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FOREWORD

In this issue:

Syndromic respiratory illness surveillance programmes coordinated by the NICD include pneumonia surveillance and two influenza-like illness (ILI) programmes: systematic ILI surveillance at public health clinics (ILI-PHC surveillance programme) and the Viral Watch programme (ILI-Viral Watch) at private practices. Two reports in this issue detail influenza surveillance in South Africa for the period 2020 – 2021 and for weeks 1 through 32 of 2022, bringing 'flu surveillance information up to date. Since the COVID-19 pandemic, 2022 has seen a return of South Africa's typical influenza season with the B/Victoria and A(H3N2) viruses predominating. Individuals, and especially those in high-risk categories, are therefore encouraged to receive the annual 'flu vaccine.

Also in this issue is the malaria vector surveillance report for 2021. The period under review confirmed the perennial presence of four malaria vector mosquito species and the occurrence of several other *Anopheles* species that may also be contributing to malaria transmission in South Africa. Given South Africa's aim to eliminate malaria in the near future, periodic assessments of malaria risk and receptivity by vector surveillance is strongly recommended.

This is the final issue for 2022 and we wish all our readers and contributors a safe and joyous holiday season.

Basil Brooke, Editor

RESPIRATORY PATHOGEN EPIDEMIOLOGY FROM THE SYSTEMATIC INFLUENZA-LIKE ILLNESS AND PNEUMONIA SURVEILLANCE PROGRAMMES, SOUTH AFRICA, 2020-2021

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Summary

Syndromic respiratory illness surveillance programmes coordinated by the National Institute for Communicable Diseases (NICD) include pneumonia surveillance and two influenza-like illness (ILI) programmes: systematic ILI surveillance at public health clinics (ILI-PHC surveillance programme) and the Viral Watch programme (ILI-Viral Watch) at private practices. Respiratory samples collected from enrolled individuals meeting case definitions at sentinel sites were tested for influenza, respiratory syncytial virus (RSV), *Bordetella pertussis* and severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) by real-time polymerase chain reaction.

Influenza was detected in all three programmes in 2020 (168/6 467, 2.6%) and 2021 (445/9 312, 4.8%). In 2020, influenza circulated mainly in the Western Cape Province prior to the start of the normal winter influenza season. In 2020, following the restrictions put in place for coronavirus disease identified in 2019 (COVID-19), influenza circulation decreased with no influenza season occurring (usually May to August). Influenza circulation was low at the start of 2021, but increased outside of the normal influenza season towards the end of 2021 (late spring, weeks 37 to 49). In the pneumonia surveillance programme in 2020, influenza A (H1N1)pdm09 (28/33, 84.8%) was most commonly detected followed by a few influenza B/Victoria (4/33, 12.1%) viruses. In 2021, the most common types and subtypes detected were influenza A (H1N1)pdm09 (108/217, 49.8%), influenza B/Victoria (57/217, 26.3%) and influenza A (H3N2) (35/217, 16.1%). Similar to influenza, no RSV season was observed in either year, although the virus was still detected in 2020 (643/6 467, 9.9%) and 2021 (522/9 312, 5.6%) in all three programmes. The most common RSV subgroup detected in the pneumonia surveillance programme in 2020 was RSV subgroup A (458/549, 83.4%), followed by RSV subgroup B (74/549,13.8%). In 2021, the most common RSV subgroups were RSV subgroup A (220/425, 51.8%) and RSV subgroup B (199/425, 46.8%). A total of 11 cases of *B. pertussis* was detected in 2020 (10/6 065, 0.2%) and 2021 (1/9 055, 0.01%) from ILI-PHC and pneumonia surveillance programmes.

SARS-CoV-2 testing commenced in March 2020 and was detected in all three programmes in 2020 (862/5 730, 15.0%) and 2021 (2 361/9 378, 25.2%). By the end of 2021, four periods of increased transmission had been observed, each driven by a different SARS-CoV-2 variant. The first was from week 21 to week 44 in 2020, peaking in week 30 and driven by the ancestral virus. The second and third periods of increased transmission were from week 52 of 2020 to week 10 in 2021, peaking in week 53 of 2020 (Beta variant), and week 24 to week 40 of 2021 peaking in week 29 (Delta variant),

respectively. The fourth period of increased transmission, driven by the Omicron BA.1 variant, started in week 46 and was ongoing at the end of 2021.

In the first two years of the COVID-19 pandemic, these surveillance programmes managed to monitor four pathogens (influenza, RSV, *B. pertussis* and SARS-CoV-2). With these surveillance programmes, we were able to report changes in transmission of respiratory pathogens and detect SARS-CoV-2 variants. This should be a sustainable platform to monitor for future changes in SARS-CoV-2 transmission and changes in epidemiology of other pathogens.

Introduction

Surveillance systems are used globally to monitor trends in diseases, detect seasonal changes and describe epidemiological characteristics of patients. Surveillance additionally assists in identifying groups at risk for severe disease, such as people with human immunodeficiency virus (HIV).[1] A well-functioning sentinel surveillance programme plays a crucial role in respiratory disease detection, control and monitoring.[2]

Respiratory diseases are a major contributor to hospitalization and death.[3] In South Africa, surveillance programmes such as the pneumonia surveillance programme and influenza-like illness (ILI-PHC surveillance programme and Viral Watch ILI-Viral Watch) programmes are managed by the National Institute for Communicable Diseases (NICD) and are used to monitor respiratory pathogens of public health importance.[4]

Data collected through these programmes are used to describe epidemiological characteristics of individuals infected with respiratory pathogens of public health importance, determine vaccine effectiveness, and to provide data and make recommendations to policymakers and stakeholders that inform monitoring and control measures. These data are summarized and distributed through regular reports and peer-reviewed publications.[4-7]

The aim of this report is to describe the epidemiology of key respiratory pathogens in South Africa using data from 2020-2021 to inform policies and practices concerning their ongoing control and management.

Methods

A summary of each surveillance programme is included below. Respiratory specimens from ILI-PHC surveillance programme and pneumonia surveillance sites were tested for four pathogens: influenza virus, respiratory syncytial virus (RSV), *Bordetella pertussis* and SARS-CoV-2. ILI-Viral Watch specimens were tested for influenza, respiratory syncytial virus (RSV), and SARS-CoV-2. Influenza, RSV and *B. pertussis* were tested in programmes from 1 January 2020 through 31 December 2021. Testing for SARS-CoV-2 was initiated in all three programmes in week 10 of 2020 (starting 2 March). Prior to March 2020, a combined nasopharyngeal (NP) and oropharyngeal swab was collected. From March 2020, only NP swabs were collected.

Description of surveillance programmes and study sites

The pneumonia surveillance programme in South Africa is a hospital-based, active, sentinel surveillance programme established in 2009. In 2020-2021 it included five provinces namely; Gauteng (GP), North West (NW), KwaZulu-Natal (KZN), Western Cape (WC) and Mpumalanga (MP), and nine hospitals (Rahima Moosa Mother and Child Hospital (GP), Helen Joseph Hospital (GP), Edendale Hospital (KZN), Mapulaneng Hospital (MP), Matikwana Hospital (MP), Klerksdorp-Tshepong Hospital Complex (NW), Red Cross Children's Hospital (WC), Mitchell's Plain Hospital (WC) and Tintswalo Hospital (MP) (added February 2021).

The ILI-PHC programme was established in 2012 and enrols outpatients with influenza-like illness at sentinel sites in four provinces (KZN, NW, MP and WC). The systematic ILI programme included individuals who were tested in public health clinics (ILI-PHC). The sites are Eastridge Clinic (WC), Mitchell's Plain Clinic (WC), Jouberton Clinic (NW), Agincourt Clinic (MP, added in November 2020) and Edendale Clinic (KZN).

The ILI-Viral Watch programme was established in 1984 and is a prospective sentinel outpatient-based surveillance programme operating through a general practitioner network.[8] This programme focuses on influenza, RSV and SARS-CoV-2, and aims to describe the epidemiology of these pathogens in outpatients and determine influenza vaccine effectiveness. This programme is active in eight provinces; Eastern Cape (EC), Free State (FS), Limpopo (LP), Northern Cape (NC), GP, NW and WC. General practitioners submit nasopharyngeal (NP) swabs from patients who meet the ILI case definition and suspected SARS-CoV-2 (outpatient) (Table 1) for laboratory testing.

Table 1. Case definitions by age group and surveillance site/programme for the clinical syndromes included in the influenza-like illness (ILI-PHC surveillance programme and ILI-Viral Watch) and pneumonia surveillance programmes, South Africa, 2020-2021.

Case definition	Criteria	Surveillance site/programme
Influenza-like illness (ILI)	**Patients of all ages** Acute fever of ≥38°C and/or self-reported fever AND cough within the last 10 days	ILI-Viral Watch and ILI-PHC surveillance programme
Severe respiratory illness (SRI)	**2 days to <3 months** Any child hospitalised with a diagnosis of suspected sepsis or physician-diagnosed LRTI irrespective of signs and symptoms. **3 months to <5 years** Any child ≥3 months to <5 years hospitalised with physician-diagnosed LRTI including bronchiolitis, pneumonia, bronchitis and pleural effusion **≥5 years** Any person hospitalised with physician diagnosed-LRTI* or suspected COVID-19	Pneumonia surveillance
Suspected pertussis	Any patient presenting with cough illness of any duration and at least one of: paroxysms of cough, post-tussive vomiting, inspiratory whoop **OR** Infants <1 year with apnoea, with or without cyanosis	ILI-PHC surveillance programme Pneumonia surveillance ILI Viral Watch (on request only)
Suspected SARS-CoV-2 (outpatient)	Any person presenting with an acute (≤14 days) respiratory tract infection or other clinical illness compatible with COVID-19** and not meeting the ILI case definition	ILI-Viral Watch and ILI-PHC surveillance programme
Suspected SARS-CoV-2 (hospitalised)	Any person admitted with a physician-diagnosis of suspected COVID-19 and not meeting pneumonia surveillance case definition	Pneumonia surveillance

*LRTI =lower respiratory tract infection and includes suspected pulmonary TB, suspected pertussis

**Symptoms include ANY of the following respiratory symptoms: cough, sore throat, shortness of breath, anosmia (loss of sense of smell) or dysgeusia (alteration of the sense of taste), with or without other symptoms (which may include fever, weakness, myalgia or diarrhoea)

Sample and data collection

For the pneumonia and ILI-PHC surveillance programme, potentially eligible patients were approached for screening by a surveillance officer. For those meeting the case definitions and consented to inclusion, a paper-based or electronic case investigation form (CIF) was completed and uploaded to the NICD SQL (structured query language) database and an NP swab was collected for testing. In the ILI-Viral Watch programme, a short CIF form was completed by a physician and submitted to the NICD, which was then captured on an NICD Microsoft Access database. HIV status was determined based on testing undertaken as part of standard-of-care or medical record review. Samples were stored at 4°C before being transported on ice packs in cooler boxes to the NICD for testing within 72 hours of collection.

Laboratory testing for influenza, RSV, *B. pertussis* and SARS-CoV-2

Influenza A and B viruses, RSV and SARS-CoV-2 were tested at the NICD using a commercial multiplex RT-PCR assay (Allpex SARS-CoV-2/FluA/FluB/RSV PCR kit, Seegene Inc., Seoul, South Korea) A specimen was considered positive for SARS-CoV-2 when the PCR cycle threshold (C_t) was <40 for ≥1 of the gene targets S, N or RdRp. SARS-CoV-2 positive specimens were characterised on the Allplex™ SARS-CoV-2 Variants I and II PCR assays (Seegene Inc., Seoul, Korea) from March 2020 to June 2021, with some samples selected for sequencing. SARS-CoV-2 positive specimens were exclusively sequenced from July 2021 using the Illumina COVIDSeq protocol (Illumina, CA, USA). Influenza A and B positive specimens were subtyped using the US Centres for Disease Control and Prevention (CDC) RT-PCR protocol and reagents.[9] RSV A and B positive specimens were subgrouped using an in-house assay.[10]

Bordetella pertussis was tested using a previously described RT-PCR method.[11] A specimen was considered positive when the *IS481* and/or *ptxS1* gene targets are detected with a ct value of <45.

Data management and analysis

Data management was centralised at the NICD. All electronic data were saved on a Microsoft Access or SQL database. Data recorded for each enrolled patient included laboratory, clinical and demographic data. Data quality, including checks for missing data and duplicate entries, were managed by the CRDM data team.

Detection rates were calculated as the number of positive tests divided by the number of samples tested. A moving epidemic curve was generated using the Moving Epidemic Method (MEM), a sequential analysis using the R Language, available from: http://CRAN.R-project.org/web/package=mem, designed to calculate the duration, start and end of the annual influenza and RSV seasons. The detection rate for the year was plotted against the moving average for previous years (2009-2019) to determine the level of activity for the year using an algorithm.[12] MEM uses the historical 40th, 90th and 97.5th percentiles to calculate thresholds of activity, defined as:

- Epidemic threshold: Median of weekly values for all baseline years
- Low activity: Between epidemic threshold and 40th percentile
- Moderate activity: Between 40th and 90th percentiles
- High activity: Between 90th and 97.5th percentile
- Very high activity: 97.5th percentile and above

The season starts when the detection rate rises above the epidemic threshold and remains above the threshold for three consecutive weeks. The season ends when the detection rate falls below the epidemic threshold for three consecutive weeks. Data from 2020 and 2021 were plotted against the thresholds set by data collected from the ILI-PHC programme between 2013 and 2019 (pre-COVID-19 pandemic) and pneumonia surveillance between 2010 and 2019. All analyses were conducted using Stata (version 16, StataCorp LP, College Station, TX, USA).

Results

Patients enrolled and tested

In ILI-Viral Watch, 661 patients were enrolled from January 2020 through December 2021. These were tested for influenza, RSV, and *B. pertussis* (659/661, 99.7%), and for SARS-CoV-2 (532/661, 80.4%). From January 2020 through December 2021, 15 809 patients were enrolled in the two syndromic surveillance programmes conducted in the public sector (ILI- PHC and pneumonia surveillance). Of these, 15 355 (97.1%) samples were tested for respiratory pathogens (Figure 1). Of those individuals that were tested, 27.9% (4 283/15 355) were enrolled in the ILI-PHC surveillance programme, and 72.1% (11 072/15 355) were enrolled in the pneumonia surveillance programme. (Figure 1). In ILI-PHC surveillance programme, 4 279 specimens were tested for influenza, RSV and *B. pertussis* and, of these, 4 045 were also tested for SARS-CoV-2. Four specimens were exclusively tested for SARS-CoV-2.

In pneumonia surveillance, 97.9% (10 841/11 072) of specimens were tested for influenza, RSV and *B. pertussis*. From March 2020, 10 531 specimens were in addition tested for SARS-CoV-2 at the NICD. Of the 11 072 individuals enrolled, 231 (2.1%) were only tested for SARS-CoV-2 at the site laboratory with no additional specimen sent to the NICD. Individuals aged ≥15 years made up the majority of both ILI-PHC surveillance programme (2 946/4 283, 68.8%) and pneumonia surveillance cases (6 650/11 072, 60.1%). Among individuals aged <15 years in the ILI-PHC, the majority were aged ≤1 year old (532/752, 70.7%) (Table 2). Among individuals aged ≥15 years in the ILI-PHC, the majority were aged 25-44 years (1 601/2 946, 53.3%) (Table 3). Among individuals aged <15 years enrolled in the pneumonia surveillance programme in 2020 and 2021, most were in the ≤1year age group (3 473/4 422, 78.5%) (Table 4). Among individuals aged ≥15 years enrolled in the pneumonia surveillance programme, the majority of patients were aged 45-64 years (2 494/6 650, 37.5%) or 25-44 years (2 381/6 650, 35.8%) (Table 5).

The overall HIV prevalence was 26.0% (2 670/10 255) among patients in the pneumonia surveillance programme and 14.5% (598/4 131) among patients in the ILI-PHC surveillance programmes (Figure 2). The HIV prevalence varied by age group and surveillance programme case definition (Figure 2) with HIV prevalence being highest in the 25-44-year age group for individuals enrolled in ILI-PHC (378/1601, 23.6%) and pneumonia surveillance (1 356/2381, 57.0%) programmes.

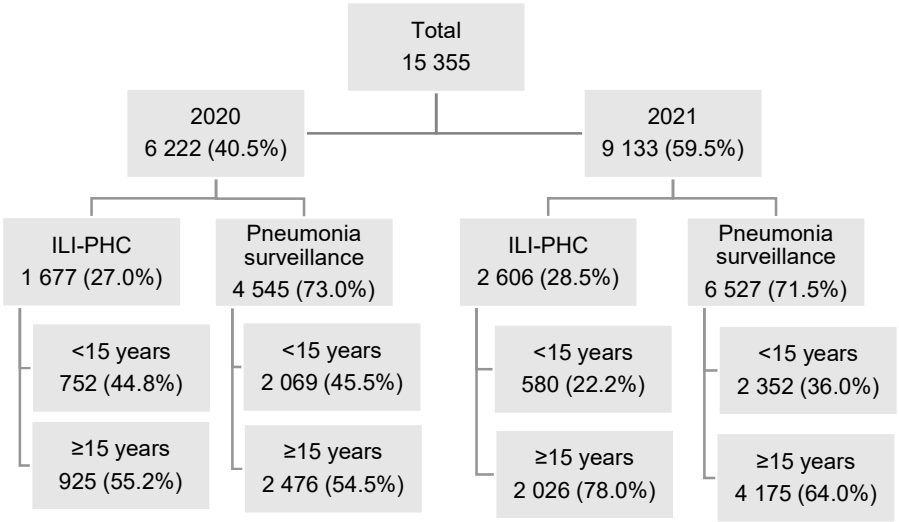

Figure 1. Numbers of samples tested for respiratory pathogens in the pneumonia surveillance and influenza-like illness in public health clinics (PHC) surveillance programme, South Africa, 2020-2021. Individuals <5 years in ILI-PHC n=904 and pneumonia surveillance n=4 131.

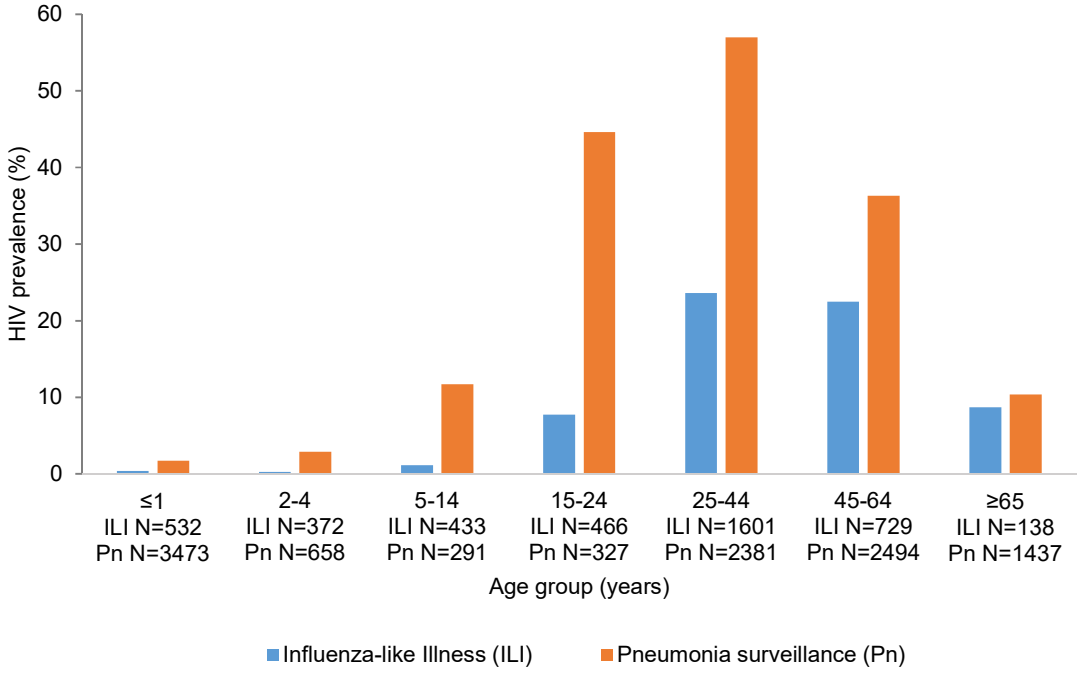

Figure 2. HIV prevalence by age group for individuals enrolled in the pneumonia surveillance and ILI-PHC surveillance programmes, South Africa, 2020-2021. The value below the age category depicts the total number enrolled in each surveillance programme per age group.

Respiratory pathogens

In 2020 and 2021 in the ILI-PHC surveillance programme, RSV was the most commonly detected pathogen in individuals <15 years old (128/1 337, 9.6%), followed by influenza (107/1 337, 8.0%), SARS-CoV-2 (76/1 337, 5.7%) and *B. pertussis* (2/1 337, 0.2%). In those patients <5 years old, RSV was most commonly detected, whereas SARS-CoV-2 was most commonly detected in individuals ≥5 -14 years old (41/76, 53.9%) (Table 2). In individuals aged ≥15 years old in the ILI-PHC surveillance programme, SARS-CoV-2 was the most commonly detected pathogen (720/2 946, 24.4%) followed by influenza (138/2 946, 4.7%) and RSV (53/2 946, 1.8%). *Bordetella pertussis* was not detected (Table 3).

Among individuals aged <15 years old enrolled in the pneumonia surveillance programme in 2020 and 2021, the most commonly detected pathogen was RSV (916/4 422, 20.7%), followed by SARS-CoV-2 (178/4 422, 4.0%), influenza (172/4 422, 3.9%) and *B. pertussis* (9/4 422, 0.2%) (Table 4). Overall, the in-hospital mortality was 1.0% (45/4 422) among patients <15 years old. Among individuals aged ≥15 years in the pneumonia surveillance programme, SARS-CoV-2 was the most commonly detected pathogen (2 149/6 650, 32.3%) followed by influenza (77/6 650, 1.2%) and RSV (58/6 650, 0.9%). *Bordetella pertussis* was not detected. Additionally, 25.8% (510/1 980) of individuals infected with

SARS-CoV-2 were people living with HIV. Of those infected with SARS-CoV-2, 17.1% (369/2 149) died. Overall, the in-hospital mortality was 13.0% (870/6 650) (Table 5).

Influenza

There was no influenza season declared in 2020 and 2021 in South Africa. In ILI-Viral Watch in 2020, influenza was detected in 83/402 (20.6%) patients. Out of the positive cases, 82/83 (98.8%) were before the national lockdown in March 2020. Of the total 83 cases, 79 (95.2%) were influenza A(H1N1)pdm09, three (3.6%) were influenza A(H3N2) and one (1.2%) influenza B/Victoria (Figure 3A). All cases detected were from the Western Cape (77/83, 92.8%) and Gauteng provinces (6/83, 7.2%). In 2021, 257 specimens were received from patients enrolled in ILI-Viral Watch in 6 provinces. Influenza was detected in 36 (14.0%) patients. Of these 36 cases, 17 (47.2%) were influenza A(H1N1)pdm09, seven (19.4%) were influenza A(H3N2), one (2.7%) was A subtype inconclusive and 11 (30.6%) influenza B/Victoria (Figure 3A). Most cases were detected from the Gauteng (183/257, 71.2%) followed by Western Cape (60/257, 23.3%), Eastern Cape (4/257, 1.6%), Free State (4/257, 1.6%), Mpumalanga (3/257 1.2%) and North West provinces (2/257, 0.8%). No specimens were received from Limpopo Province. An unseasonal increase in cases was detected towards the end of 2021.

In the ILI-PHC surveillance programme, of the 1 674 specimens tested in 2020, 52 (3.1%) were positive for influenza and detected before the national lockdown in March 2020. Of the 52 influenza positive specimens, 36 (69.2%) were identified as influenza A(H1N1)pdm09, one (1.9%) influenza A subtype inconclusive, 12 (23.1%) as influenza B/Victoria and three (5.8%) B lineage inconclusive due to low viral load (cycle threshold value (C_t) ≥35) for further characterization (Figure 3B). Of the 2 605 specimens tested in 2021, 193 (7.4%) were positive for influenza. Of the 193 influenza positive specimens, 100 (51.8%) were identified as influenza A(H1N1)pdm09, 28 (14.5%) were identified as influenza A(H3N2), five (2.6%) influenza A subtype inconclusive, 55 (28.5%) as influenza B/Victoria and five (2.6%) B lineage inconclusive due to too low viral load for further characterization (C_t≥35). (Figure 3B). Cases peaked in week 47 of 2021, with 23/62 (37.1%) influenza positive specimens. In 2020, the detection rate of influenza remained below the epidemic threshold, and transmissibility remained low during this period (Figure 3D). In contrast with 2020, the detection rate of influenza in 2021 remained below threshold until week 44 to week 48 where the detection rate was briefly in the low-moderate activity zone.

In the pneumonia surveillance programme in 2020, influenza was detected in 0.8% (33/4 391) of enrolled patients, and 28 (84.8%) were influenza A(H1N1)pdm09, one (0.3%) influenza A subtype inconclusive and four (12.1%) were influenza B/Victoria (Figure 6A). There was an increase in cases between weeks 6 through to week 15 with all specimens from the Western Cape Province, mostly influenza A(H1N1)pdm09 (24/33, 72.2%). All influenza cases were detected before the national lockdown in March 2020. In 2021, 3.4% (217/6 450) of enrolled patients had influenza detected, 108 (49.8%) were influenza A(H1N1)pdm09, 35 (16.1%) were influenza A(H3N2), 10 (4.6%) influenza A subtype inconclusive, 57 (26.3%) influenza B/Victoria, 6 (2.8%) B lineage inconclusive, 1 (0.5%) B lineage undetermined (Figure 3C). Moreover, the detection rate increased into the low activity zone for one week in week 12 in 2020 and again in week 38 to week 52 in 2021 (Figure 3E).

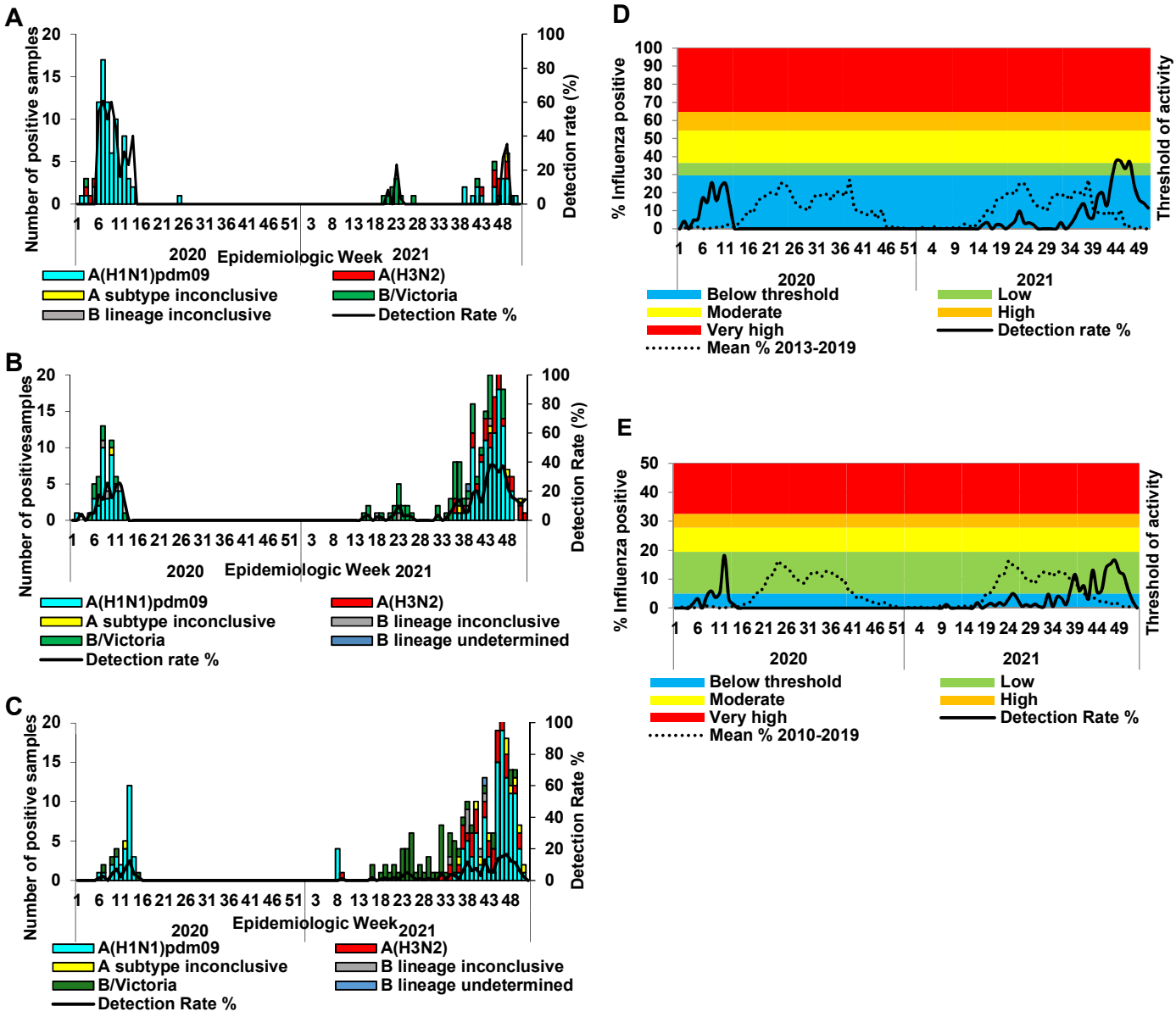

Figure 3. Number of influenza positive samples by influenza subtype and lineage and detection rate by week A) ILI-Viral Watch[1,7], B) ILI-PHC[2,7], C) pneumonia surveillance[3,7], and D) influenza percentage detections and epidemic threshold by epidemiological week for all age groups using the MEM[4] method, ILI-PHC[2,5] and E) pneumonia surveillance public hospitals[3,6], South Africa, 2020-2021.

[1]Specimens from patients with ILI-Viral Watch at 90 sentinel sites in 8 provinces

[2]Specimens from patients with ILI-PHC at 5 sentinel sites in 4 provinces

[3]Specimens from patients with influenza-like illnesses at 7 sentinel sites in 5 provinces

[4]MEM- Moving Epidemic Method

[5]Thresholds based on 2013-2019 data

[6]Thresholds based on 2010-2019 data

[7]Inconclusive: insufficient viral load in sample ($C_t \geq 35$) and unable to characterise further

Detection rate only reported for weeks with >10 specimens submitted

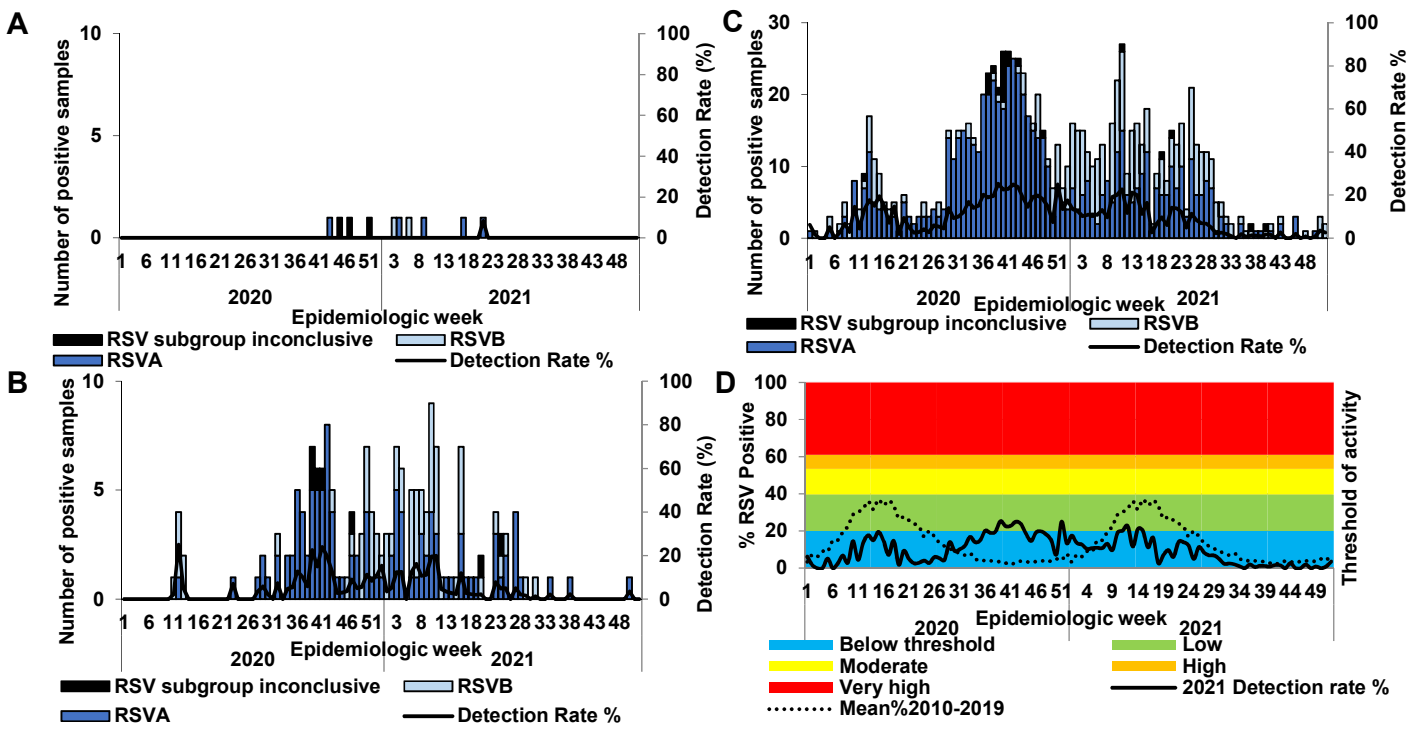

Figure 4. Number of RSV positive samples by subgroup and detection rate by week A) ILI-Viral Watch[1,6], B) ILI-PHC[2,6], C) pneumonia surveillance[3,6], and D) RSV detection rate and epidemic threshold by epidemiological week for all age groups using the MEM[4] method, pneumonia surveillance public hospitals[3,5], South Africa, 2020-2021.

[1]Specimens from patients with ILI-Viral Watch at 90 sentinel sites in 8 provinces

[2]Specimens from patients with ILI-PHC at 5 sentinel sites in 4 provinces

[3]Specimens from patients with influenza-like illnesses at 7 sentinel sites in 5 provinces

[4]MEM- Moving Epidemic Method

[5]Thresholds based on 2010-2019 data

[6]Inconclusive: insufficient viral load in sample ($C_t \geq 35$) and unable to characterise further

Detection rate only reported for weeks with >10 specimens submitted

As with influenza, no RSV season was declared during the 2020-2021 period. In the ILI-Viral Watch programme in 2020, RSV was detected in 1.0% (4/402) of specimens. Three of these patients were from Western Cape Province (3/4, 75.0%) and one was from Gauteng Province (1/4, 25.0%), (Figure 4A). In 2021, 257 specimens were received and tested. RSV was detected in six (2.3%) specimens. Of the six RSV positive patients, four (4/6, 66%) were from Gauteng Province and two (2/6, 33.3%) were from Western Cape Province.

In the ILI-PHC surveillance programme in 2020, RSV was detected in 5.4% (90/1 674) of patients tested (Figure 4B). Of the 90 RSV positive specimens, RSV subgroup A predominated (63/90, 70.0%), with sporadic detections of RSV subgroup B (22/90, 24.4%). There were 5/90 (5.6%) specimens that were inconclusive in subgroup due to too low viral load to allow further characterization. In 2021, RSV was detected in 3.4% (91/2 605) of patients tested (Figure 4B). Of these, 47/91 (51.6%) were characterised as RSV subgroup A, 41/91 (45.1%) were RSV subgroup B and 3/91 (3.3%) were inconclusive for subgroup due to low viral load.

RSV in the pneumonia surveillance programme circulated throughout 2020 and 2021 but had no defined season compared to previous years. Of the 4 391 specimens tested in 2020, 12.5% (549) were positive for RSV. RSV subgroup A (458/549, 83.4%) predominated (Figure 4C). Of the 6 450 specimens tested in 2021, 425 (6.5%) were positive for RSV. Of these positive specimens, 220/425 (51.8%) were characterised as RSV subgroup A, 199/425 (46.8%) were RSV subgroup B and 6/425 (1.4%) could not be subgrouped (RSV subgroup inconclusive). The RSV detection rate in pneumonia surveillance remained below the seasonal threshold for most of 2020-2021, except for brief durations in the low activity threshold between week 36 to week 43 in 2020 and week 11 and 14 in 2021 (Figure 4D).

Bordetella pertussis

Few *B. pertussis* cases were detected in the ILI-PHC surveillance programme and pneumonia surveillance programmes (2 and 9 cases, respectively) during 2020-2021. No *B. pertussis* cases were detected in the ILI-Viral Watch programme, as specimens were not routinely tested for this organism.

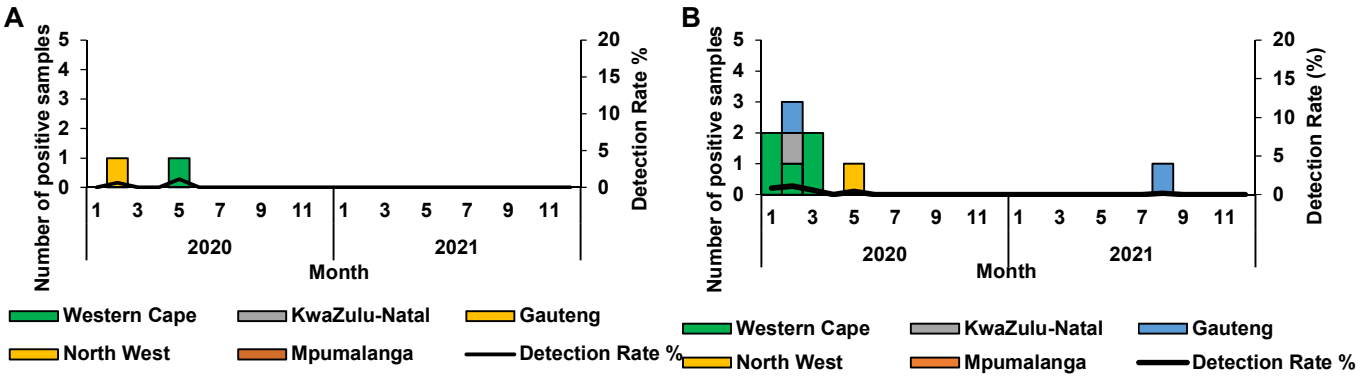

Figure 5. Number of B. pertussis positive samples and detection rate by province and month, A) ILI-PHC and B) pneumonia surveillance programme, South Africa, 2020-2021.

Among those who were enrolled in the ILI-PHC surveillance programme in 2020, 1 674 patients were tested for *B. pertussis*, two (0.1%) tested positive, one each from North West and Western Cape provinces. Both *B. pertussis* cases were detected in February and May and in individuals aged ≥5 years (Figure 5A). During 2021, 2 605 patients met the case definition, but none tested positive.

Among those who were enrolled in the pneumonia surveillance programme in 2020, 4 391 patients were tested for *B. pertussis*, of which eight (0.2%) tested positive. Of these, five (62.5%) were from Western Cape Province while North West, Gauteng and KwaZulu-Natal provinces had one case each (1/8, 12.5%) (Figure 5B). During 2021, 6 450 specimens were tested for *B. pertussis*, and one (0.02%) tested positive. This case was identified in Gauteng Province in a female aged ≤1 years old.

Severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2)

The first case of SARS-CoV-2 from the three surveillance programmes was detected in week 14 (week starting, 30 March 2020) in the ILI-Viral Watch programme. During March 2020 through December 2021, 3 676 cases were identified from all three surveillance programmes.

In the ILI-Viral Watch programme, 276 specimens were submitted for testing for SARS-CoV-2, and 37/276 (13.4%) of specimens were positive (Figure 6A). SARS-CoV-2 was detected in 63/257 (24.5%) specimens tested (Figure 6A). Of the 63 SARS-CoV-2 positive individuals, the majority were from Gauteng Province (48/63, 76.2%) followed by Western Cape Province (15/63, 23.8%).

In the ILI-PHC surveillance programme from March through December 2020, SARS-CoV-2 was detected in 15.3% (221/1 442) of specimens tested (Figure 6B). The majority of cases were reported from the Western Cape (95/221, 43.0%) and the North West provinces (75/221, 33.9%). From week 48 (week starting 23 November 2020) the detection rate started to increase for the second period of increase in transmission and peaked at 64.3% (9/14) in week 53 (week starting 27 December 2020). In 2021, SARS-CoV-2 was detected in 22.1% (575/2 605) of patients tested (Figure 6B). The majority of cases were detected from the North West (306/576, 53.1%) and KwaZulu-Natal provinces (108/576, 18.8%). During 2020-2021, four periods of increase in transmission were observed with detection rates peaking at 48.8%, 64.3%, 59.6% and 75.0% in weeks 22 of 2020 to week 36 of 2020, weeks 48 of 2020 to week 8 of 2021, weeks 25 of 2021 to week 39 of 2021 and weeks 48 of 2021 to week 52 of 2021.

In the pneumonia surveillance programme from March through December 2020, SARS-CoV-2 was detected in 15.1% (604/4 013) of patients tested (Figure 6C). The first case among pneumonia cases was detected in week 17 (week starting 20 April 2020). The majority of cases were detected from KwaZulu-Natal (167/604, 27.6%), Gauteng (156/604, 25.8%) and North West (143/604, 23.7%) provinces (Figure 6C).

In 2021, 26.4% (1 723/6 521) of SARS-CoV-2 cases were detected in patients tested. The majority of cases were in Gauteng (434/1 724, 25.2%) and North West provinces (364/1 724, 21.1%) (Figure 6C). During 2020-2021, four periods of increased transmission were observed with detection rates peaking at 48.4%, 56.9%, 59.19% and 61.6% in week 22 in 2020 to week 40 of 2020, week 50 of 2020 to week 10 of 2021, week 24 of 2021 to week 38 of 2021 and week 47 of 2021 to week 52 of 2021, respectively.

In the ILI-PHC surveillance programme (Figure 6C) and pneumonia surveillance programme (Figure 6E), ancestral virus was detected in the first period of increase in transmission. The Beta variant was predominant in the second period of increase in transmission. The third period of increase in transmission was dominated by the Delta variant. Finally, the Omicron (21K/BA.1) variant was detected in the fourth period of increase in transmission.

Ten SARS-CoV-2 and influenza co-infections and 33 SARS-CoV-2 and RSV co-infections were detected during 2020-2021 in the ILI-PHC and pneumonia surveillance programmes.

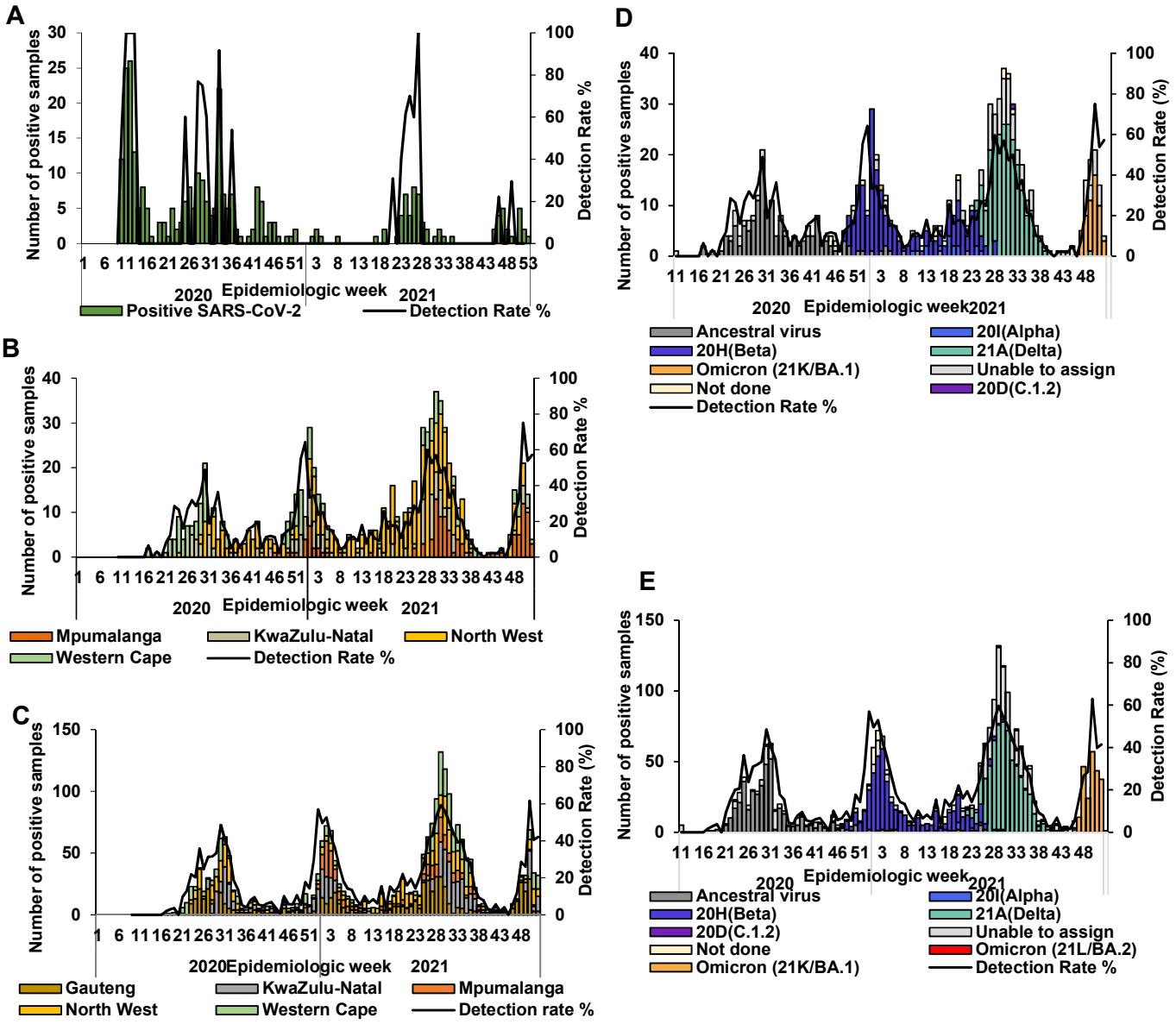

Figure 6. Numbers of SARS-CoV-2 positive samples by detection rate per week, A) ILI-Viral Watch[1], B) ILI-PHC[2], C) pneumonia surveillance programme[3], and by variant and detection rate per week D) ILI-PHC[2,4] and E) pneumonia surveillance programme[3,4], South Africa, 2020-2021.

[1]Specimens from patients with influenza-like illness at 90 sentinel sites in 8 provinces.

[2]Specimens from patients at 5 sentinel sites in 4 provinces who met suspected SARS-CoV-2 case definition and/or ILI-PHC surveillance programme case definition.

[3]Specimens from patients at 7 sentinel sites in 5 provinces who met suspected SARS-CoV-2 case definition and/or pneumonia surveillance case definition

[4]Unable to assign: no lineage assigned due to poor sequence quality **OR** low viral load ($C_t \geq 35$) **OR** variant PCR could not assign variant and no sequencing result

Detection rate only reported for weeks with >10 specimens submitted

Table 2. Demographic and clinical characteristics of patients aged <15 years enrolled in the ILI-PHC surveillance programme and testing positive for influenza, RSV, *Bordetella pertussis* and SARS-CoV-2, South Africa, 2020-2021.

	Enrolled n/N (%)	Influenza, n/N (%)	RSV, n/N (%)	*B. pertussis*, n/N (%)	SARS-CoV-2 n/N (%)
Characteristic					
Year					
2020	757/1,337 (57)	50/107 (47)	73/128 (57)	2/2 (100)	36/76 (47)
2021	580/1,337 (43)	57/107 (53)	55/128 (43)	0/2 (0)	40/76 (53)
Age group (years)					
≤1 years	532/1,337 (40)	27/107 (25)	74/128 (58)	0/2 (0)	24/76 (32)
2-4 years	372/1,337 (28)	40/107 (37)	40/128 (31)	0/2 (0)	11/76 (14)
5-14 years	433/1,337 (32)	40/107 (37)	14/128 (11)	2/2 (100)	41/76 (54)
Sex					
Male	704/1,337 (53)	48/107 (45)	69/128 (54)	0/2 (0)	36/76 (47)
Female	633/1,337 (47)	59/107 (55)	59/128 (46)	2/2 (100)	40/76 (53)
Province					
Mpumalanga	118/1,337 (9)	5/107 (5)	18/128 (14)	0/2 (0)	8/76 (11)
North West	104/1,337 (8)	7/107 (7)	5/128 (4)	1/2 (50)	5/76 (7)
KwaZulu-Natal	260/1,337 (19)	18/107 (17)	18/128 (14)	0/2 (0)	15/76 (20)
Western Cape	855/1,337 (64)	77/107 (72)	87/128 (68)	1/2 (50)	48/76 (63)
HIV-infected	8/1,287 (1)	2/105 (2)	0/122 (0)	0/2 (0)	1/75 (1)
Malnutrition*	50/899 (6)	5/66 (8)	6/115 (5)	0/0 (0)	0/35 (0)
Premature**	27/902 (3)	2/67 (3)	1/114 (1)	0/0 (0)	2/35 (6)
Underlying illness***	38/1,337 (3)	2/107 (2)	2/128 (2)	0/2 (0)	4/76 (5)

* Malnutrition defined by <-2 Z-scores (-2 standard deviations) of the mean weight for age in months and gender. This also includes any children recorded as having Kwashiorkor or Marasmus

**Premature defined as born before 37 completed weeks of gestation

***Underlying illness included any of: Asthma, other chronic lung diseases, chronic heart disease (valvular heart disease, coronary heart disease, or heart failure excluding hypertension), stroke, seizures, anaemia, liver disease (cirrhosis or liver failure), renal disease (nephrotic syndrome, chronic renal failure), immunocompromising conditions excluding HIV infection (organ transplant, immunosuppressive therapy, immunoglobulin deficiency, malignancy, autoimmune disease), diabetes, pregnancy, burns, obesity, asplenia, neurological disease (spinal cord injury, neuromuscular conditions)

Total number of specimens tested for influenza, RSV and *B. pertussis* n=1 337, and SARS-CoV-2 n=1 184

Table 3. Demographic and clinical characteristics of patients aged ≥15 years enrolled in the ILI-PHC surveillance programme and testing positive for influenza, RSV, *Bordetella pertussis* and SARS-CoV-2, South Africa, 2020-2021.

	Enrolled n/N (%)	Influenza, n/N (%)	RSV, n/N (%)	*B. pertussis*, n/N (%)	SARS-CoV-2 n/N (%)
Characteristic					
Year					
2020	920/2,946 (31)	2/138 (1)	17/53 (32)	0/0 (0)	185/720 (26)
2021	2,026/2,946 (69)	136/138 (99)	36/53 (68)	0/0 (0)	535/720 (74)
Age group (years)					
15-24	466/2,946 (16)	14/138 (10)	8/53 (15)	0/0 (0)	102/720 (14)
25-44	1,601/2,946 (54)	96/138 (70)	30/53 (57)	0/0 (0)	388/720 (54)
45-64	729/2,946 (25)	26/138 (19)	11/53 (21)	0/0 (0)	193/720 (27)
>65	138/2,946 (5)	2/138 (1)	2/53 (4)	0/0 (0)	36/720 (5)
Unknown	12/2,946 (0)	0/138 (0)	2/53 (4)	0/0 (0)	1/720 (0)
Sex					
Male	1,275/2,946 (43)	69/138 (50)	24/53 (45)	0/0 (0)	277/720 (38)
Female	1,671/2,946 (57)	69/138 (50)	29/53 (55)	0/0 (0)	443/720 (62)
Province					
Mpumalanga	257/2,946 (9)	2/138 (1)	3/53 (6)	0/0 (0)	85/720 (12)
North West	1,359/2,946 (46)	64/138 (46)	26/53 (49)	0/0 (0)	375/720 (52)
KwaZulu-Natal	580/2,946 (20)	30/138 (22)	18/53 (34)	0/0 (0)	135/720 (19)
Western Cape	750/2,946 (25)	42/138 (30)	6/53 (11)	0/0 (0)	125/720 (17)
HIV-infected	590/2,844 (21)	30/137 (22)	16/49 (33)	0/0 (0)	143/700 (20)
Underlying illness*	346/2,946 (12)	18/138 (13)	6/53 (11)	0/0 (0)	71/720 (10)

*Underlying illness included any of: Asthma, other chronic lung diseases, chronic heart disease (valvular heart disease, coronary heart disease, or heart failure excluding hypertension), stroke, seizures, anaemia, liver disease (cirrhosis or liver failure), renal disease (nephrotic syndrome, chronic renal failure), immunocompromising conditions excluding HIV infection (organ transplant, immunosuppressive therapy, immunoglobulin deficiency, malignancy, autoimmune disease), diabetes, pregnancy, burns, obesity, asplenia, neurological disease (spinal cord injury, neuromuscular conditions)

Total number of specimens tested for influenza, RSV and *B. pertussis* n=2 942, and SARS-CoV-2 n=2 861

Table 4. Demographic and clinical characteristics of patients aged <15 years enrolled in the pneumonia surveillance programme and testing positive for influenza, RSV, Bordetella pertussis and SARS-CoV-2, South Africa, 2020-2021.

	Enrolled n/N (%)	Influenza, n/N (%)	RSV, n/N (%)	*B. pertussis*, n/N (%)	SARS-CoV-2 n/N (%)
Characteristic					
Year					
2020	2,070/4,422 (47)	32/172 (19)	539/916 (59)	8/9 (89)	51/178 (29)
2021	2,352/4,422 (53)	140/172 (81)	377/916 (41)	1/9 (11)	127/178 (71)
Age group (years)					
≤1 years	3,473/4,422 (79)	112/172 (65)	847/916 (92)	8/9 (89)	148/178 (83)
2-4 years	658/4,422 (15)	43/172 (25)	62/916 (7)	1/9 (11)	16/178 (9)
5-14 years	291/4,422 (7)	17/172 (10)	7/916 (1)	0/9 (0)	14/178 (8)
Sex					
Male	2,562/4,422 (58)	104/172 (60)	475/916 (52)	4/9 (44)	102/178 (57)
Female	1,860/4,422 (42)	68/172 (40)	441/916 (48)	5/9 (56)	76/178 (43)
Province					
Mpumalanga	346/4,422 (8)	17/172 (10)	43/916 (5)	0/9 (0)	11/178 (6)
Gauteng	754/4,422 (17)	33/172 (19)	145/916 (16)	2/9 (22)	46/178 (26)
North West	333/4,422 (8)	12/172 (7)	58/916 (6)	1/9 (11)	15/178 (8)
KwaZulu-Natal	486/4,422 (11)	19/172 (11)	52/916 (6)	1/9 (11)	19/178 (11)
Western Cape	2,503/4,422 (57)	91/172 (53)	618/916 (67)	5/9 (56)	87/178 (49)
Symptom duration (≤ 10 days)	4,294/4,422 (97)	165/172 (96)	906/916 (99)	6/9 (67)	168/178 (94)
HIV-infected	113/4,041 (3)	2/149 (1)	6/846 (1)	0/9 (0)	7/161 (4)
Malnutrition*	719/4,077 (18)	33/152 (22)	118/905 (13)	1/9 (11)	38/161 (24)
Premature**	389/4,115 (9)	10/150 (7)	84/908 (9)	2/9 (22)	21/164 (13)
Underlying illness***	308/4,422 (7)	12/172 (7)	40/916 (4)	0/9 (0)	15/178 (8)
Hospital duration ≤5 days	4,058/4,422 (92)	160/172 (93)	863/916 (94)	7/9 (78)	147/178 (83)
ICU admission	77/4,412 (2)	2/172 (1)	17/914 (2)	1/9 (11)	4/177 (2)
In-hospital mortality	45/4,422 (1)	1/172 (1)	1/916 (0)	0/9 (0)	4/178 (2)

* Malnutrition defined by <-2 Z-scores (-2 standard deviations) of the mean weight for age in months and gender. This also includes any children recorded as having Kwashiorkor or Marasmus

**Premature defined as born before 37 completed weeks of gestation

***Underlying illness included any of: Asthma, other chronic lung diseases, chronic heart disease (valvular heart disease, coronary heart disease, or heart failure excluding hypertension), stroke, seizures, anaemia, liver disease (cirrhosis or liver failure), renal disease (nephrotic syndrome, chronic renal failure), immunocompromising conditions excluding HIV infection (organ transplant, immunosuppressive therapy, immunoglobulin deficiency, malignancy, autoimmune disease), diabetes, pregnancy, burns, obesity, asplenia, neurological disease (spinal cord injury, neuromuscular conditions)

Total number of specimens tested for influenza, RSV and *B. pertussis* n=4 355, and SARS-CoV-2 n=4 115

Table 5. Demographic and clinical characteristics of patients aged ≥15 years enrolled in the pneumonia surveillance programme and testing positive for influenza, RSV, Bordetella pertussis and SARS-CoV-2, South Africa, 2020-2021.

	Enrolled n/N (%)	Influenza, n/N (%)	RSV, n/N (%)	*B. pertussis*, n/N (%)	SARS-CoV-2 n/N (%)
Characteristic					
Year					
2020	2,475/6,650 (37)	1/77 (1)	10/58 (17)	0/0 (0)	553/2,149 (26)
2021	4,175/6,650 (63)	76/77 (99)	48/58 (83)	0/0 (0)	1,596/2,149 (74)
Age group (years)					
15-24	327/6,650 (5)	3/77 (4)	5/58 (9)	0/0 (0)	71/2,149 (3)
25-44	2,381/6,650 (36)	30/77 (39)	24/58 (41)	0/0 (0)	579/2,149 (27)
45-64	2,494/6,650 (38)	34/77 (44)	20/58 (34)	0/0 (0)	939/2,149 (44)
>65	1,437/6,650 (22)	10/77 (13)	5/58 (9)	0/0 (0)	558/2,149 (26)
Unknown	11/6,650 (0)	0/77 (0)	4/58 (7)	0/0 (0)	2/2,149 (0)
Sex					
Male	2,986/6,650 (45)	29/77 (38)	26/58 (45)	0/0 (0)	812/2,149 (38)
Female	3,664/6,650 (55)	48/77 (62)	32/58 (55)	0/0 (0)	1,337/2,149 (62)
Province					
Mpumalanga	981/6,650 (15)	7/77 (9)	10/58 (17)	0/0 (0)	248/2,149 (12)
Gauteng	1,917/6,650 (29)	29/77 (38)	17/58 (29)	0/0 (0)	544/2,149 (25)
North West	1,294/6,650 (19)	16/77 (21)	10/58 (17)	0/0 (0)	492/2,149 (23)
KwaZulu-Natal	1,509/6,650 (23)	9/77 (12)	9/58 (16)	0/0 (0)	500/2,149 (23)
Western Cape	949/6,650 (14)	16/77 (21)	12/58 (21)	0/0 (0)	365/2,149 (17)
Symptom duration (≤ 10 days)	4,903/6,650 (74)	67/77 (87)	41/58 (71)	0/0 (0)	1,810/2,149 (84)
HIV-infected	2,557/6,214 (41)	41/73 (56)	28/52 (54)	0/0 (0)	510/1,980 (26)
Underlying illness*	2,230/6,650 (34)	18/77 (23)	13/58 (22)	0/0 (0)	865/2,149 (40)
Hospital duration ≤5 days	5,175/6,650 (78)	59/77 (77)	41/58 (71)	0/0 (0)	1,703/2,149 (79)
ICU admission	63/6,597 (1)	0/76 (0)	0/52 (0)	0/0 (0)	47/2,135 (2)
In-hospital mortality	870/6,650 (13)	8/77 (10)	6/58 (10)	0/0 (0)	369/2,149 (17)

*Underlying illness included any of: Asthma, other chronic lung diseases, chronic heart disease (valvular heart disease, coronary heart disease, or heart failure excluding hypertension), stroke, seizures, anaemia, liver disease (cirrhosis or liver failure), renal disease (nephrotic syndrome, chronic renal failure), immunocompromising conditions excluding HIV infection (organ transplant, immunosuppressive therapy, immunoglobulin deficiency, malignancy, autoimmune disease), diabetes, pregnancy, burns, obesity, asplenia, neurological disease (spinal cord injury, neuromuscular conditions)

Total number of specimens tested for influenza, RSV and *B. pertussis* n=6 486, and SARS-CoV-2 n=6 416

Discussion

For the first time since the surveillance programmes were established, there was no influenza season observed during the South African winter period in 2020 and 2021. In all surveillance programmes influenza circulation, predominated by influenza A(H1N1)pdm09, was mostly limited to the Western Cape Province during the first few weeks of 2020 and was likely introduced by international travel. However, a sustained increase of A(H1N1)pdm09 in the late spring and early summer season was observed toward the end of 2021.

The absence of the influenza season in South Africa was similar to reports from other Southern Hemisphere countries.[13] COVID-19, the disease caused by SARS-CoV-2, with similar respiratory transmission to influenza, necessitated implementation of various levels of lockdown, physical and social distancing, promotion of good hand hygiene and compulsory wearing of masks. These measures likely contributed to the lack of transmission of influenza and other respiratory pathogens.[14] Compared to years prior to 2020 [15,16], the influenza cases in children remained the same throughout 2020 and 2021 in the ILI-PHC surveillance programme. However, the cases substantially increased in adults between 2020 and 2021. Due to low circulation of influenza in the country, it was not possible to assess influenza vaccine effectiveness for 2020 and 2021.

Similarly, there was no RSV season observed in 2020 and 2021, which usually precedes the influenza season in South Africa. This was also seen in other Southern Hemisphere countries.[17] Increased transmission of RSV was however noted from July to October in 2020 and March to May in 2021. The increase in RSV circulation from July 2020 could have been due to relaxation of COVID-19 restrictions and reopening of schools.[14]

There was very little *B. pertussis* detected (0.1% & 0.2% in 2020 and 2021, respectively) by the surveillance programmes compared to pre-pandemic years. The overall detection rate of pertussis decreased in comparison to the previous years (2018 and 2019: which identified 2.1% (98/4 630) and 0.8%, 33/4 383 cases respectively).[15,16] This is similar to what was observed in other countries such as England[18] and France.[19]

In 2020, the surveillance programmes were expanded to include surveillance for COVID-19. Although the pneumonia surveillance and ILI-PHC surveillance programmes in the public sector were sustained, the ILI-Viral Watch surveillance programme in the private sector was affected by the COVID-19 pandemic, with very few sites sending samples for testing compared to 2018 and 2019.[15,16] The lower number of submissions from this programme was likely due to a shift to tele-consultations by a number of general practitioners during 2020, or to doctors referring patients directly to laboratories instead of taking specimens themselves.

Using the surveillance programme data, the first period of increase in transmission of COVID-19 peaked in week 30 of 2020 and the second wave peaked in week 2 of 2021. The third period of increase in transmission peaked in week 28 of 2021 and the fourth period of increase in transmission peaked

in week 50 of 2021 which was similar to what was reported by the national surveillance of laboratory–confirmed cases of COVID-19[20] and DATCOV[21], these being national surveillance for COVID-19 hospitalisations. Compared to these, smaller numbers were reported through the syndromic surveillance programmes. The dominant variants detected in each period of increase in transmission corresponded to what was reported nationally by the Network for Genomic Surveillance in South Africa.[22] This is an indication that the pneumonia and systematic influenza-like illness programmes can be used to report on changes in transmission of respiratory pathogens, as well as to detect different variants of SARS-CoV-2, and should be considered a sustainable platform to monitor SARS-CoV-2.

Conclusion

In the first two years of the COVID-19 pandemic, these surveillance programmes managed to monitor four pathogens: influenza, RSV, *B. pertussis* and SARS-CoV-2. Changes in the transmission of respiratory pathogens and the detection of SARS-CoV-2 variants were reported through these surveillance programmes. It is envisioned that these programmes will be a sustainable platform to monitor increases in SARS-CoV-2 transmission and changes in epidemiology of other respiratory pathogens.

Recommendations

- The non-pharmaceutical interventions utilised during the COVID-19 pandemic (social distancing (staying home when ill), wearing of masks, hand washing/sanitising) can be utilised by persons experiencing respiratory symptoms, especially when mixing with individuals at risk of severed respiratory disease.
- Annual community awareness campaigns should be conducted by Department of Health and private sector partners including health insurance companies prior to the RSV (February - May) and influenza (May - August) seasons to highlight common symptoms, danger signs and when to seek clinical care, risk groups for severe disease, prevention strategies and to advocate for vaccination against influenza. Campaigns should include radio and poster campaigns as well as community health worker education sessions. Furthermore, clinics should be prepared in the RSV and influenza seasons for an increase in patients experiencing respiratory symptoms, assist patients with care and have basic medication ready to use. Additional information can be accessed in guidelines for influenza[23], COVID-19[24] and pertussis.[25]
- Syndromic respiratory surveillance should be sustained (and expanded, should resources be available), to allow ongoing systematic monitoring of trends disease, impact of interventions

and risk factors for severe illness for respiratory pathogens including SARS-CoV-2. Weekly and annual reports inform policy makers (such as Department of Health or WHO) of accurate trends in disease.

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INFLUENZA SURVEILLANCE IN SOUTH AFRICA:

WEEKS 1 - 32, 2022

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Summary

This report summarizes the results of influenza surveillance in South Africa for the period of week 1 through week 32, 2022, and was compiled by the World Health Organization (WHO) National Influenza Centre (NIC) housed at the Centre for Respiratory Diseases and Meningitis (CRDM) of the National Institute for Communicable Diseases (NICD). During 2022, influenza activity was observed from week 1 through 32, with an increased period of activity in the normal winter influenza season. Influenza circulation was dominated by A(H1N1)pdm09, followed by A(H3N2) and B/Victoria. While some antigenic drift was observed, strains fell within the same phylogenetic clades as 2022 Southern Hemisphere vaccine strains. This report includes data from individuals meeting syndromic case definitions within three respiratory illness surveillance programmes: Viral Watch influenza-like illness (VW) surveillance in outpatients (n=732) at private general practitioners, influenza-like illness (ILI) surveillance in outpatients (n=1028) at public health clinics and pneumonia surveillance in hospitalized patients (n=4340). Together, the three surveillance programmes contributed data from all nine provinces in South Africa. Influenza activity was observed from weeks 1 through 32, with an overall detection rate for 2022 from 3 January through 14 August of 11% (648/6100). Using the Moving Epidemic Method (MEM), the levels of activity reached moderate and low levels in the ILI and pneumonia surveillance programmes, respectively. Influenza single infections were dominated by influenza A(H1N1)pdm09 (62%, 387/621), followed by A(H3N2) (30%, 186/621) and B/Victoria (8%,

48/621). Dual infections were detected in three individuals [A(H1N1)pdm09 and B lineage inconclusive, A(H1N1)pdm09 and A(H3N2), A(H1N1)pdm09 and B/Victoria]. Influenza B/Yamagata was not detected. Subtype/lineage could not be determined for 4% (24/648) of infections, due to low viral load. Despite a low vaccine coverage (12%, 61/521) in the Viral Watch programme, vaccine effectiveness for any influenza, influenza A(H1N1)pdm09 and influenza A(H3N2) adjusted for age and season was 65% (95%CI: 30%, 82%), 46% (95% CI: -20%, 76%) and 91% (95%CI: 31%, 99%), respectively. Vaccine effectiveness for influenza B/Victoria could not be determined due to small numbers. Cell culture-derived influenza virus isolates were obtained with an 85% (155/183) success rate. Haemagglutinin inhibition (HAI) assays performed at the NICD demonstrated that 46% (29/63) of tested A(H1N1)pdm09, 100% (22/22) of A(H3N2) and 100% (9/9) of B/Victoria viruses were recognized by antisera raised against current vaccine and vaccine-like strains. All samples tested for (12/12 A(H1N1) pdm09 and 3/3 A(H3N2)) were susceptible to zanamivir, oseltamivir, peramivir and laninamivir. No known resistance mutations were detected among the 91 sequenced viruses. Genetic analysis of the haemagglutinin gene of South African 2022 influenza viruses was available for 80 A(H1N1)pdm09, 9 A(H3N2) and 2 B/Victoria viruses. Influenza A(H1N1)pdm09 viruses clustered into two major genetic subgroups namely 6B.1A.5a.1 and 6B.1A.5a.2, with the majority (59/80, 74%) belonging to the 6B.1A.5a.2 clade together with the 2022 A(H1N1)pdm09 vaccine strain for the Southern Hemisphere (A/Victoria/2570/2019). All A(H3N2) strains clustered within the 3C.2a1b.2a.2 clade along with the current Southern Hemisphere A(H3N2) vaccine strain (A/Darwin/9/2021). Both B/Victoria viruses clustered in the V1A.3a.2 subclade together with the current Southern Hemisphere influenza vaccine strain (B/Austria/1359417/2021). Following easing of COVID-19 restrictions, South Africa experienced the first typical influenza season since the start of the pandemic. The influenza season was ongoing as of week 43 of 2022, with a biphasic pattern in which infections later in the season were dominated by B/Victoria and A(H3N2) viruses. Individuals, especially those in high risk categories, are encouraged to receive the annual influenza vaccine.

Introduction

Influenza epidemics in South Africa usually occurring between April and October, with a peak during the winter months.[1,2] The following strains were recommended for the trivalent and quadrivalent inactivated influenza vaccine (IIV) 2022 Southern Hemisphere influenza season:

Egg-based tri/quadri-valent vaccines including:

- an A/Victoria/2570/2019 (H1N1)pdm09-like virus;
- an A/Darwin/9/2021 (H3N2)-like virus;
- a B/Austria/1359417/2021 (B/Victoria lineage) like- virus; and
- a B/Phuket/3073/2013-like (B/Yamagata lineage) virus (quadrivalent vaccine only)

These recommendations included a change to the A(H3N2) and B/Victoria lineage component of egg-based vaccines strains compared with the 2021 Southern Hemisphere trivalent and quadrivalent IIV. For A(H3N2) vaccine virus component, A/Hong Kong/2671/2019-like virus was replaced with A/Darwin/9/2021-like virus and for B/Victoria lineage vaccine virus component, B/Washington/02/2019-like virus was replaced with a B/Austria/1359417/2021-like virus. In South Africa, the trivalent IIV was only available in the public sector (at designated clinics and hospitals), the quadrivalent IIV was available mostly in the private sector with limited doses in public sector, generally from March or April.

Methods

South Africa has three influenza surveillance programmes coordinated by the Centre for Respiratory Diseases and Meningitis (CRDM) at the National Institute for Communicable Diseases (NICD). These programmes include (i) Viral Watch influenza-like illness (VW) surveillance in outpatients at private general practitioners, (ii) systematic influenza-like illness (ILI) surveillance in outpatients at public health clinics, and (iii) national pneumonia surveillance in public health hospitals (Table 1).

Table 1. Characteristics of influenza and respiratory surveillance programmes in South Africa, 2022.

Programme	Viral Watch	Influenza-like illness surveillance	National syndromic surveillance for pneumonia
Start year	1984	2012	2009
Provinces*	EC, FS, GP, LP, MP, NC, NW, WC	KZN, NW, WC, MP	GP, KZN, MP, NW, WC, EC
Number of sites	98	5	13
Type of site	General practitioners	Public primary health care clinics	Public hospitals
Case definition	An acute respiratory illness with fever (≥38°C), cough and symptom onset ≤10 days or **Suspected SARS-CoV-2:** Any person presenting with an acute (≤14 days) respiratory tract infection or other clinical illness compatible with COVID-19**	An acute respiratory illness with fever (≥38°C), cough and symptom onset ≤10 days or **Suspected SARS-CoV-2:** Any person presenting with an acute (≤14 days) respiratory tract infection or other clinical illness compatible with COVID-19**	Acute (symptom onset ≤10 days) or chronic (symptom onset >10 days) lower respiratory tract infection requiring hospitalisation or **Suspected SARS-CoV-2:** Any person admitted with a physician-diagnosis of suspected COVID-19 and not meeting SRI case definition
Specimens collected	Throat swabs and/or nasal/nasopharyngeal swabs	Combined oropharyngeal and nasopharyngeal swabs	Combined oropharyngeal and nasopharyngeal swabs

*EC: Eastern Cape; FS: Free State; GP: Gauteng; KZN: KwaZulu-Natal; LP: Limpopo; MP: Mpumalanga; NC: Northern Cape; NW: North West; WC: Western Cape ** Symptoms include ANY of the following respiratory symptoms: cough, sore throat, shortness of breath, anosmia (loss of sense of smell) or dysgeusia (alteration of the sense of taste), with or without other symptoms (which may include fever, weakness, myalgia, or diarrhoea)

Nasopharyngeal/nasal swabs were tested using the Allplex™ SARS-CoV-2/influenza/RSV commercial kit (Seegene, Seoul, Korea) and the US Centres for Disease Control and Prevention (CDC) subtyping method (with reagents sourced through the International Reagent Resource, IRR Portal).

Influenza transmission thresholds were calculated using the Moving Epidemic Method (MEM), a sequential analysis using the R language (http://CRAN.R-project.org/web/package=mem) designed to calculate the duration, start and end of the annual influenza epidemic.[3,4] MEM uses the 40th, 90th and 97.5th percentiles established from available years of historical data to calculate thresholds of activity. Thresholds of activity for influenza are defined as follows: below threshold, low activity, moderate activity, high activity and very high activity. Thresholds from ILI surveillance at primary healthcare clinics (outpatients) are used as an indicator of disease transmission in the community and thresholds from pneumonia surveillance (inpatients) are used as an indicator of impact of disease on health care provision.

The effectiveness of the trivalent/quadrivalent seasonal influenza vaccine (TIV/QIV) to prevent influenza- associated medically attended acute respiratory illness was assessed using a test-negative case control study design. Patients meeting the case definition for influenza-like illness presenting to an outpatient influenza sentinel surveillance programme (Viral Watch) in South Africa during the 2022 influenza season were included in the analysis.

During 2022, influenza virus isolation was attempted on clinical specimens testing positive for influenza on rRT-PCR with a high viral load (C_t value ≤30). Madin-Darby Canine Kidney (MDCK) cells were used for virus isolations. Influenza virus cultures and original specimens were shared with the WHO Collaborating Centres for Influenza Surveillance and Research (WHO-CC) in Australia, United Kingdom and United States for antigenic and genetic characterization. Haemagglutination inhibition (HAI) assays were performed at the NIC in South Africa. Turkey red blood cells were used as indicator cells in the HA and HAI assays. All the HAI assays were completed using the IRR 2021-2022 WHO influenza reagent kit for identification of influenza isolates (CDC International Reagent Resource). HAIs were performed for all isolates with HAI titers.

Phenotypic susceptibility testing to zanamivir, oseltamivir, peramivir and laninamivir was performed for South African samples at the WHO Collaborating Centre in Australia (VIDRL). Genotypic analysis for resistance mutation detection was performed on CLC Genomics Workbench (Qiagen, Hilden, Germany) using the following GenBank references: A/California/07/2009 (CY121680) for A(H1N1)pdm09, A/Wisconsin/67/2005 (CY163680) for A(H3N2) and B/Brisbane/60/2008 (KX058884) for B/Victoria. The phenotypic effect of detected substitutions was predicted using Flusurver (https://flusurver.bii.a-star. edu.sg/).

All influenza sequences analysed were obtained from GISAID on 25 August 2022. Viruses with incomplete sequence data for the haemagglutinin (HA) gene were excluded from the analysis. Genetic characterisation was carried out by phylogenetic analysis (using the Aliview alignment editor and IQTREE v1.6.12 software with ultrafast bootstraps) of the HA gene. Groups and sub-groups were identified by specific amino acid mutations relative to a designated reference strain on NextClade.

Results

From 3 January 2022 (week 1) through 14 August 2022 (week 32), 6179 individuals were enrolled and respiratory specimens from 6100 (99%) individuals were tested through the three surveillance programmes (Table 2). Influenza infections were identified in 648 individuals, resulting in an overall detection rate of 11% (648/6100). Influenza detections occurred from week 1 through 32. Influenza single infections were dominated by influenza A(H1N1)pdm09 (62%, 387/621), followed by A(H3N2) (30%, 186/621) and B/Victoria (8%, 48/621). Dual infections were detected in three individuals [A(H1N1)pdm09 and B lineage inconclusive, A(H1N1)pdm09 and A(H3N2), A(H1N1)pdm09 and B/Victoria]. Influenza B/Yamagata was not detected. Inconclusive results for subtyping occurred in 4% (24/648) of samples. The latter samples had a primary identification reverse transcription real-time polymerase chain reaction (rRT-PCR) cycle threshold (C_t) value greater than 35 and subsequent characterisation PCR was not performed to determine the subtype/lineage. The 2022 influenza season started in week 17 (week starting 25 April 2022) when the influenza detection rate among patients in the pneumonia surveillance programme breached the epidemic threshold as determined by the Moving Epidemic Method (MEM), and was continuing at the time of this report (week 32). The mean onset of influenza season in South Africa in 2005-2019 was week 17 (3rd week of April), ranging from week 16 to week 25.

Table 2. Numbers of influenza infections identified in all syndromic influenza surveillance programmes, South Africa, 3 January – 14 August 2022 (weeks 1-32).

Programme	Number of specimens tested	Number influenza positive (% of all specimens tested)	Influenza A				Influenza B				#Dual infection
			Total A	Subtype inconclusive*	A(H1N1) pdm09	A(H3N2)	Total B	Lineage inconclusive*	B/ Victoria	B/ Yamagata	
			n (% of total influenza positives)								
Viral Watch	732	257 (35)	235 (91)	5 (2)	152 (59)	78 (30)	20 (8)	2 (1)	18 (7)	0	2 (1)
Influenza-like illness surveillance	1028	152 (15)	138 (91)	3 (2)	91 (60)	44 (29)	13 (9)	1 (1)	12 (8)	0	1 (1)
Pneumonia surveillance	4340	239 (6)	216 (90)	8 (3)	144 (60)	64 (27)	23 (10)	5 (2)	18 (8)	0	0
Total	**6100**	**648 (11)**	**589 (91)**	**16 (2)**	**387 (60)**	**186 (29)**	**56 (9)**	**8 (1)**	**48 (7)**	**0**	**3 (0)**

*Inconclusive: insufficient viral load in sample and unable to characterise further; #Dual infections: A(H1N1)pdm09 and B lineage inconclusive, A(H1N1)pdm09 and A(H3N2); A(H1N1)pdm09 and B/Victoria

Viral Watch programme

Specimens from 732 patients were received and tested from VW practitioners located in 7 of the 8 provinces participating in surveillance (Table 3), with the majority of specimens received from Gauteng (468/732, 64%) and Western Cape (178/732, 24%) provinces. Influenza was detected in 257 (35%) patients, of which 91% (235/257) were influenza A, 8% (20/257) were influenza B and 1% (2/257) were dual infections (Figure 1, Table 3). Among the influenza A infections for which a subtype could be determined, 66% (152/230) were A(H1N1)pdm09 and 34% (78/230) were A(H3N2). All influenza B infections for which a lineage was determined were B/Victoria (18/18). The two dual infections detected were (i) A(H1N1)pdm09 and B lineage inconclusive and (ii) A(H1N1)pdm09 and A(H3N2).

Table 3. Numbers of influenza infections by subtype/lineage, and total number of specimens tested by province in the Viral Watch surveillance programme, South Africa, 3 January – 14 August 2022 (Weeks 1-32).

Province	A(H1N1) pdm09	A (H3N2)	A subtype inconclusive*	B /Victoria	B /Yamagata	B lineage inconclusive*	Dual infection#	Total cases	Total specimens tested	Detection rate (%)
Eastern Cape	20	6	0	4	0	2	0	32	45	**71**
Free State	7	0	0	0	0	0	0	7	8	**88**
Gauteng	80	24	4	8	0	0	2	118	468	**25**
Limpopo	2	2	1	1	0	0	0	6	8	**75**
Mpumalanga	7	0	0	1	0	0	0	8	19	**42**
Northern Cape	0	0	0	0	0	0	0	0	0	**-**
North West	3	0	0	0	0	0	0	3	6	**50**
Western Cape	33	46	0	4	0	0	0	83	178	**47**
Total	**152**	**78**	**5**	**18**	**0**	**2**	**2**	**257**	**732**	**35**

*Inconclusive: insufficient viral load in sample and unable to characterise further; # A(H1N1)pdm09 and B lineage inconclusive, A(H1N1)pdm09 and A(H3N2)

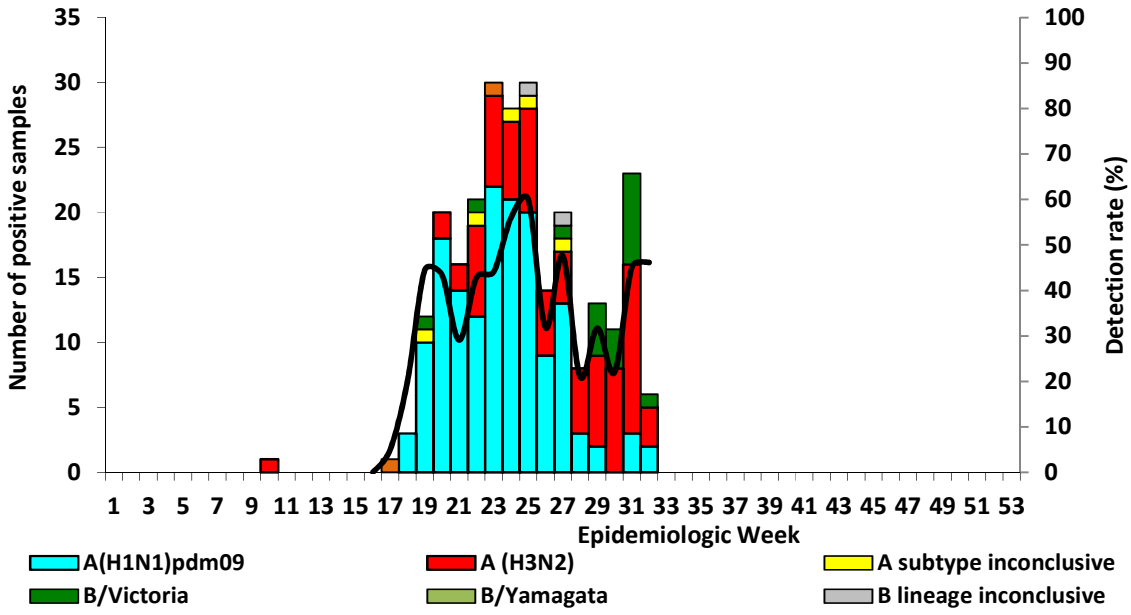

Figure 1. Number of influenza infections by influenza subtype/lineage and detection rate by epidemiologic week - Viral Watch programme for influenza-like illness surveillance, South Africa, Weeks 1 to 32, 2022 (n=257). Inconclusive: insufficient viral load in sample and unable to characterise further. Dual infections: A(H1N1)pdm09 and B lineage inconclusive, A(H1N1)pdm09 and A(H3N2).

Influenza-like illness (ILI) surveillance programme at primary health care clinics

Specimens from 1028 patients with ILI were received from five primary health care clinics located in four provinces. In total, 152 (15%) individuals tested positive for influenza. Among the single infections that could be further characterised, influenza A(H1N1)pdm09 accounted for 62% (91/147), A(H3N2) for 30% (44/147) and influenza B/Victoria for 8% (12/147) of cases. One individual had a dual infection [A(H1N1)pdm09 and B/Victoria]. Influenza B/Yamagata was not detected in 2022 (Table 4, Figure 2). The influenza detection rate increased from week 13, peaking in week 23 (48%, 25/52), and subsequently decreased (Figure 2). Individuals aged ≥5 years accounted for 76% (116/152) of influenza infections.

Table 4. Number of influenza cases by subtype/lineage, and total number of specimens collected by province for the influenza-like illness surveillance programme at primary healthcare clinics, South Africa, Weeks 1-32, 2022 (n=152).

Province	A(H1N1) pdm09	A (H3N2)	A subtype inconclusive*	B/ Victoria	B/ Yamagata	B lineage inconclusive*	Dual infection#	Total cases	Total specimens	Detection rate
KwaZulu-Natal	22	26	0	1	0	0	0	49	295	**17**
Mpumalanga	20	0	0	10	0	1	0	31	167	**19**
North West	24	0	1	0	0	0	0	25	217	**12**
Western Cape	25	18	2	1	0	0	1	47	349	**13**
Total	**91**	**44**	**3**	**12**	**0**	**1**	**1**	**152**	**1028**	**15**

Surveillance sites included primary health care clinics in 4 provinces: KwaZulu-Natal (Edendale Clinic), Mpumalanga (Agincourt Clinic), North West (Jouberton Clinic) and Western Cape (Eastridge Clinic and Mitchell's Plain Clinic). *Inconclusive: insufficient viral load in sample and unable to characterise further (primary test PCR C_t value >35). #Dual infection: A(H1N1)pdm09 and B/Victoria.

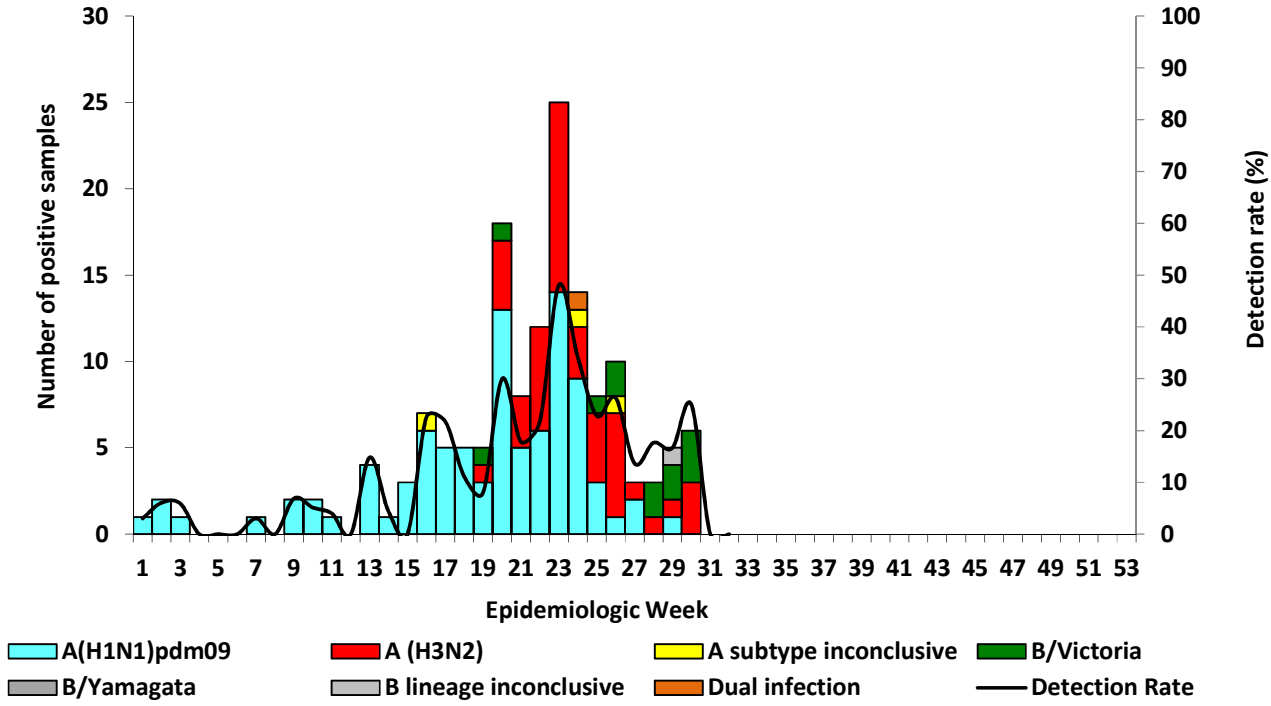

Figure 2. Number of influenza cases by subtype/lineage and detection rate by epidemiologic week - Influenza-like illness (ILI) surveillance programme at primary health care clinics, South Africa, Weeks 1 to 32, 2022 (n=152). Inconclusive: insufficient viral load in sample and unable to characterise further. Dual infection: A(H1N1)pdm09 and B/Victoria

Using the MEM, with a baseline determined from previous years (2012-2019), the estimated level of influenza disease transmission in the community reached a level of moderate activity in week 23 of 2022 in the ILI surveillance programme at public healthcare clinics (Figure 3).

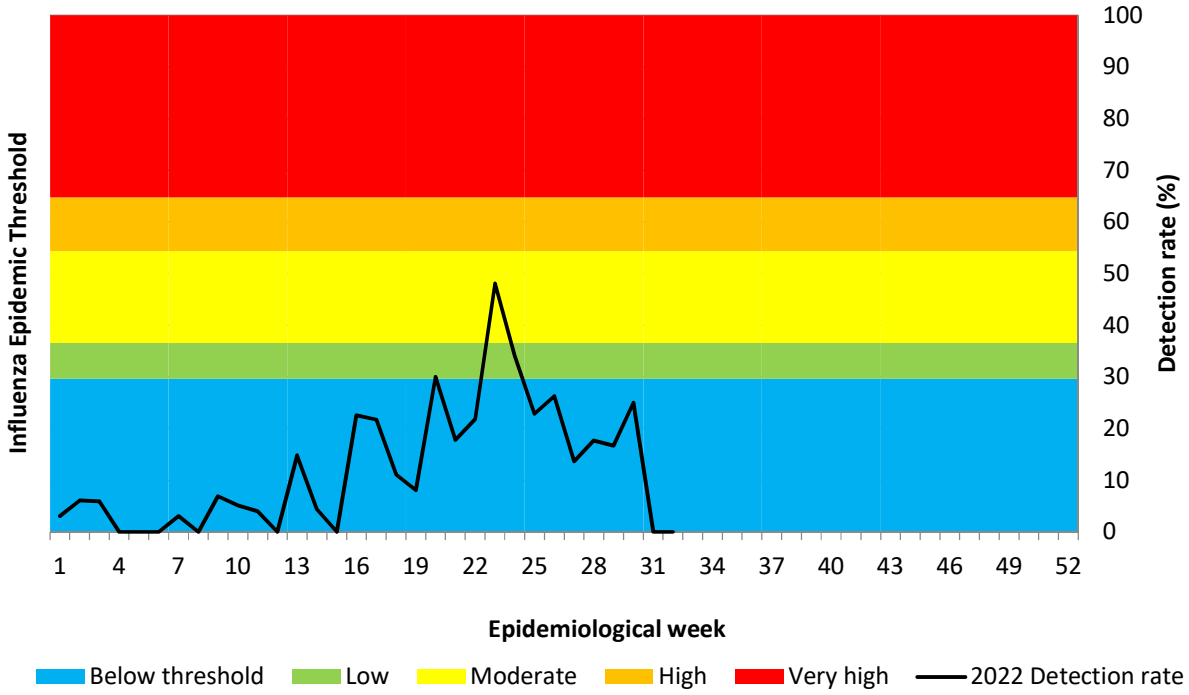

Figure 3. Influenza detection rate and epidemic thresholds*, influenza-like illness surveillance at primary health care clinics, South Africa, 3 January – 14 August 2022 (Weeks 1-32).
*Influenza transmission thresholds based on 2012-2019 data and calculated using the Moving Epidemic Method (MEM)

Pneumonia surveillance programme

Specimens from 4340 patients hospitalised with severe respiratory illness were received from the thirteen sentinel hospitals located in six provinces, and 239 (6%) influenza cases were detected. Among influenza-positive samples which could be further characterised, 64% (144/226) were A(H1N1)pdm09, 28% (64/226) were A(H3N2) and 8% (18/226) were B/Victoria (Table 5). Influenza B/Yamagata infection was not detected in the pneumonia surveillance programme in weeks 1 through 32 of 2022. The influenza detection rate increased from week 16, with a peak detection rate of 16% (30/188) in week 25, and subsequently decreased (Figure 4). Individuals aged ≥5 years accounted for 56% (134/239) of influenza cases. The impact of the 2022 influenza disease was estimated to be low from week 17 through week 27, and was below the threshold outside of this time period (Figure 5).

Table 5. Number of influenza infections by subtype/lineage, and total number of specimens collected by province for the pneumonia surveillance programme, South Africa, 3 January – 14 August 2022 (Weeks 1-32).

Province	A(H1N1) pdm09	A(H3N2)	A subtype inconclusive*	B/ Victoria	B/ Yamagata	B lineage inconclusive*	Total cases	Total specimens tested	Detection rate %
Eastern Cape	8	1	1	6	0	2	18	212	**8**
Gauteng	37	13	1	3	0	3	57	1110	**5**
KwaZulu-Natal	26	12	1	1	0	0	40	630	**6**
Mpumalanga	29	4	1	6	0	0	40	592	**7**
North West	27	1	0	0	0	0	28	368	**8**
Western Cape	17	33	4	2	0	0	56	1428	**4**
Total	**144**	**64**	**8**	**18**	**0**	**5**	**239**	**4340**	**6**

Surveillance sites included hospitals in six provinces: Gauteng (Helen Joseph Hospital, Rahima Moosa Hospital, Tembisa Hospital), KwaZulu-Natal (Edendale Hospital), Mpumalanga (Mapulaneng, Matikwana and Tintswalo Hospitals), North West (Klerksdorp-Tshepong Hospital Complex) and Western Cape (Red Cross Children's Hospital, Tygerberg Hospital and Mitchell's Plain Hospital). *Inconclusive: insufficient viral load in sample and unable to characterise further

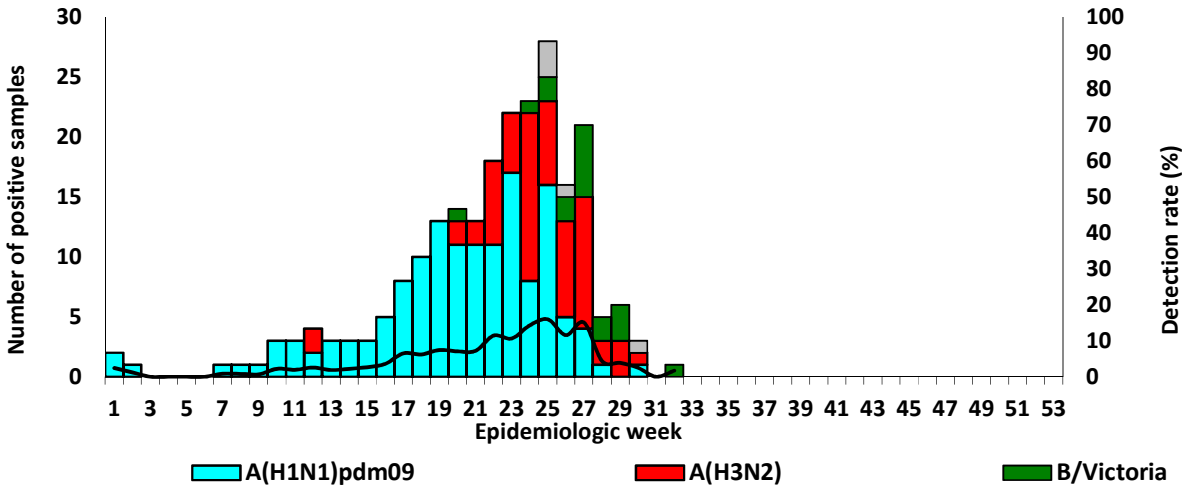

Figure 4. Number of influenza cases by subtypes/lineages and detection rate by epidemiologic week – National pneumonia surveillance, South Africa, 3 January – 14 August 2022 (Weeks 1-32). Inconclusive: insufficient viral load in sample and unable to characterise further.

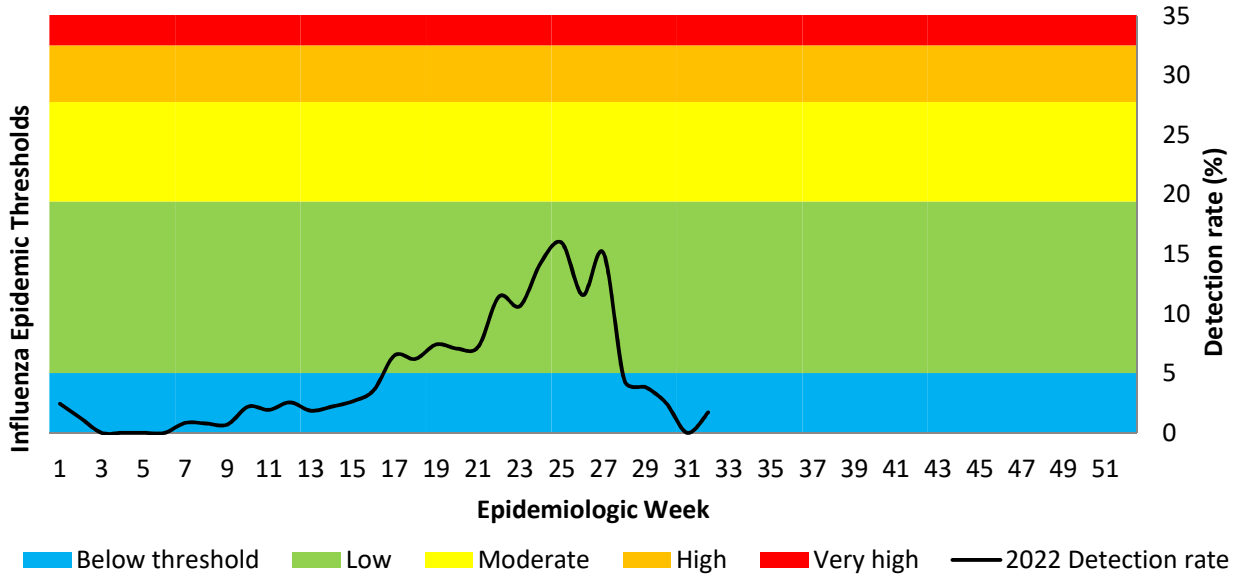

Figure 5. Influenza detection rate and epidemic thresholds*, National pneumonia surveillance programme, South Africa, 3 January – 14 August 2022 (Weeks 1-32).
*Influenza transmission thresholds based on 2010-2019 data and calculated using the Moving Epidemic Method (MEM)

Vaccine effectiveness

Of the 521 surveillance cases enrolled in the VW programme during the season and included in vaccine effectiveness (VE) analysis (aged >6 months with known vaccination status), 207 (40%) were classified as cases (influenza test positive) and 314 (60%) as controls (influenza test negative). Vaccine coverage was 12% (61/521) overall in the VW programme (Table 6): 6% (12/207) and 16% (49/314) among cases and controls respectively. Coverage was highest in the ≥65 years age group (53%) and lowest among cases aged <18 years (8.8%).

The overall (any influenza) VE estimate, adjusted for age and seasonality was 65% (95% confidence interval (CI): 30%, 82%) (Table 7). Influenza A(H1N1)pdm09 VE estimate, adjusted for age and seasonality was 46% (95% CI: -20%, 76%). The influenza A(H3N2) estimate, adjusted for age and seasonality was 91% (95% CI: 31%, 99%). VE was not able to be determined for B/Victoria due to small numbers.

Table 6. Vaccine coverage and vaccine effectiveness (VE) by age group and timing within season, 2022.

	Vaccine coverage			Unadjusted VE
	Cases n/N (%)	Controls n/N (%)	Total n/N (%)	% (95% confidence interval)
All	12/207 (6)	49/314 (16)	61/521 (12)	66.7 (35.7, 82.8)
<18 years	4/72 (6)	10/86 (12)	14/158 (9)	55.3 (-49.1, 86.6)
18-64 years	7/128 (6)	28/201 (14)	47/329 (10)	62.3 (15.5, 84.8)
≥65 years	1/7 (1)	11/27 (41)	18/34 (53)	75.8 (-130.4, 97.5)
Early-season (week 16-21)	3/47 (6)	9/96 (9)	12/143 (8)	34.1 (-155.8, 83.2)
Mid-season (week 22-27)	7/113 (6)	20/117 (17)	27/230 (12)	68.0 (20.9, 87.0)
Late-season (week 28-31)	2/47 (4)	20/101 (20)	22/148 (15)	82.0 (19.4, 96.0)

Table 7. Vaccine coverage and vaccine effectiveness (VE) by influenza subtype, adjusted by age and seasonality, 2022.

	Vaccine coverage			Adjusted VE
	Cases n/N (%)	Controls n/N (%)	Total n/N(%)	% (95% confidence interval)
Any influenza	12/207 (5.8)	49/314 (15.6)	61/521 (11.7)	64.5 (29.9-82.0)
Influenza A(H1N1)pdm09	9/122 (7.4)	49/314 (15.6)	58/436 (13.3)	45.8 (-20,75.5)
Influenza A (H3N2)	1/58 (1.7)	49/331 (14.8)	50/389 (12.8)	90.8 (31.4,98.8)

Influenza virus isolation

During 2022, influenza virus isolation was attempted on 183 clinical specimens with an overall isolation rate of 85% (155/183) (Table 8). The isolation success rate was highest for A(H1N1)pdm09 viruses (89%). In total, 93 A(H1N1)pdm09, 44 A(H3N2) and 18 B/Victoria and viruses were isolated. Influenza virus isolation in embryonated hens' eggs remains challenging and was not attempted.

Table 8. Summary of influenza virus isolations in Madin-Darby Canine Kidney (MDCK) cell cultures, South Africa, 3 January – 14 August 2022 (Weeks 1-32).

Programme	Specimens cultured	Successful cultures	Number of cultures/ attempted (%)		
			A(H1N1)pdm09	A(H3N2)	B/Victoria
Viral Watch	34	23	16/20 (80)	5/7 (71)	2/7 (29)
Influenza-like illness surveillance	59	53	26/29 (90)	20/23 (87)	7/7 (100)
Pneumonia surveillance	90	79	51/56 (91)	19/21 (90)	9/13 (69)
Total	**183**	**155**	**93/105 (89)**	**44/51 (86)**	**18/27 (67)**

Influenza specimens shared with WHO Collaborating Centres

Among virus cultures and original specimens from 132 individuals shared with WHO Collaborating Centres, 65% (86/132) were A(H1N1) pdm09, 24% (32/132) were A(H3N2) and 11% (14/132) were B/Victoria (Table 9).

Table 9. Summary of influenza virus specimens collected in South Africa and shared with WHO-Collaborating Centers for Influenza Surveillance and Research, 3 January – 14 August 2022 (Weeks 1-32).

WHO-CC	A(H1N1)pdm09	A(H3N2)	B/Victoria	Total
Australia	40	17	5	62
United Kingdom	37	2	1	40
United States	9	13	8	30
Total	**86**	**32**	**14**	**132**

Antigenic characterisation of influenza virus isolates

Results for antigenic characterisation of influenza A(H1N1)pdm09, A(H3N2) and B/Victoria are summarised in Table 10. HAIs were performed for all isolates with HAI titers (n=94). A total of 94 virus cultures were characterised antigenically, including 63 A(H1N1)pdm09, 22 A(H3N2) and 9 B/Victoria cultures. 46% (29/63) of A(H1N1)pdm09 viruses had A/Indiana/02/2020-like reactivity and 100% (22/22) of A(H3N2) had A/Tasmania/503/2020-like activity. For the B/Victoria viruses, 100% (9/9) were classified as B/Washington/02/2019-like reactors.

Table 10. Summary of haemagglutination inhibition (HAI) assay results, South Africa, 3 January – 14 August 2022 (Weeks 1-32).

Programme	Number of cultures with HAI titres	A(H1N1)pdm09		A(H3N2)		B/Victoria	
		A/Indiana/02/2020		A/Tasmania/503/2020		B/Washington/02/2019	
		Normal reactors	Low reactors	Normal reactors	Low reactors	Normal reactors	Low reactors
Viral Watch	11	0	9	1	0	1	0
Influenza-like illness	34	10	8	11	0	5	0
Pneumonia surveillance	49	19	17	10	0	3	0
Total n/N (% per virus)	94	29/63 (46)	34/63 (54)	22/22 (100)	0/22 (0)	9/9 (100)	0/9 (0)

HAI assay results from samples shared with the WHO Collaborating Centre in Australia (VIDRL) showed that for A(H1N1) pdm09, 17/30 (57%) were A/Victoria/2570/2019-like and 13/30 (43%) were A/Victoria/2570/2019 low reactors; for A(H3N2), 3/3 (100%) were A/Darwin/6/2021 low reactors; and for B/Victoria, 1/1 (100%) were B/Austria/1359417/2021-like.

Neuraminidase inhibitor susceptibility

For phenotypic susceptibility testing to zanamivir, oseltamivir, peramivir and laninamivir, all samples (12/12 A(H1N1) pdm09 and 3/3 A(H3N2)) showed normal inhibition with all antivirals tested. The mutational analysis on NA segments from sequenced viruses [A(H1N1)pdm09 (n=80), A(H3N2) (n=9), B/Victoria (n=2)], showed that known typical drug-resistance substitutions were not detected.

Genetic characterisation of influenza viruses

The 2022 viruses (n=91) included for genetic characterisation were sequenced at the WHO-CCs for Influenza Surveillance and Research in Australia and the United Kingdom [A(H1N1)pdm09 (n=53), A(H3N2) (n=4) and B/Victoria (n=2)]. Additional sequences deposited by the Vaccine and Infectious Disease Analytics-University of the Witwatersrand (WITS-VIDA) [A(H1N1)pdm09 (n=17) and A(H3N2) (n=5)] and 10 viruses sequenced by the NICD [A(H1N1)pdm09 (n=10)], were also included. Sequences of 2021 viruses were from WHO-CCs (n=157), WITS-VIDA (n=4) and NICD (n=5).

Influenza A(H1N1)pdm09

Genetic analysis of the HA gene of South African influenza A(H1N1)pdm09 viruses indicated that all 50 viruses collected in 2021 and 80 collected in 2022 belong to clade 6B (Figure 6). Strains further clustered into two major genetic subgroups namely 6B.1A.5a.1 (containing Q189E, R113K, D187A

amino acid mutations) and 6B.1A.5a.2 (containing Q189E, K142R, K45Q, T277A, P137S amino acid mutations). The majority of the of the 2021 viruses (44/50, 88%) belonged to the 6B.1A.5a.1 clade. In contrast, the majority of the of the 2022 viruses (59/80, 74%) belonged to the 6B.1A.5a.2 clade together with the 2022 A(H1N1)pdm09 vaccine strain for the Southern Hemisphere (A/Victoria/2570/2019) while only 21 (26%) clustered within the 6B.1A.5a.1 clade (Figure 6).

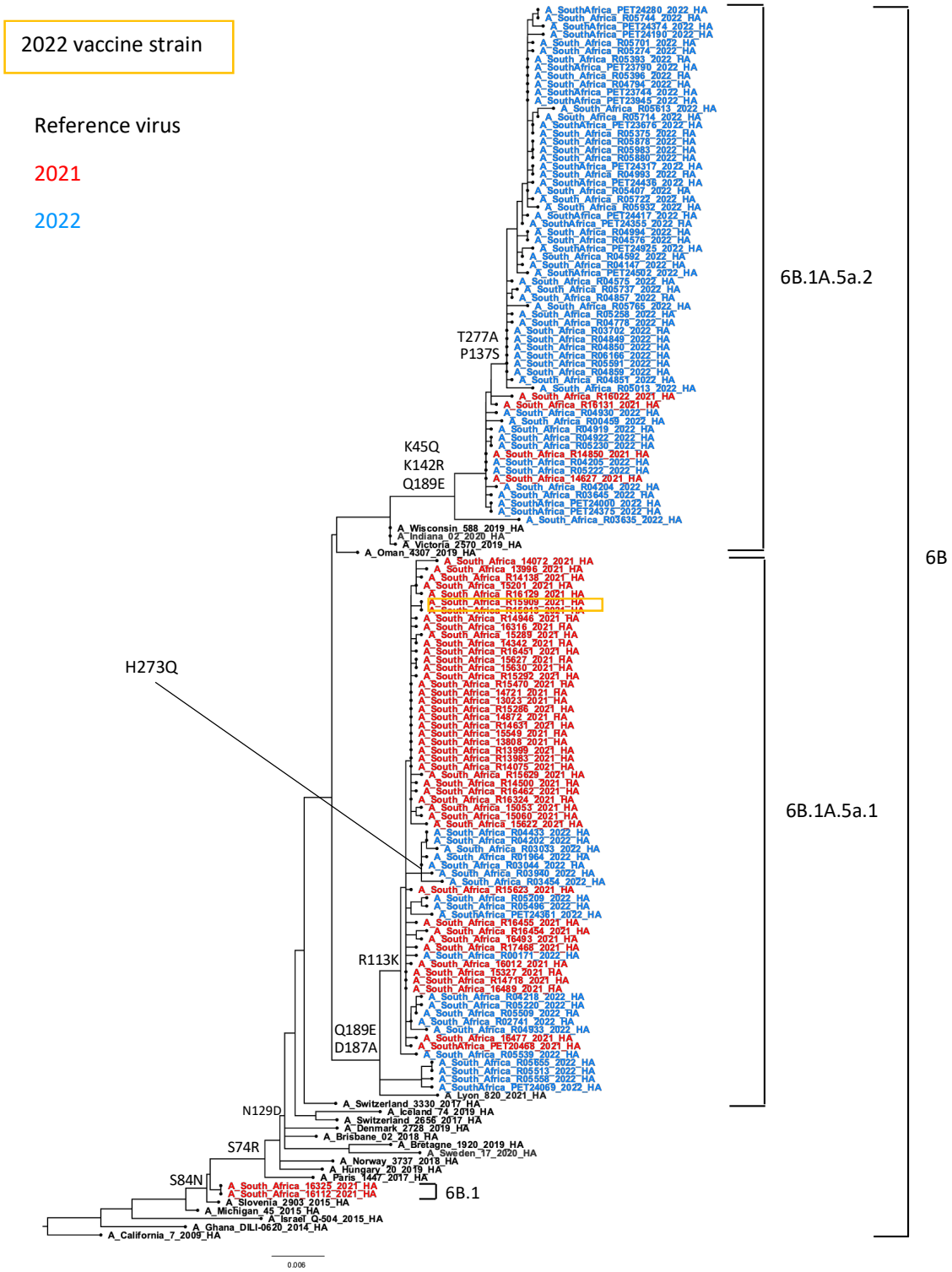

Figure 6. Maximum likelihood phylogenetic tree (ML tree, TPM2u+F+G4 (Best model), No. of Bootstrap replications n=2000, constructed with IQTREE) of the haemagglutinin gene of influenza A(H1N1)pdm09 viruses. The 2022 Southern Hemisphere vaccine strain is indicated by the green block, South African 2021 viruses in red (n=50) and South African 2022 viruses in blue (n=80), and reference strains in black. A/California/07/2009 (H1N1) was used as the root.

Influenza A(H3N2)

Genetic analysis of the HA gene of 2021 and 2022 South African influenza A(H3N2) viruses (n=31) indicated that they belonged to the 3C.2a clade (Figure 7). The 2021 strains (n=22) belonged to three different genetic subgroups with each subgroup characterised by different amino acid substitutions in the HA [3C.2a1b.1b (K310R), 3C.2a1b.1a (T131N, R261Q) and 3C.2a1b.2a.2 (S205F)]. All 2022 (n=9) strains clustered within the 3C.2a1b.2a.2 clade along with the current Southern Hemisphere A(H3N2) vaccine strain (A/Darwin/9/2021).

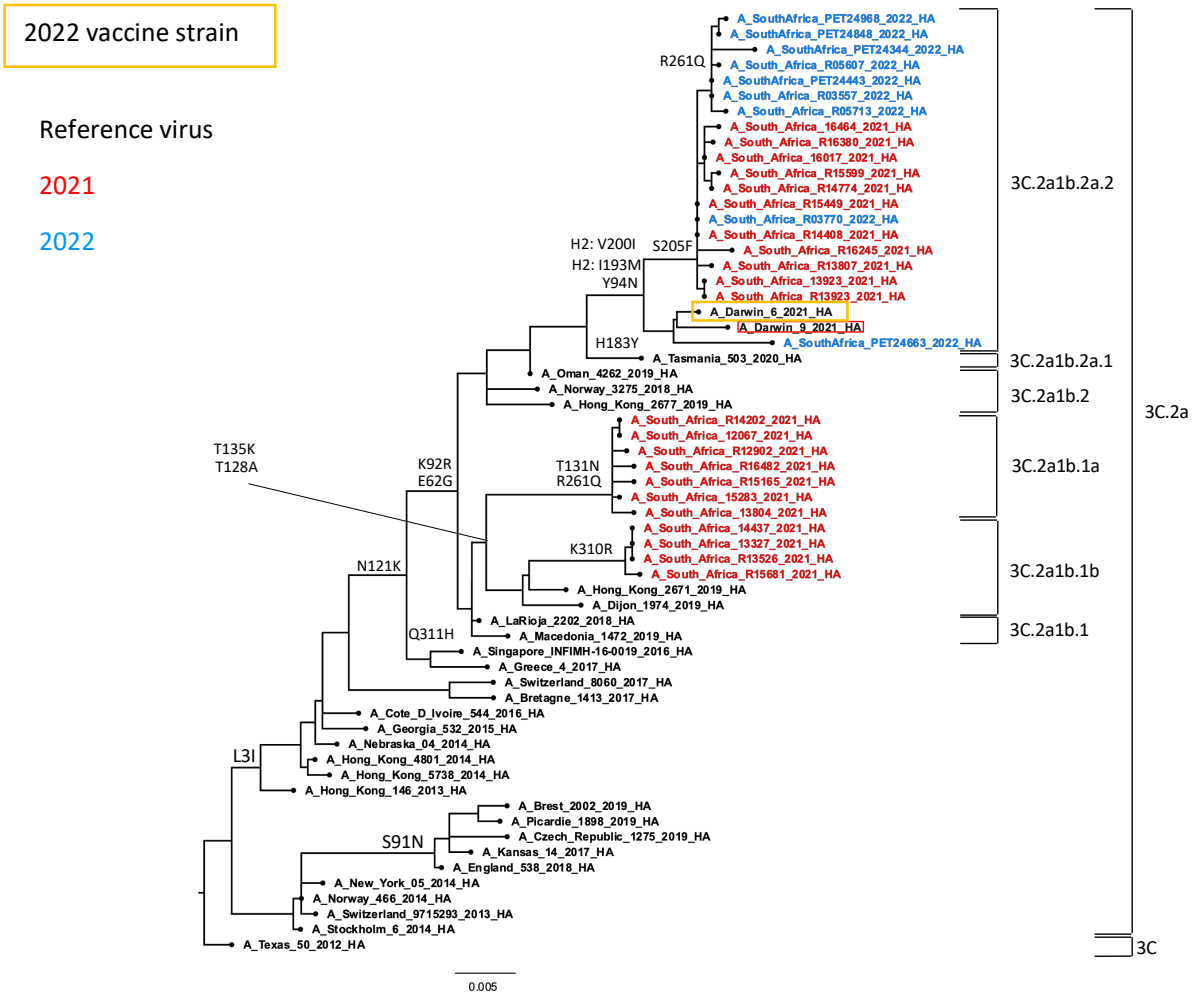

Figure 7. Maximum likelihood phylogenetic tree (ML tree, TPM2u+F+G4 (Best model), No. of Bootstrap replications n=2000, constructed with IQTREE) of the haemagglutinin gene of influenza A(H3N2) viruses. The 2022 Southern Hemisphere vaccine strain is indicated in a green box. South African 2021 viruses are in red (n=22), South African 2022 viruses are in blue (n=9) and reference strains are in black. A/Texas/50/2012 (H3N2) was used as the root.

Influenza B

Genetic analysis of 96 influenza B/Victoria viruses from 2021 and 2022 showed that all belonged to clade V1A (Figure 8). The V1A.3a.2 subclade (K203R, P144L, N126S, T221A, T182A, A202V) consisted of all the 2021 viruses (n=94) and two of the 2022 viruses (with an additional E198G substitution). The current Southern Hemisphere influenza vaccine strain (B/Austria/1359417/2021) also clustered within the same subclade.

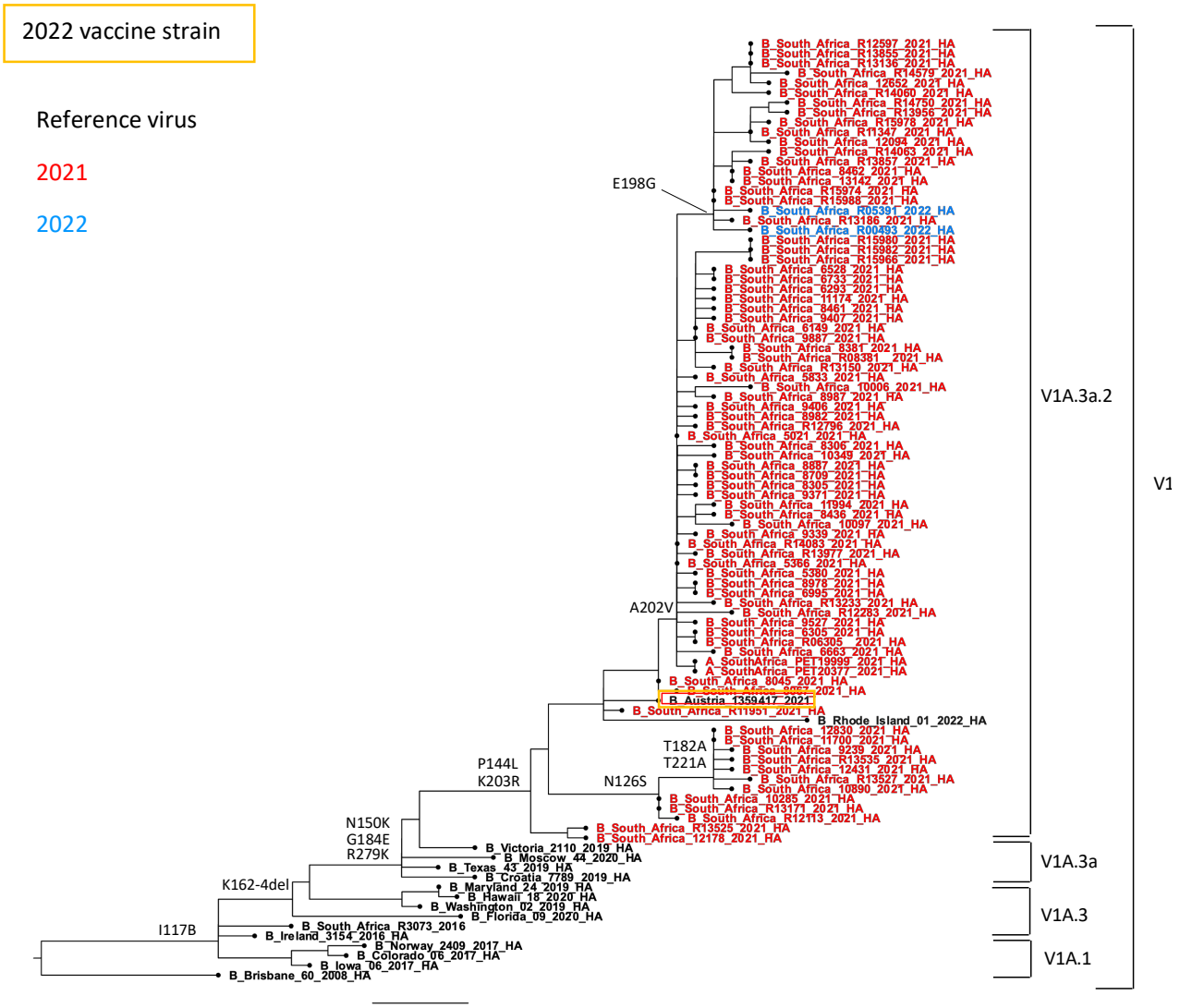

Figure 8. Maximum likelihood phylogenetic tree (ML tree, TPM2u+F+G4 (Best model), No. of Bootstrap replications n=2000, constructed with IQTREE) of the haemagglutinin gene of influenza B/Victoria viruses. The 2022 Southern Hemisphere vaccine strain is indicated in a green box, South African 2021 viruses are in red (n=94) and South African 2022 viruses are in blue (n=2), reference strains are in black. B/Brisbane/60/2008 was used as the root.

Discussion

As of the end of week 32 2022, influenza activity had been observed from week 1 through 32, with an increased period of activity in the normal winter influenza season, and was ongoing at the time of this report. Levels of activity reached moderate and low levels in the ILI and pneumonia surveillance programmes, respectively. However, as the baseline for MEM is established using data from prior to the COVID-19 pandemic, and that COVID-19 now contributes to the enrolled number of ILI and pneumonia surveillance cases, this may result in an underestimation of the detection rate and resulting thresholds, and the determination of the influenza season may therefore be biased.

Influenza circulation was dominated by A(H1N1)pdm09, followed by A(H3N2) and B/Victoria. Influenza B/Yamagata was not detected. Haemagglutinin inhibition assays demonstrated that 46% of tested A(H1N1)pdm09, 100% of A(H3N2) and 100% of B/Victoria viruses were recognised by antisera raised against current vaccine and vaccine-like strains. All samples tested were susceptible to zanamivir, oseltamivir, peramivir and laninamivir, and no known resistance mutations were detected. While some antigenic drift was observed, strains fell within the same phylogenetic clades as 2022 Southern Hemisphere vaccine strains.

Despite a low vaccine coverage (12%) in the Viral Watch programme, vaccine effectiveness for any influenza, influenza A(H1N1)pdm09 and influenza A(H3N2) adjusted for age and season was 65% (95% CI: 30%, 82%), 46% (95% CI: -20%, 76%) and 91% (95% CI: 31%, 99%), respectively. Vaccine effectiveness for influenza B/Victoria could not be determined due to small numbers at the time of this report. Vaccine effectiveness calculations will be updated when the 2022 influenza season has ended.

Following easing of COVID-19 restrictions, South Africa experienced the first typical influenza season since the start of the pandemic. The influenza season was ongoing as of week 43 of 2022, with a biphasic pattern in which infections later in the season were dominated by B/Victoria and A(H3N2) viruses.[5]

Recommendations

- Individuals are encouraged to receive the annual flu vaccine. Ideally the flu vaccine should be taken early (March/April each year) before the flu season so that it has sufficient time to protect a person. However, it is never too late to vaccinate as long as the flu virus is circulating.
- Groups recommended to receive influenza vaccination include:

- Healthcare workers
- Persons aged ≥ 65 years
- Persons with underlying chronic health conditions
- HIV–infected adults
- Pregnant women at any stage of pregnancy including up to 6 weeks postpartum
- Residents of old-age homes, chronic care and rehabilitation institutions
- Persons aged 6 months to ≤18 years on long-term aspirin therapy
- Any persons wishing to minimise the risk of influenza acquisition

- People who are sick with flu-like symptoms can prevent spread by:
  - Covering their mouth when coughing with a tissue or cough into the elbow
  - Wearing a mask
  - Washing their hands frequently with soap and water or cleaning hands using an alcohol-based sanitiser
  - Staying at home and trying to keep a distance from others

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Epidemiology team: Boitumelo Chuene, Cheryl Cohen, Hanif Carrim, Jackie Kleynhans, Jocelyn Moyes, Mvuyo Makhasi, Nicola Chiwandire, Sibongile Walaza, Thulisa Mkhencele

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The following public health facilities and laboratories participated in the pneumonia and ILI surveillance programmes:

ILI Clinic-based surveillance
Agincourt Health Care Centre, Agincourt, Mpumalanga Province
Eastridge Clinic and Mitchell's Plain Clinic, Western Cape Province
Jouberton Clinic, North West Province
Edendale Gateway Clinic, KwaZulu-Natal Province

Pneumonia surveillance
Edendale Hospital, KwaZulu-Natal Province
Helen Joseph and Rahima Moosa Mother and Child Hospitals and Tembisa Hospital, Gauteng Province
Klerksdorp and Tshepong Hospital Complex, North West Province
Tintswalo Hospital, Mapulaneng and Matikwana Hospitals, Mpumalanga Province
The Red Cross Childrens' War Memorial Hospital, Mitchell's Plain Hospital and Tygerberg Hospital Western Cape Province

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MALARIA VECTOR SURVEILLANCE REPORT, SOUTH AFRICA, JANUARY – DECEMBER 2021

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Summary

Malaria in South Africa is seasonal and primarily occurs in the Limpopo, Mpumalanga and KwaZulu-Natal provinces. Malaria vectors are controlled by indoor spraying of residual insecticides (IRS) and limited larval source management. Vector surveillance in collaboration with the National Institute for Communicable Diseases (NICD) during 2021 revealed the presence of four malaria vector species - *Anopheles arabiensis* (n=4,873, 43%), *An. merus* (n=709, 6%), *An. parensis* (n=1,175, 10%) and *An. vaneedeni* (n=335, 3%). These have previously been shown to contribute to ongoing residual malaria transmission in South Africa. Several closely related non-vector *Anopheles* species were also collected. The specimens analysed were collected from KwaZulu-Natal (69.7%, n=7,967), Mpumalanga (6.5%, n=747) and Limpopo (23.7%, n=2,714) provinces. The surveillance information by province and municipality shows that IRS-based vector control needs to be maintained at a high rate of coverage in high-incidence areas, and that spraying should ideally be completed before the onset of each malaria season. Consideration can be given to a more targeted or reactive approach in areas where no local cases have been recorded for three or more years. Given that all sporozoite positive (and therefore malaria infective) adult *Anopheles* females collected in the recent years were found resting outdoors,

and given that there are no large-scale vector control tools targeting outdoor-resting mosquitoes, larviciding, including the treatment of winter breeding sites, should continue to be used as a complimentary method to enhance the effect of IRS in areas where locally-acquired cases occur and in other receptive areas at risk for malaria. Consideration should also be given to the distribution of dual active ingredient insecticide treated bed nets to migrant / mobile communities that are not protected by the IRS programmes.

Introduction

South Africa's malaria affected areas include the low altitude border regions of Limpopo, Mpumalanga and KwaZulu-Natal (KZN) provinces. These regions typically experience active malaria transmission, especially during the peak malaria season that spans the summer months of November to April. The total number of malaria cases in the 2020/2021 malaria season in South Africa was 4961, while the 2021/2022 malaria season saw 2386 cases.[1]

Each of South Africa's malaria endemic provinces have developed well-coordinated malaria prevention operations including routine vector control which is primarily based on the application of indoor residual insecticide spraying (IRS) and, to a lesser extent, larval source management.[2] Although IRS has proven efficacy spanning many decades, residual malaria transmission continues and is likely caused by outdoor feeding and resting *Anopheles* vector mosquitoes that are less susceptible to indoor applications of insecticide.[3,4,5] In addition, populations of the major malaria vector species, *Anopheles funestus* and *An. arabiensis*, have developed resistance to insecticides, especially in northern KwaZulu-Natal.[2,6] The pyrethroid resistance phenotype in *An. arabiensis* in this region is however of low intensity currently and is not considered to be operationally significant yet. This is in contrast to the pyrethroid-carbamate resistance profile in *An. funestus* which is of high intensity, highly significant epidemiologically and was at least partly causative of the malaria epidemic experienced in South Africa during the period 1996 to 2000.[7]

Residual malaria transmission, comparatively high incidence and burgeoning insecticide resistance in malaria vector populations within South Africa's borders necessitate ongoing and enhanced vector surveillance to inform best practices for control. This is pertinent in terms of South Africa's malaria elimination agenda[8] and the ongoing COVID-19 pandemic, making it especially important to reduce disease burden as far as possible.[9] Currently, surveillance is routinely conducted by the entomology teams of Mpumalanga, KwaZulu-Natal and Limpopo provinces with support from partner institutions including the National Institute for Communicable Diseases (NICD), the Wits Research Institute for

Malaria (WRIM) of the University of the Witwatersrand, the UP Institute for Sustainable Malaria Control (UP ISMC) of the University of Pretoria, and the South African Medical Research Council.

This report summarises malaria vector surveillance in South Africa in 2021 based on specimens referred to the Vector Control Reference Laboratory (VCRL) of the Centre for Emerging Zoonotic and Parasitic Diseases (CEZPD), NICD, as well as specimens collected and analysed by personnel from the University of Pretoria.

Methods

Anopheles mosquitoes were collected from sentinel sites in KwaZulu-Natal, Mpumalanga and Limpopo provinces (Figure 1). These specimens were either collected by VCRL and UP ISMC, or referred to the VCRL by partner institutions and provincial malaria control programme entomology teams from January to December 2021.

Adult *Anopheles* mosquitoes were collected by human-baited net traps, human landing catches, cattle kraal, house search, CDC-light traps, BG-sentinel traps, CO_2 net traps, and outdoor placed clay pots, drums, cloth tubes, modified buckets and tyres. Other specimens were collected as larvae and were reared to adults for subsequent analysis. One or more of these collection techniques were deployed at each sentinel site (Figure 1). Adult specimens were preserved on silica gel in 1.5ml microcentrifuge tubes and were identified as far as possible using external morphological characters by VCRL, partner institution and or provincial malaria control programme personnel. Specimens identified as members of the *An. gambiae* complex or *An. funestus* group were subsequently identified to species using standard polymerase chain reaction (PCR) assays by VCRL and UP ISMC.[10,11,12] An ELISA assay was used to detect the presence of *Plasmodium falciparum* circumsporozoites in selected female specimens.[13,14] The VCRL is a SANAS accredited laboratory and the ISO 17025:2017 standard was used to ensure the quality of results of all specimens received and analysed.

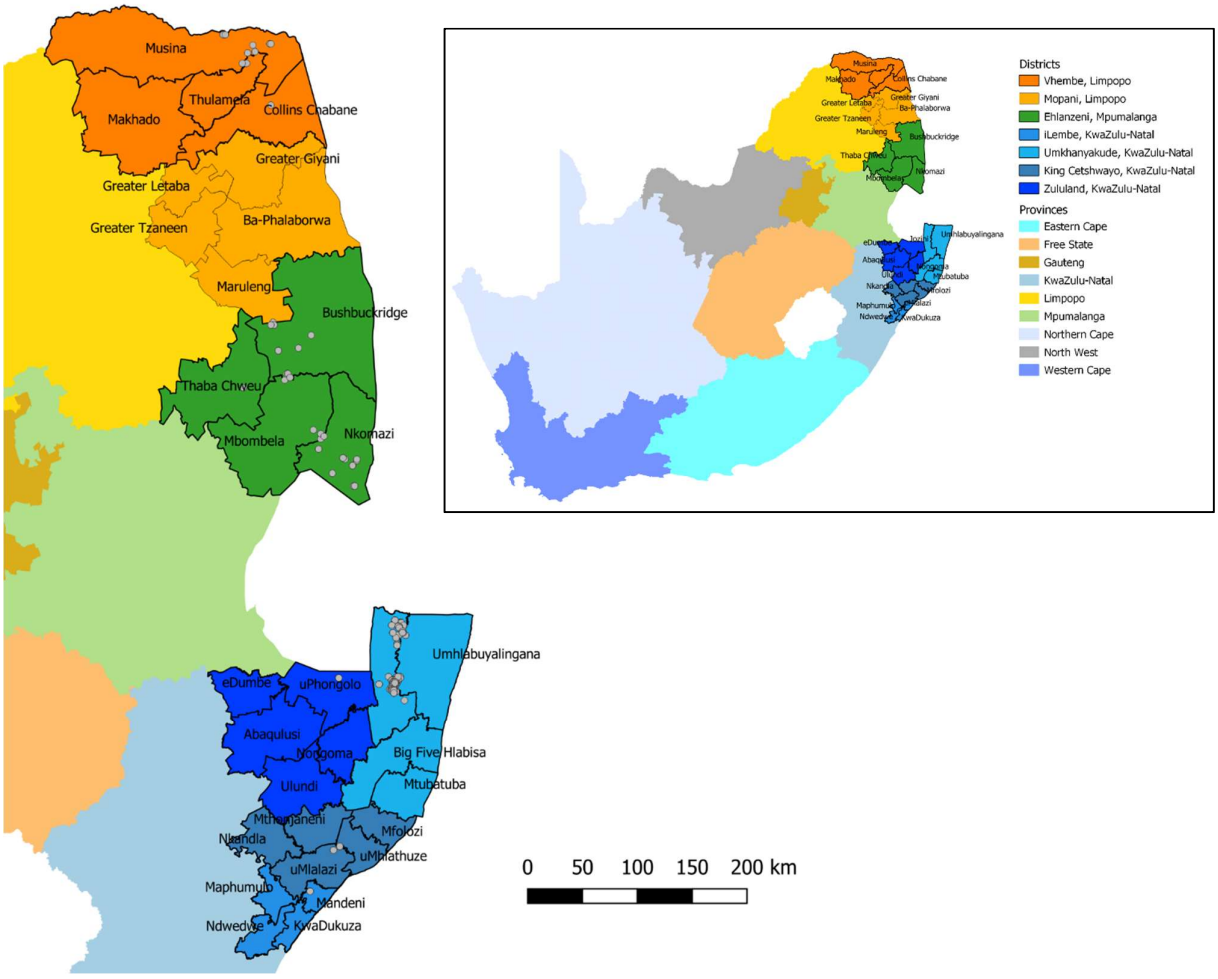

Figure 1. Sentinel sites (grey dots) in KwaZulu-Natal, Mpumalanga and Limpopo provinces from which *Anopheles* specimens were collected, South Africa, 2021.

Results

A total of 11,428 *Anopheles* mosquitoes was collected from sentinel sites in the Ilembe, Umkhanyakude, King Cetshwayo and Zululand districts of KwaZulu-Natal Province, the Ehlanzeni district of Mpumalanga Province and the Vhembe and Mopani districts of Limpopo Province. Most of the specimens were collected from KwaZulu-Natal (69.7%, n=7,967) followed by Limpopo (23.7%, n=2,714) and Mpumalanga (6.5%, n=747) provinces (Table 1). These were subsequently clustered as either *An. gambiae* complex (52%, n=5,990), *An. funestus* group (18%, n=2,007) or other *Anopheles* species (30%, n=3,431). *Anopheles arabiensis* predominated the collections (43%, n=4,873), especially in KwaZulu-Natal, although substantial numbers of *An. merus*, *An. parensis*, *An. listeri*, *An. marshallii* complex and *An. pretoriensis* were also collected. *Anopheles merus* and *An. listeri* predominated in

Mpumalanga and Limpopo provinces, respectively (Table 1). None of the 136 adult female mosquitoes from KZN selected for detection of *P. falciparum* circumsporozoites by ELISA were positive (Table 2)

Table 1. Numbers of *Anopheles* specimens collected by species and province, South Africa, 2021.

***Anopheles* species complex, group or other**	**Species**	**KwaZulu-Natal**	**Mpumalanga**	**Limpopo**	**Total**
***An. gambiae* complex**	*An. arabiensis*	4,715	105	53	4,873
	An. merus	244	462	3	709
	An. quadriannulatus	59	99	250	408
***An. funestus* group**	*An. leesoni*	163	1	157	321
	An. parensis	1,175			1,175
	An. rivulorum	110		39	149
	An. rivulorum-like	0		27	27
	An. vaneedeni	329	5	1	335
Other *Anopheles* species	*An. coustani*	129	9	75	213
	An. demeilloni	23		96	119
	An. gibbinsi			141	141
	An. listeri			721	721
	An. longipalpis		1		1
	An. maculipalpis	26	1		27
	An. marshallii complex	652			652
	An. natalensis			2	2
	An. pharoensis	85			85
	An. pretoriensis	3	15	615	633
	An. rhodesiensis			69	69
	An. rufipes	101	49	420	570
	An. squamosus	72			72
	An. tenebrous	72		43	115
	An. ziemanni	9		2	11
Total		7,967	747	2,714	11,428

Table 2. Numbers of *Anopheles* female specimens collected as adults from KwaZulu-Natal Province in 2021, and tested for the presence of *Plasmodium falciparum* circumsporozoites by ELISA.

***Anopheles* species complex, group or other**	**Species**	**Number of species tested negative for the presence of *P. falciparum***	**Number of species tested positive for the presence of *P. falciparum***
***An. gambiae* complex**	*An. arabiensis*	8	0
	An. merus	1	0
Other *Anopheles* species	*An. coustani*	17	0
	An. demeilloni	1	0
	An. maculipalpis	1	0
	An. marshallii complex	1	0
	An. pharoensis	32	0
	An. pretoriensis	2	0
	An. rufipes	35	0
	An. squamosus	38	0
Total		136	0

The malaria vectors *An. arabiensis* and *An. merus* (members of the *An. gambiae* species complex) were collected from sentinel sites in all the endemic provinces (Figure 2). In KwaZulu-Natal Province, populations of these species were found in the Jozini and Umhlabuyalingana municipalities of the Umkhanyakude District, uPhongolo municipality of Zululand District and the uMlalazi municipalities of the King Cetshwayo District. In Mpumalanga, populations of these species were found in all the municipalities of the Ehlanzeni District. In Limpopo Province, these species were found in the Collins Chabane and Musina municipalities of the Vhembe district.

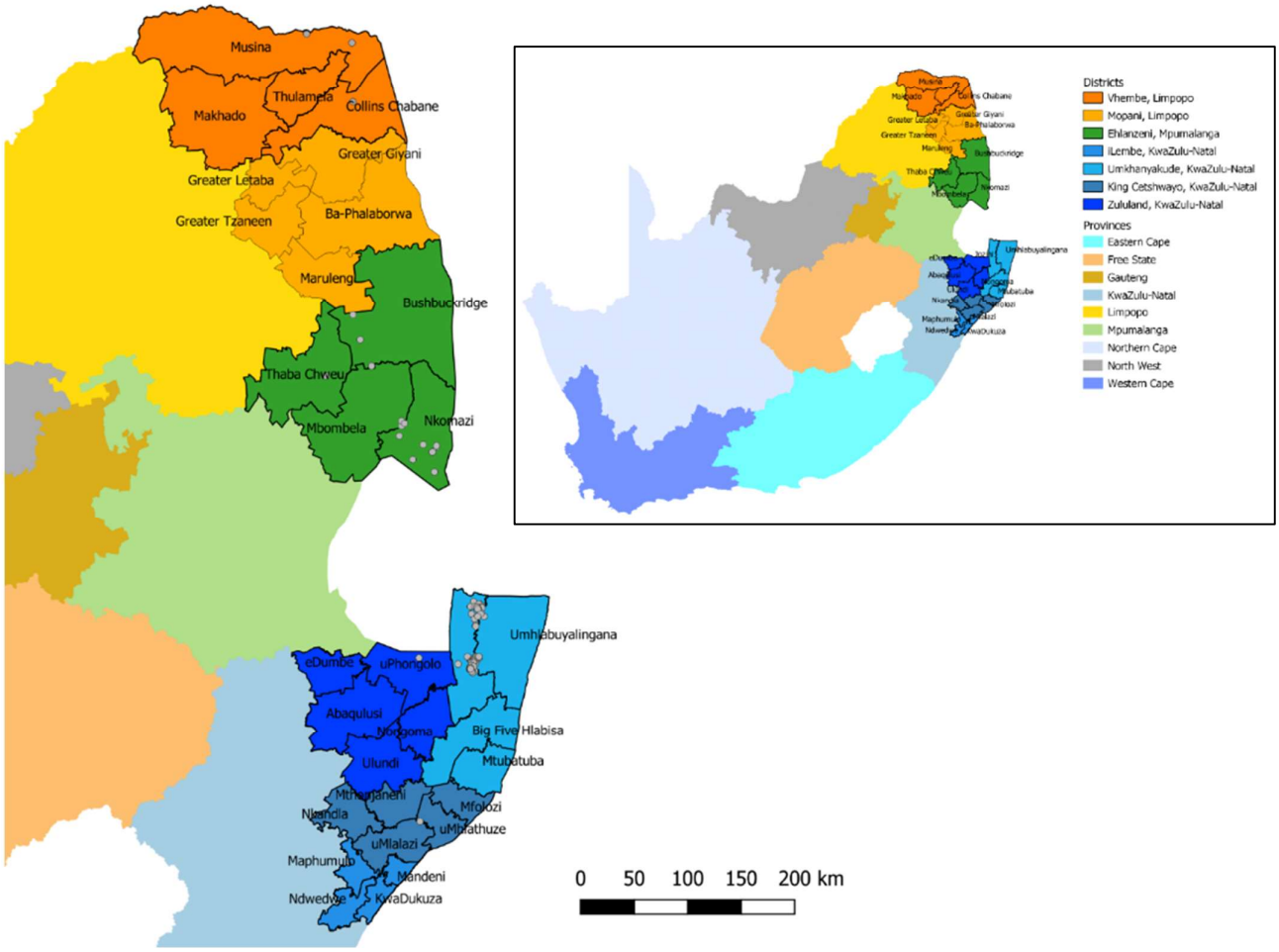

Figure 2. Sentinel sites (grey dots) in KwaZulu-Natal, Mpumalanga and Limpopo provinces from which samples of *Anopheles arabiensis* and *An. merus* (*Anopheles gambiae* complex) were collected, South Africa, 2021.

The potential secondary malaria vector species *An. vaneedeni* [3] was collected from sentinel sites in all three endemic provinces while *An. parensis*, also a potential secondary vector[15], was only collected in KwaZulu-Natal Province (Table 1). Other potential malaria vector species within the *An. funestus* group that were collected from sentinel sites in these three provinces included *An. leesoni* and *An. rivulorum* (Table 1). Collection sites for all known and suspected vector species within the *An. funestus* group are shown in Figure 3. Specimens of these species were collected in the Jozini and Umhlabuyalingana municipalities of the Umkhanyakude District, the uMlalazi municipality of the King Cetshwayo District and the Mandeni municipality of Ilembe District, KwaZulu-Natal Province, in Nkomazi and Bushbuckridge of the Ehlanzeni District of Mpumalanga Province and in the Musina and Thulamela municipalities of the Vhembe District of Limpopo Province.

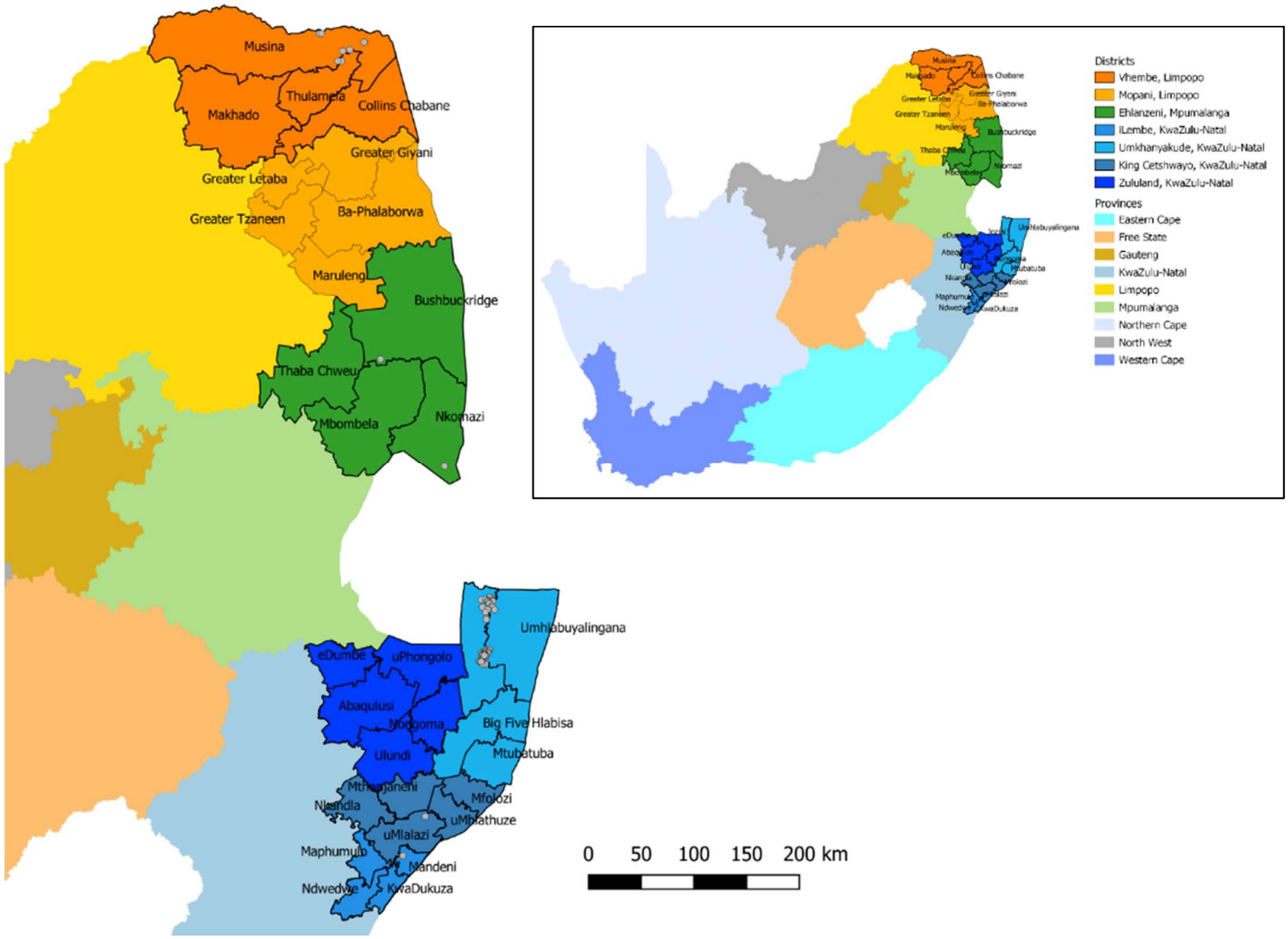

Figure 3. Sentinel sites (grey dots) in KwaZulu-Natal, Mpumalanga and Limpopo provinces from which samples of the known and potential secondary malaria vectors *Anopheles vaneedeni*, *An. parensis*, *An. rivulorum* and *An. leesoni* (*An. funestus* group) were collected, South Africa, 2021.

Anopheles coustani, *An. demeilloni*, *An. longipalpis*, *An. marshallii* complex, *An. pharoensis*, *An. pretoriensis*, *An. rufipes*, *An. squamosus* and *An. ziemanni* have been incriminated as malaria vectors in other regions of Africa[16,17,18,19, 20] but not in South Africa. The distribution of these potential vector species is shown in Figure 4. Specimens of these species were collected in the Jozini and Umhlabuyalingana municipalities in the Umkhanyakude District as well as uPhongolo municipality of the Zululand district of KwaZulu-Natal Province, in Bushbuckridge and Nkomazi municipalities of the Ehlanzeni District of Mpumalanga Province and in the Musina and Thulamela municipalities of the Vhembe district of Limpopo Province.

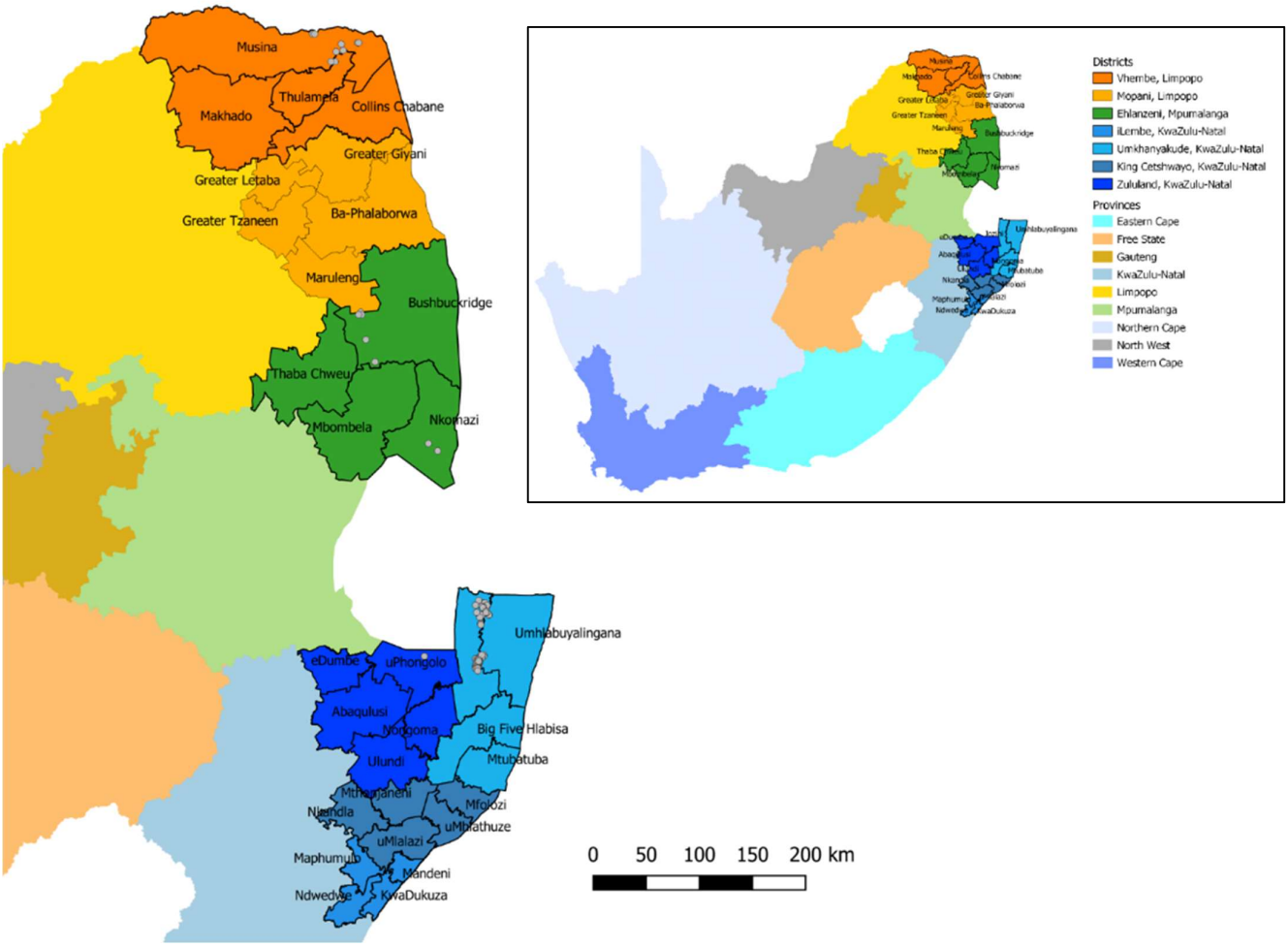

Figure 4. Sentinel sites (grey dots) in KwaZulu-Natal, Mpumalanga and Limpopo provinces from which samples of miscellaneous *Anopheles* species (species not belonging to the *An. gambiae* complex or *An. funestus* group) were collected. These sites included the collection of potential secondary malaria vectors *Anopheles coustani*, *An. demeilloni*, *An. longipalpis*, *An. marshallii* complex, *An. pharoensis*, *An. pretoriensis*, *An. rufipes*, *An. squamosus*, and *An. ziemanni*, South Africa, 2021.

The number of anophelines collected by species during specific seasons was highly variable across the three endemic provinces. For example, *An. arabiensis* was prevalent throughout the year in KwaZulu-Natal Province while *An. merus* was particularly prevalent throughout the year in the Mpumalanga Province (Figure 5). *Anopheles quadriannulatus* predominated the *Anopheles gambiae* complex collections from Limpopo Province during late summer (January to February) and autumn. *Anopheles parensis* was prevalent throughout the year in KwaZulu-Natal Province. Of the *An. funestus* group,

Anopheles vaneedeni predominated in Mpumalanga Province and *An. leesoni* dominated the collections in Limpopo Province during spring and early summer (December) (Figure 6). Miscellaneous *Anopheles* species collections in KwaZulu-Natal Province indicate that *Anopheles rufipes* and *An. pharoensis* predominated in late summer and autumn, respectively, while *An. marshallii* complex predominated in winter and spring (Figure 7). *Anopheles pretoriensis* and *An. rufipes* were evident in winter and spring, while in early summer *An. rufipes* predominated the collections of miscellaneous species in Mpumalanga Province. *Anopheles listeri* and *An. rufipes* predominated the miscellaneous species in spring and early summer, respectively, in Limpopo Province.

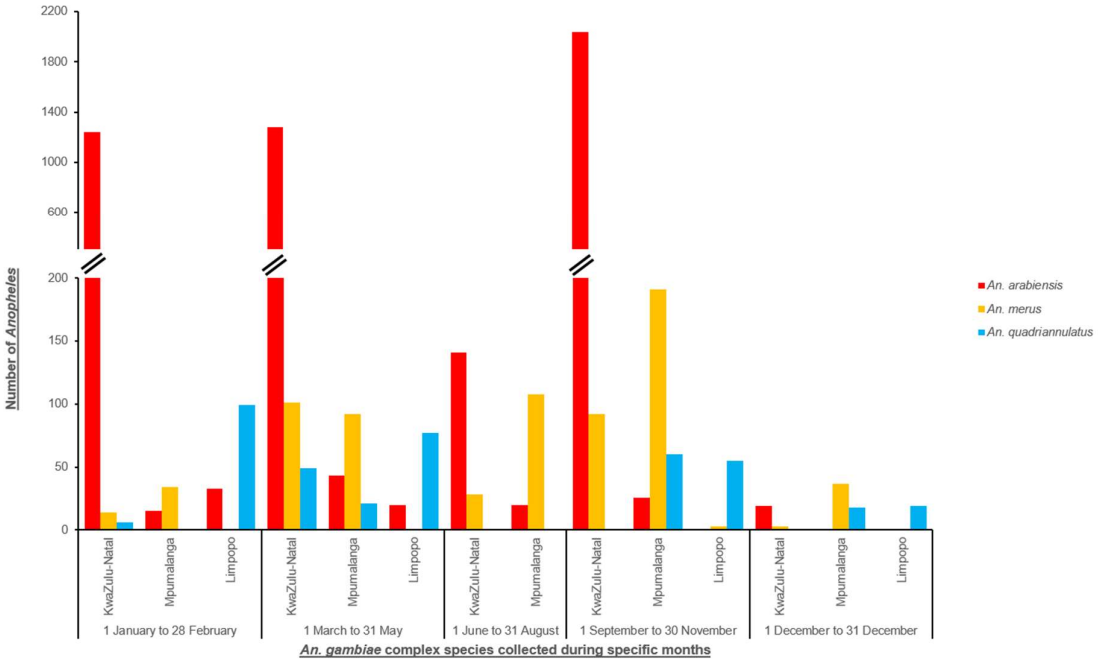

Figure 5. Distribution (in absolute numbers) of *Anopheles gambiae* complex specimens collected by species, province and season, South Africa, 2021.

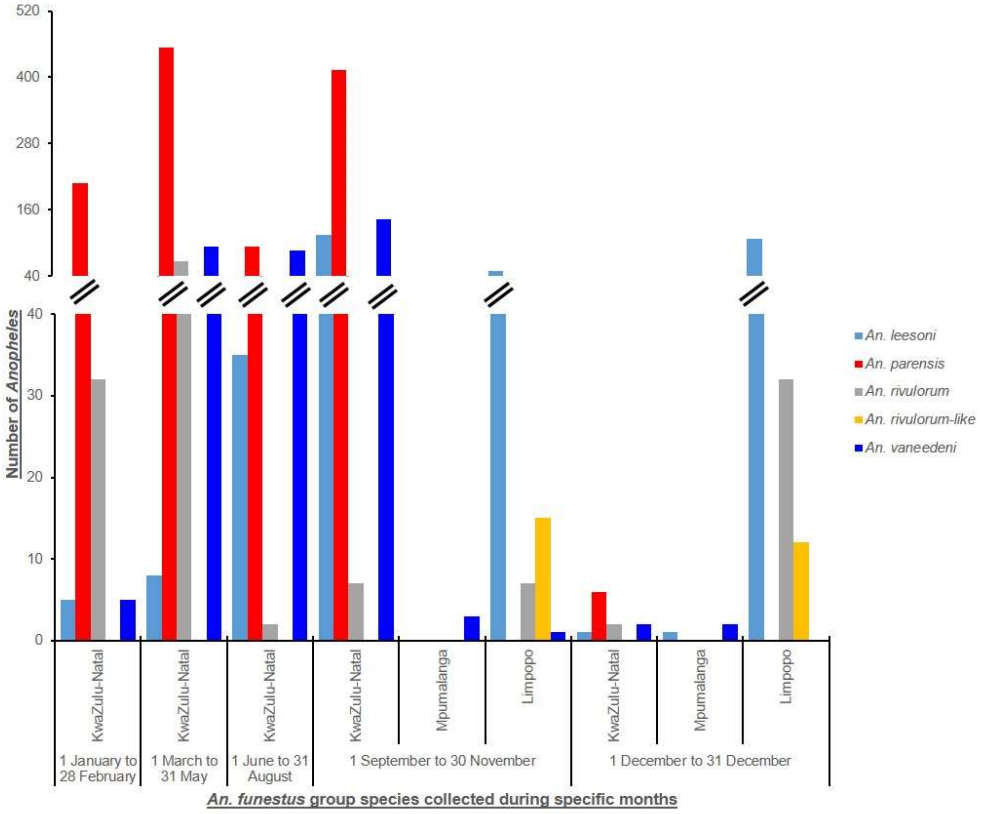

Figure 6. Distribution (in absolute numbers) of *Anopheles funestus* group specimens collected by species, province and season, South Africa, 2021.

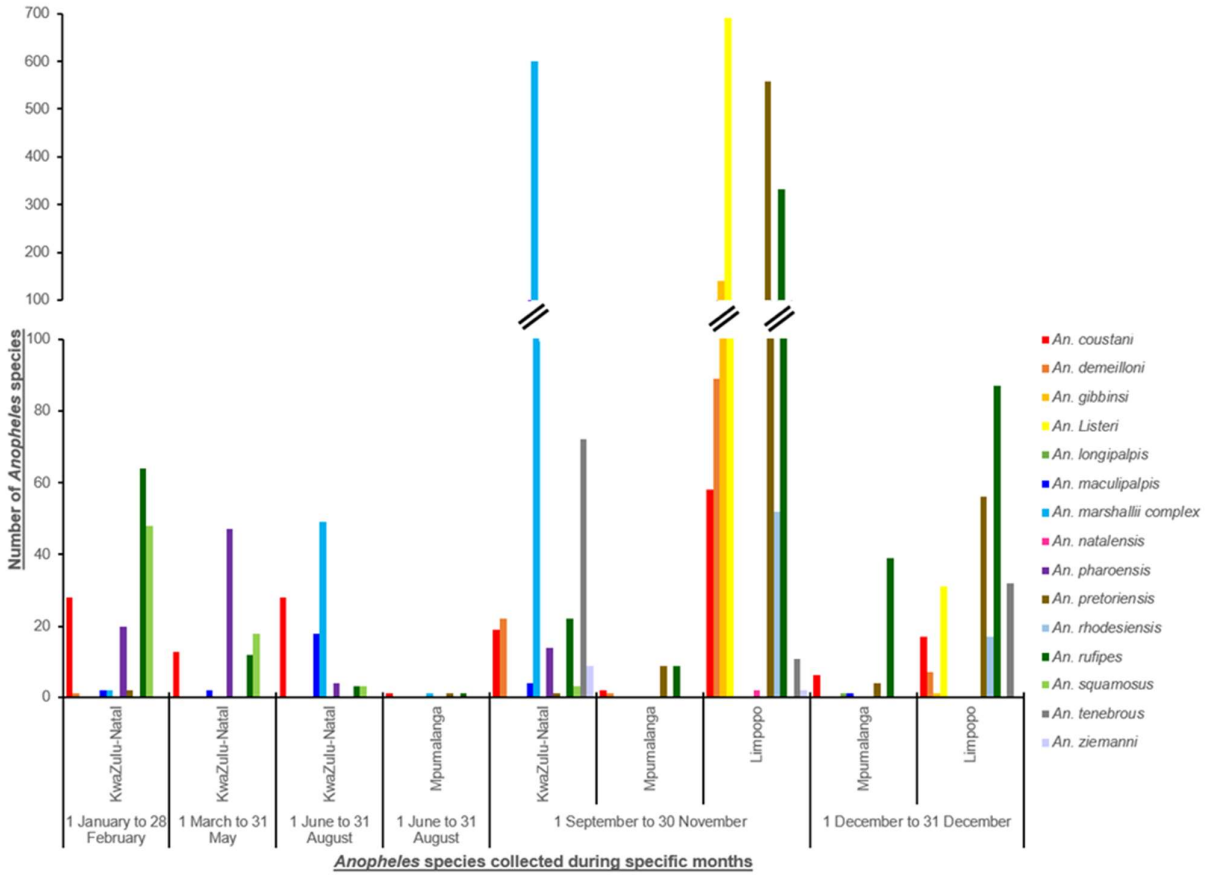

Figure 7. Distribution (in absolute numbers) of miscellaneous *Anopheles* specimens collected by species, province and season, South Africa, 2021

Anopheline specimens were collected either as larvae or adults. Collection methods and intensity of effort varied between the endemic provinces. In all three provinces, CO_2 tent traps and human landing catches were used to collect adult mosquitoes, and larvae were collected from breeding sites. CDC-light traps and CO_2 tent traps were primarily used to collect adult mosquitoes in Limpopo, yielding (53%, n=506) and (46%, n=443) respectively. In Mpumalanga, the majority of adult *Anopheles* were collected via human landing catches (65%, n=62). In KZN, the majority of adult *Anopheles* were collected using clay pots (64%, n=4,704) followed by tyre (23%, n=1,735), cloth tube (3.7%, n=276) and CO_2 tent trap (3.5%, n=256).

Within the *An. gambiae* complex, adult *An. arabiensis* and *An. merus* were collected using all the sampling methods listed in Table 3. *Anopheles arabiensis* adults were predominantly collected from clay pots (62%, n=2,786) and tyres (27%, n=1,199), while the *An. merus* adults were predominantly collected from clay pots (63%, n-=110) and human landing catches (22%, n=39) (Figure 8). Within the

An. funestus group, *An. parensis*, *An. vaneedeni*, *An. rivulorum* and *An. leesoni*, were collected by all the collection methods listed in Table 3 with the exception of male swarm collection. *Anopheles parensis* adults were predominantly collected via clay pots (67%, n=776) and tyres (27%, n=317), and 72% of the *An. vaneedeni* adults were collected from clay pots (n=235). *Anopheles leesoni* (n=120) and *An. rivulorum* (n=64) adults were also predominantly collected from clay pots (Figure 9).

The potential secondary malaria vectors *Anopheles coustani*, *An. demeilloni*, *An. longipalpis*, *An. marshallii* complex, *An. pharoensis*, *An. pretoriensis*, *An. rufipes*, *An. squamosus* and *An. ziemanni* were collected using all the sampling methods listed in Table 3 with the exception of drum and male swarm collection. *An. coustani* (n=42), *An. demeilloni* (n=60) and *An. pretoriensis* (n=108) adults were predominantly collected from CDC-light traps. The *An. longipalpis* (n=1), *An. pharoensis* (n=39) and *An. squamosus* (n=27) adults were predominantly collected from CO_2 tent traps, while *An. marshallii* complex (n=488), *An. rufipes* (n=36) and *An. ziemanni* (n=5) were predominantly collected from clay pots (Figure 10).

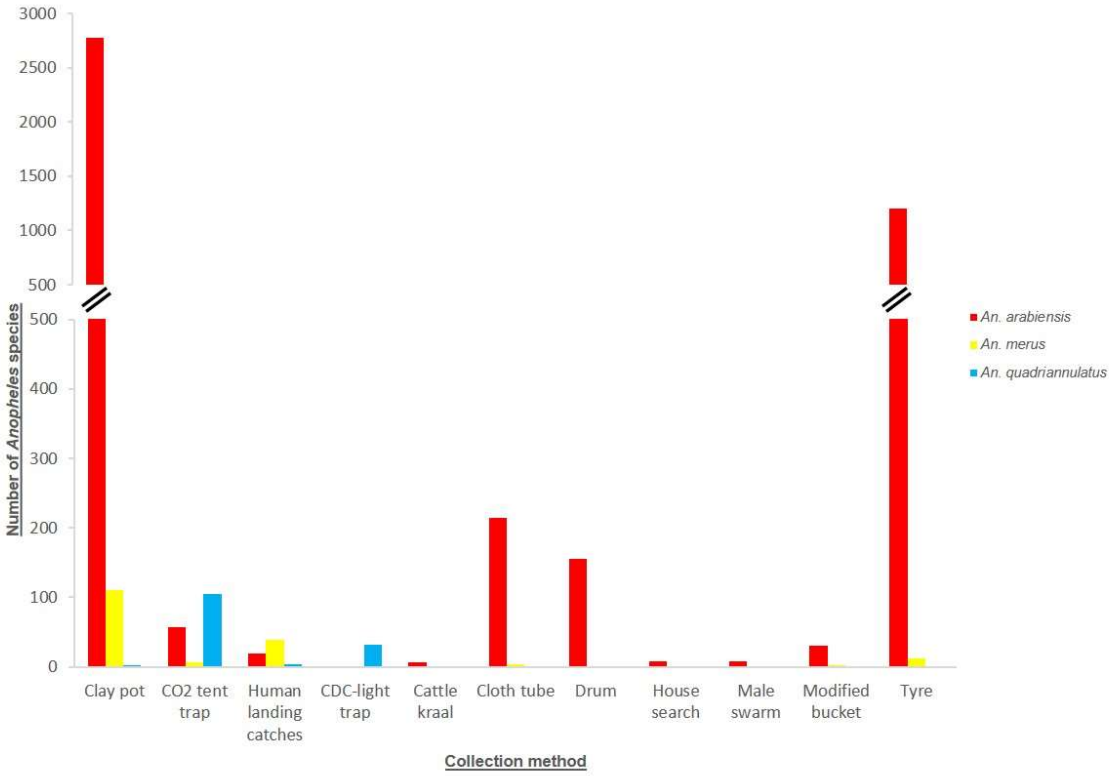

Figure 8. Distribution (in absolute numbers) of *Anopheles gambiae* complex specimens by sampling method, South Africa, 2021.

Table 3. Numbers of *Anopheles* specimens collected by sampling method, South Africa, 2021.

Anopheles species complex, group or other	Species	Clay pot		CO_2 tent trap			Human landing catches			CDC-light trap	Cattle kraal	Cloth tube	Drum	House search	Male swarm	Modified bucket	Tyre
		KwaZulu-Natal	Mpumalanga	KwaZulu-Natal	Mpumalanga	Limpopo	KwaZulu-Natal	Mpumalanga	Limpopo	Limpopo	KwaZulu-Natal	KwaZulu-Natal	KwaZulu-Natal	KwaZulu-Natal	KwaZulu-Natal	KwaZulu-Natal	
An. gambiae complex	*An. arabiensis*	2766	10	47	2	8		16	3		6	215	155	8	8	30	1199
	An. merus	109	2	1	4	2		39		1		4				2	12
	An. quadriannulatus	2		3		102		2	2	32		1					
An. funestus group	*An. leesoni*	120		9	1	111				45	2						27
	An. parensis	776		8							2	20	36	1		4	317
	An. rivulorum	64		3		25				9	7			2		2	17
	An. rivulorum-like					15				12							
	An. vaneedeni	234	1	8	2	1		2		0	4	7				25	44
Other *Anopheles* species	*An. coustani*	33		25	2	22	2	3		42				1		2	4
	An. demeilloni	19	1	1		31				60							1
	An. gibbinsi					35				106							
	An. listeri					12				49							
	An. longipalpis				1												
	An. maculipalpis	10		11							3					2	
	An. marshallii complex	488		23	1						11	13					110
	An. natalensis																
	An. pharoensis	10		39							7	1					1
	An. pretoriensis	3			3	59				108							
	An. rhodesiensis									3							
	An. rufipes	36	1	10	2	14				29	1	10		1		7	
	An. squamosus	4		27							1			1			
	An. tenebrous	25		39		6				10	1	4				1	2
	An. ziemanni	5		2								1					1

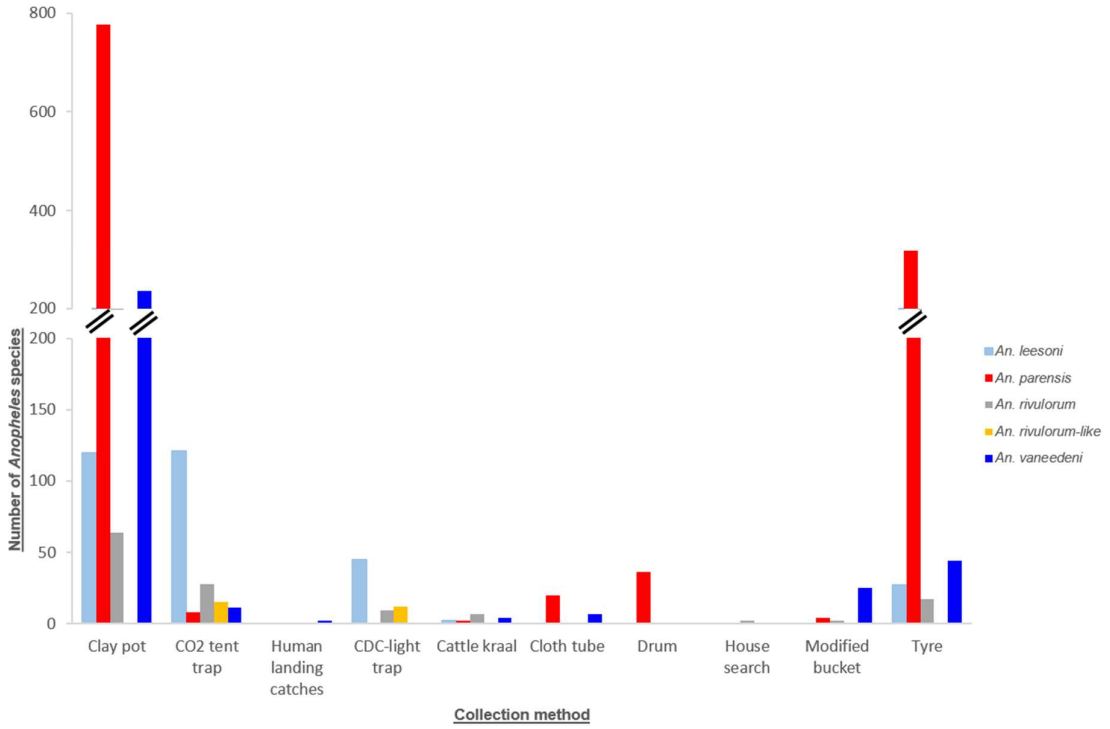

Figure 9. Distribution (in absolute numbers) of *Anopheles funestus* group specimens by sampling method, South Africa, 2021.

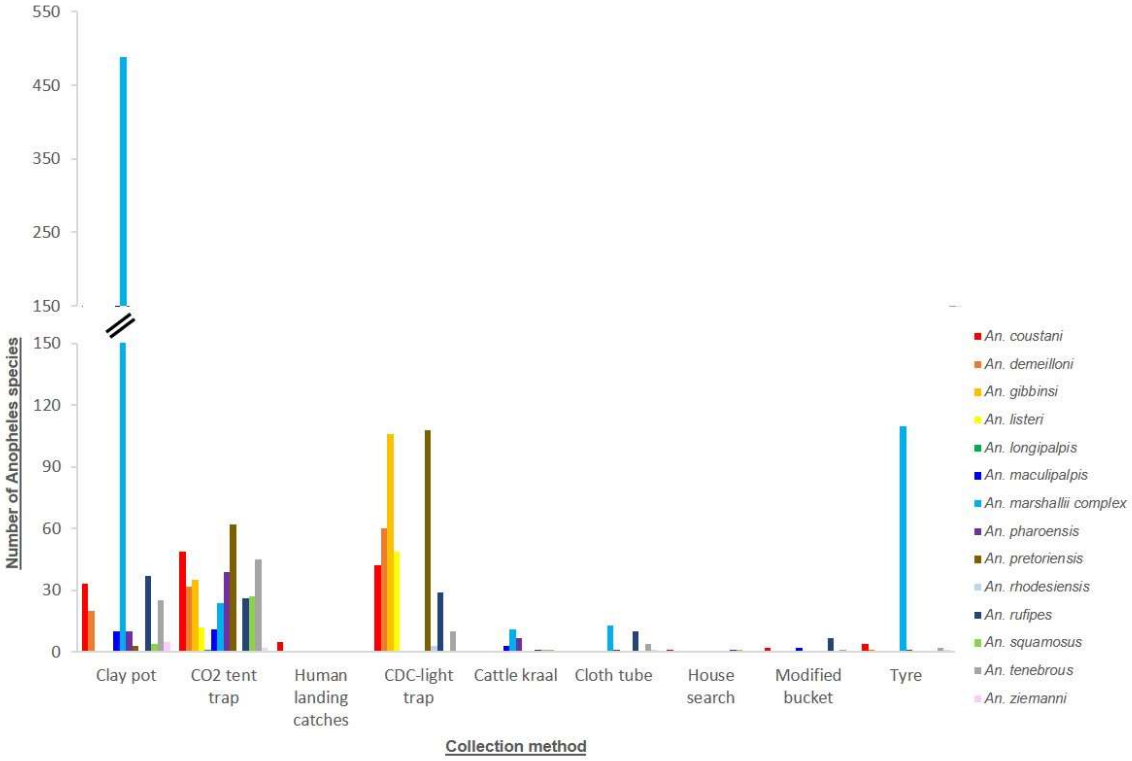

Figure 10. Distribution (in absolute numbers) of miscellaneous *Anopheles* specimens by sampling method, South Africa, 2021.

Discussion

Malaria vector surveillance in 2021 in the KwaZulu-Natal, Mpumalanga and Limpopo provinces of South Africa revealed 15 *Anopheles* species of interest in malaria transmission. The collections included species previously incriminated as vectors in South Africa (*An. arabiensis*, *An. parensis* and *An. vaneedeni*) as well as species incriminated as vectors in other African localities (*An. merus*, *An. leesoni*, *An. rivulorum*, *An. marshallii*, *An. coustani*, *An. demeilloni*, *An. longipalpis*, *An. pharoensis*, *An. pretoriensis*, *An. rufipes*, *An. squamosus* and *An. ziemanni).*[16,17,18,19, 20]

The major vector *An. arabiensis* was the predominant species collected during 2021, accounting for 60% of the specimens collected from KwaZulu-Natal Province. This species was also present in the Mpumalanga and Limpopo Provinces accounting for 14% and 2% of the specimens collected. *Anopheles arabiensis* is currently the major malaria vector in South Africa following the near eradication of *An. funestus* by intensive IRS campaigns over the last two decades.[2, 21] Since *An. arabiensis* females are at least partially inclined to feed and rest outdoors, they are less susceptible to control by IRS.[4,5] This species is therefore the primary vector of residual malaria in South Africa[4], but not the only contributor.

Anopheles merus was collected from all three endemic provinces, with the highest numbers in 2021 coming from Mpumalanga Province, similar to collections in 2019 and 2020. Although *An. merus* has not been definitively implicated in malaria transmission in South Africa to date, its confirmed vector status in other regions such as southern Mozambique (sporozoite rates for *An. merus* in the Boane District being 4.2%)[22] suggests that it is most likely an important secondary malaria vector in South Africa as well. This species is primarily a coastal saltwater breeder, although it has also been collected from fresh-water larval habitats in southern Africa including sites in South Africa.[23]

Anopheles parensis and *An*. *vaneedeni* have been incriminated as secondary malaria vectors in South Africa[3,15], while other members of the *An. funestus* group (*An. rivulorum* and *An. leesoni*) have been implicated as secondary malaria vectors in East Africa. *Anopheles vaneedeni* and *An. leesoni* were collected from all three endemic provinces while *An. parensis* was only detected in KwaZulu-Natal Province during 2021, which was also the case in 2019 and 2020. *Anopheles vaneedeni* likely contributes to residual malaria transmission in South Africa given its tendency to rest outdoors and to feed on humans amongst other vertebrate hosts.[3] *Anopheles parensis* is primarily zoonotic and may rest indoors and outdoors. This species will also occasionally feed on humans[24] and can potentially contribute to residual malaria transmission in South Africa. The major vector *An. funestus s.s.*, the predominant malaria vector species in neighbouring Mozambique and Zimbabwe,

was not detected in South Africa in 2021. This can be attributed to ongoing IRS programmes in the malaria-endemic provinces year on year.

Other species that occur in South Africa and that have been incriminated as malaria vectors in various African localities include *An. marshallii*, *An. coustani*, *An. demeilloni*, *An. longipalpis*, *An. pharoensis*, *An. pretoriensis*, *An. rufipes*, *An. squamosus* and *An. ziemanni* .[16, 17, 18, 19, 20] It is possible that one or more of these species plays a role in residual malaria transmission in South Africa. *Anopheles rufipes*, *An. pretoriensis* and *An. coustani* were present in all three endemic provinces in South Africa in 2021.

Anopheles population densities are expected to fluctuate between seasons. They are generally highest during the summer months, congruent with increased rainfall[4], translating into higher malaria transmission rates during summer and especially late summer. Some species however, especially *An. arabiensis* in northern KwaZulu-Natal Province, were present at comparatively high numbers during the dry winter months. This is likely due to continuous and intensive collections throughout the year in northern KZN by personnel of the Sterile Insect Technique project.[4]

Specimens of the *Anopheles* species directly incriminated as vectors in South Africa - *An. arabiensis*, *An. parensis* and *An. vaneedeni* - were predominantly collected from clay pots. Other potential secondary vectors were predominantly collected from clay pots, tyres, CO_2 tent traps and CDC-light traps. Combinations of these and other collection methods can therefore be used to maximize adult *Anopheles* specimen collections. Owing to substantial variation in the intensity and frequency of collection effort by locality and method, it is not possible to accurately assess which methods are in fact the most productive from this data. The surveillance objectives (key surveillance indicators) by province, district and sentinel site should guide choice of collection methods, as each method has its advantages and disadvantages.

The occurrence of primary and secondary vector species in all three of South Africa's malaria-endemic provinces shows that they remain highly receptive to malaria despite ongoing IRS operations each year. During 2021, the highest number of local malaria cases was recorded in Limpopo Province, from where only 53 (2%) *An. arabiensis* specimen were collected. This suggests that secondary vector species play an important role in ongoing malaria transmission there, which is likely true for the other endemic provinces as well.

Conclusion

Several malaria vector species occur in the north-eastern lowveld regions of South Africa, with their relative abundances remaining comparatively high through the dry winter months in some instances. Despite

coordinated provincial IRS programmes that usually achieve high spray coverage rates (80% or more of targeted structures in endemic areas), populations of these species persist and at least three of them - *An. arabiensis*, *An. vaneedeni* and *An. parensis* – have previously been implicated in ongoing residual transmission in South Africa (*An. merus* is also a highly likely contributor). The reasons for this are multiple and certainly include outdoor-biting and outdoor-resting components of these species.

Recommendations

- Malaria vector surveillance in South Africa's endemic provinces should be maintained on a weekly to monthly basis, especially during summer and autumn, by provincial entomology teams with the support of partner institutions ((NICD, Wits Research Institute for Malaria (WRIM), University of Pretoria Institute for Sustainable Malaria Control (UP ISMC) and South Africa Medical Research Council (SAMRC)).
- Malaria vector surveillance activities should prioritise the collection of insecticide susceptibility data, especially for populations of major vector species. These data should be collected annually in collaboration with partner institutions. Priority insecticides include deltamethrin, DDT, pirimiphos methyl and clothianidin if possible.
- Other vector bionomics including feeding, resting and breeding behaviours, and assessments of blood source and *Plasmodium* infectivity should continue to be assessed by entomology teams in collaboration with partner institutions.
- Malaria vector surveillance should be conducted biannually (by provincial entomology team personnel) in those districts or municipalities in endemic provinces that are currently malaria free. This provides important information on malaria receptivity and the risk of re-introduction.
- Malaria vector surveillance data should be entered into the provincial DHIS2 systems as they become available. Senior entomology team members with the support of information officers should do this. Partner institutions are strongly encouraged to share their surveillance data with the national and provincial control programmes for inclusion in the DHIS2 databases.
- Annual IRS-based vector control operations should achieve a high rate of coverage (>95%) in areas of active transmission based on incidence data from preceding malaria seasons, and the occurrence of major and secondary vector species.
- IRS activities as conducted by provincial malaria control personnel should ideally be completed before the onset of each malaria season i.e. October – November.
- Consideration should be given to a more targeted or reactive IRS approach in areas where no local cases have been recorded for three or more years. Such an approach can utilise the foci clearing operating procedures.

- Larval source management[25], including the treatment of winter *Anopheles* breeding sites, should be used to enhance the effect of IRS in high incidence areas. Spray team personnel under the guidance of the entomology teams should do this during or immediately after IRS operations, and during the winter months before IRS operations commence.
- Insecticide resistance management practices should be maintained and periodically revised based on vector surveillance information and the market availability/affordability of third generation insecticides. These include products containing one or a combination of the following active ingredients: pyrethroids, pirimiphos methyl and clothianidin.
- Additional vector control methods including dual active ingredient insecticide-treated bed net distribution to migrant communities, community outreach in terms of personal protection methods, housing design and screening, and environmental management (such as drainage of non-utilised water bodies used by mosquitoes for breeding) should be considered pre- and post-malaria elimination at the local level.

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