July 2021 - SCIENCE BRIEF NO. 6
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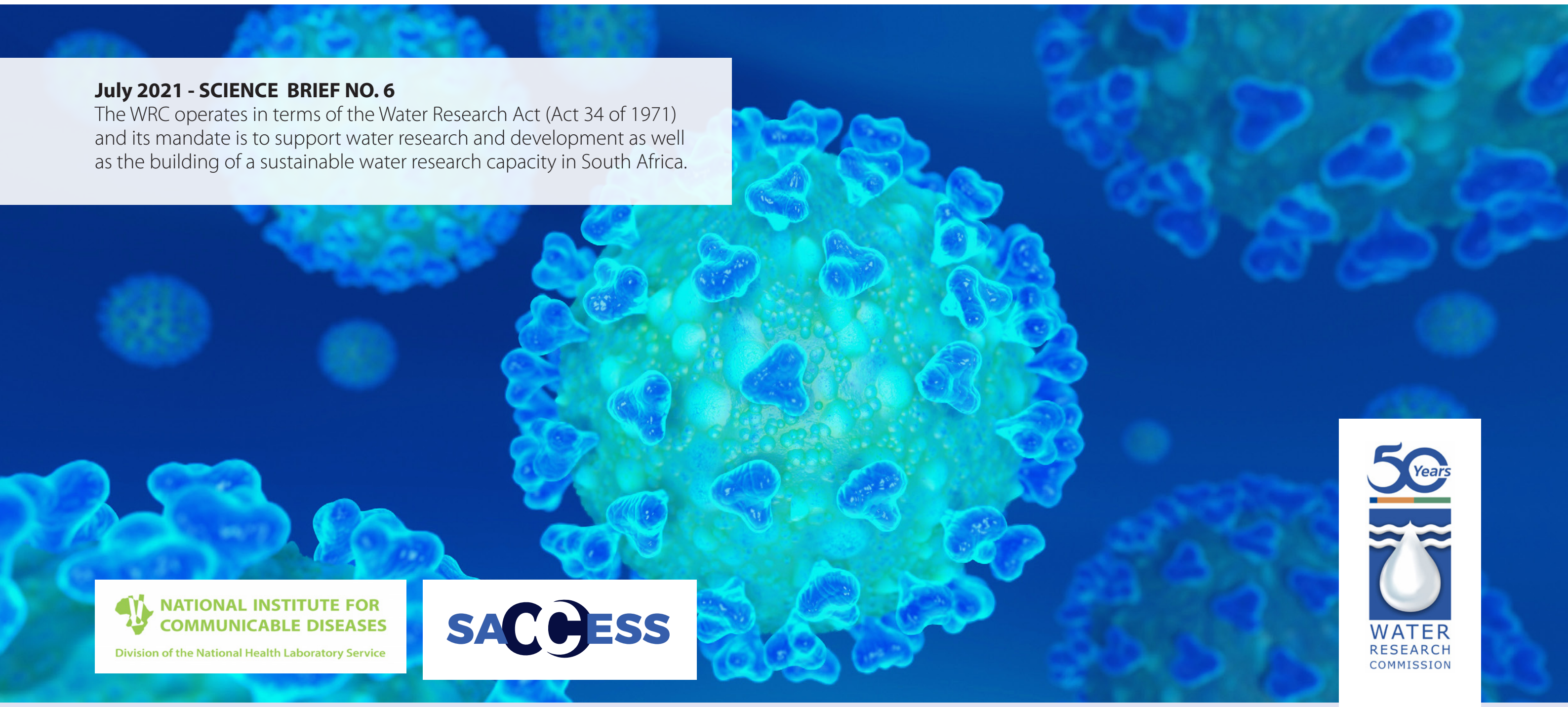

A longitudinal view and trends on the detection and sequencing of SARS-CoV-2 at sentinel wastewater treatment sites by the South African Collaborative COVID-19 Environmental Surveillance System (SACCESS) network, 2020-2021

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- *Since the inception of the wastewater-based epidemiology (WBE) Surveillance Programme for SARS-COV-2 in March 2020, seven laboratories tested 513 specimens from 38 wastewater treatment plants across 9 provinces, covering sentinel sites where 760 731/1 973 972 (38,5%) of laboratory-confirmed COVID-19 cases were reported.*
- *The geospatially allocated line list of laboratory-confirmed clinical cases from commencement of the South African pandemic (4 March 2020) until the end of week 25 (26 June 2021) yielded 1 973 972 cases. Among 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.*
- *SARS-CoV-2 was detected in 350 (68%) samples tested.*
- *Detection of SARS-CoV-2 was more frequent during clinical 'waves' reported.*
- *Viral load in wastewater tended to increase during the third wave in parallel with increasing clinical cases.*
- *Detection of SARS-CoV-2 with quantitation of viral loads from wastewater may be a helpful additional data source to support population level monitoring of SARS-CoV-2 epidemiology.*
- *Results from sentinel sites across South African urban areas indicated correspondence at some sites with increased numbers of clinical cases in Gauteng, KwaZulu-Natal, Free State and Northern Cape provinces and support the further exploration of these data to monitor SARS-CoV-2 epidemiology using wastewater-based testing.*
- ***The results to date and the trends have demonstrated the significance and cost efficiency of WBE in complementing the management of the pandemic offering early warning and identifying hotspots.***

Background

Globally, detection and monitoring of SARS-CoV-2 epidemiology through wastewater was proposed as early as April 2020[1,2]. First reports describing the feasibility and practical usefulness of this approach emerged simultaneously from a number of countries around 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]. Longitudinal tracking of SARS-CoV-2 viral load in wastewater is shown to correlate 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].

Wastewater-based epidemiology (WBE) is emerging as a useful modality to monitor SARS-CoV-2 epidemiology and support public health decision-making regarding COVID-19 response activities. Based on the global developments in the field and the opportunity WBE offers in terms of an early warning and hotspot indicator of the viral infections in communities, several south African institutions, such the Water Research Commission (WRC), National Institute for Communicable Diseases (NICD), South African Medical Research Council (MRC), University of Pretoria, Durban University of Technology (DUT) and private laboratories launched programmes in support of WBE surveillance of the SARS-CoV2 virus. A further outcome was the formation of the South African Collaborative COVID-19 Environmental Surveillance System (SACCESS) network of laboratories, to report results of sentinel surveillance in urban centres and metros using PCR-based detection and quantitation of SARS-CoV-2 virus in wastewater samples, alongside corresponding district or metro epidemic curves. <u>NICD was nominated to play the coordinating role and with funding the support from the WRC the National Surveillance programme is incrementally being scaled.</u>

Detection of SARS-CoV-2 with quantitation of viral load from wastewater can 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.

In South Africa, SARS-CoV-2 epidemiology is monitored through laboratory testing of clinical cases (by reverse-transcriptase polymerase chain reaction (RT-PCR) tests and rapid antigen tests), COVID-19 related hospital admissions and COVID-19–related deaths. Laboratory testing data is relayed by testing laboratories to the National Institute for Communicable Diseases (NICD). Hospital admissions and death data is submitted by the DATCOV system to the NICD. 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.

A number of independent initiatives to test for SARS-CoV-2 in wastewater commenced in early 2020. 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 development of common testing methodology, identify and address challenges, and share best practices related to qualitative, quantitative and RNA sequencing of SARS-CoV-2 in wastewater. In South Africa, treatment of wastewater is the responsibility of local government. Approximately 1 050 wastewater treatment works (WWTPs) are administered by metropolitan councils and local government and treat both 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.

As at the beginning of July 2021, South Africa has experienced two 'waves' of COVID-19, and is experiencing a third 'wave'. The initial rise in case numbers began in the Northern Cape and Free State provinces in March and early April of 2021. As of 6 July 2021, the rise in cases meets the criteria for a 'third wave' in all provinces. Presently, the case incidence rate in Gauteng Province is currently highest at 45/100 000, with over 34% of daily tests reportedly positive.

We aimed to identify epidemiological trends of SARS-CoV-2 in South African urban wastewater treatment plants and compared PCR detection and quantitation results from influent sewage with trends in clinical epidemiology. We also describe the establishment of the SACCESS network

Methods

<u>Outbreak context and clinical case epidemiology</u>

Since the first case of SARS-CoV-2 was detected on 3 March 2020, South African laboratories have conducted over 13 million RT-PCR and antigen tests. Just over 2 million cases and 61 000 deaths have been recorded in South Africa as of 6 July 2021. 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 linelist 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.

<u>Establishment of the laboratory testing network</u>

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, 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-March 2021 as funding for partners became available. Results from testing funded through the WRC and NICD are reported here.

SARS-CoV-2 detection and quantitation methodology

At identified wastewater treatment facilities, one litre grab samples of influent were collected and transported at <5°C to the testing facility. Table 1 summarises testing modalities used in this study. 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 amplifies 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

Testing modality	Test modalities	Interpretive principles to support public health responses
Detection of SARS-CoV-2	Concentration of virus RNA 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) or from ▪ 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

Interpretation of results

Table 1 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. Concentration, extraction and RT-PCR detection methodology used by laboratory partners.

Name of 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)

Name of Laboratory partner	Method for virus concentration	Method for nucleic acid extraction	RT-PCR assay
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, 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, additional sites in Gauteng (seven sites), Limpopo (two sites), Mpumalanga (three sites), North West (three sites), the Northern Cape (one site) and Western Cape (three sites) provinces were added, to bring 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 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); 7 514 in Emalahleni DM, 5 166 in Mbombela DM; 4 762 in Matlosana DM, 2 931 in JB Marks Local Municipality (LM); 10 047 in Rustenburg LM; 7 533 in Sol Plaatje LM, 200 310 in City of Cape Town Metro).

Provincial trends

Over 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 these 383 samples, SARS-CoV-2 was identified in 105 samples in 2020 (74%) and 245 in 2021 (66%), with each site submitting an average of 13.5 samples over the two years sampling period (range 2-21). In 2020 and 2021 respectively, 24 (17%) and 45 (12%) of samples had invalid results. Of 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 displayed in Figures 1-6 together with epidemiologic curves for clinical cases detected 2020-2021 for the districts or metros where each WWTW is 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 City of Johannesburg, a single plant in City of Tshwane and three plants in Ekurhuleni 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 the 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 has a lower viral load. In Nelson Mandela Metro, samples from week 24 show 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 appears 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 (Figure 6A), testing commenced in week 7 of 2021 (February 2021) and all samples have tested negative until week 15 (mid-April) at a single plant, and week 19 in a second plant, in keeping with an increase in 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

In this description of results from detection of SARS-CoV-2 from wastewater at South African sentinel sites, we have demonstrated some concordance of qualitative and quantitative results with clinical epidemiologic curves. We have demonstrated the potential of the 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 point (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.

Detection results from our network reflect that SARS-CoV-2 is reliably present in wastewater during 'waves', and during the third wave, quantitation results have reflected the current upward trend observed amongst clinical cases. However certain anomalies in our results 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-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 presently unclear.

Presently, our results are helpful to retrospectively confirm the presence of SARS-CoV-2 in wastewater and hence in metros of South Africa. However, our data challenge the network partners to exploit and build on the opportunity provided by this network to supply meaningful and timely data to public health decision makers at provincial and national level as has been done elsewhere[6]. These 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 guide the implementation of public health measures to curb transmission and contain the economic and social impact of SARS-CoV-2. Work has been done globally attempting to calculate population burdens of disease from quantitative PCR results[10]. This requires knowledge of duration and quantity of SARS-CoV-2 shedding from infected persons as well as the rate of decay of RNA in wastewater. 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 potential to monitor population trends in 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 network may prove useful to provide surveillance data for other waterborne or excreted communicable diseases including agents of gastroenteritis, hepatitis and other respiratory illness such as influenza[12] and for 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 tested 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 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, our detection results from sentinel sites across South African urban areas indicating correspondence at some sites with 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 wastewate- based testing.

Acknowledgements

This WRC brief is adapted from an article published in the NICD Bulletin – Volume 19, 20 July 2021.

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

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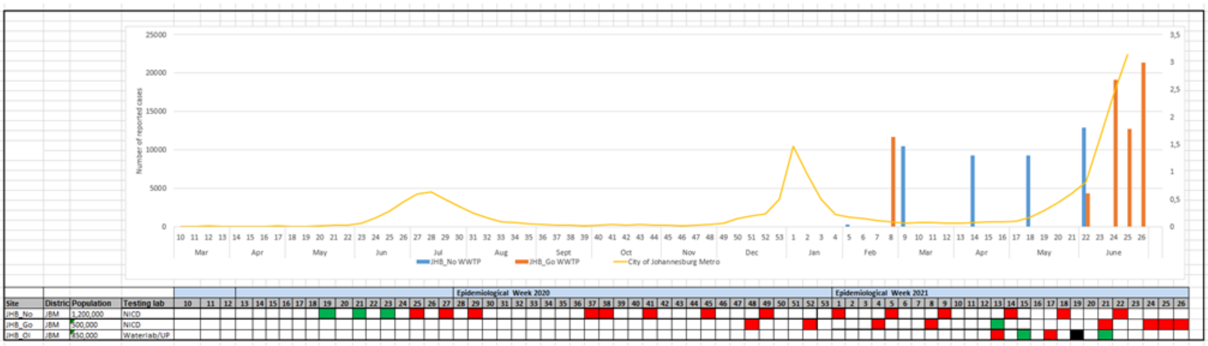


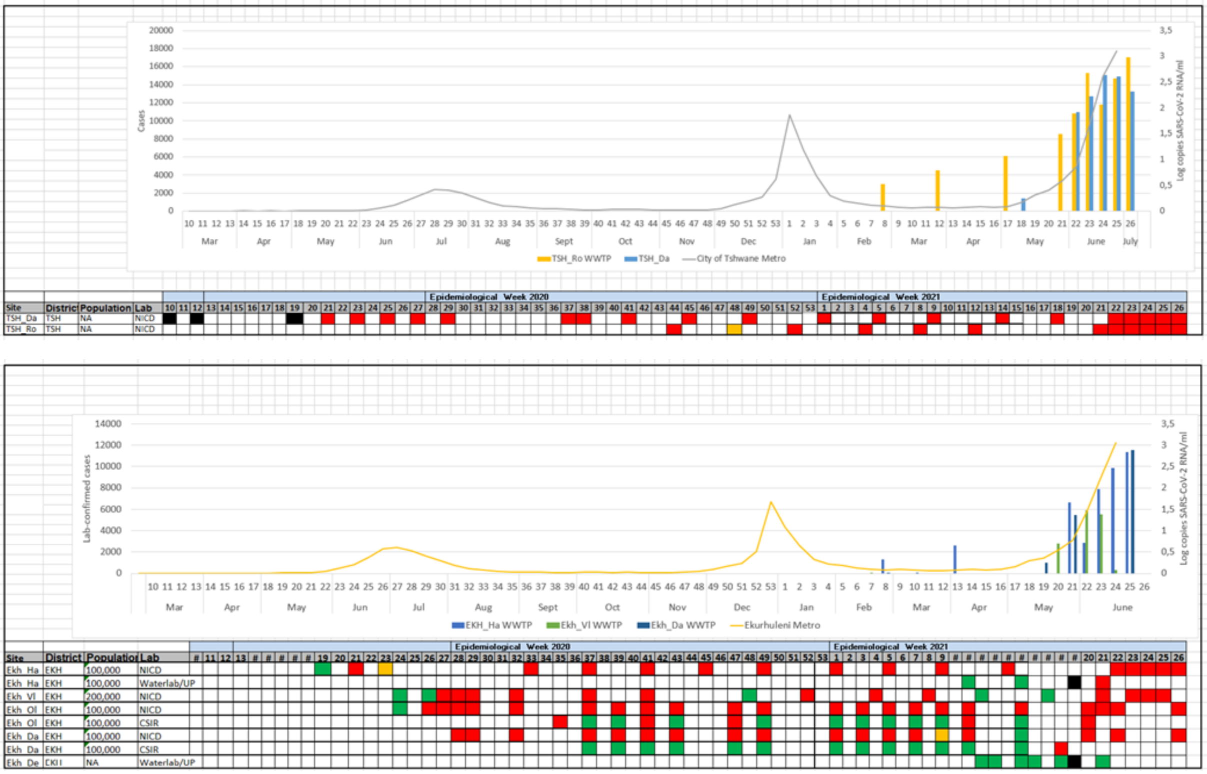


Figure 1. Epidemiological curves of laboratory-confirmed SARS-CoV-2 cases, SARS-CoV-2 viral loads in wastewater and SARS-CoV-2 PCR detection results in wastewater by district, Gauteng Province, 2020-2021.

Districts shown in include City of Johannesburg (A), City of Tshwane (B) and Ekurhuleni Metropolitan area (C). Figure: Yellow curve represents laboratory-confirmed COVID-19 cases (left axis) by epidemiological week 2020-2021 (horizontal axis). Blue 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. 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

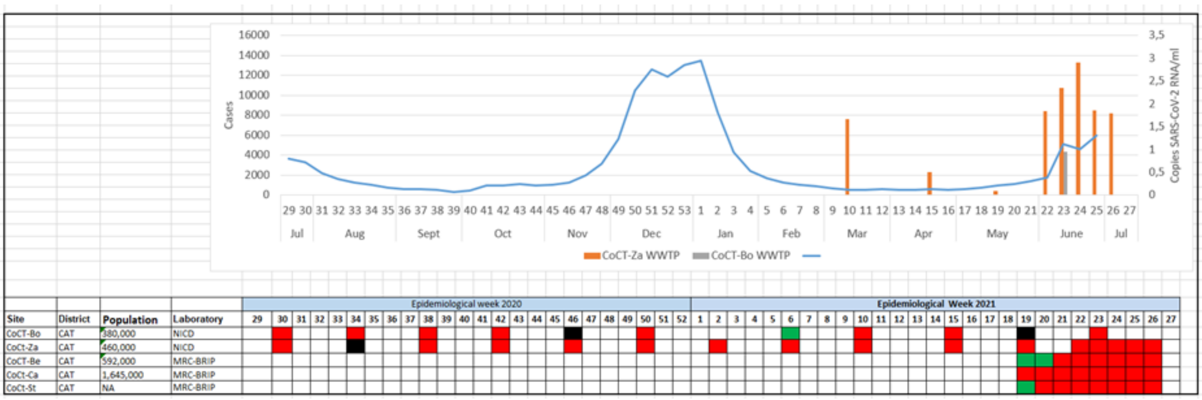


Figure 2. Epidemiological curves of laboratory-confirmed SARS-CoV-2 cases, SARS-CoV-2 viral loads in wastewater and SARS-CoV-2 PCR detection results in wastewater for the City of Cape Town, Western Cape province, 2020-2021.

Figure: Yellow curve represents laboratory-confirmed COVID-19 cases (left axis) by epidemiological week 2020-2021 (horizontal axis). Blue 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

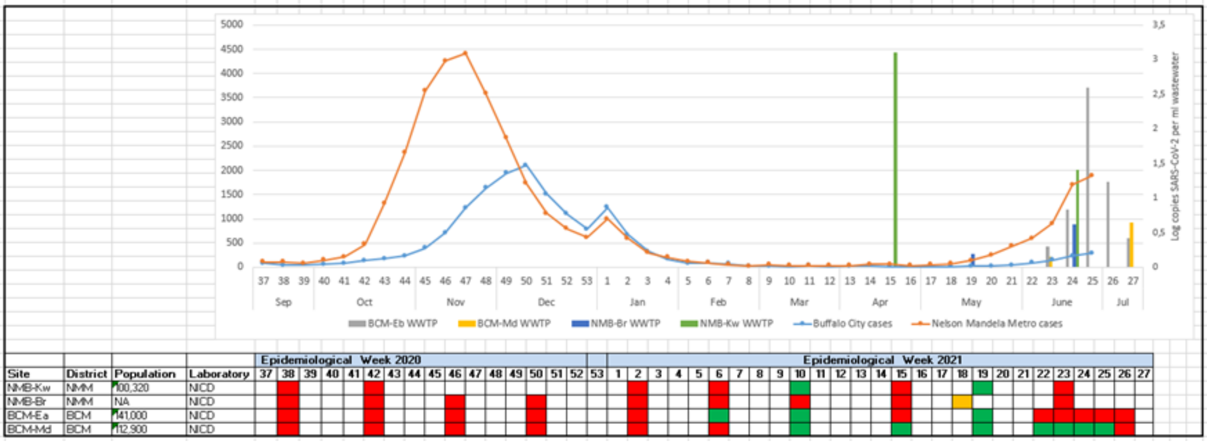


Figure 3. Epidemiological curves of laboratory-confirmed SARS-CoV-2 cases, SARS-CoV-2 viral loads in wastewater and SARS-CoV-2 PCR detection results in wastewater for Nelson Mandela Metropolitan area (NMMB), and Buffalo City Metropolitan area (BCM) in the Eastern Cape Province, 2020-2021.

Figure: Yellow curve represents laboratory-confirmed COVID-19 cases (left axis) by epidemiological week 2020-2021 (horizontal axis). Blue 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. 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

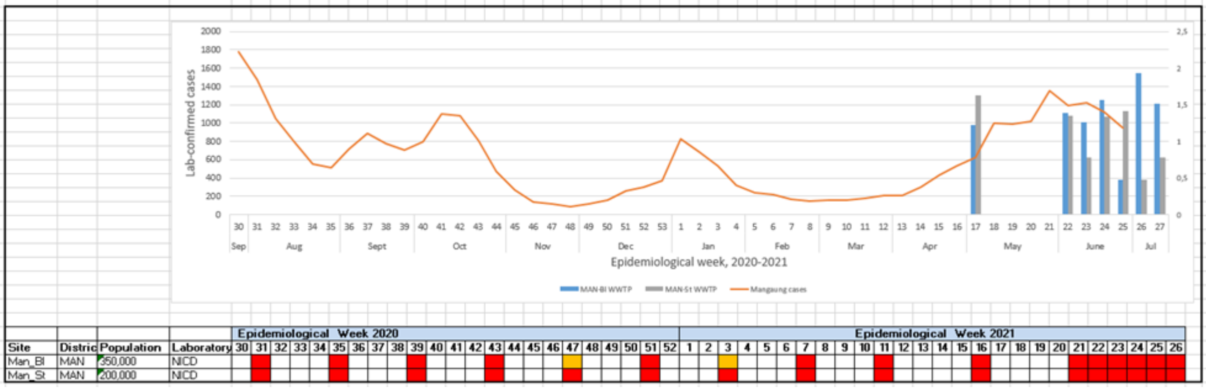
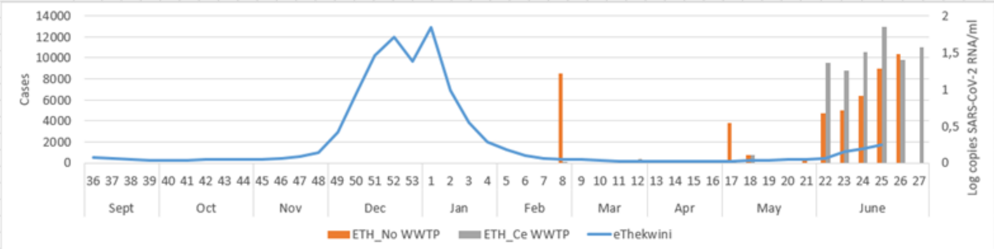


Figure 4. Epidemiological curves of laboratory-confirmed SARS-CoV-2 cases, SARS-CoV-2 viral loads in wastewater and SARS-CoV-2 PCR detection results in wastewater for Mangaung Metropolitan area in Free State Province, 2020-2021.

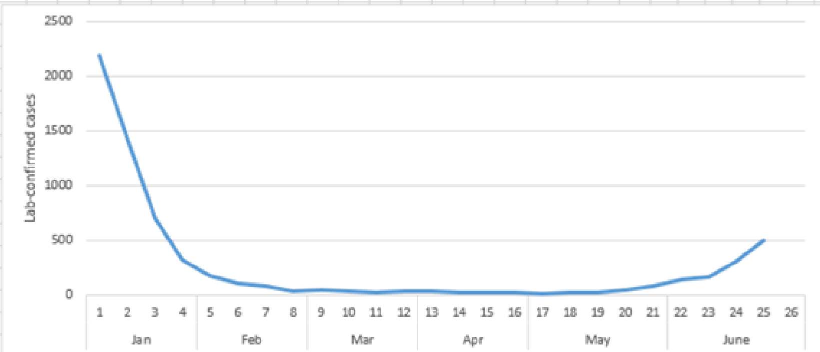
Figure: Yellow curve represents laboratory-confirmed COVID-19 cases (left axis) by epidemiological week 2020-2021 (horizontal axis). Blue bars represent quantitative viral loads in copies per millilitre (right axis) in specimens collected in given epidemiological week 2020-2021 (horizontal axis). Table: Green squares represent negative PCR results, red squares represent positive results and black squares represent invalid results. 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



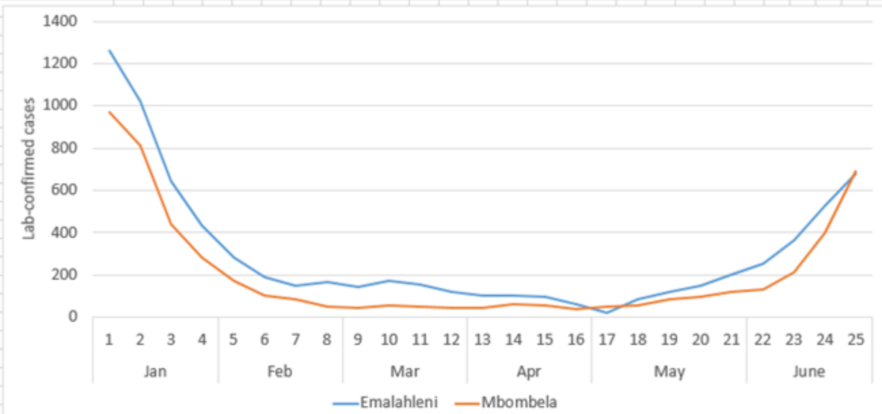
				Epidemiological Week 2020																			Epidemiological Week 2021																									
Site	Distric	Population	Laboratory	35	36	37	38	39	40	41	42	43	44	45	46	47	48	49	50	51	52	53	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26
ETH_No	ETH	316,425	NICD																																													
ETH_Ce	ETH	350,000	NICD																																													

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, 2020-2021.

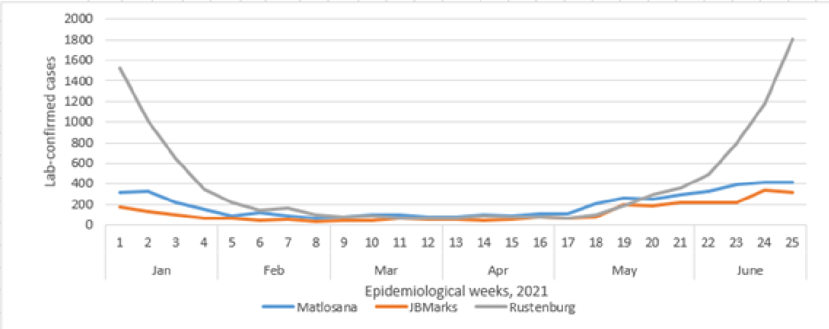
Figure: Yellow curve represents laboratory-confirmed COVID-19 cases (left axis) by epidemiological week 2020-2021 (horizontal axis). Blue bars represent quantitative viral loads in copies per millilitre (right axis) in specimens collected in given epidemiological week 2020-2021 (horizontal axis). Table: 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



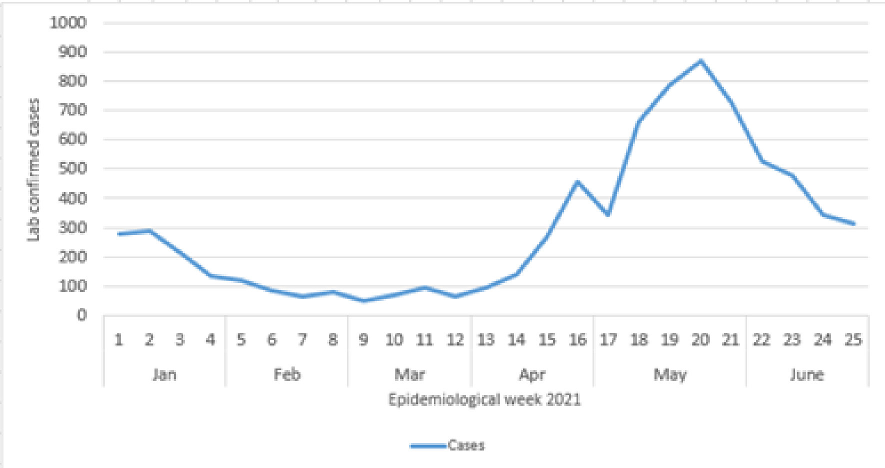
				Epidemiological Week 2021																									
Site	District	Population	Laboratory	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26
POL_Le	POL	12,285	Waterlab/UP																										
POL_Po	POL	NA	Waterlab/UP																										



				Epidemiological Week 2021																										
Site	District	Population	Laboratory	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	25	26
MBO_Ki	MBO	NA	Waterlab/UP																											
KBO_Ki	MBO	NA	Lumegen																											
EHL_Ri	EHL	NA	Lumegen																											



				Epidemiological Week 2021																									
Site Name	District	Population	Laboratory	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
MAT_Kl	MATLOSANA	185,348	Lumegen																										
JBM_Po	JBMARKS	250,000	Lumegen																										
BOJ_Ru	BOJANALA	109,665	Lumegen																										



				Epidemiological Week 2021																									
Site	Subdistrict	Population	Laborator	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
So_Ho	Sol Plaatjie	248,000	Lumegen																										

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, 2020-2021.

Figure: Yellow curve represents laboratory-confirmed COVID-19 cases (left axis) by epidemiological week 2020-2021 (horizontal axis). Blue 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