Wastewater-based genomic epidemiology for SARS-CoV-2 surveillance in South Africa

6 October 2023

Sample collection dates up to 29 September 2023
(Epidemiological week 39)

F. Els*, N.S Msomi*, N.L Ndlovu, V. Mabasa, E. Phalane, M.E Macheke, S. Gwala, T. Mangena,
L. Monametsi, L.P Rabotapi, S. Maposa, M. Yousif[#], K. McCarthy[#]

*Joint first authors
#Joint last authors

Centre for Vaccines and Immunology,
National Institute for Communicable Diseases,
A division of the National Health Laboratory Service

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Summary: SARS-CoV-2 transmission and genomics based on evaluation of wastewater at sentinel sites across RSA

Wastewater levels

Epidemiological weeks 33-39

- From weeks 33-39, the cumulative SARS-CoV-2 levels measured at wastewater treatment works (WWTW) **has remained around 2 log (100) genome copies/ml of wastewater**. This has followed on from the increases observed in weeks 31-33, when the cumulative SARS-CoV-2 levels in wastewater at sentinel sites in South Africa showed increases to levels above 2 log (100) genome copies/ml of wastewater, up from below one log copy/ml in epidemiological week 22 (first week in June 2023).

- In weeks **33-39 increases and/or higher levels have been seen in Gauteng (Daspoort and Hartebeesfontein WWTW), KwaZulu Natal (Central WWTW), and Free State (Bloemspruit and Sterktwater WWTW).**

- Correlation with syndromic surveillance for influenza-like illness (ILI) and severe acute respiratory infection findings (SARI) is required to determine the clinical and public health significance of ongoing transmission. https://www.nicd.ac.za/diseases-a-z-index/disease-index-covid-19/surveillance-reports/weekly-respiratory-pathogens-surveillance-report-week/

Wastewater genomics

Epidemiological weeks 31-36

- Genomics results were obtained for weeks 34-36 for the heat map and mutational profile.

- **Omicron lineages XBB.1.5 followed by XBB.1.16 and XBB sub-lineages** remain the dominant lineages circulating in clinical samples in June, July and August 2023

- The **Omicron lineage BA.2.86** is circulating in KwaZulu-Natal in eThekwini (in the catchments of Northern and Central WWTWs), and in Gauteng, in the City of Johannesburg (in catchments of Northern and Goudkoppies WWTWs), in Ekurhuleni (in the catchments of Olifantsfontein, Vlakplaats, and Hartebeesfontein WWTWs), and the City of Tshwane (in the catchment of Daspoort WWTP). It is also circulating in Eastern Cape in Buffalo City (in the catchment of Mdantsane WWTW), in Western Cape, in the City of Cape Town (in the catchment of Borches Quarry WWTW), and Free State, in Mangaung (catchments Bloemspruit and Sterkwater WWTWs).

Interpretation: Ongoing transmission of SARS-CoV-2 due to Omicron lineages including the new lineage BA.2.86.

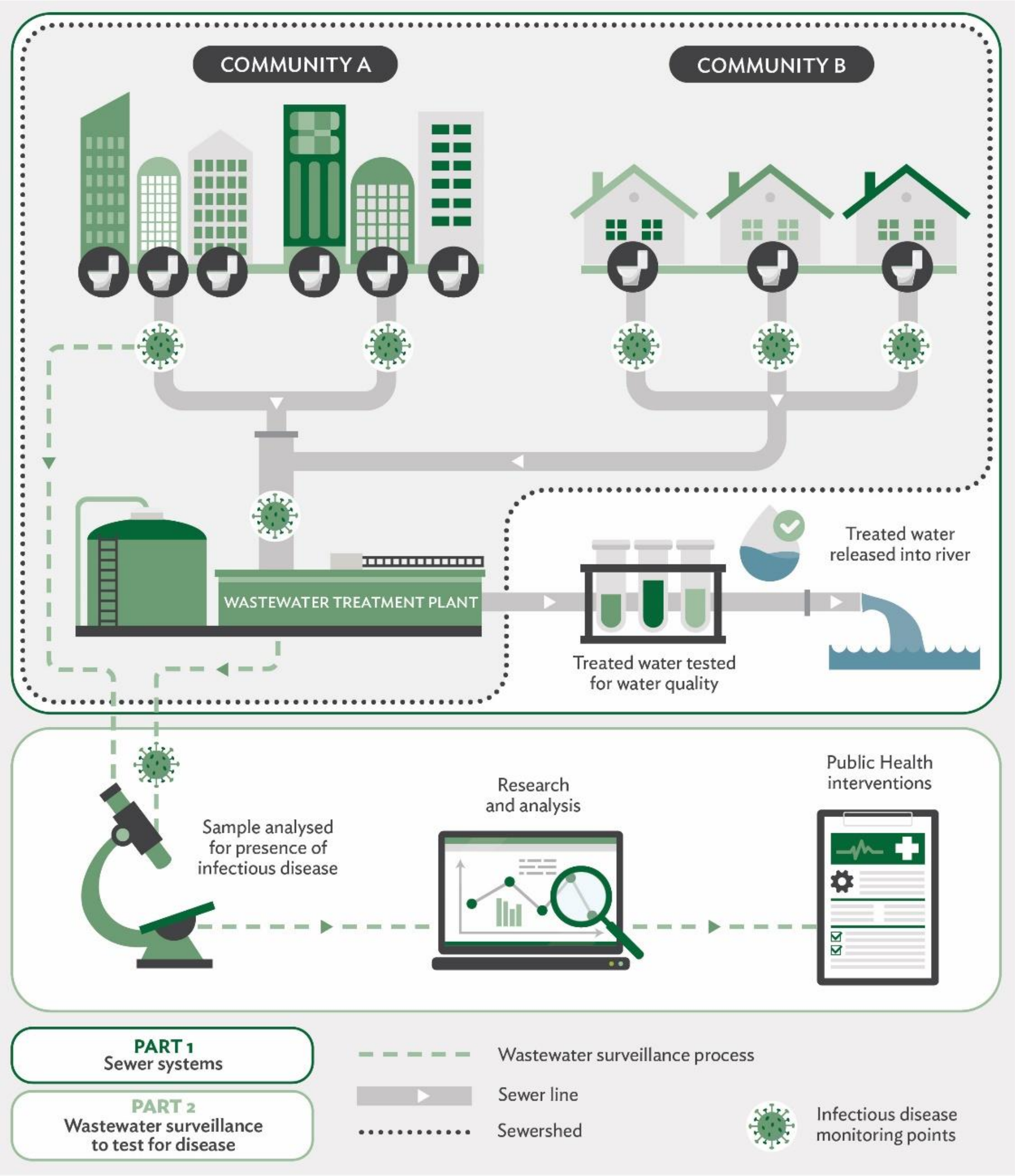
Wastewater-based Epidemiology for COVID-19

How is wastewater tested for SARS-CoV-2?

For a full description of this process, see the photoessay developed in collaboration with the Gauteng City Region Observatory
https://www.gcro.ac.za/outputs/photo-essays/detail/photo-essay-sewersheds-what-can-wastewater-tell-us-about-community-health/

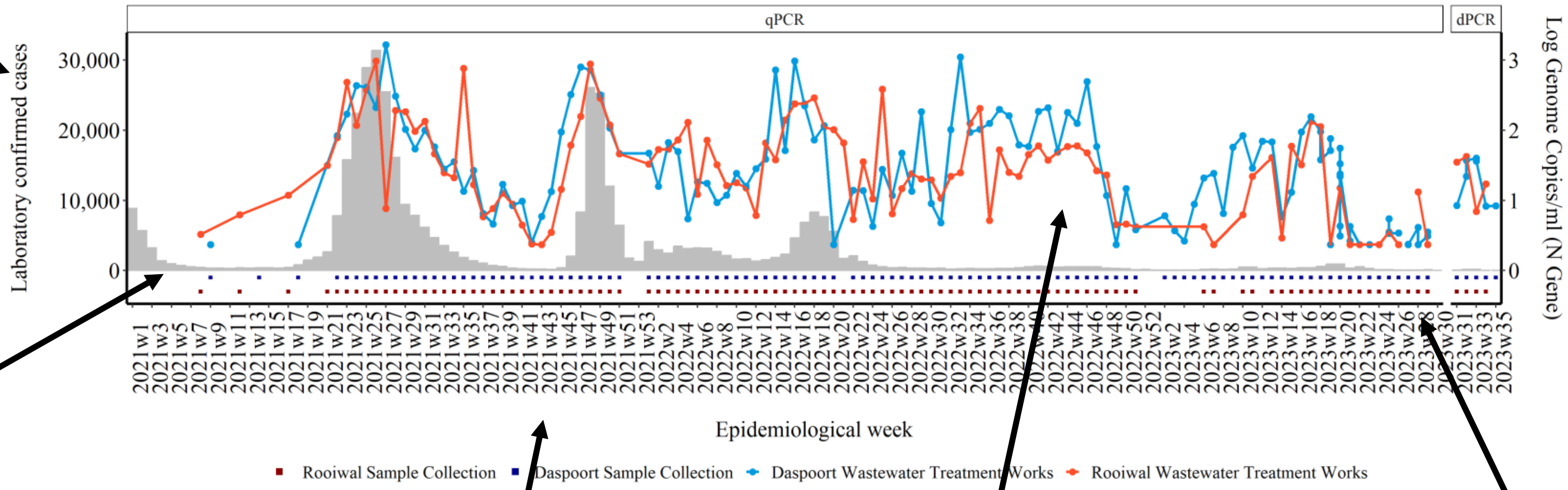
For a technical description and analysis of wastewater levels and results see
https://pubmed.ncbi.nlm.nih.gov/37506905/
https://www.medrxiv.org/content/10.1101/2022.12.15.22283506v1 (accepted by Nature Communications, publication pending)

SARS-CoV-2 is not transmitted by faeco-oral route. Wastewater with SARS-CoV-2 is not infectious



Wastewater-based Epidemiology for COVID-19

What does wastewater testing for SARS-CoV-2 mean?



Facets:

Indicates the platform used to test samples

Left vertical axis:

Number of lab confirmed cases in *the metro or district where the water treatment plant is located*

Right vertical axis:

Log (ie 10 to the power x) copies of SARS-CoV-2 genome per ml of wastewater. So log 2=10^2 =100 copies per millilitre, log 3=10^3=1000 copies per millilitre

Bars:

Number of lab confirmed clinical cases in specimens submitted to NICD from persons in the metro/subdistrict where the plant is located

Horizontal axis:

Epidemiological weeks from 2021 to 2023

Coloured lines:

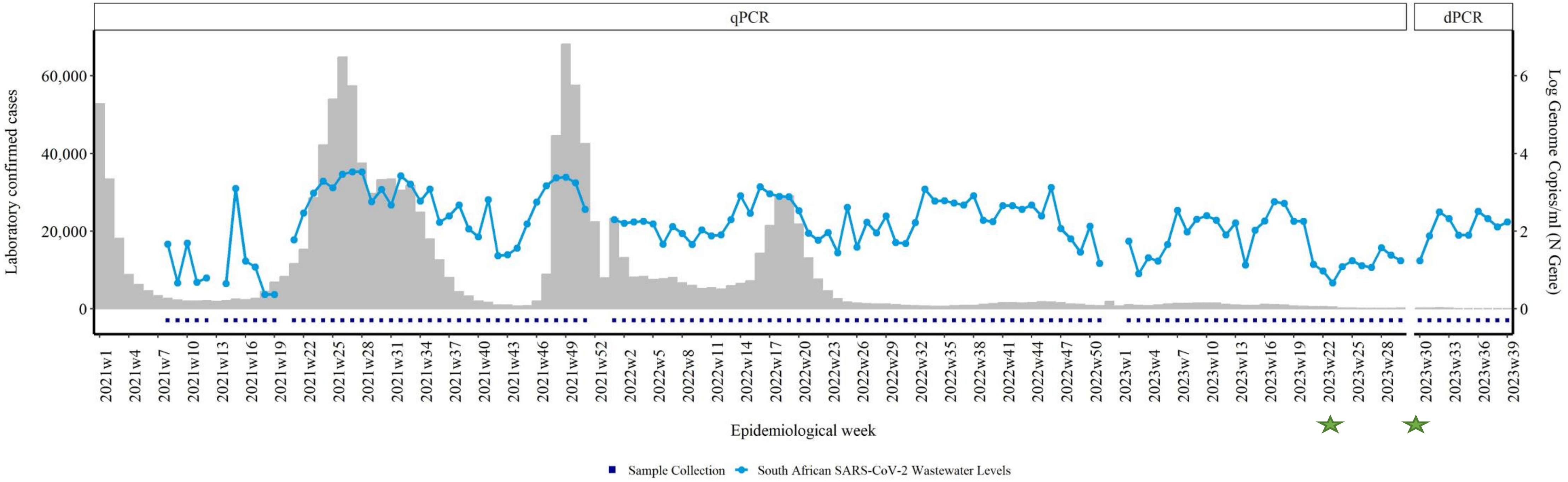
Changes in wastewater SARS-CoV-2 results over time for different treatment facilities

Coloured squares:

Epi weeks during which samples were collected

South Africa at a glance:

Summed total of clinical and genome copies



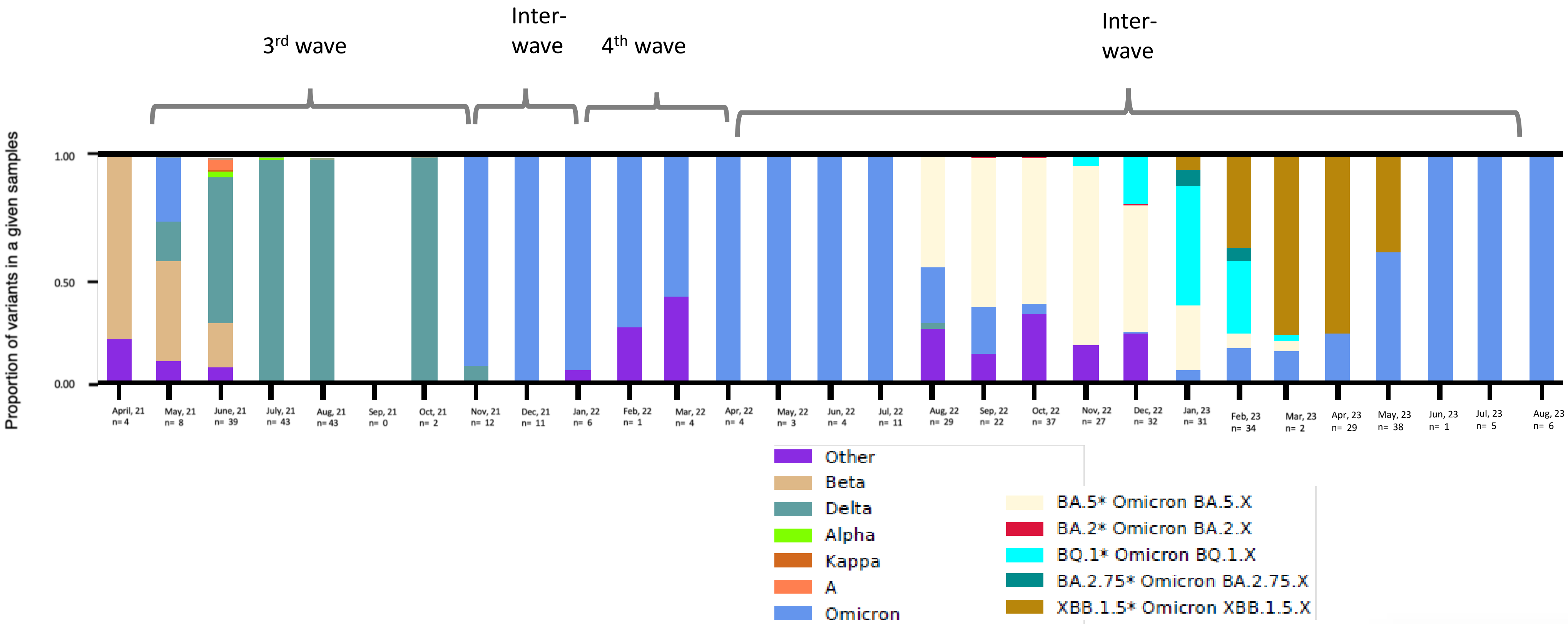
★ Chloroform start and end date

Changes in levels of SARS-Cov-2 (line graph) in in-flowing untreated wastewater from plants tested by NICD, compared with laboratory-confirmed cases from Tshwane, Johannesburg, Ekurhuleni, eThekwini, Mangaung, Nelson Mandela, Buffalo City, and City of Cape Town (grey bars), by epidemiological week, 2021-2023.

South Africa at a glance:
Circulating variants as determined by Freyja deconvolution of sequence data

- SARS-CoV-2 variants in wastewater as determined by the ‘Freyja’ tool (Scripps Institute)
    - Allows determination of variants in each wastewater sample
- Results from sequencing data ending in epi week 32 (10 August 2023)
    - Omicron lineages XBB.1.5 and Omicron were circulating from June to August, with Omicron dominance in August.

Inferred variants in wastewater samples from South African wastewater treatment plants by month, between April 2021- August 2023



South Africa at a glance:
Circulating lineages as determined by Freyja deconvolution of sequence data

- Results from sequencing data ending in epi week 32 (10 August 2023)
    - Omicron lineages XBB.1.5 and Omicron were circulating from June to August, with Omicron dominance in August.
    - Omicron XBB sub-lineages were in circulation throughout August.
    - The predominant lineages circulating in clinical samples in the recent week are XBB.1.5 followed by XBB.1.16 and XBB sub-lineages.

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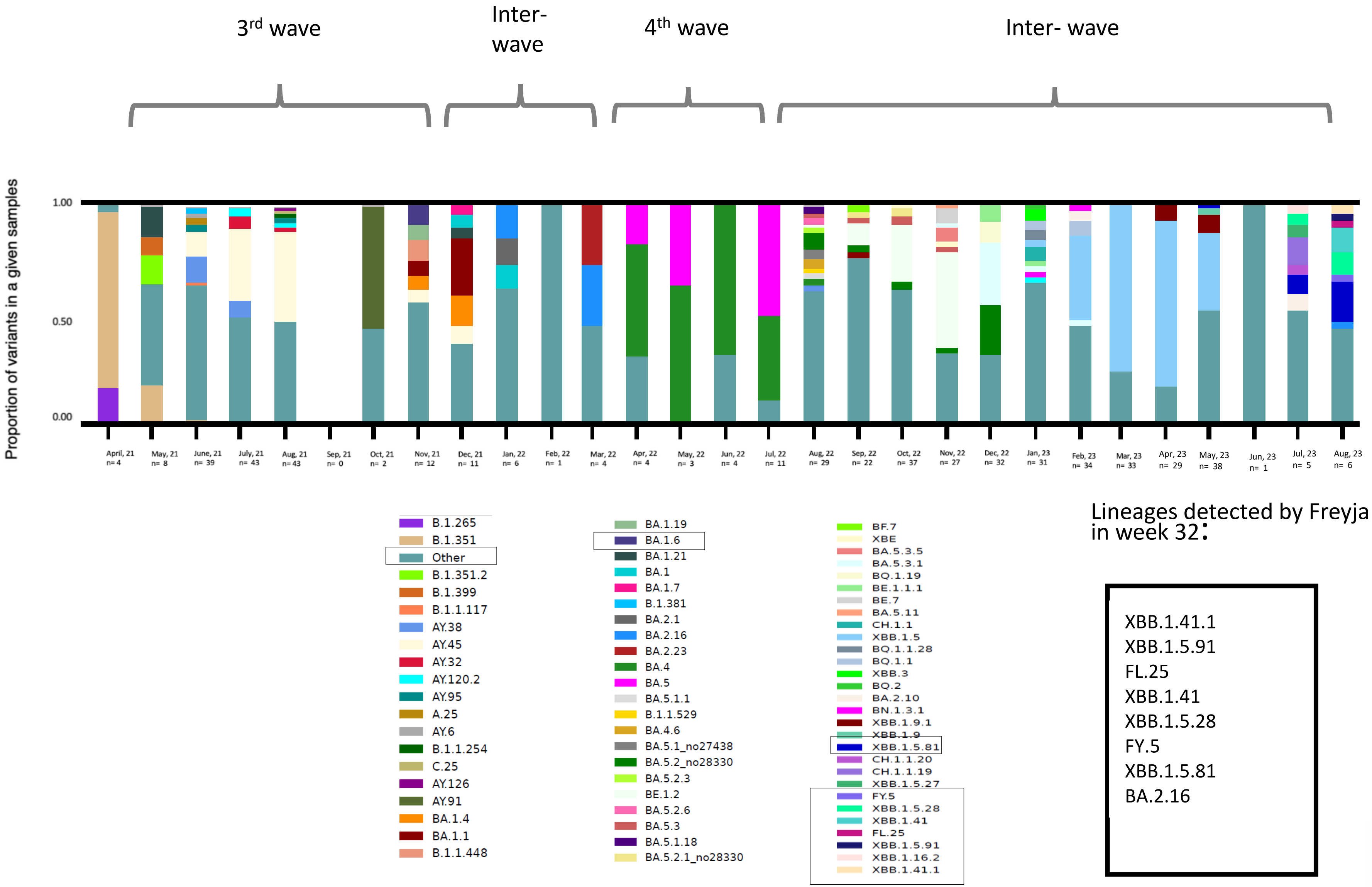

Lineages detected by Freyja in week 32:

XBB.1.41.1
XBB.1.5.91
FL.25
XBB.1.41
XBB.1.5.28
FY.5
XBB.1.5.81
BA.2.16

BA.2.86 is likely contained in 'other' as numbers are small

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What mutations in the spike protein tell us about the circulating lineages

Summary:

Summary of lineages associated with most common mutations associated

Epi Week:

Epidemiological week during which samples were collected

Site Names:

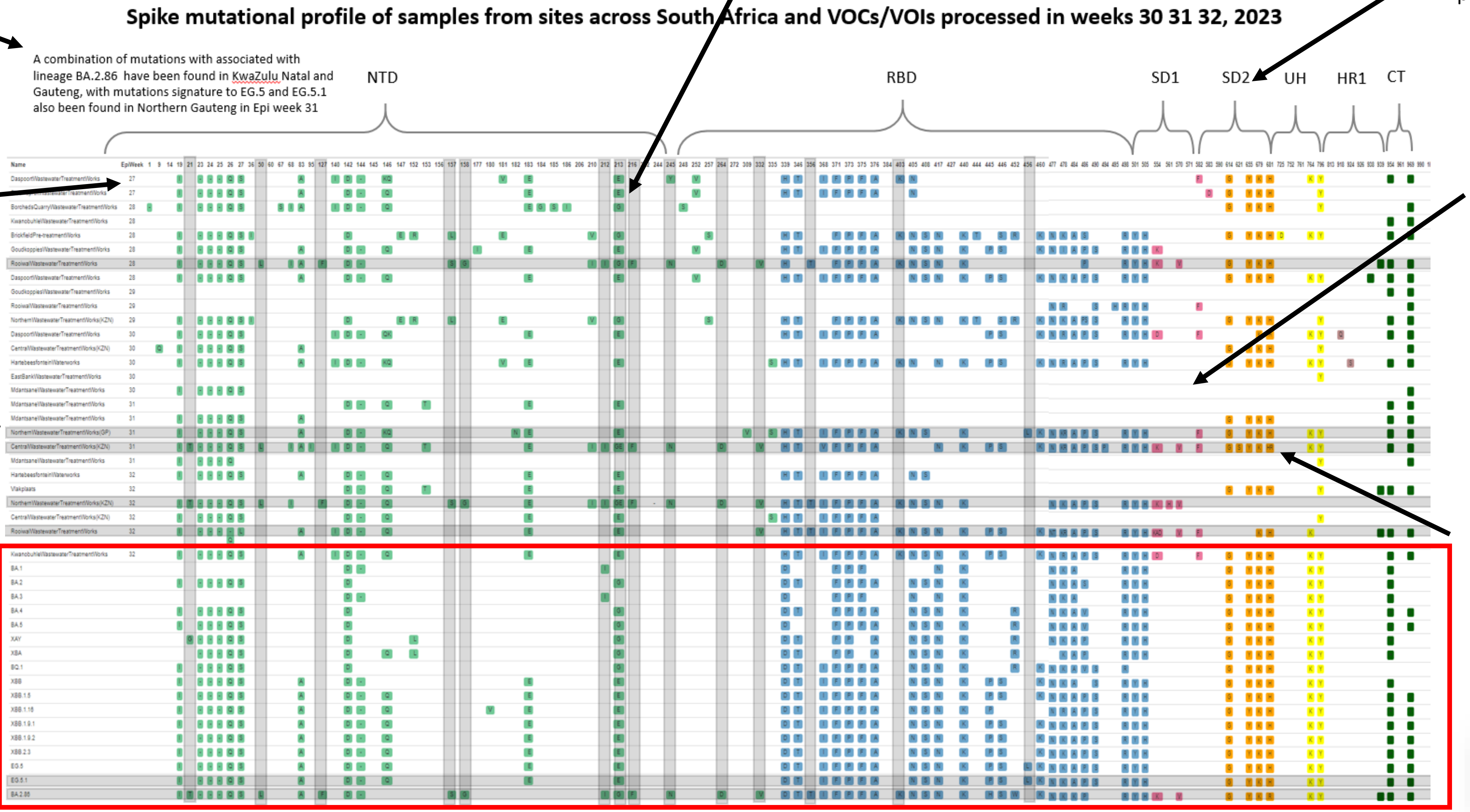
Sites from which wastewater samples were collected

Reference lineages:

Reference lineages with signature mutations with which wastewater samples are compared for lineage determination

Highlighted

Highlighting mutations found in wastewater samples that correspond to the reference lineages



Spike mutational profile of samples from sites across South Africa and VOCs/VOIs processed in weeks 30 31 32, 2023

A combination of mutations with associated with lineage BA.2.86 have been found in KwaZulu Natal and Gauteng, with mutations signature to EG.5 and EG.5.1 also been found in Northern Gauteng in Epi week 31

Top x-axis:

Position of the spike protein

Blank rows or columns

Positions along the spike protein in which there are no changes in the spike protein amino acids of the samples compared to the Wuhan reference genome

Coloured blocks

Indicate changes (mutations) in the amino acids of the spike protein

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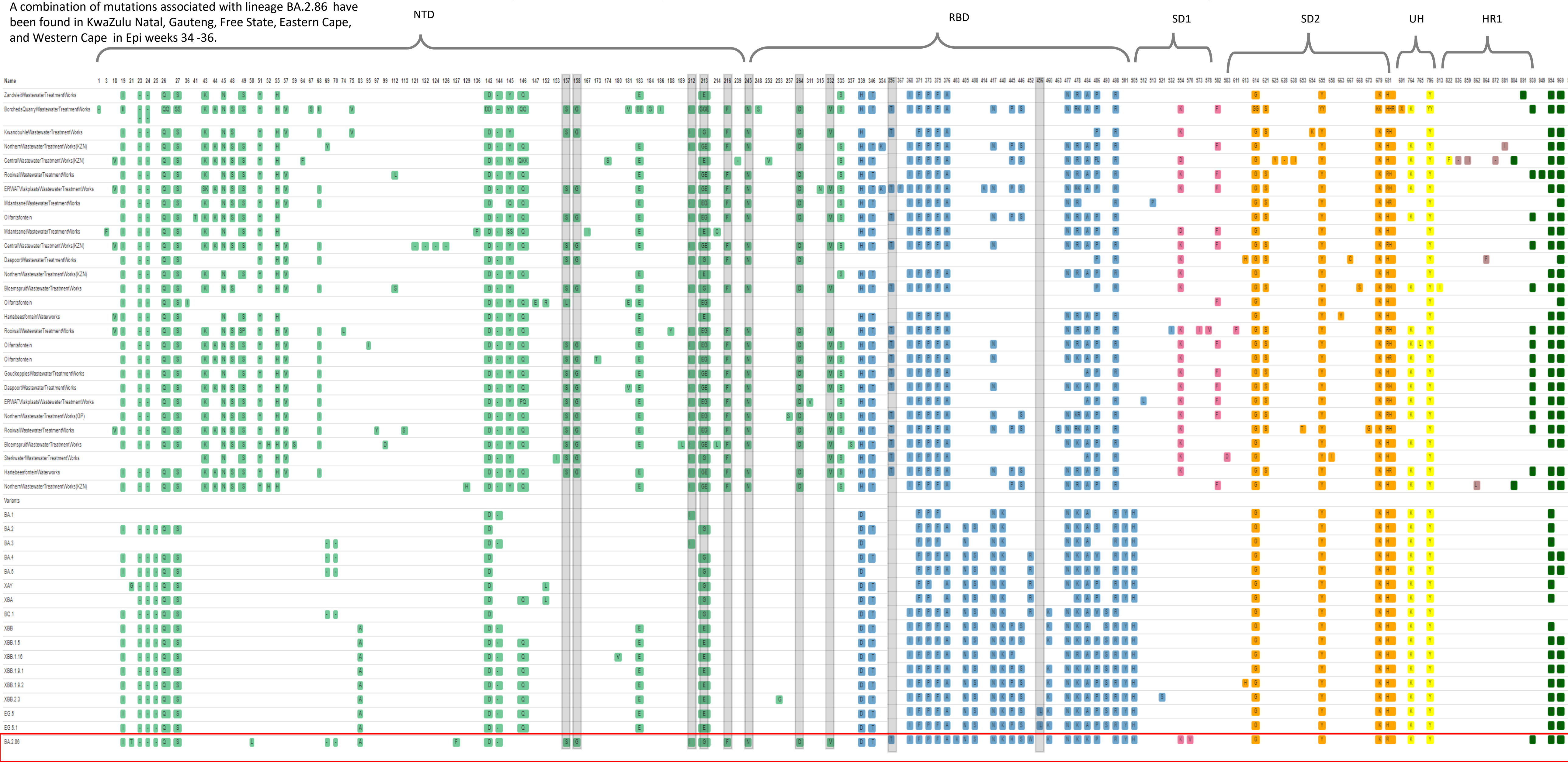
Spike mutational profile of samples from sites across South Africa and VOCs/VOIs processed in weeks 34 - 36, 2023

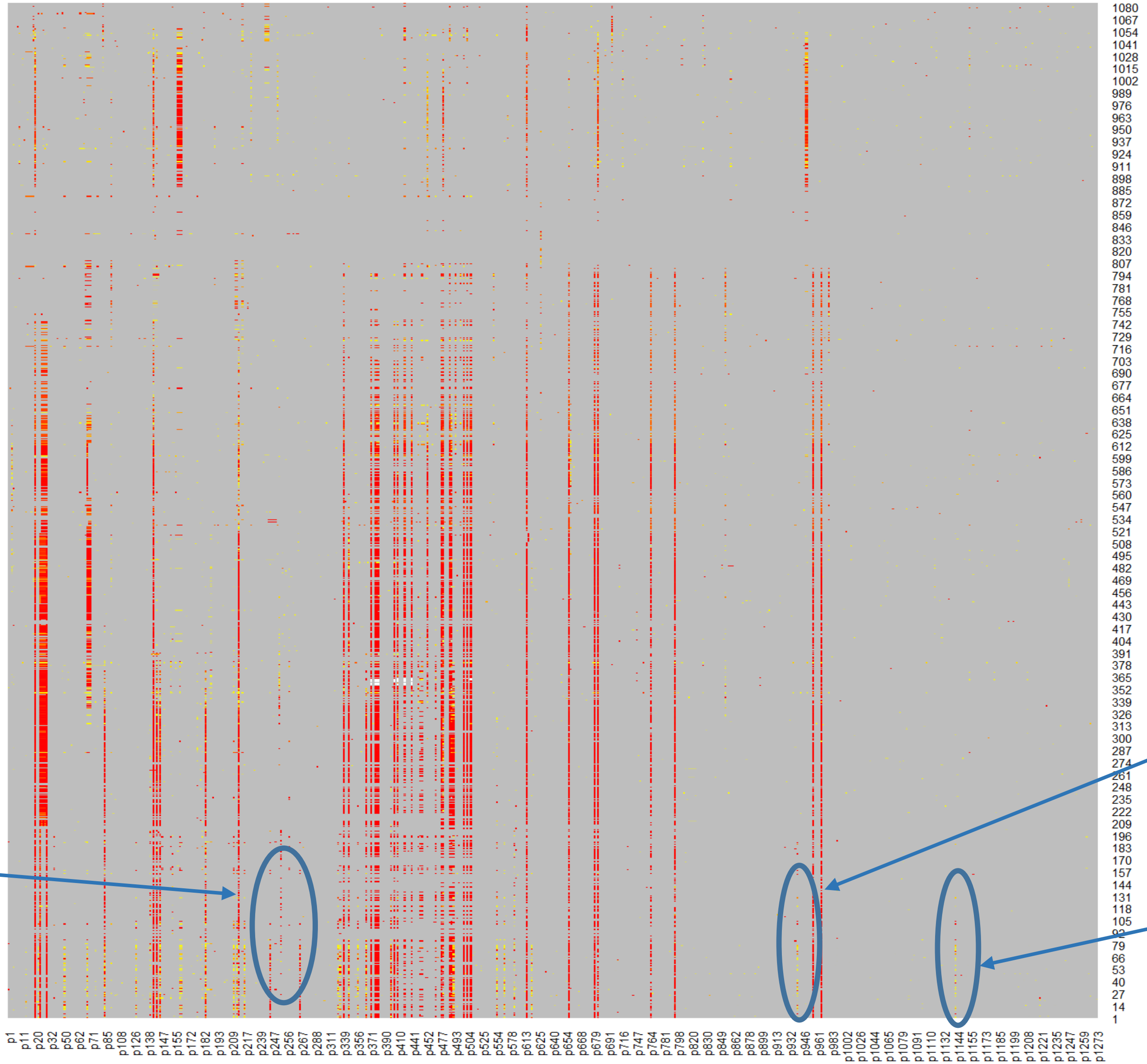
A combination of mutations associated with lineage BA.2.86 have been found in KwaZulu Natal, Gauteng, Free State, Eastern Cape, and Western Cape in Epi weeks 34 -36.



Amino acid mutations and frequency – Spike protein

XBB* is a recombinant of BA.2.10.1 and BA.2.75 that is characterised by one or more of the following mutations in the spike protein: V83A, Y144-, H146Q, Q183E, V213E,G252V, G339H, R346T, L368I, V445P, G446S, N460K, F486S, F490S

List of variants and sub-lineages of interest and concern (https://www.who.int/en/activities/tracking-SARS-CoV-2-variants)



BA.2.86 is a highly mutated sub-lineage of BA.2, recently circulating in Denmark, Israel and the United States of America and is characterised by one or more of the following mutations in the spike protein: R21T, S50L, H69-, V70-, V127F, F157S, R158G, N211-, L212I, V213G, L216F, H245N, A264D, I332V, K356T, R403K, V445H, N450D, L452W, N481K, V483-, E484K, E554K, A570V, P6215, 1670V, P681R, S939F, P1143L, Ins16:MPLF*

G252V

Mutation in spike protein associated with XBB* sub-lineages

S939F

Spike protein mutation associated with the BA.2.86 lineage

P1143L

Spike protein mutation associated with the BA.2.86 lineage

Heatmap showing patterns of emerging mutations in the spike region of SARS-CoV-2, collected from April, 2021 - August, 2023. Mutations appearing in yellow have a low read frequency, those appearing in orange have a medium read frequency and those appearing in red have a high read frequency. Mutations are included and updated weekly.

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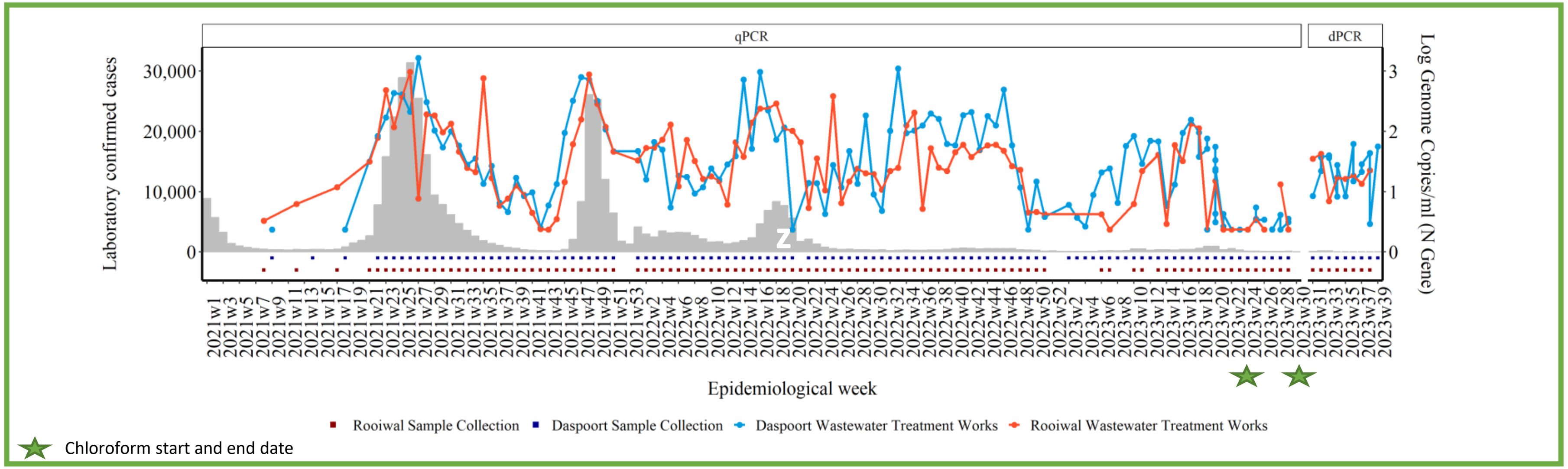
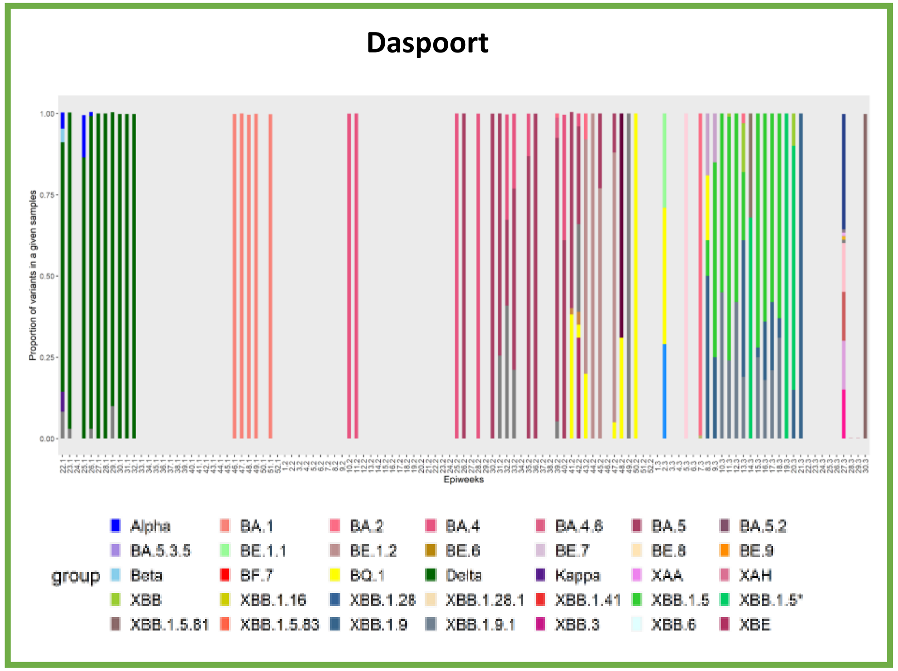
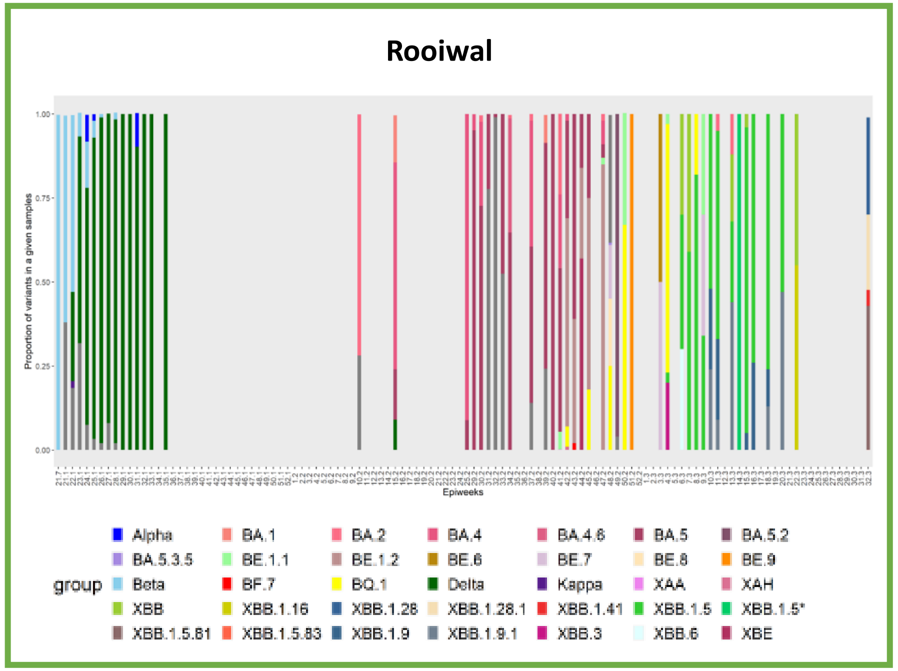
Gauteng - Tshwane



Daspoort



Rooiwal



SARS-CoV-2 levels and Genomic Results in Epi week 39:

- The SARS-CoV-2 levels in Daspoort WWTW increased 2-fold from Epi week 37 to Epi week 39 to moderate levels.
- No new results for Epi week 39 in Rooiwal WWTW. However, in Epi week 37 SARS-CoV-2 levels increased slightly.

**** Sequencing data ending in Epi week 32 in Rooiwal and 30 in Daspoort***

- Omicron lineages FD.2, XBB.1.5.49, XBB.1.5.17, XBB.1.5.15, XBB.1.5.25, FY.3, FY.1, XBB.1.22.1, XBB.1.22, FY.5 were circulating in Daspoort during Epi week 27. With XBB.1.5.81 dominating in Epi week 30.

- Omicron lineages XBB.1.5.81, XBB.1.28, XBB.1.28.1, XBB.1.4, XBB.1.5.83 were circulating in Rooiwal during epi week 32.

SNP Analysis:

- In Daspoort, mutations associated with XBB.1.5 (V83A, Q183E, R346T, L368I, F486P, F490S) were detected during Epi week 30.
- A combination of mutations (V127F, F157S, R158G, L212I, V213G, L216F, H245N, A264D, I332V, K356T, R403K, V483-, E554K, A570V, P621S, 1670V, S939F) associated with lineage BA.2.86 were found in Rooiwal, Gauteng

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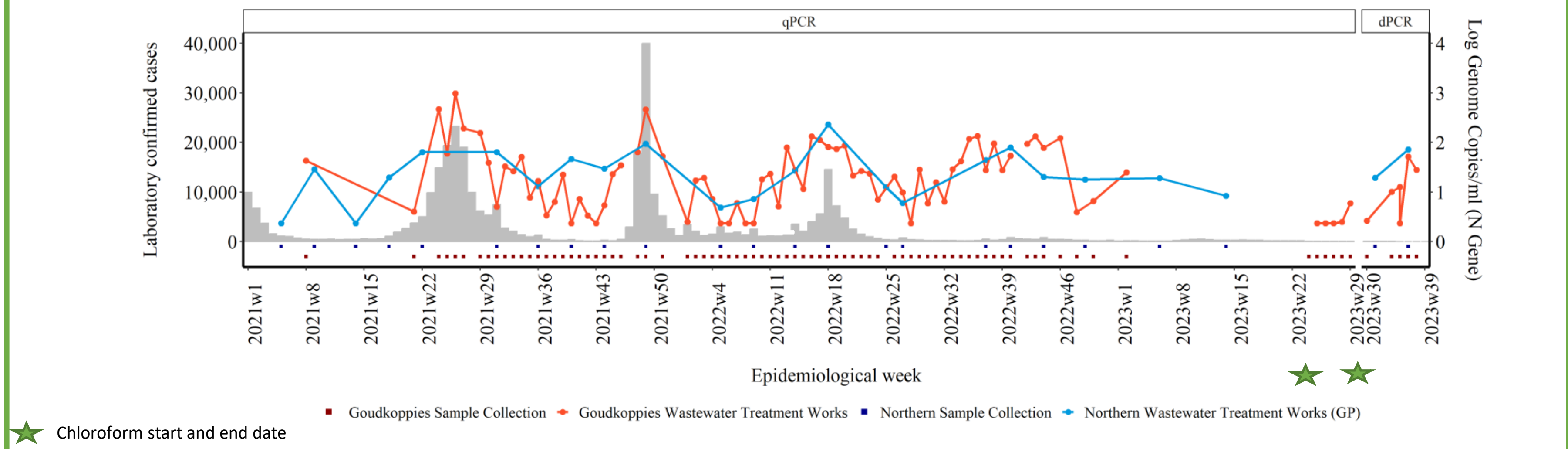
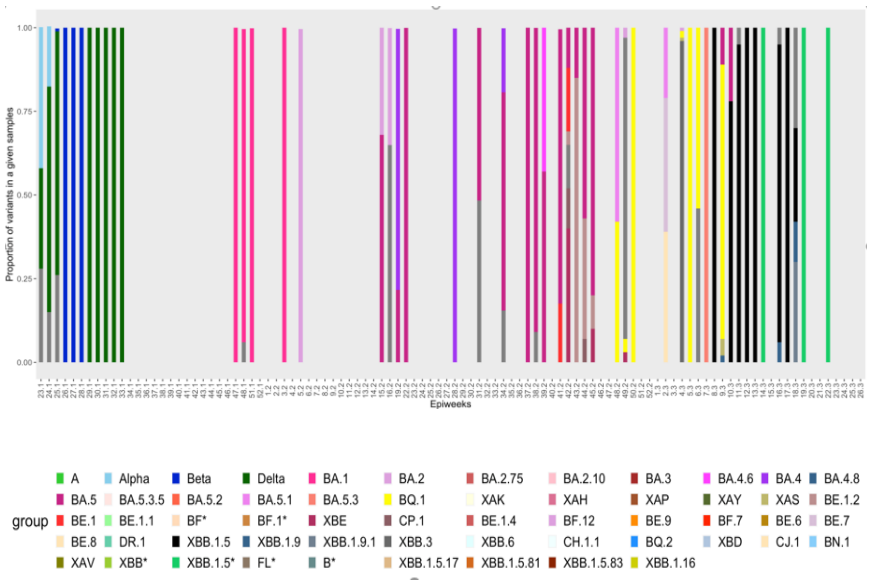
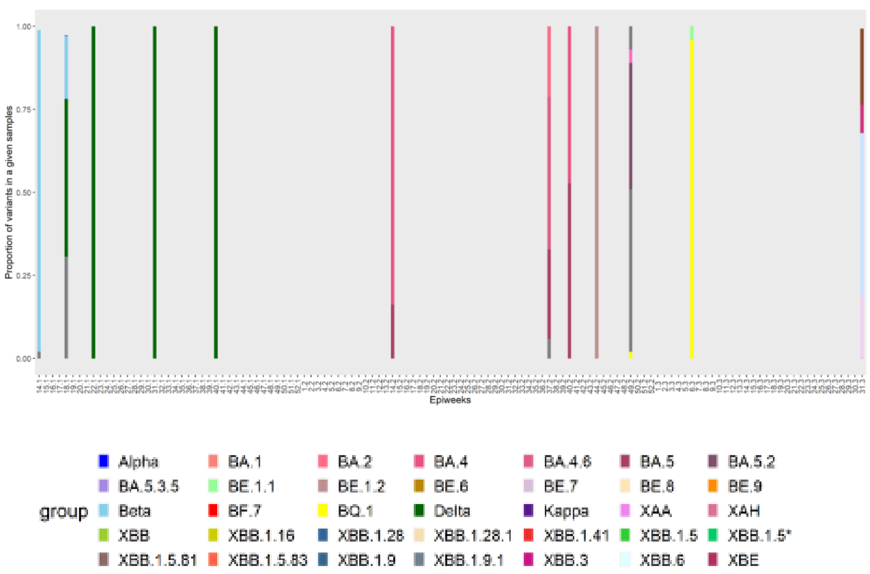
Gauteng - Johannesburg



Goudkoppies



Northern



SARS-CoV-2 levels and Genomic Results in Epi week 39:

- No new results for Epi week 39 in Goudkoppies WWTW. However, the SARS-CoV-2 levels in Goudkoppies WWTW increased from Epi week 30, and levels were moderate from Epi week 34.
- The SARS-CoV-2 levels increased to moderate in Northern WWTW in Epi week 36. No new results from Epi week 39 are available.

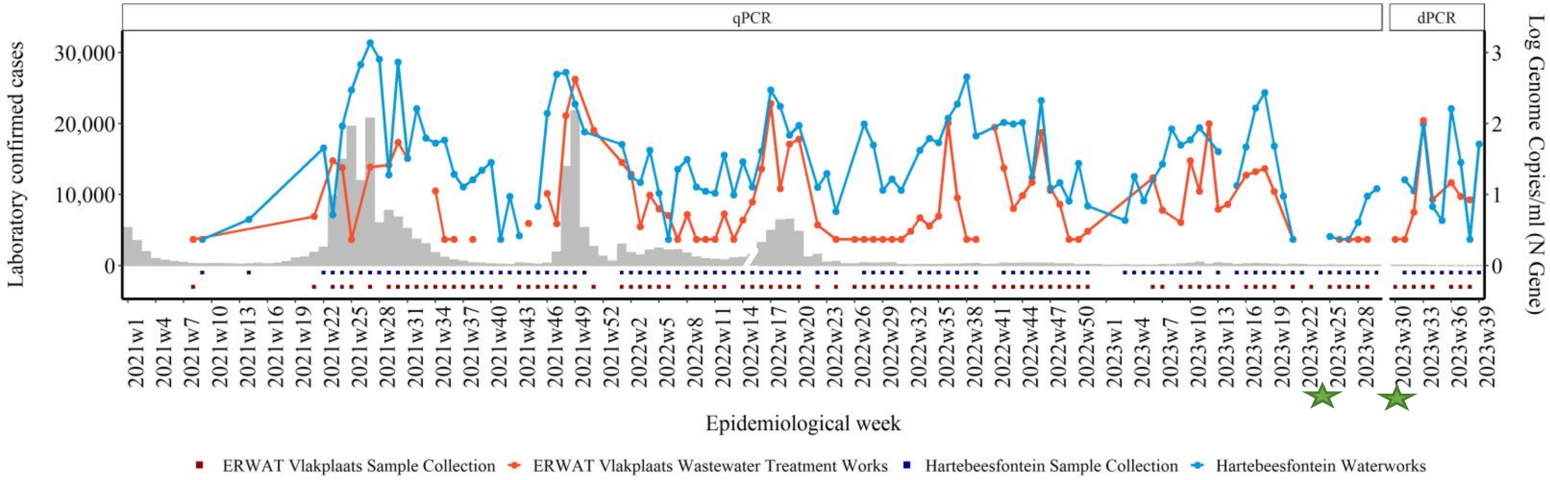
**** Sequencing data ending in Epi week 26 in Goudkoppies and 31 in Northern.***

- SARS-CoV-2 sequencing coverage in the Goudkoppies samples collected during Epi weeks 30-32 are too low for meaningful interpretation
- Omicron lineages XBB.1.5.28, XBB.1.41.1, XBB.2.4, XBB.1.42.1 and XBB.2.9 were circulating during Epi week 31 in Northern Gauteng.

SNP Analysis:

- SNP analysis could not be performed as the SARS-CoV-2 sequencing coverage in the Goudkoppies samples collected during Epi weeks 30-32 are too low for meaningful interpretation
- In Northern Gauteng, mutations associated with EG.5 and EG.5.1 (F456L) and XBB.1.5 (V83A, Q183E, R346T, L368I, F486P, F490S) were detected during Epi week 31.

Gauteng - Ekurhuleni



★ Chloroform start and end date

SARS-CoV-2 levels and Genomic Results in Epi week 39:

- The SARS-CoV-2 levels in Hartebeesfontein WWTW increased significantly from low levels in Epi week 33, to moderate levels in Epi week 39.
- No new result for Epi week 39, however, as of Epi week 37, SARS-CoV-2 levels decreased in Vlakplaats WWTW, after a slight increase in Epi week 36.

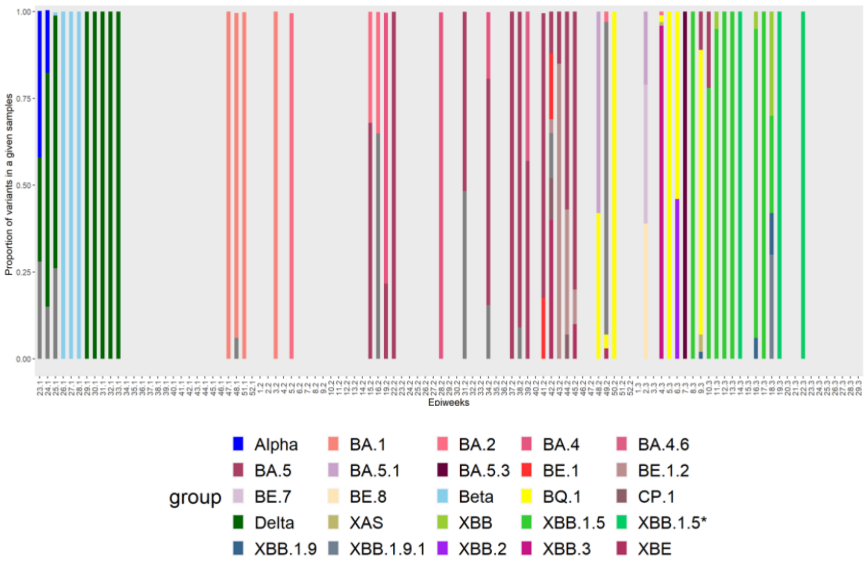
**** Sequencing data ending in Epi week 29 in Erwat and 32 in Hartebeesfontein.***

- SARS-CoV-2 sequencing coverage in the Vlakplaats samples collected during Epi weeks 30-32 are too low for meaningful interpretation
- Omicron lineages XBB.1.5.28, XBB.2.9, XBB.2.4, XBB.2.8 and XBB.1.27 were circulating in Epi weeks 30 at the Hartebeesfontein water treatment plant

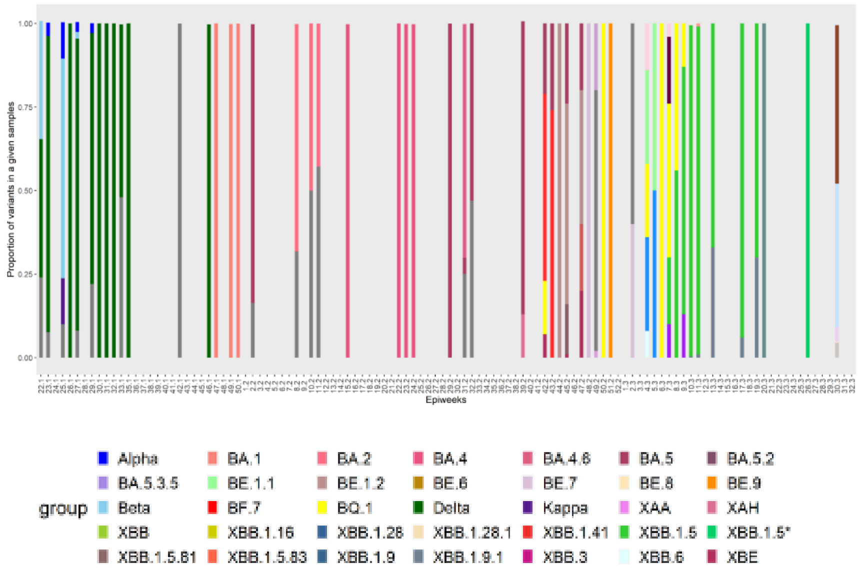
SNP Analysis:

- SNP analysis could not be performed as the SARS-CoV-2 sequencing coverage in the Vlakplaats samples collected during Epi weeks 30-32 are too low for meaningful interpretation
- A combination of mutations associated with the XBB 1.15 lineage (V83A, Q183E, R346T, L368I, F486P, F490S) were detected in Hartrbeesfontein during Epi weeks 32.

ERWAT Vlakplaat



Hartebeesfontein



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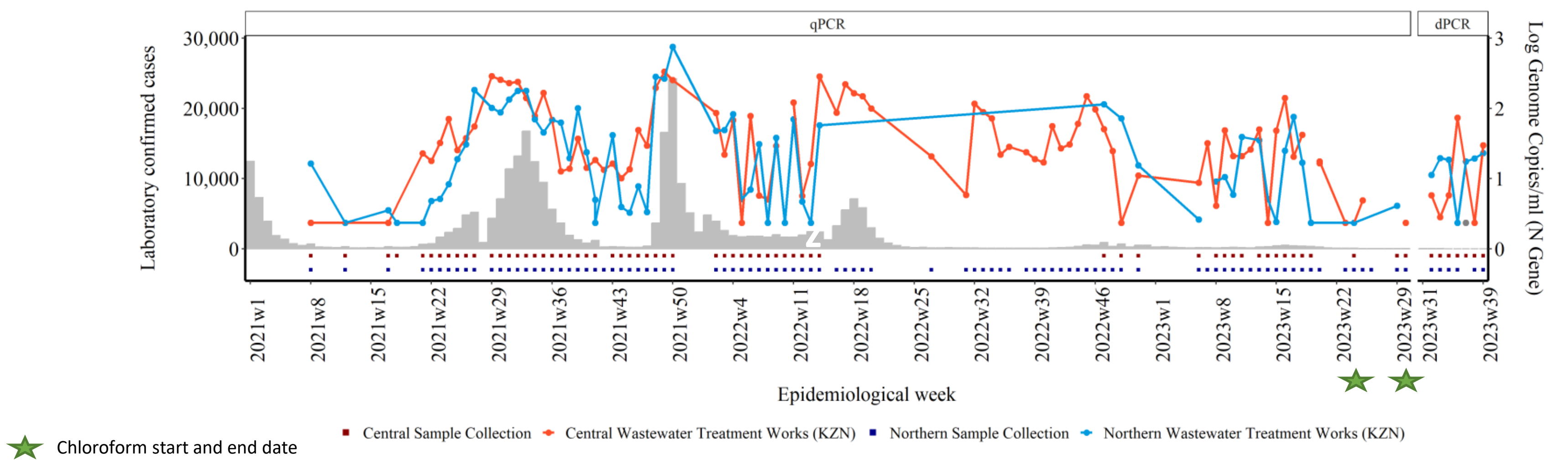
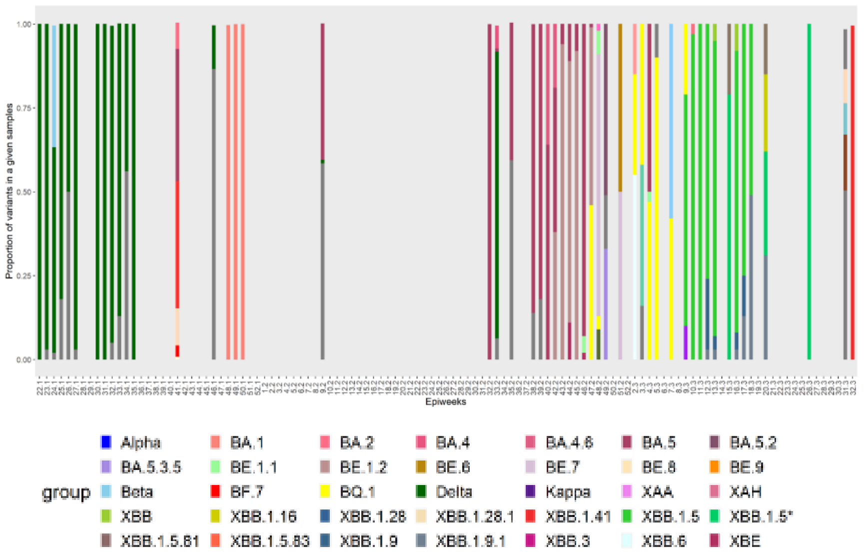
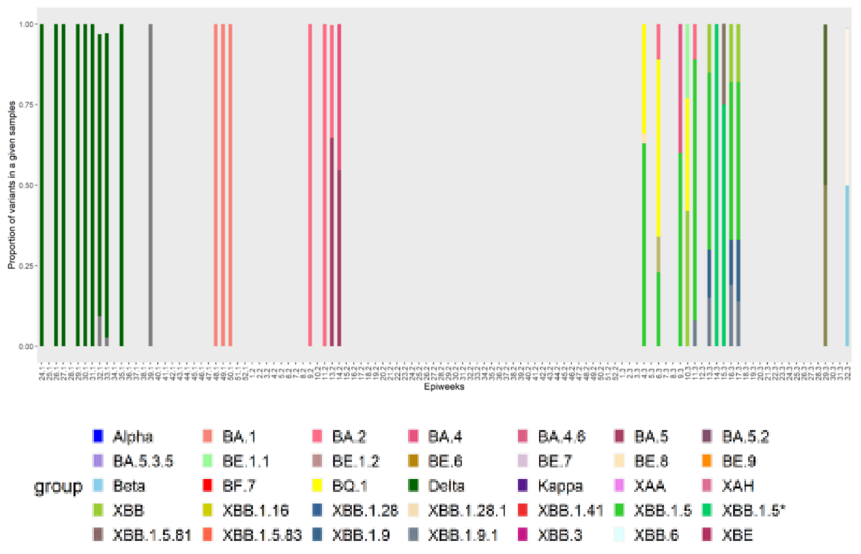
KwaZulu-Natal - eThekwini



Central



North



SARS-CoV-2 levels and Genomic Results in Epi week 39:

- SARS-CoV-2 levels in Central WWTW in Epi week 39 showed a sharp increase from low levels in Epi week 38 (1 log copy/ml) to moderate levels (2 log copies/ml).
- SARS-CoV-2 levels increased from low to moderate in week Epi 36 in Northern WWTW and remain low in Epi week 39.

**** Sequencing data ending in Epi week 32 in Central and North.***

- XBB.1.5.91, XBB.1.41.1, BA.2.1, EG.2, FL.2.4, XBB.1.41 were circulating in Epi week 31, with XBB.1.41 dominating in Epi week 32 at the Central eThekwini water treatment plant

- In eThekwini North, FL.25, BA.2.16, BA.2.15 were circulating during Epi week 32

SNP Analysis:

- In both Central and Northern eThekwini mutations associated with BA.2.86 (R21T, S50L, V127F, F157S, R158G, L212I, V213G) and XBB sub-lineages (V83A, Q183E, R346T, L368I, F486P, F490S) were detected during Epi weeks 31 and 32

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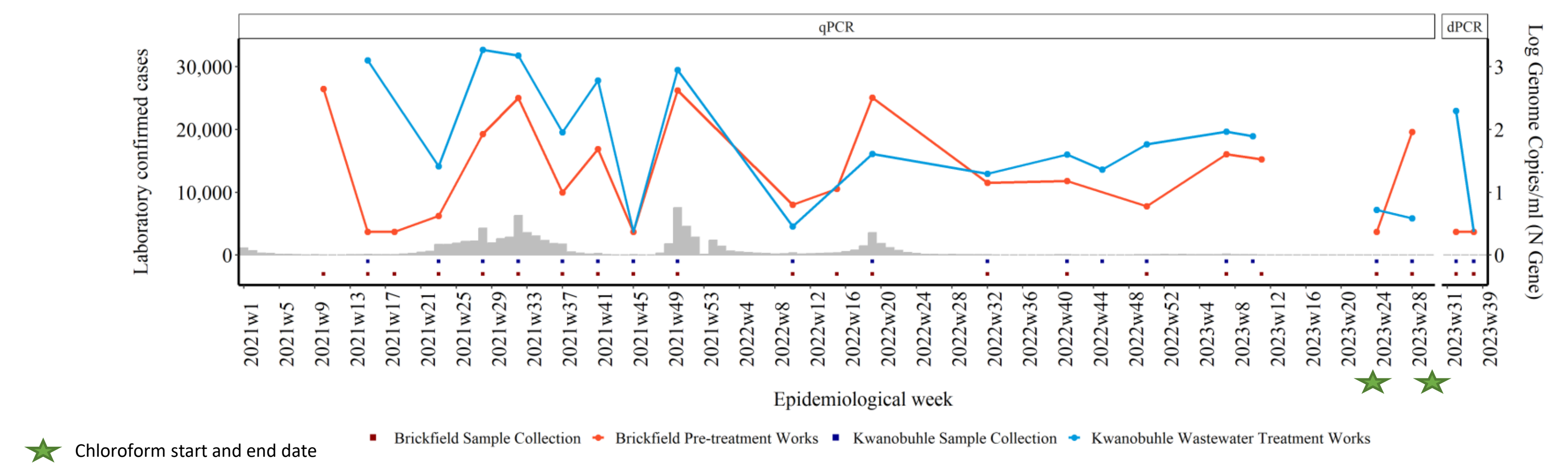
Eastern Cape – Nelson Mandela



SARS-CoV-2 levels and Genomic Results in Epi week 39:

- As of Epi week 37, a sharp decline in SARS-CoV-2 levels were seen in Kwanobuhle WWTW. No new results are available for Epi week 39.
- SARS-CoV-2 levels increased from low to intermediate from Epi week 24 to Epi week 31 in Brickfield Pre-treatment works. As of Epi week 37, SARS-CoV-2 levels remain low. No new results are available for Epi week 39.

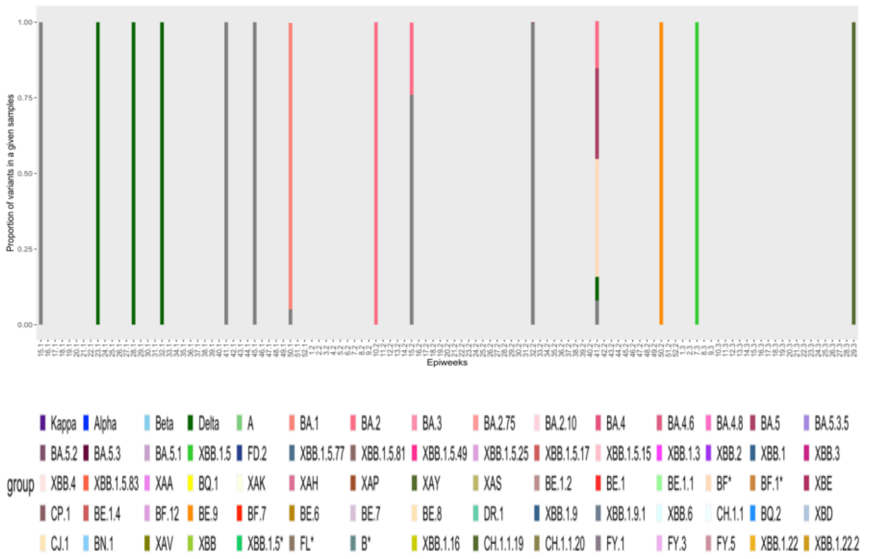
**** Sequencing data ending in Epi week 29 in Brickfield and 32 in Kwanobuhle.***

- SARS-CoV-2 sequencing coverage in the Brickfield samples collected during Epi weeks 30-32 are too low for meaningful interpretation
- Omicron lineages XBB.1.5.81, XBB.1.5.32 and XBB.1.5.27 were circulating in Kwanobuhle during epi week 32.

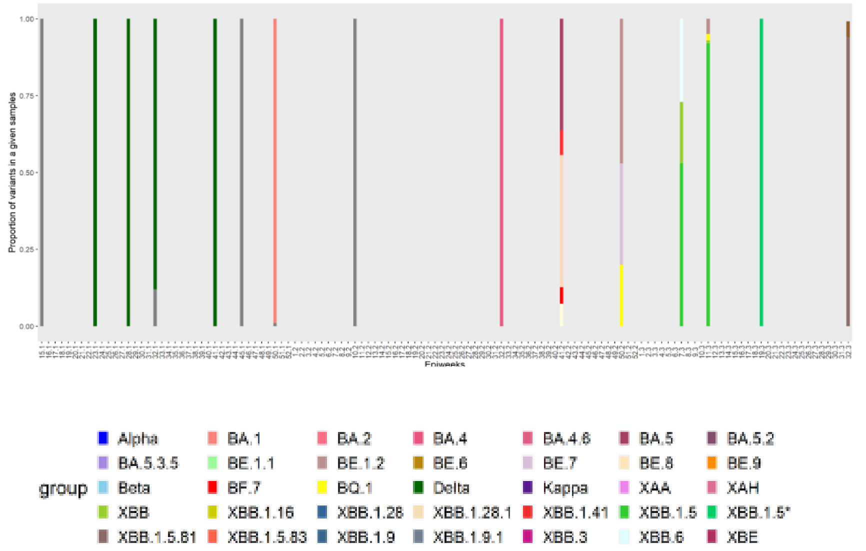
SNP Analysis:

- SNP analysis could not be performed as the SARS-CoV-2 sequencing coverage in the Brickfield samples collected during Epi weeks 30-32 are too low for meaningful interpretation
- Mutations associated with XBB.1.5 (V83A, Q183E, R346T, L368I, F486P, F490S) have been found in Kwanobuhle during Epi weeks 30 and 31. A mutation (R403K) associated with BA.2.86 was also detected in Kwanobuhle during Epi week 32

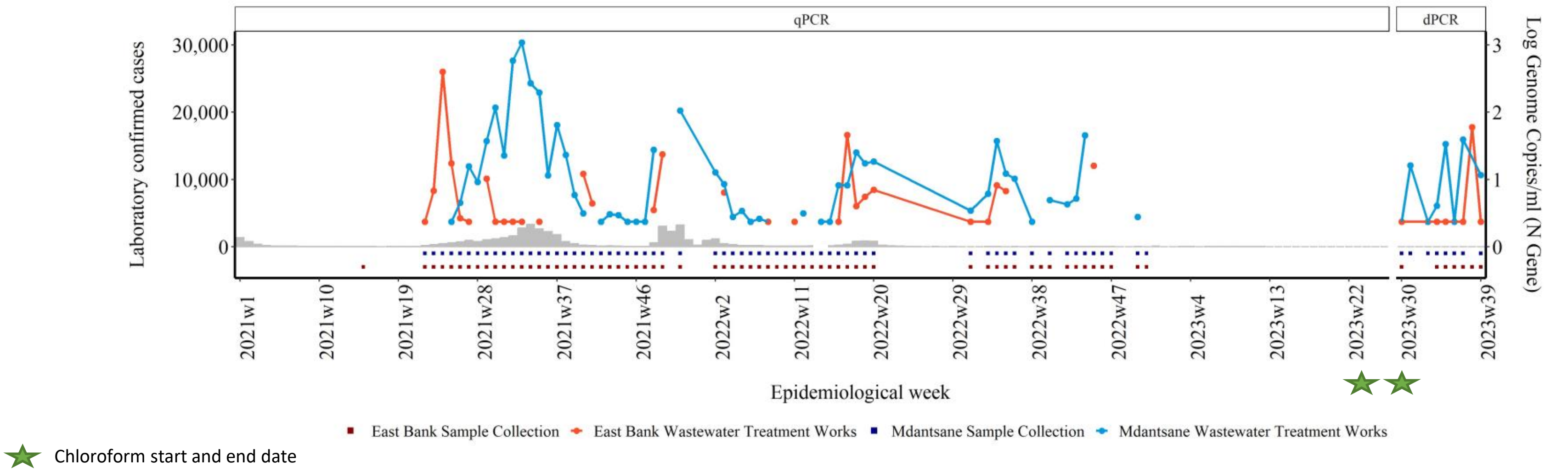
Brickfield



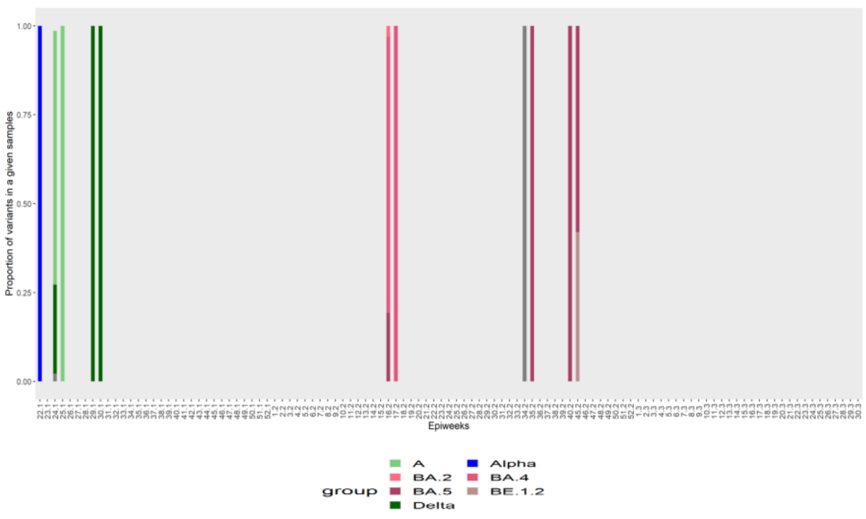
Kwanobuhle



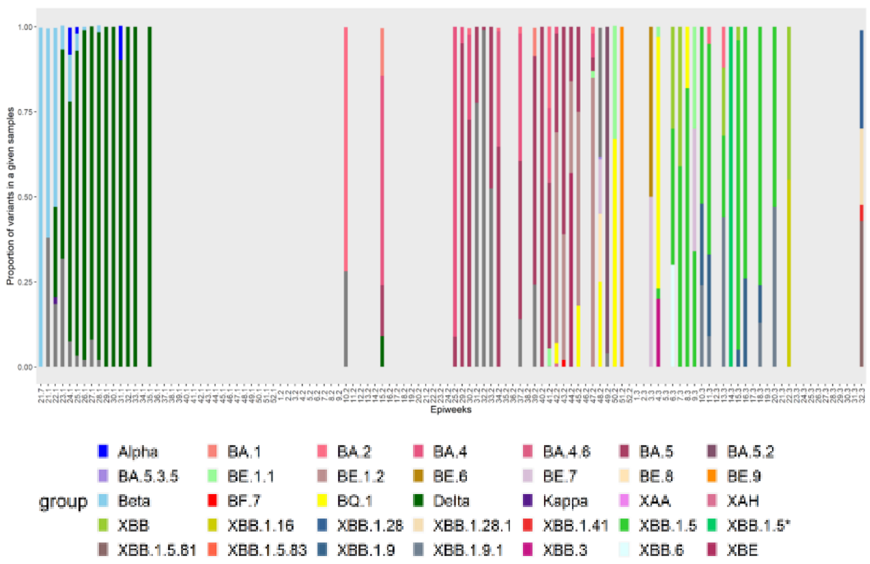
Eastern Cape – Buffalo City



Eastbank



Mdantsane



SARS-CoV-2 levels and Genomic Results in Epi week 39:

- In Epi week 39, SARS-CoV-2 levels in Mdantsane WWTW remain low after an increase in SARS-CoV-2 levels was seen in Epi week 35.
- SARS-CoV-2 levels in East Bank WWTW in Epi week 39 remain low after an increase was seen in Epi week 38.

**** Sequencing data ending in Epi week 30 in Eastbank and 31 in Mdantsane.***

- SARS-CoV-2 sequencing coverage in the Eastbank samples collected during Epi weeks 30-32 are too low for meaningful interpretation .

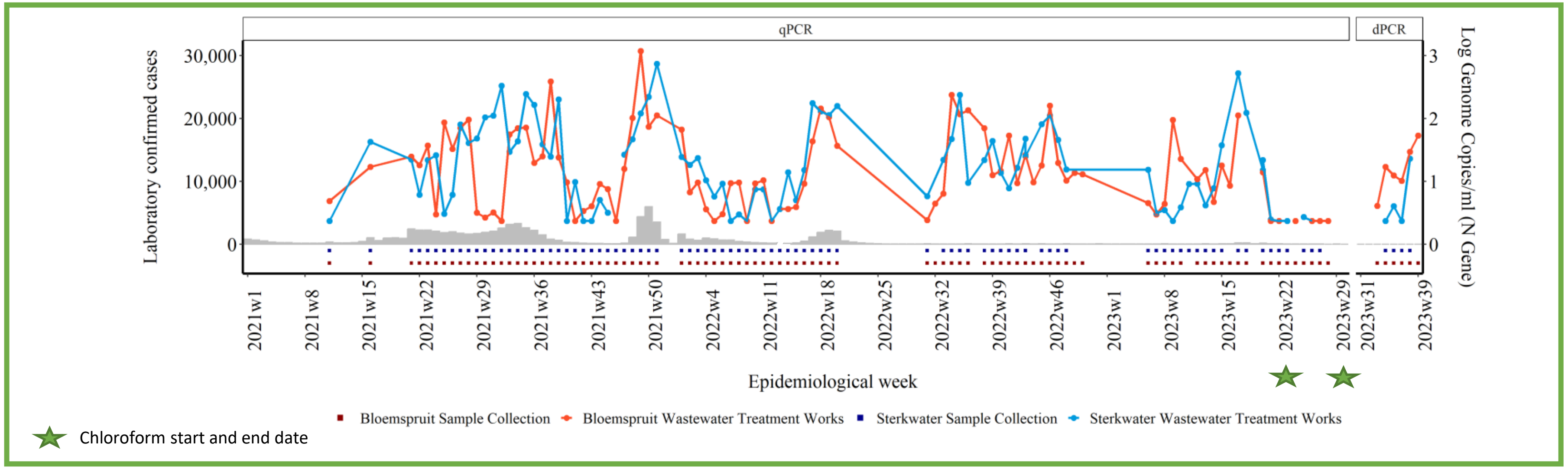
- Omicron lineage XBB.1.5.88 was the dominant lineage circulating in Mdantsane during Epi week 31, with XBB.1.5.32, XBB.1.5.56, and XBB sublineages also in circulation

SNP Analysis:

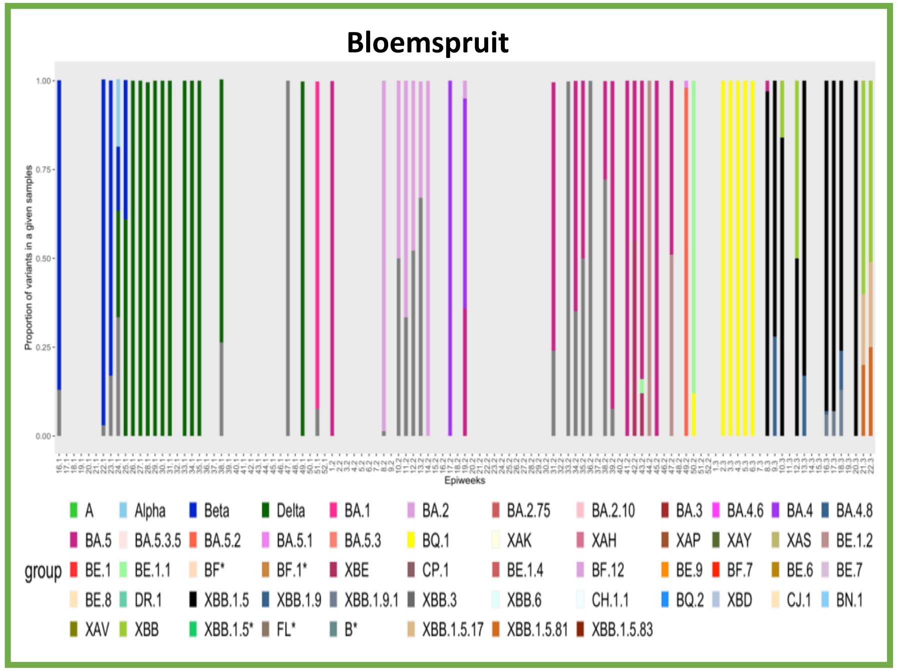
- SNP analysis could not be performed as the SARS-CoV-2 sequencing coverage in the Eastbank samples collected during Epi weeks 30-32 are too low for meaningful interpretation

- A combination of mutations (V83A, Q183E, R346T, L368I, F486P, F490S) associated with lineage XBB 1.15 have been found in Mdantsane during Epi weeks 30 and 31.

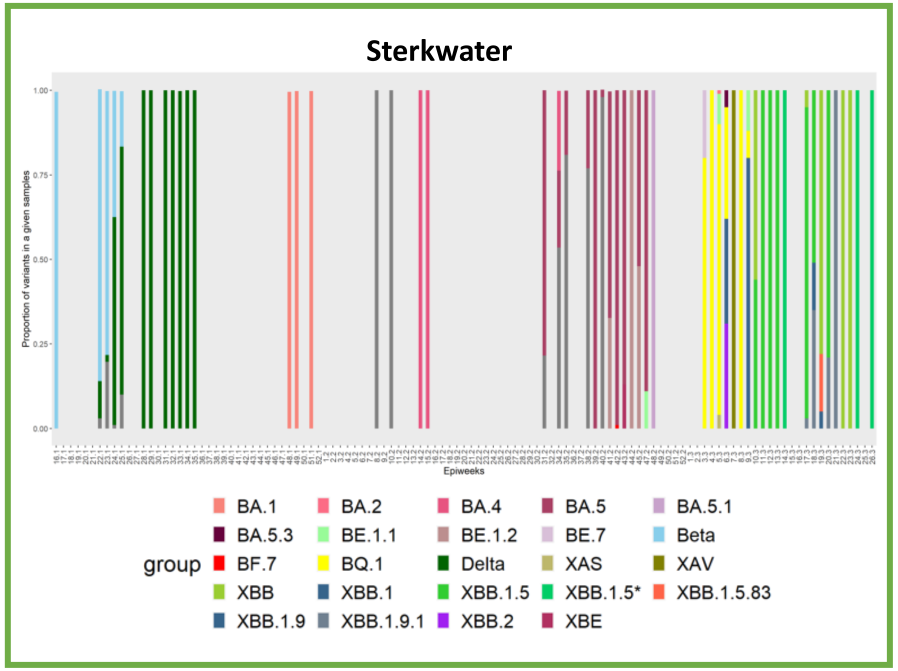
Free State – Mangaung



Bloemspruit



Sterkwater



SARS-CoV-2 levels and Genomic Results in Epi week 39:

- In Bloemspruit WWTW, an increase to moderate SARS-CoV-2 levels were seen in Epi week 39.
- A sharp increase in SARS-CoV-2 levels were seen in Sterkwater WWTW, and levels remain low to moderate in Epi week 39.

*** Sequencing data ending in Epi week 22 in Bloemspruit and 26 in Sterkwater.**

- SARS-CoV-2 sequencing coverage in the Bloemspruit and Sterkwater samples collected during Epi weeks 30-32 are too low for meaningful interpretation

SNP Analysis:

- SNP analysis could not be performed as the SARS-CoV-2 sequencing coverage in the Bloemspruit and Sterkwater samples collected during Epi weeks 30-32 are too low for meaningful interpretation

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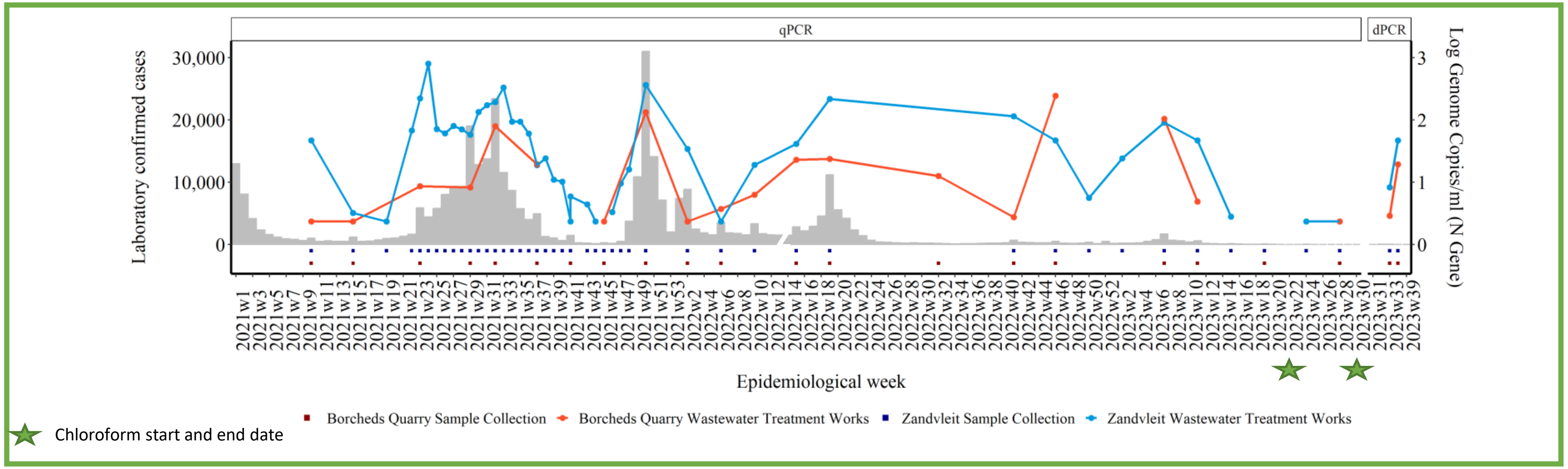
Western Cape – City of Cape Town



SARS-CoV-2 levels and Genomic Results in Epi week 39:

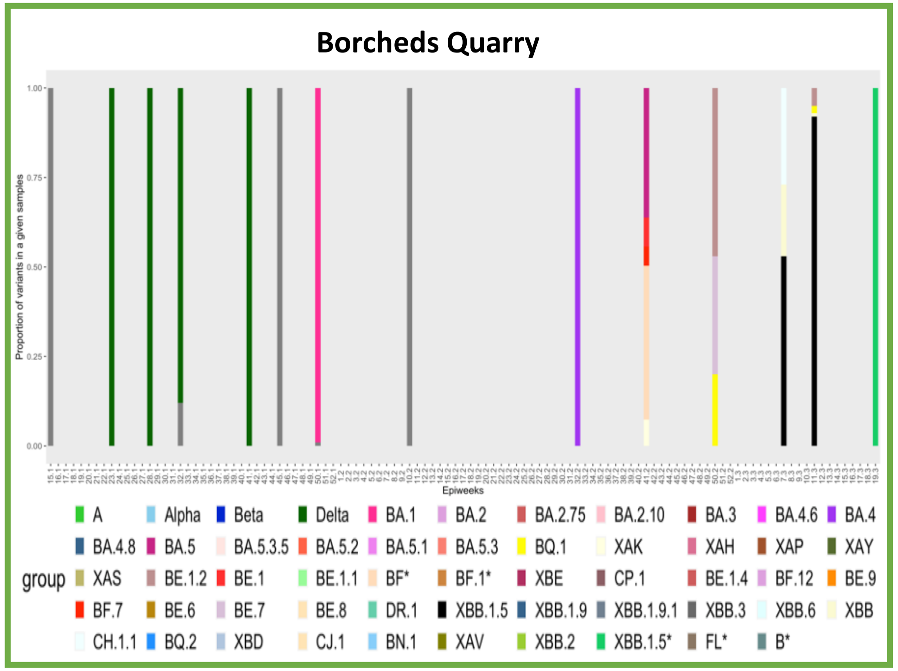
- No new results for Epi week 39. However, in Epi week 37, SARS-CoV-2 levels in Borcheds Quarry WWTW increased 2-fold to moderate levels.
- No new results for Epi week 39. However, a 2-fold increase in SARS-CoV-2 levels were observed in Zandevleit WWTW in Epi week 37.

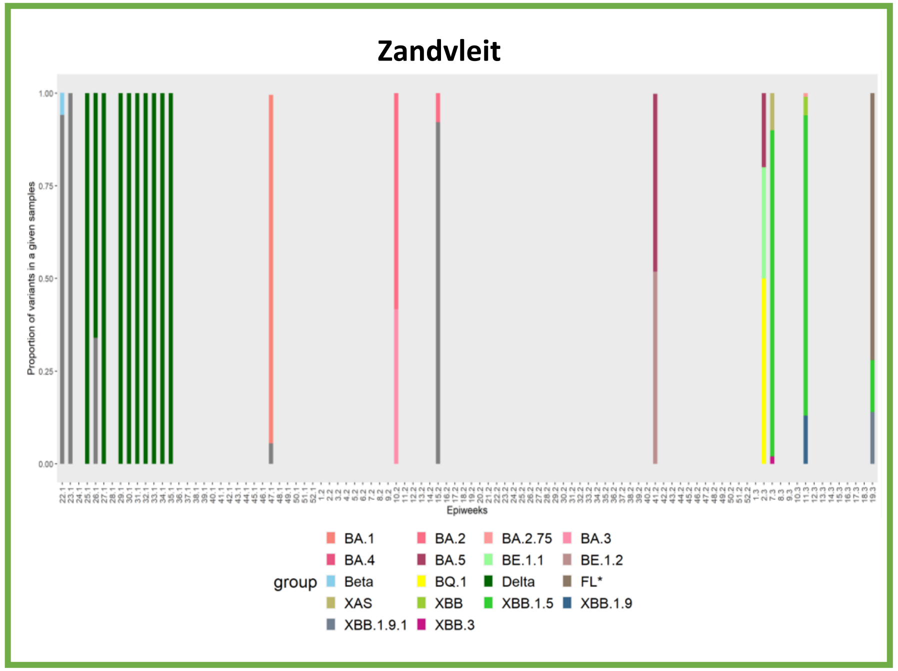
**** Sequencing data ending in Epi week 22 in Bloemspruit and 26 in Sterkwater.***

- Freyja lineage determination could not be performed as no samples were collected from both Cape Town wastewater treatment plants during Epi weeks 30-32.

SNP Analysis:

- SNP analysis could not be performed as no samples were collected from both Cape Town wastewater treatment plants during Epi weeks 30-32.


Borcheds Quarry


Zandvleit

COLLABORATORS

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