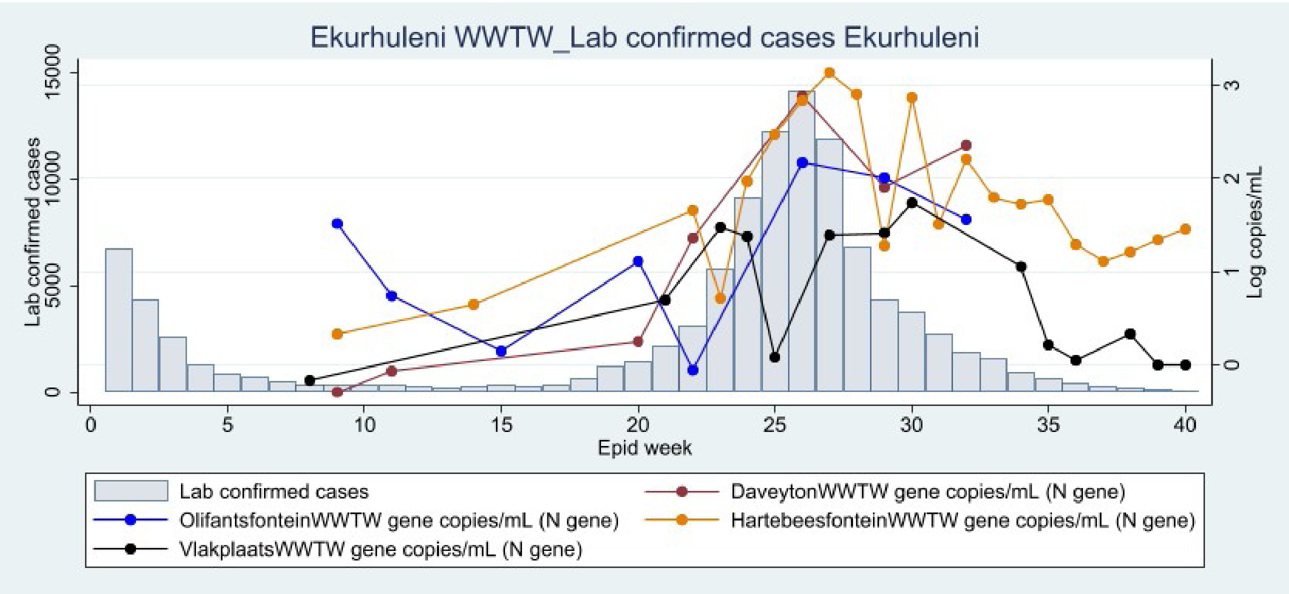


Figure 1. A-C Laboratory confirmed cases of SARS-CoV-2 (bars) and levels of SARS-CoV-2 in log copies/ml of wastewater (coloured lines) for selected metropolitan areas in Gauteng Province during epidemiological weeks 1-40, 2021.

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 26. SARS-CoV-2 levels in all plants were seen to decrease from week 32 to week 35, corresponding to a decrease in clinical cases. However, levels of SARS-CoV-2 in wastewater from one WWTFs in Ekurhuleni and one WWTF in Johannesburg have increased from week 36. Health authorities should strengthen surveillance in Johannesburg (Northern WWTF) and the north eastern Ekurhuleni including Tembisa (Hartebeesfontein WWTF). This may suggest increasing transmission of SARS-CoV-2 in this catchment area.

KwaZulu-Natal Province

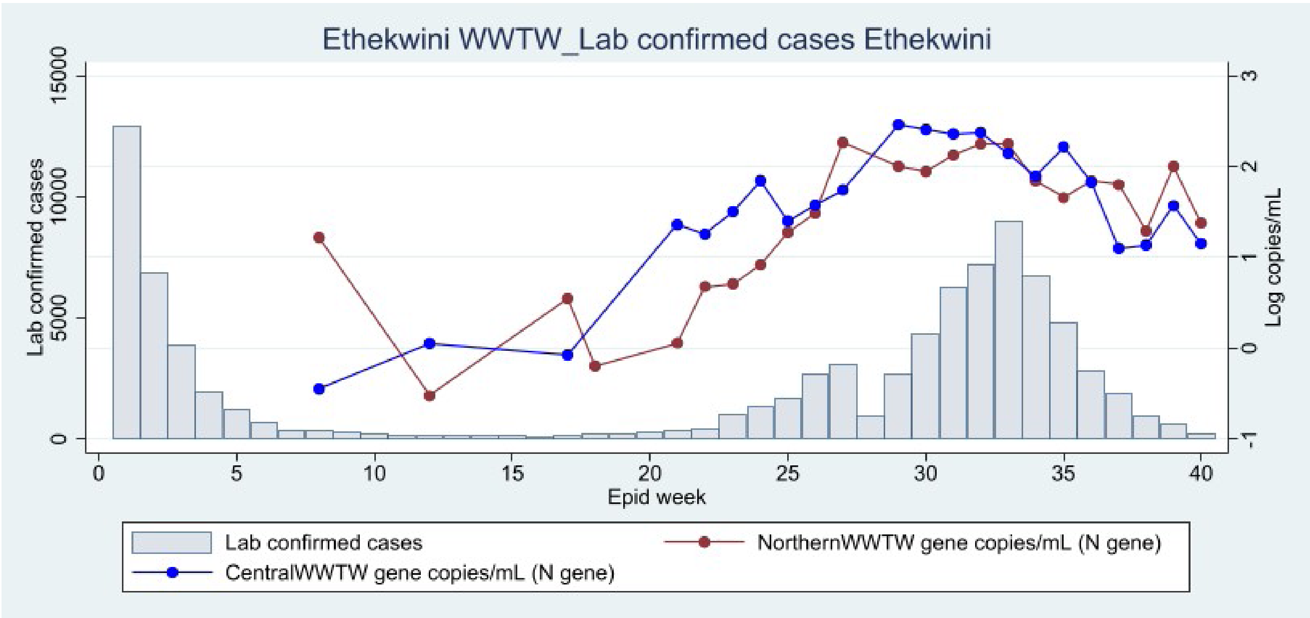


Figure 2. Laboratory confirmed cases of SARS-CoV-2 (bars) and levels of SARS-CoV-2 in log copies/ml of wastewater (coloured lines) in Ethekwini, KwaZulu-Natal Province during epidemiological weeks 1-40, 2021.

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 2). 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 showed a steady decrease in wastewater levels, a transient increase in week 38, and a further decrease in week 40, which may reflect a decreased clinical burden of SARS-CoV-2 in the catchment areas of these wastewater treatment plants.

Free State Province

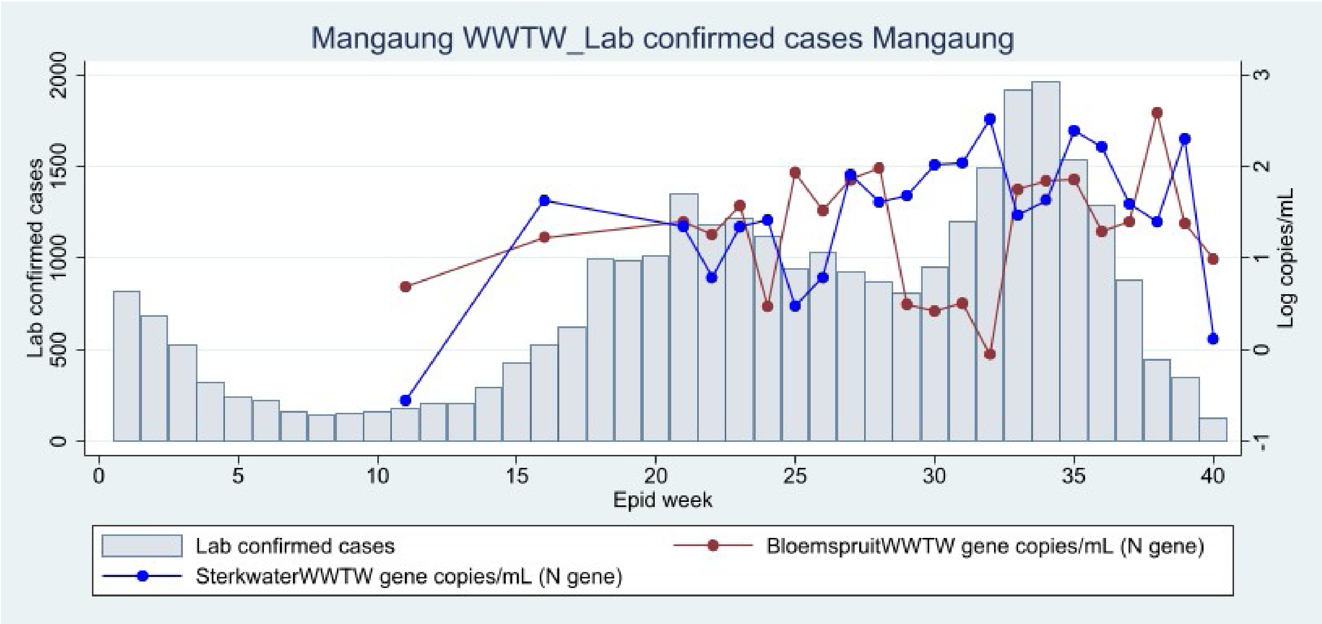
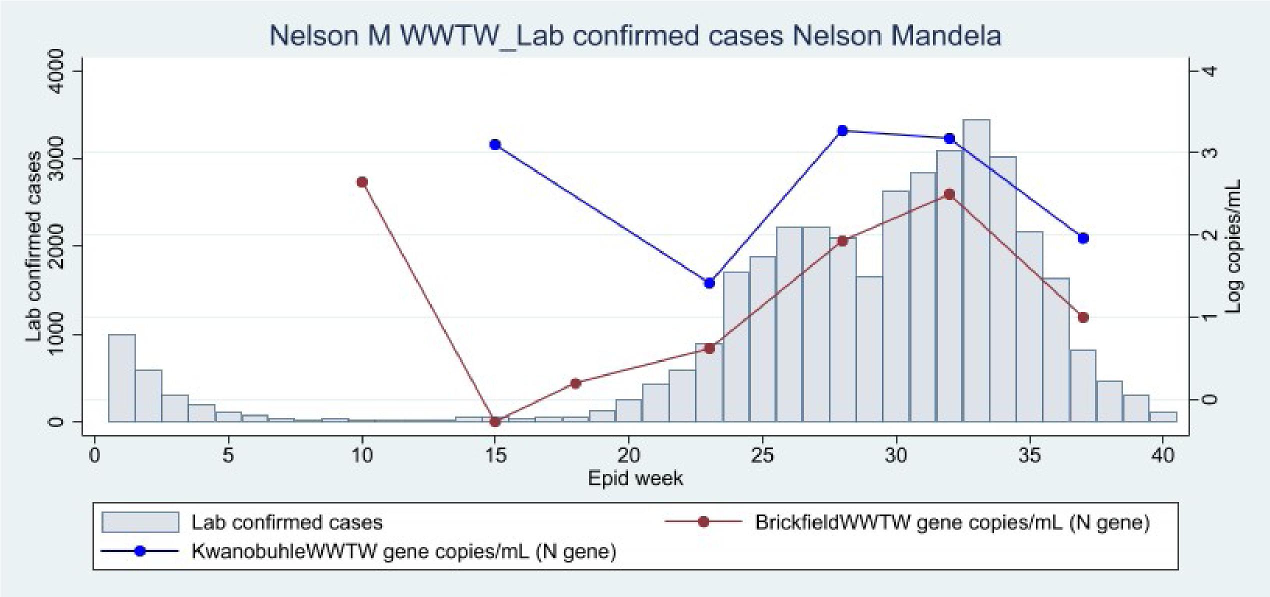


Figure 3. Laboratory confirmed cases of SARS-CoV-2 (bars) and levels of SARS-CoV-2 in log copies/ml of wastewater (coloured lines) in Mangaung, Free State Province during epidemiological weeks 1-40, 2021.

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) was observed, and has reflected an increase in clinical cases from week 29. In south-eastern Mangaung (Bloemspruit WWTF), SARS-CoV-2 levels showed an increase from week 33. However, SARS-CoV-2 levels are seen to decreasing in both WWTFs in Mangaung between weeks 38 and 40, with a corresponding decrease in clinical cases.

Eastern Cape Province

A



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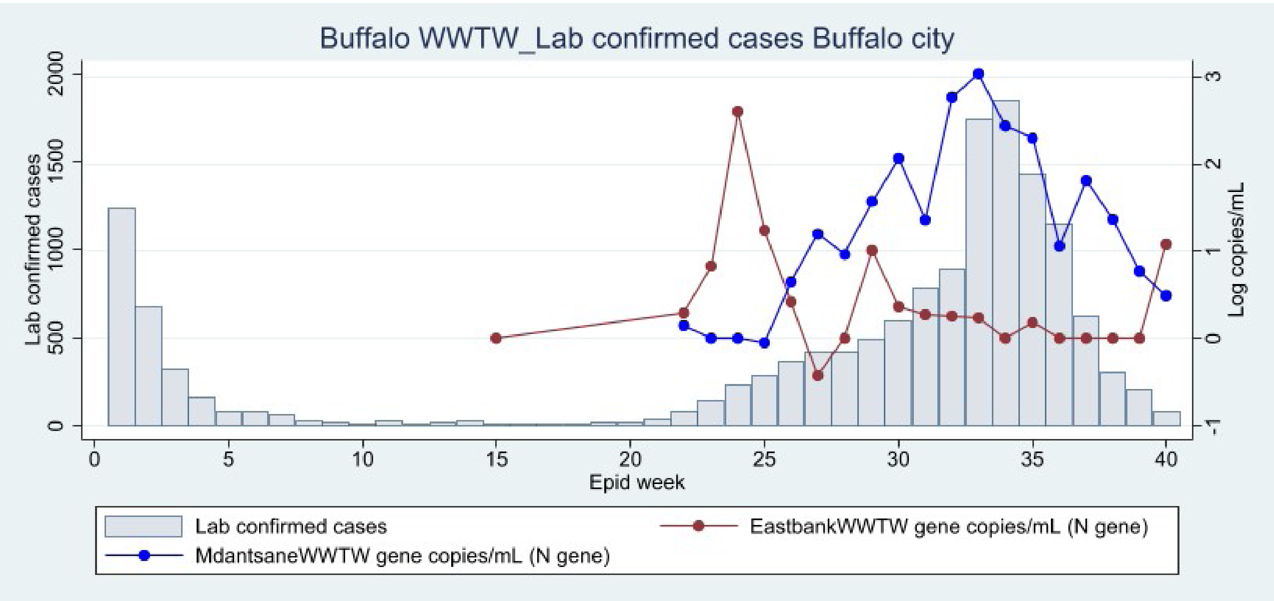

Figure 4. Laboratory confirmed cases of SARS-CoV-2 (bars) and levels of SARS-CoV-2 in log copies/ml of wastewater (coloured lines) in Nelson Mandela Metro (A) and Buffalo City Metro (B), Eastern Cape Province during epidemiological weeks 1-40, 2021.

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 steadily increased from 23 to week 33, paralleling the increase in clinical cases. However, from week 33 to 40, there has been a steady decrease in SARS-CoV-2 levels in Mdantsane WWTP. In East Bank WWTP, there has been a steady increase in SARS-CoV-2 levels from week 38 despite the decline in the number of clinical cases. Health authorities should strengthen surveillance in the area drained by Eastbank WWTP.

Western Cape Province

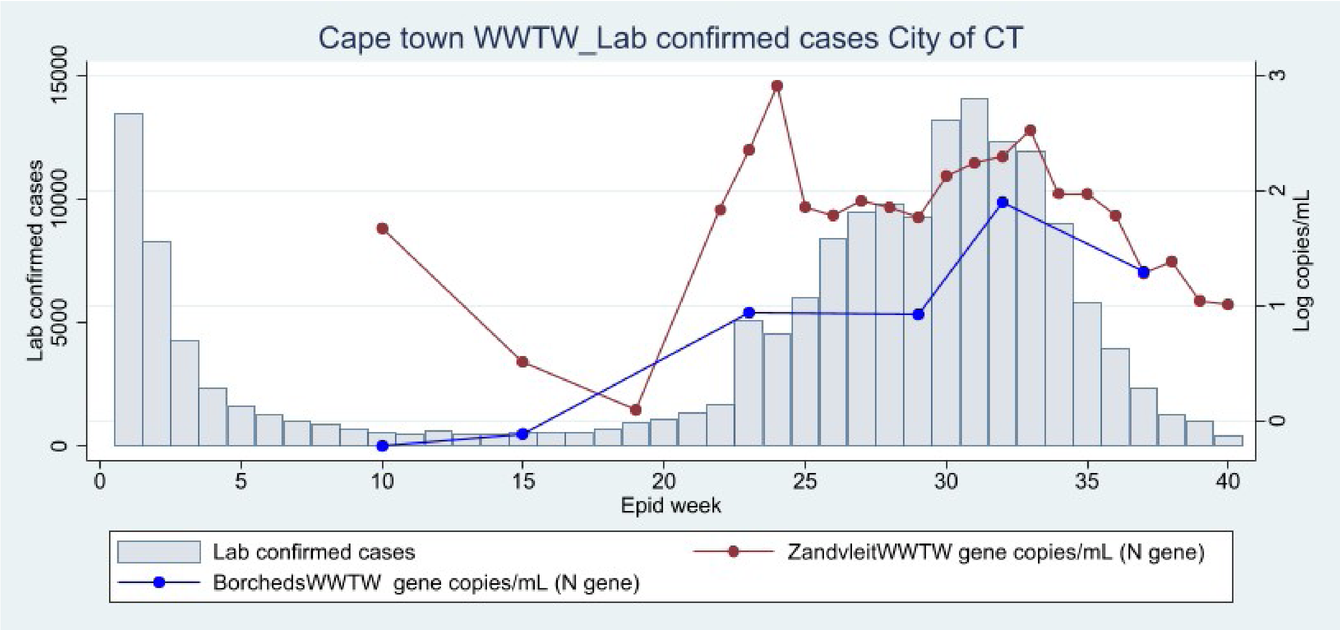

Figure 5. Laboratory confirmed cases of SARS-CoV-2 (bars) and levels of SARS-CoV-2 in log copies/ml of wastewater (coloured lines) in City of Cape Town, Western Cape Province during epidemiological weeks 1-34, 2021.

In the Western Cape Province, the NICD commenced quantitative testing in week 10, 2021. SARS-CoV-2 levels at a single facility (Borcherds quarry WWTF)   rose in parallel with clinical cases and decreased at week 33. In the second facility (Zandvliet WWTF), there has been a steady decline in SARS-CoV-2 levels in wastewater since week 33, corresponding to decrease in clinical cases. 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.

ACKNOWLEDGEMENTS

The contributions of local government and wastewater treatment staff to sample collection and transport is acknowledged and appreciated. The Water Research Commission is thanked for their vision and support. The NICD SARS-CoV-2 epidemiology and IT team members are thanked for sharing district and sub-district case burdens in order to generate graphs. These team members include Andronica Moipone Shonhiwa, Genevie Ntshoe, Joy Ebonwu, Lactatia Motsuku, Liliwe Shuping, Mazvita Muchengeti, Jackie Kleynhans, Gillian Hunt, Victor Odhiambo Olago, Husna Ismail, Nevashan Govender, Ann Mathews, Vivien Essel, Veerle Msimang, Tendesayi Kufa-Chakezha, Nkengafac Villyen Motaze, Natalie Mayet, Tebogo Mmaborwa Matjokotja, Mzimasi Neti, Tracy Arendse, Teresa Lamola, Itumeleng Matiea, Darren Muganhiri, Babongile Ndlovu, Khuliso Ravhuhali, Emelda Ramutshila, Salaminah Mhlanga, Akhona Mzoneli, Nimesh Naran, Trisha Whitbread, Mpho Moeti, Chidozie Iwu, Eva Mathatha, Fhatuwani Gavhi, Masingita Makamu, Matimba Makhubele, Simbulele Mdleleni, Tsumbedzo Mukange, Trevor Bell, Lincoln Darwin, Fazil McKenna, Ndivhuwo Munava, Muzammil Raza Bano, Themba Ngobeni.

The NICD Centre for Respiratory Disease and Meningitis are thanked for their assistance in setting up and troubleshooting PCR testing, and ongoing supportive collaboration.