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FOREWORD

Monitoring the progression of the COVID-19 pandemic is primarily based on the collation of SARS-CoV-2 clinical data from surveillance platforms and networks, such as the DATCOV sentinel surveillance system in South Africa. These data are provided by testing facilities/laboratories and healthcare facilities. The sampling of wastewater is emerging as a useful additional modality to monitor SARS-CoV-2 epidemiology and inform public health responses to the pandemic. The South African Collaborative COVID-19 Environmental Surveillance System (SACCESS) network of laboratories has been established to detect and quantify SARS-CoV-2 virus in wastewater samples. Results from the SACCESS network to date show that quantification of viral load from wastewater may be a helpful additional data source to support population-level monitoring of SARS-CoV-2 epidemiology.

This is the first special issue of our COVID-19 series for volume 19, and we trust this information will be of interest to epidemiologists, pathologists, laboratorians and all those involved in monitoring the COVID-19 pandemic. As the third COVID-19 wave rages in South Africa, we encourage our readers to be especially vigilant by adopting all publicized measures to reduce the risk of infection and rate of transmission.

Prof Basil Brooke - Editor

MONITORING THE THIRD WAVE: DETECTION OF SARS-CoV-2 AT SENTINEL WASTEWATER TREATMENT SITES BY THE SOUTH AFRICAN COLLABORATIVE COVID-19 ENVIRONMENTAL SURVEILLANCE SYSTEM (SACCESS) NETWORK, 2020-2021

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SUMMARY

Wastewater sampling and surveillance is emerging as a useful modality to monitor SARS-CoV-2 epidemiology and inform public health responses to the COVID-19 pandemic. The formation of the South African Collaborative COVID-19 Environmental Surveillance System (SACCESS) network of laboratories is described here, as are the results of sentinel surveillance in urban centres and metros using PCR-based detection and quantitation of SARS-CoV-2 virus in wastewater samples. These are reported alongside corresponding district or metro epidemic curves. Grab samples from influent wastewater were transported on ice to participating laboratories where concentration, RNA extraction and PCR detection with or without quantitation were performed. Since inception in March 2020, seven laboratories tested 513 specimens from 38 wastewater treatment plants across all nine of South Africa's provinces. These cover sentinel sites from where 760,731/1,973,972 (38,5%) of laboratory-confirmed COVID-19 cases in South Africa have been reported (30 June 2021). SARS-CoV-2 was detected in 350

(68%) samples. Detection of SARS-CoV-2 was more frequent during clinical 'waves'. Viral load in wastewater tended to increase during the third wave in parallel with increasing clinical cases. Detection of SARS-CoV-2 with quantification of viral load from wastewater may be a helpful additional data source to support population-level monitoring of SARS-CoV-2 epidemiology. Additional geospatial and epidemiological investigations should be done to support the use-cases of wastewater-based epidemiology for COVID-19.

BACKGROUND

The detection and monitoring of SARS-CoV-2 epidemiology through wastewater was first proposed in April 2020.[1,2] Initial reports describing the feasibility and practical usefulness of this approach emerged simultaneously from several countries during August 2020.[3,4] Recent evidence has shown that SARS-CoV-2 can be detected in wastewater prior to the appearance of clinical cases[5], and longitudinal tracking of SARS-CoV-2 viral load in wastewater correlates with the burden of clinically diagnosed cases.[6] Sequencing of SARS-CoV-2 RNA fragments in wastewater has identified variants of concern as well as mutations not detected in clinical cases.[7]

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.

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.

South Africa has been severely affected by the COVID-19 pandemic, having experienced two waves as of the beginning of July 2021, and the onset of a third wave at time of writing. The aim of this project was therefore to identify epidemiological trends of SARS-CoV-2 in South African urban wastewater treatment plants and compare PCR detection and quantitation results from influent sewage with trends in clinical epidemiology. Details of the establishment of the SACCESS network are also given.

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 6 July 2021. The de-duplicated and geospatially allocated national line list of laboratory-confirmed cases of SARS-CoV-2 (identified by RT-PCR or antigen test) were 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, the South African Medical Research Council (SAMRC), the Council for Scientific and Industrial Research, the University of Pretoria, the Institute for Water and Wastewater Technology at the Durban University of Technology, the National Institute for Occupational Health and a number of private laboratories (Waterlab, Greenhill and Lumegen Laboratories) commenced with independent projects involving qualitative and quantitative detection of SARS-CoV-2 in local WWTPs. A proof of concept was published by the SAMRC in 2021.[8]
In August 2020, laboratories doing this work collectively established the SACCESS network to support communication of challenges and sharing best practice. Through funding and partnership with the Water Research Commission (WRC), the NICD and partners identified WWTPs for sampling and SARS-CoV-2 testing to maximise coverage across metros and sentinel sites in provinces with smaller populations. A number of webinars were held to share methodologies for concentration, extraction and PCR detection of SARS-CoV-2, and a compendium of methodologies was published.[9] Testing of WWTPs commenced between June 2020 and March 2021 as funding for partners became available. Results from testing funded through the WRC and NICD are reported here.

SARS-CoV-2 detection, quantitation methodology and interpretation of results

At identified wastewater treatment facilities, one litre grab samples of influent were collected and transported at <5oC to the testing facility. Table 1 summarises testing modalities used in this study, and provides interpretive principles to support the application of wastewater detection, quantification of SARS-CoV-2 RNA to public health surveillance and response activities. A positive PCR test result was defined as detection of any SARS-CoV-2 gene target (amongst the N, E or RdRP genes). A negative PCR test was defined as a positive internal control without a positive N, E or RdRP gene target. An invalid test result was 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. Table 2 provides the concentration, extraction and PCR detection methodology for each network laboratory. 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) was 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 were calculated, were 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. 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

Table 2. Concentration, extraction and RT-PCR detection methodology used by laboratory partners, South African Collaborative COVID-19 Environmental Surveillance System (SACCESS) network.

Laboratory partner	Method for virus concentration	Method for nucleic acid extraction	RT-PCR assay
NICD	Centricon® Plus-70 centrifugal	QIAamp® viral RNA mini kit	Allplex™ 2019-nCoV Assay
CSIR	Polyethylene Glycol	Omega Bio-tek ENZA total RNA Kit II	2019-nCoV CDC EUA Kit
NIOH	Skim milk flocculation	MagMAX Viral and Pathogen Nucleic Acid Isolation Kit	Thermofisher (TaqPath kit)
Waterlab/UP	Skim milk flocculation	QIAamp® Ultrasens® Virus kit	Allplex™ 2019-nCoV Assay
SAMRC-TB	Ultra centrifugation	ZymoBIOMICS kit	2019-nCoV CDC EUA Kit
SAMRC-BRIP	Centrifugation	RNeasy PowerSoil	2019-nCoV CDC EUA Kit
Lumegen	Tangential Flow Filtration	Qiagen extraction Kit	Thermofisher (TaqPath kit)

RESULTS

Overview of wastewater and clinical SARS-CoV-2 test results

In 2020, WWTW from 19 sites across five provinces submitted samples for testing, including nine 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).

In 2021, seven additional sites in Gauteng, two in Limpopo, three in Mpumalanga, three in North West, one in the Northern Cape and three in the Western Cape provinces were added, bringing the total to 38 sites. Two sites in Gauteng were duplicated, being tested by the NICD and another laboratory.

The de-duplicated and geospatially allocated line list of laboratory-confirmed clinical cases from commencement of the South African COVID-19 pandemic (4 March 2020) until the end of week 25 (26 June 2021) yielded 1,973,972 cases. Amongst these, 760,731 (38,5%) were located in a metropolitan area or local municipality where wastewater-based surveillance for SARS-CoV-2 was being conducted at the time (15,940 in Buffalo City, 36,370 in Nelson Mandela Metro Bay, 30,133 in Mangaung Free State, 135,701 in City of Johannesburg, 110,148 in City of Tshwane, 75,934 in Ekurhuleni, 29,825 in West Rand, 81,931 in eThekwini, 6,496 in Capricorn District Municipality (DM), 7514 in Emalahleni DM, 5,166 in Mbombela DM, 4,762 in Matlosana DM, 2,931 in JBMarks Local Municipality (LM), 10,047 in Rustenburg LM, 7,533 in Sol Plaatjie LM, 200,310 in City of Cape Town Metro).

Provincial trends

During 2020 and 2021 to date, 142 and 371 wastewater samples were submitted from 18 and 38 WWTW respectively, and were tested by or reported to the NICD by partner laboratories. Of 383 samples, SARS-CoV-2 was identified in 105 in 2020 (74%) and 245 in 2021 (66%), with each site submitting an average of 13.5 samples over the two years (range 2-21). In 2020 and 2021, 24 (17%) and 45 (12%) samples had invalid results respectively. Of the samples tested in 2021, 88 of 245 (36%) positive samples had quantitative tests conducted. The SARS-CoV-2 N-gene was found in 85/88. Detection and quantitation results are shown in Figures 1-6 together with epidemiologic curves for clinical cases detected during 2020-2021 for the districts or metros where each WWTW are located.

In Gauteng province (Figure 1A-C), all samples were negative up until week 21 of 2020, (commencing 17 May 2020), when samples from two plants (Ekurhuleni and City of Tshwane) tested positive, coinciding with the onset of the first wave. All sites continued to test positive through the inter-wave period (August-December 2020) and through the second wave (ending week 7, February 2021). A number of sites in City of Tshwane, Johannesburg and Ekurhuleni tested negative during weeks 11-16 (mid-March until end April 2021). Since week 17 (mid-April) all nine WWTP across Gauteng that submitted samples have tested positive, signifying an increase in population burden of disease across the province. Quantitative results from two plants in the City of Johannesburg, a single plant in the City of Tshwane and three plants in Ekurhuleni District were first tested in week 16 (end April 2021) and show consistently increasing concentrations of SARS-CoV-2 in log RNA copies/ml of influent wastewater, consistent with observed increases in reported cases in these districts.

In the Western Cape Province (Figure 2), samples from two WWTP have consistently tested positive. However, quantitative results in wastewater have shown an increase in viral copies per millilitre in June, in parallel with the increasing numbers of clinical cases.

In the Eastern Cape Province (Figure 3), sample results are available from week 38 of 2020 (mid-September 2020), and all tested positive until week 6 and/or week 10 of 2021 (March-April 2021), coinciding with the end of the second wave. Quantitative results from plants in Buffalo city have increased but a recent sample from week 26 had a lower viral load. In Nelson Mandela Metro, samples from week 24 showed high viral load, in keeping with climbing clinical cases. A single positive sample with a high viral load was detected in week 15 and is of uncertain significance as few clinical cases were detected that week.

In Free State Province (Figure 4), testing commenced in week 31 of 2020. SARS-CoV-2 RNA has been detected at both sites continuously since then, in keeping with the high clinical case load (greater than 200 cases per week). Quantitative results from week 17 have been consistently elevated, but one plant appeared to have a decreasing viral load in weeks 26 and 27.

All samples from KwaZulu-Natal Province (Figure 5) from week 36, 2020 to date have tested positive since commencement of testing in week 36 of 2020. Viral quantification has shown consistent increases from week 19 (mid May 2021) onwards, signifying an increase in cases.

In Limpopo Province (Figure 6A), testing commenced in week 7 of 2021 (February 2021) and all samples tested negative until week 15 (mid-April) at a single plant, and week 19 in a second plant, in keeping with an increased case load. In Mpumalanga (Figure 6B), testing commenced in week 6 (February 2021). Positive results were reported consistently from week 14 (early April 2021), in keeping with an increase in local cases.

In North West Province (Figure 6C), samples from week 19 (mid May 2021) have consistently tested positive. In Kimberley in the Northern Cape Province (Figure 6D), positive samples were detected from week 13 (early April) until week 22 (early June), with the most recent sample (week 24, late June) having tested negative, possibly matching the decline in clinical cases.

DISCUSSION

SARS-CoV-2 data from wastewater at South African sentinel sites show some concordance of qualitative and quantitative results with clinical epidemiologic curves, showing the potential of the SACCESS network to provide descriptive epidemiological data pertaining to geographic variation and burden of disease.

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 (personal communication, PHIRST study, Cheryl Cohen, NICD). 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.

Results from the SACCESS network show that SARS-CoV-2 is present in wastewater during clinical waves, and during the third wave, quantitation results have reflected the current upward trend observed amongst clinical cases. Certain anomalies in these results however require careful attention. Whilst SARS-CoV-2 cases were present in all metros and areas under surveillance during the inter-wave period, continued positive results even during times of apparent low population burden may signify high sensitivity of detection mechanisms, higher than reported numbers of cases in sewage drainage areas or, less likely, false positive laboratory test results. A single high concentration of SARS-CoV-2 was detected in Nelson Mandela Metro Bay in week 15, when only 60 clinical cases were reported. We consider an error in the quantitative PCR result unlikely, therefore this result may reflect a localised transmission event which went undetected or high numbers of asymptomatic cases. In recent weeks (22-27, June to early July 2021) although the general trend of SARS-CoV-2 concentration is upward, fluctuations of quantitation results have been observed. The significance of these fluctuations is unclear.

These data help to retrospectively confirm the presence of SARS-CoV-2 in wastewater and hence in South African metros, and they challenge network partners to exploit and build on the opportunity provided by SACCESS to supply meaningful and timely information to public health decision makers at provincial and national levels, as has been done elsewhere.[6] The data include temporal and geographical changes in qualitative and quantitative detection patterns within metros by determining population sizes and caseloads within sewage drainage areas, mapping of sewage reticulation networks by metropolitan officials with nodal sampling, and testing of wastewater to identify localised areas of high SARS-CoV-2 prevalence. These data may also guide the implementation of public health measures to curb transmission and contain the economic and social impact of SARS-CoV-2.

Calculating population burdens of disease from quantitative PCR results requires information on the duration and quantity of SARS-CoV-2 shedding from infected persons as well as the rate of decay of RNA in wastewater.[10] With frequent sampling, it is possible to determine the effective reproductive rate of SARS-CoV-2 using wastewater based epidemiology.[11] Genomic sequencing of SARS-CoV-2 viral fragments in wastewater also has the potential to monitor population trends in the circulation of variants.[7] Trends in quantitation of viral RNA may also allow inferences to be made regarding the success of vaccine roll-out. Lastly, the SACCESS network may prove useful in providing surveillance data for other waterborne or excreted communicable diseases including agents of gastroenteritis, hepatitis and other respiratory illness such as influenza,[12] and for the monitoring of antimicrobial resistance.[13]

Limitations of wastewater-based epidemiology include restriction of the surveillance methodology to urban populations with functional sewage reticulation networks, inability to measure disease amongst non-sewered populations (e.g. in informal settlements or rural areas) and reliance on repeated measurements from the same plant with identical methodology to demonstrate temporal trends. 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. The use-case scenarios of SARS-CoV-2 wastewater-based epidemiology to support public health interventions at local, provincial and national level in South Africa need to be established.

In conclusion, wastewater surveillance at sentinel sites across South African urban areas indicates a clear correspondence with SARS-CoV-2 epidemiological trends at some sites. Increased numbers of clinical cases in Gauteng, KwaZulu-Natal, Free State and Northern Cape provinces support the further exploration of the use of these data to monitor SARS-CoV-2 epidemiology using wastewater-based testing.

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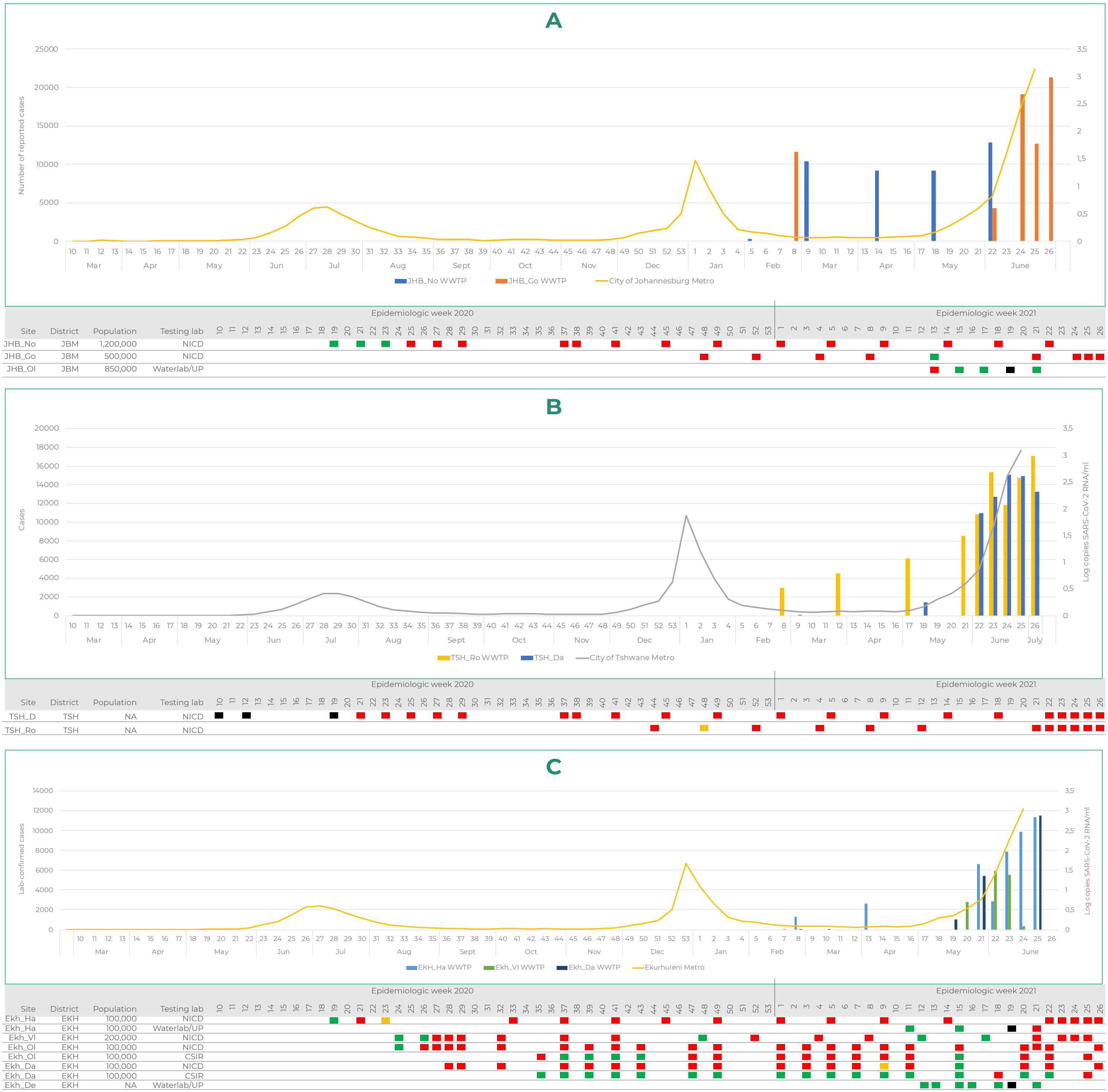


Figure 1. Epidemiological curves of laboratory-confirmed SARS-CoV-2 cases, SARS-CoV-2 viral loads in wastewater and SARS-CoV-2 PCR detection results in wastewater by district, Gauteng Province, South Africa, 2020-2021. Districts shown in include City of Johannesburg (A), City of Tshwane (B) and Ekurhuleni Metropolitan area (C). Figure: The curve represents laboratory-confirmed COVID-19 cases (left axis) by epidemiological week 2020-2021 (horizontal axis). The bars represent quantitative viral loads in copies per millilitre (right axis) in specimens collected in given epidemiological week 2020-2021 (horizontal axis). Table: Green squares represent negative PCR results, red squares represent positive results, yellow squares represent presumptive positive results and black squares represent invalid results. JHB=City of Johannesburg. TSH=City of Tshwane. EKH=Ekhuruleni. WWTP=wastewater treatment plant. *Population is the approximate size of the population served by the wastewater treatment plant. Lab is the name of the testing laboratory.

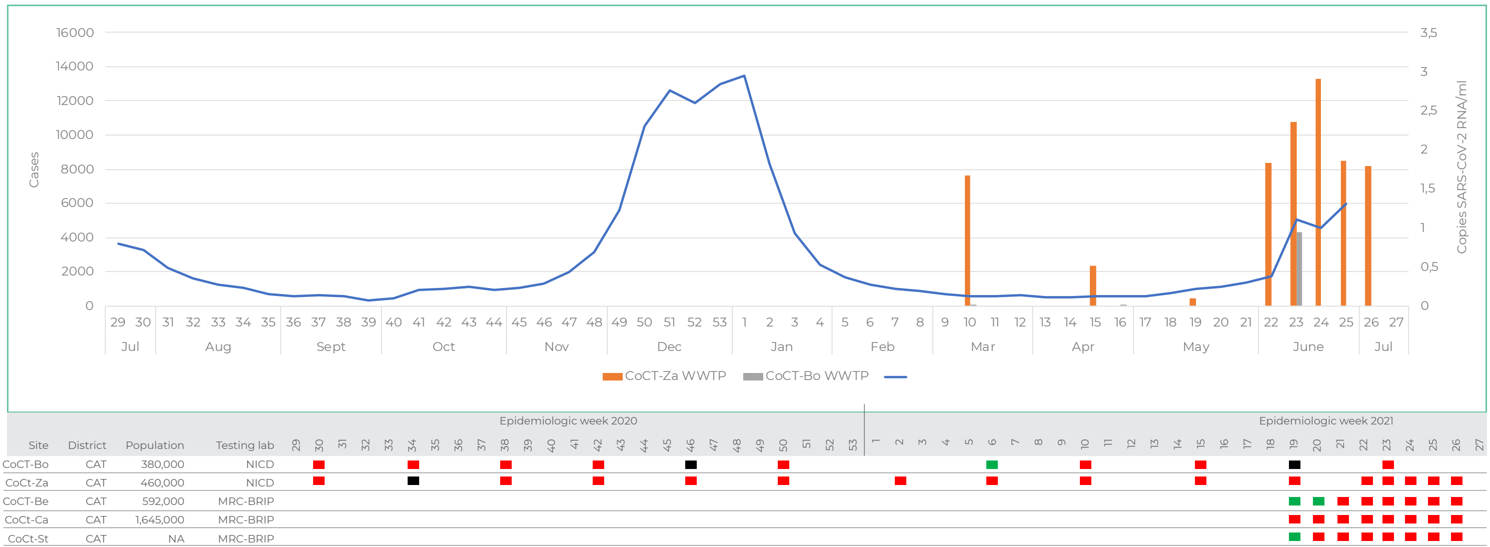


Figure 2. Epidemiological curves of laboratory-confirmed SARS-CoV-2 cases, SARS-CoV-2 viral loads in wastewater and SARS-CoV-2 PCR detection results in wastewater for the City of Cape Town, Western Cape Province, South Africa, 2020-2021. Figure: The curve represents laboratory-confirmed COVID-19 cases (left axis) by epidemiological week 2020-2021 (horizontal axis). The bars represent quantitative viral loads in copies per millilitre (right axis) in specimens collected in given epidemiological week 2020-2021 (horizontal axis). Table: Green squares represent negative PCR results, red squares represent positive results and black squares represent invalid results. CoCT=City of Cape Town. WWTP=wastewater treatment plant.*Population is the approximate size of the population served by the wastewater treatment plant. *Lab is the name of the testing laboratory.

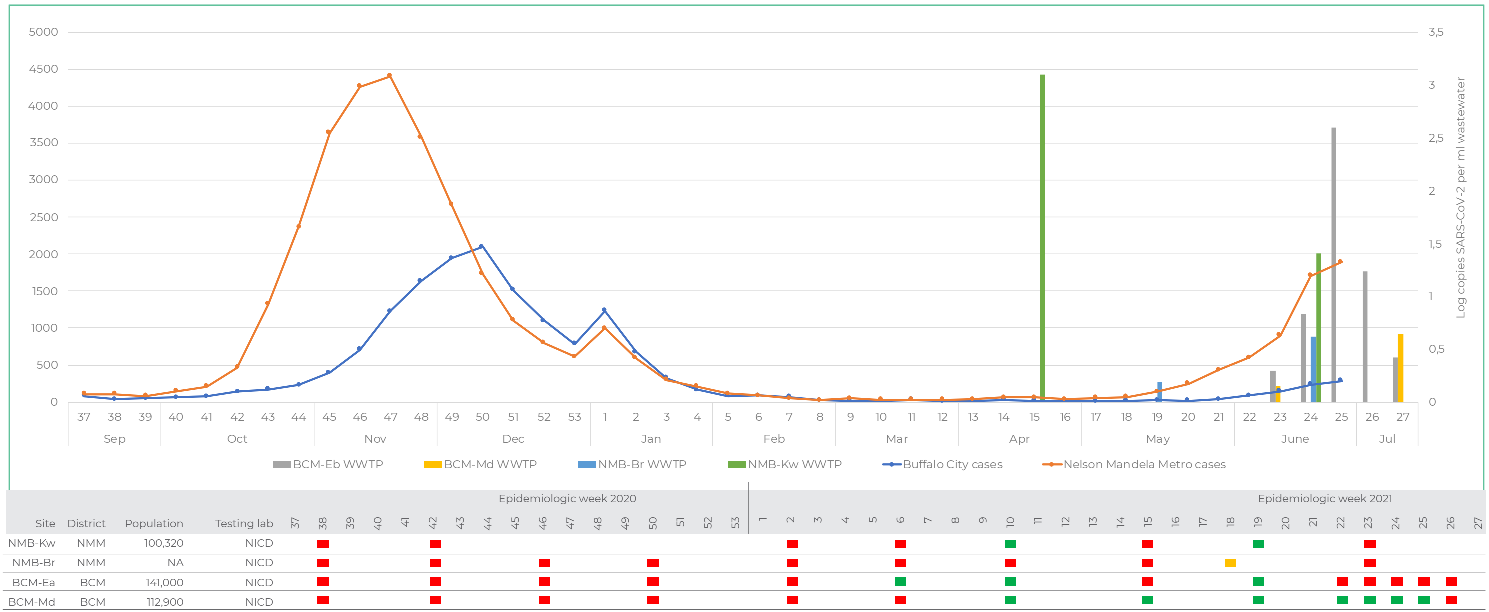


Figure 3. Epidemiological curves of laboratory-confirmed SARS-CoV-2 cases, SARS-CoV-2 viral loads in wastewater and SARS-CoV-2 PCR detection results in wastewater for Nelson Mandela Metropolitan area (NMMB), and Buffalo City Metropolitan area (BCM) in the Eastern Cape Province, South Africa, 2020-2021. Figure: The curves represents laboratory-confirmed COVID-19 cases (left axis) by epidemiological week 2020-2021 (horizontal axis). The bars represent quantitative viral loads in copies per millilitre (right axis) in specimens collected in given epidemiological week 2020-2021 (horizontal axis). Table: Green squares represent negative PCR results, red squares represent positive results, yellow squares represent presumptive positive results and black squares represent invalid results. NMB=Nelson Mandela Bay. BCM=Buffalo City Metro. WWTP=wastewater treatment plant.*Population is the approximate size of the population served by the wastewater treatment plant. *Lab is the name of the testing laboratory.

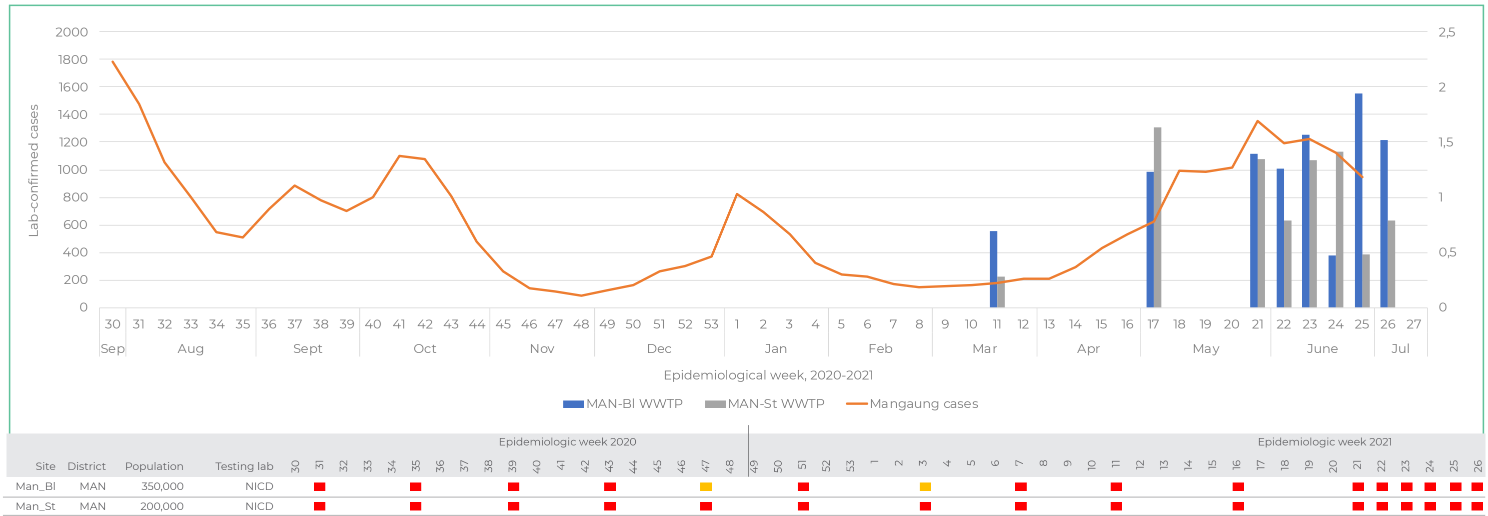


Figure 4. Epidemiological curves of laboratory-confirmed SARS-CoV-2 cases, SARS-CoV-2 viral loads in wastewater and SARS-CoV-2 PCR detection results in wastewater for Mangaung Metropolitan area in Free State Province, South Africa, 2020-2021. Figure: The curve represents laboratory-confirmed COVID-19 cases (left axis) by epidemiological week 2020-2021 (horizontal axis). The bars represent quantitative viral loads in copies per millilitre (right axis) in specimens collected in given epidemiological week 2020-2021 (horizontal axis). Table: Green squares represent negative PCR results, red squares represent positive results, yellow squares represent presumptive positive results black squares represent invalid results. MAN=Mangaung Metro. WWTP=wastewater treatment plant. *Population is the approximate size of the population served by the wastewater treatment plant. *Lab is the name of the testing laboratory.

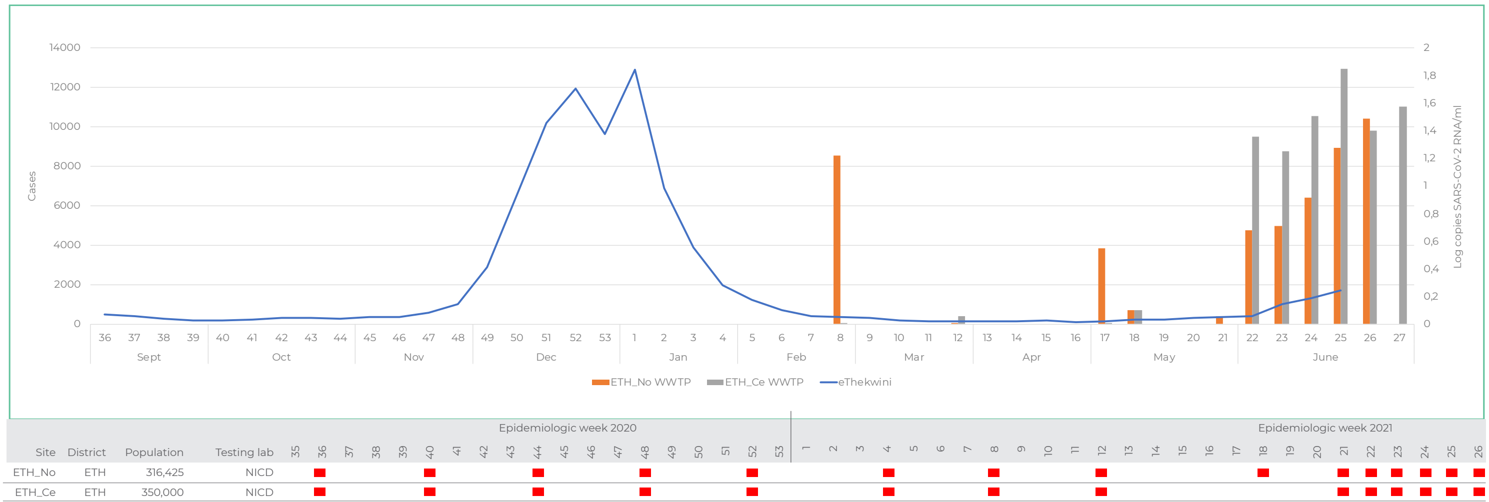
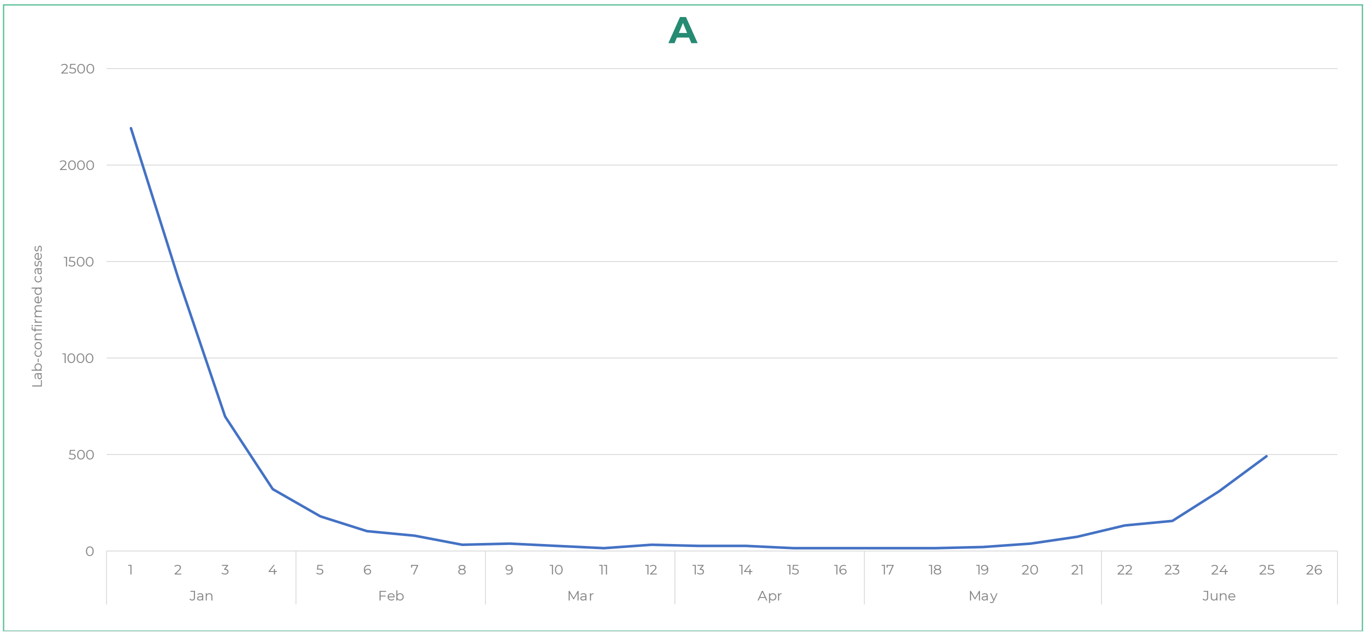
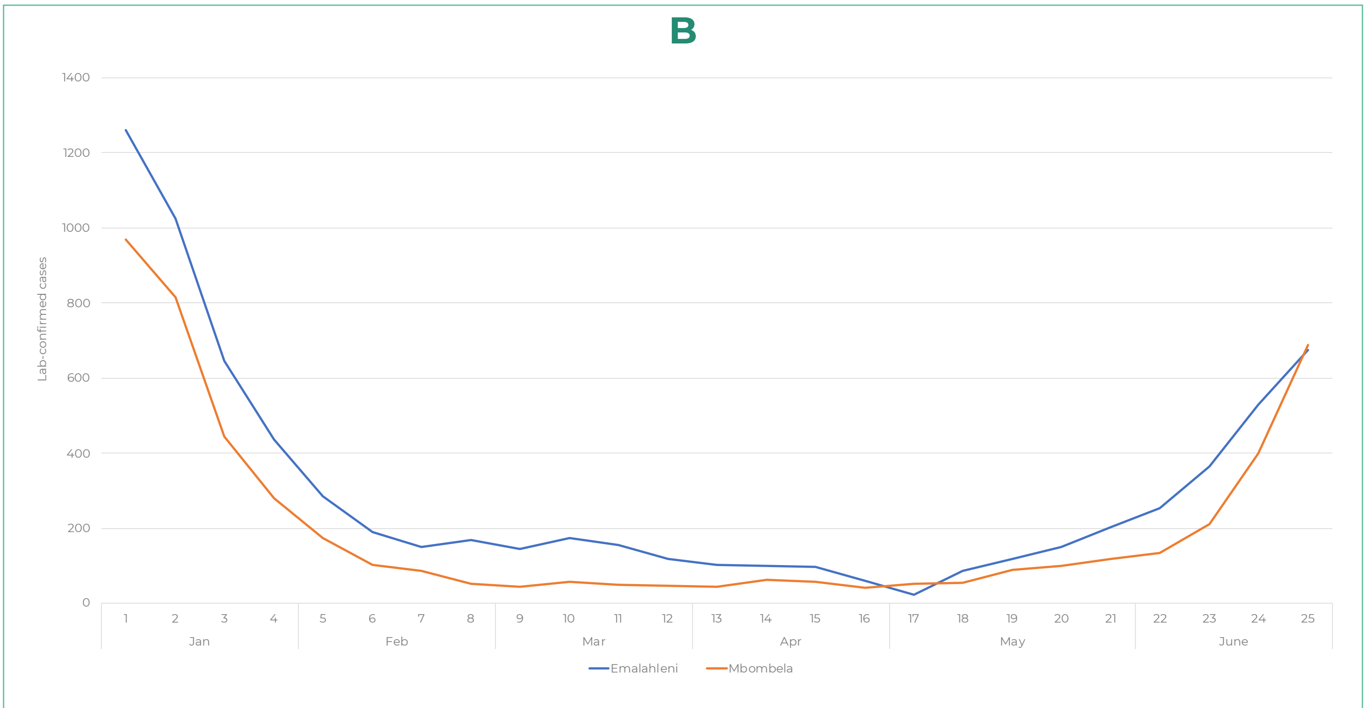


Figure 5. Epidemiological curves of laboratory-confirmed SARS-CoV-2 cases, SARS-CoV-2 viral loads in wastewater and SARS-CoV-2 PCR detection results in wastewater for eThekwini Metropolitan area in KwaZulu-Natal Province, South Africa, 2020-2021. Figure: The curve represents laboratory-confirmed COVID-19 cases (left axis) by epidemiological week 2020-2021 (horizontal axis). The bars represent quantitative viral loads in copies per millilitre (right axis) in specimens collected in given epidemiological week 2020-2021 (horizontal axis). Table: Green squares represent negative PCR results, red squares represent positive results and black squares represent invalid results. ETH=Ethekwini Metro. WWTP=wastewater treatment plant. *Population is the approximate size of the population served by the wastewater treatment plant. *Lab is the name of the testing laboratory.



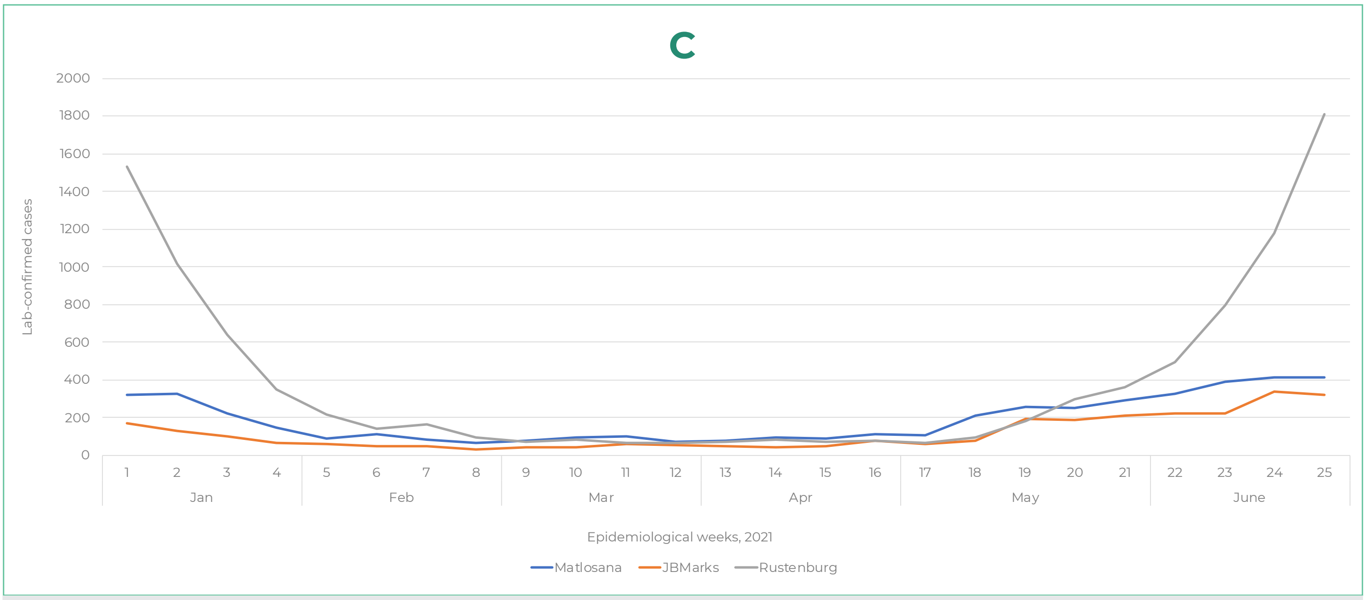
Epidemiologic week 2021

Site	District	Population	Testing lab	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26
POL_Le	POL	12,285	Waterlab/UP													■		■		■		■		■					
POL_Po	POL	NA	Waterlab/UP							■				■		■		■		■		■		■	■			■	

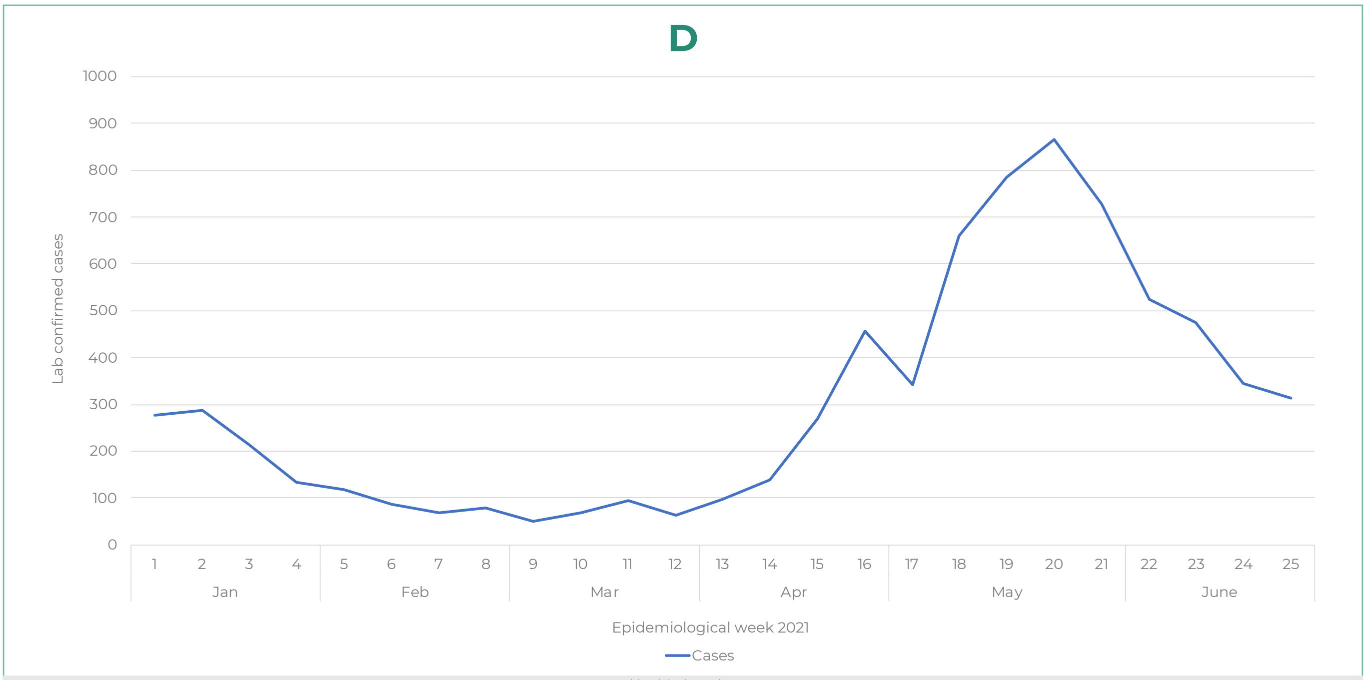


Epidemiologic week 2021

Site	District	Population	Testing lab	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26
MBO_Ki	MBO	NA	Waterlab/UP													■		■		■	■	■		■					
KBO_Ki	MBO	NA	Lumegen						■		■		■		■		■						■		■		■		
EHL_Ri	EHL	NA	Lumegen						■		■		■		■		■		■				■		■		■		



Site	District	Population	Testing lab	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26
				Epidemiologic week 2021																									
MAT_Kl	MATLOSANA	185,348	Lumegen				■ (black)			■ (black)		■ (red)		■ (black)		■ (red)		■ (black)		■ (black)		■ (red)		■ (red)		■ (red)		■ (red)	
JBM_Po	JBMARKS	250,000	Lumegen					■ (black)		■ (black)			■ (black)		■ (black)	■ (black)	■ (red)	■ (green)		■ (green)		■ (red)					■ (red)	■ (red)	
BOJ_Ru	BOJANALA	109,665	Lumegen						■ (black)		■ (black)		■ (black)		■ (black)		■ (green)		■ (green)		■ (green)		■ (red)		■ (red)		■ (red)		



Site	District	Population	Testing lab	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26
				Epidemiologic week 2021																									
So_Ho	Sol Plaatjie	248,000	Lumegen							■ (black)		■ (black)		■ (black)		■ (red)		■ (red)	■ (red)		■ (black)		■ (red)		■ (red)		■ (green)		

Figure 6 A-D. Epidemiological curves of laboratory-confirmed SARS-CoV-2 cases, SARS-CoV-2 viral loads in wastewater and SARS-CoV-2 PCR detection results in wastewater for from Limpopo (A), Mpumalanga (B), North West (C) and Northern Cape (D) provinces, South Africa, 2020-2021. Figure: The curve represents laboratory-confirmed COVID-19 cases (left axis) by epidemiological week 2020-2021 (horizontal axis). Table: Green squares represent negative PCR results, red squares represent positive results and black squares represent invalid results. POL=Polokwane, MBO=Mbombela, EHL=Ehlanzeni. WWTP=wastewater treatment plant. *Population is the approximate size of the population served by the wastewater treatment plant. *Lab is the name of the testing laboratory.