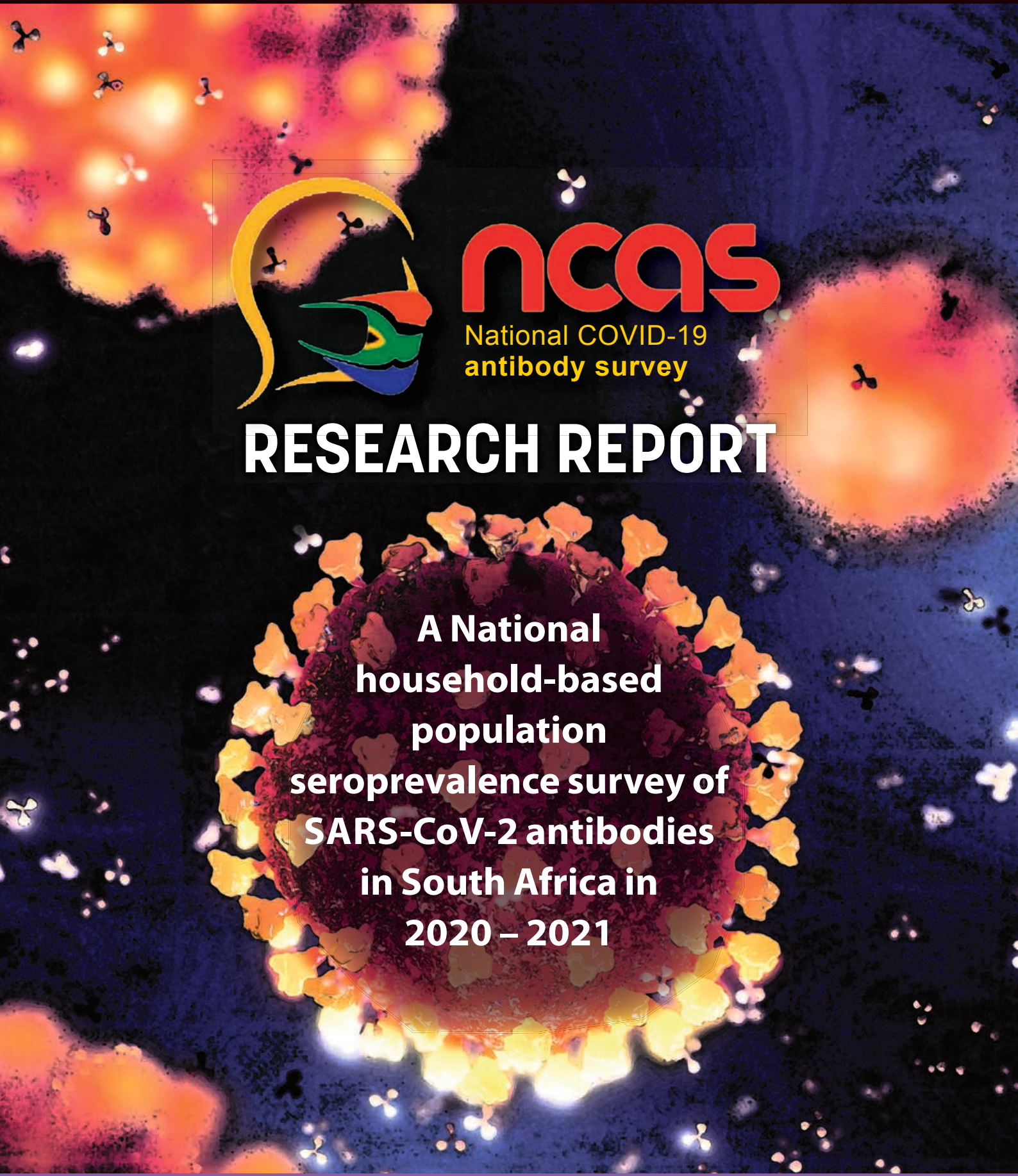

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National COVID-19
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SURVEY INVESTIGATORS AND STUDY TEAM

Human Sciences Research Council (HSRC)	
Professor Leickness Simbayi	Principal investigator
Professor Sizulu Moyo	Principal investigator
Professor Khangelani Zuma	Co-Principal investigator
Dr Nompumelelo Zungu	Co-Principal investigator
Dr Edmore Marinda	Co-Principal investigator
Dr Sean Jooste	Co-Principal investigator
Mr Johan van Zyl	Project Director
Dr Whadi-ah Parker	Project Director
Dr Musawenkosi Mabaso	Co-investigator
Dr Shandir Ramlagan	Co-investigator
Ms Alicia North	Co-investigator
Dr Inbarani Naidoo	Co-investigator
Mr Adlai Davids	Co-investigator
Dr Jacqueline Mthembu	Co-investigator
National Institute for Communicable Diseases (NICD)	
Professor Adrian Puren	Co-Principal investigator
Ms Beverly Singh	Co-investigator
Ms Ewalde Cutler	Co-investigator
Professor Penny Moore	Co-investigator
Ms Mirriam Fortuin	Co-investigator
Ms Vimbai S. Madzorera	Co-investigator
Dr Jinal Bhiman	Co-investigator
South African Medical Research Council (SAMRC)	
Dr Tarylee Reddy	Co-investigator
Mr Ishen Seocharan	Co-investigator
Dr Nobubelo Ngandu	Co-investigator
Professor Samuel Manda	Co-investigator
Dr Brodie Daniels	Co-investigator
Professor Ameena Goga	Senior Technical Advisor
University of the Witwatersrand (Wits)	
Professor Lynn Morris	Co-Principal investigator
National Health Laboratory Services (NHLS)	
Dr Kamy Chetty	Senior Technical Advisor
Professor Elizabeth Mayne	Co-investigator
Epicentre	
Ms Cherie Cawood	Project Director
Momentum Metropolitan Holdings Ltd. (MMI)	
Dr Boshoff Steenekamp	Senior Technical Advisor

FOREWORD

The global COVID-19 pandemic and its impact was unprecedented and challenged health systems including those in the developed world. The readiness and preparedness to respond to such a health crisis were overshadowed by the speed and magnitude of the spread of this previously unknown SARS-CoV-2 virus causative virus.

The Solidarity Fund was established immediately after confirmation of the first case of COVID-19 in South Africa in March 2020 as a platform to pool funding resources and other contributions from the general public, civil society, and public and private sectors to augment and strengthen the South African government's COVID-19 response, *'in solidarity'*. The Fund operated as a rapid response mechanism, assisting the country in addressing the key areas that would have the most significant impact on reducing the devastating effects of the pandemic on the health and wellbeing of citizens.

It was soon clear that a rapid and proactive response was essential to get ahead of this rampant novel virus. The epidemiology of the pandemic was evolving daily and although countries were learning about transmission, disease impact, morbidity, and mortality, from each other, South Africa needed to have a local understanding of the actual prevalence of infections and of those most affected to prioritize interventions that would have the greatest impact. It was therefore essential to gather data for an evidence-based response to get ahead of the curve, and to appropriately prepare the health system. Importantly, we needed a knowledge base upon which to promote the use of evidence for decision-making to strengthen the country's focused response.

With this in mind, the Solidarity Fund, in supportive collaboration with the Ministerial Advisory Committee on COVID-19, contracted the Human Sciences Research Council (HSRC) and its partners to conduct the first national COVID-19 antibody prevalence survey, in the country, to provide insights about the spread of the virus, the most vulnerable groups, and the evolution of the pandemic in the country.

This report details the survey and presents findings on the status of COVID-19 infections in the country from November 2020 to June 2021, a period that includes Wave 2 and Wave 3 of the pandemic in South Africa. We had always known that the daily statistics were dependent on those who came forward for testing and that the true number of cases in the communities, and therefore the national prevalence was unknown. Not surprisingly, the study showed that the overall estimated number of COVID-19 infections at the end of the study was approximately 5 times higher than the recorded number of cases based on PCR testing. This data provided a holistic picture of the extent of the pandemic in the country, and its impact across age and sex and localities. Sharing this data with government (national, provincial, and local), policymakers, modelling groups, researchers, and academics for the benefit of all South Africans was therefore crucial. The preliminary survey findings were presented to decision-makers, including government representatives from early 2021 to support decision-making in the then rapidly shifting pandemic. The survey findings helped refine and focus the national response, providing valuable information on the prevalence of asymptomatic cases, which helped with scoping for prevention, testing, and vaccination strategies.

The Solidarity Fund is proud to have been part of this research study at a time when such information was critical for decision-making. The results informed programmes and influenced prioritization of resources to areas of greatest need and high impact. We wish to congratulate the HSRC team that developed, implemented and reported the findings of the study, and we trust that the reader takes away the same valuable lessons we also learned from the study. The survey is also a contribution to data and information to better understand the pandemic in South Africa at the time and provides lessons for research for predicted future epidemics.

Dr Gugulethu Ngubane

Executive Head of Health Response

The Solidarity (COVID-19 Response) Fund

EXECUTIVE SUMMARY

Background

South Africa is one of the countries on the African continent that was most affected by the COVID-19 pandemic. Seroprevalence surveys of SARS-CoV-2 antibodies provide valuable information about the extent of the pandemic given the existence of asymptomatic cases. We report findings of a nationwide household-based population survey of SARS-CoV-2 seropositivity in South Africans 12 years and older during the period November 2020 to June 2021.

Methods

The survey used a cross-sectional multi-stage stratified cluster survey design and was undertaken over two separate periods (November 2020 – February 2021 and April – June 2021). Trained interviewers administered a questionnaire that collected sociodemographic, health status, and behavioural information. Nurses and phlebotomists collected venous blood samples from participants, and these were tested for SARS-CoV-2 antibodies using the Abbott Architect anti-SARS-CoV-2 immunoglobulin class G (IgG) chemiluminescent microparticle immunoassay, with the final status determined by the Euroimmun Anti-SARS-CoV-2 ELISA (IgG) Euroimmun® antibody assay. A subset of specimens positive on the Abbott® assay was selected for further analysis using a pseudotyped neutralisation assay if they were also positive on an ELISA binding assay using a full-length spike from the ancestral D614G variant. Summary statistics were used to describe SARS-CoV-2 seroprevalence and characteristics of the study population. Bivariate and multivariate logistics regression analyses was used to identify sociodemographic, health status and behavioral factors associated with SARS-CoV-2 seropositivity.

Results

The SARS-CoV-2 seroprevalence using the Euroimmun assay was (19.6%, 95% CI 17.9–21.3). Seroprevalence varied by province and was higher in the Free State (26.8%, 95% CI 22.0–32.1), Eastern Cape (26%, 95% CI 22.5-29.9) and lower in Mpumalanga (13.6%, 95% CI 8.9–20.2) and Limpopo (11.6%, 95% CI 7.6–17.4). Among the metros, seroprevalence estimates were highest in Mangaung (29.0%, 95% CI 19.8-40.4), Nelson Mandela Bay (26.0%, 95% CI 19.8-33.4) and the City of Cape Town (25.4%, 95% CI 20.5-31.0) metropolitan areas. The cumulative estimated number of infections based on seropositivity in the study population at the end of the survey period was 8 675 265 (95% CI 7 508 393–9 842 137). Increased odds of seropositivity were associated with being female [aOR=1.44 (95% CI 1.23–1.70), p<0.001] and having hypertension [aOR=1.28 (95% CI 1.00–1.64), p=0.048] while those aged 18 – 35 years old had lower odds of seropositivity [aOR=0.69 (95% CI 0.53–0.90), p=0.007] compared to those aged 12 – 17 years old. There were no significant differences in other variables. Of the 754 samples tested for neutralising antibodies, 45% neutralised both the ancestral D614G and the Beta strains, 28% neutralised the ancestral D614G only, 18% neutralised the Beta strain only, and 9% failed to neutralise either strain. The proportion number of samples that neutralised the Beta variant increased over time (<55% November to >75% January), while those that neutralised the ancestral strain declined (>90% November to <70% January, over time). Neutralisation potency against both SARS-COV-2 virus strains was low for most of the samples, with titres rarely reaching >1:1000.

Conclusion

This study provided nationally representative estimates of the prevalence of SARS-CoV-2 antibodies in South Africa over the period November 2020 to June 2021 for people 12 years and older. An estimated 8 675 265 people aged 12 years and older had been exposed to the virus by June 2021 – an estimate that is approximately 5 times the cumulative number of infections based on PCR testing for all ages at the same time. The survey was completed before widespread vaccinations against the virus and therefore indicated the level of community susceptibility at the time.

Women were significantly more likely to be infected indicating their vulnerability and highlighting the need for additional support for women given factors that likely increase their risk. People aged 18-35 years old were less likely to be infected than young people (12-17 years old) indicating a need for youth focused strategies which were limited at the time of the survey. The findings also highlighted the risk of infections in rural areas, in particular on farms where living conditions may have accelerated the spread of the virus. Less than half of the samples tested neutralised both the original and the more transmissible Beta strain of the SARS-CoV-2 virus, which indicated the importance and the urgency of vaccination roll out.

Introduction

The World Health Organization (WHO) declared the coronavirus disease (COVID-19) caused by the severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) as a pandemic on 11 March 2020. The first case of SARS-CoV-2 in South Africa was reported on 5 March 2020. By the end of November 2021, South Africa had experienced three waves (periods of increased transmission) of the pandemic (June – August 2020, December 2020 – February 2021 and June – September 2021). During the early stages of the pandemic, surveillance was largely based on the detection of active cases using polymerase chain reaction (PCR) testing. Uncertainty remained about the prevalence of the virus in communities because some individuals were not able to access testing facilities, while others were asymptomatic and therefore remained diagnosed. (Xu *et al.*, 2020, Pollán *et al.*, 2020). Studies that reported on SARS-CoV-2 infections in cloistered or specific settings, showed that many infections were asymptomatic and would remain undetected if testing was prompted by the presence of symptoms alone. These studies used PCR testing and revealed that the proportion of persons who tested positive for COVID-19 whilst asymptomatic ranged between 17.9% and 87.9% (Mizumoto *et al.*, 2020, Sutton *et al.*, 2020, Reuters, 2020, Gudbjartsson *et al.*, 2020). The reverse transcriptase PCR test detects viral particles early on during the active progression of the disease but cannot be used to determine if someone had the infection in the past (Wang *et al.*, 2020). Assays that detect antibodies to SARS-CoV-2 can indicate past infections and may potentially indicate if a person has developed immunity against SARS-CoV-2, although the duration of this immunity is still not well known. It was soon evident that it was important to understand the prevalence of antibodies (seropositivity) in the general population, in addition to the prevalence determined by PCR tests (Kissler *et al.*, 2020) to inform decisions about responding to the pandemic. Several early surveys in European settings estimated the presence of antibodies to SARS-CoV-2 to be higher than figures generated through PCR surveillance, up to 85 times higher (Reuters, 2020, Gudbjartsson *et al.*, 2020, Bendavid *et al.*, 2020, Milliken, 2020, Pollán *et al.*, 2020).

Given that asymptomatic individuals can transmit infections (Arons *et al.*, 2020, Furukawa *et al.*, 2020, Emery *et al.*, 2020), understanding the proportion of asymptomatic infections was also important in improving understanding of the pandemic, susceptibility of communities – particularly where vaccination targets had not yet been achieved – and to inform strategies to limit the spread of the virus in addition to other preventive measures. Early data indicated that in the absence of an effective vaccination programme, intermittent social distancing, and non-pharmaceutical infection control interventions (NPIs) would be required for at least two years to control the transmission and reduce the likelihood of resurgences. With vaccines initially unavailable for most African countries, South Africa started its vaccination programme on 17 February 2021 and by 17 July 2021, 5.07 million doses had been administered, with more than 1.68 million people (4.2% of the adult population) being fully vaccinated (Bhekisisa, 2021). Targets for population immunity were initially projected to be reached by the end of 2021 (NICD, 2021b).

An improved understanding of the association between COVID-19 infection and sociodemographic, behavioural and health factors (including comorbidities and health system capacity) was essential for strengthening preventive and mitigation strategies. Targeted testing and population screening in early 2020 in Iceland found that women and children under the age of 10 years had lower incidences of COVID-19 when compared to males and persons aged 10 years and older, respectively (Gudbjartsson *et al.*, 2020). Preliminary findings from a COVID-19 seroprevalence survey of 3 000 people in New York State showed that 13.9% of the respondents had antibodies to the virus, with seroprevalence higher among females than males, and higher among Latin and African Americans than Whites (Higgins-Dunn *et al.*, 2020). Geographic variability was also evident, with significantly different proportions in New York City (21%), Long Island (16.7%), and in areas of upstate New York (3.6%) revealing the presence of antibodies. Early international and local studies demonstrated that hospital admissions and deaths due to COVID-19 complications also varied between socio-demographic groups and those with and without certain underlying medical conditions (Higgins-Dunn *et al.*, 2020, WCDOH, 2021). Increased risk of severe COVID-19 with poor outcomes is associated with underlying health conditions such as cardiovascular disease, chronic kidney disease, chronic respiratory disease, chronic liver disease, hypertension, diabetes, HIV/AIDS, and active tuberculosis (Walker *et al.*, 2021, Robbiani *et al.*, 2020, Clark *et al.*, 2020). Survival with these conditions in the context of COVID-19 varies by age and sex and is in turn influenced by underlying socioeconomic conditions and the performance of the health system in a given setting (Walker *et al.*, 2021, Clark *et al.*, 2020). The human (behavioural) response to the pandemic can also drastically shape its timing and intensity.

Protective and enduring immune responses to viral infections arise from the combined actions of lymphocytes: B cells (responsible for humoral antibody immunity) and T cells (responsible for cellular immunity and helping B cell responses). Although nearly all people who recover from COVID-19 produce antibodies that target the virus, some do not make enough neutralising antibodies to mount an ideal immune response, while others have poor to modest neutralising activity (Robbiani *et al.*, 2020). Understanding these immune responses and dynamics is important in understanding the correlates of protection in the South African population. Following infection, antibodies to the virus typically appear in the second or third week of illness with the levels Immunoglobulin M (IgM) waning by week 6, while Immunoglobulin G (IgG) is detected for a longer period. The decline in antibody responses is likely to be determined by the severity of the disease with a more rapid decline in responses in asymptomatic and mild disease compared to severe disease. The use of different antigens and epitopes and different assay formats, such as direct or indirect tests, is likely to affect the interpretation of different antibody kinetics since different isotypes and antibodies target different antigens and epitopes (Di Germanio *et al.*, 2021b, Dobano *et al.*, 2021, Føns and Krogfelt, 2021). The selection of test format is thus critical in surveillance. The SARS-CoV-2 genome encodes for 30 proteins – including the four structural proteins, namely, the spike (S), membrane (M), envelope (E), and nucleocapsid (NC) proteins. The more common targets in the test platforms are those that target the spike sub-unit (S1) that allows for viral entry, the receptor binding domain (RBD) of S1 that binds to the human cellular receptor angiotensin-converting enzyme 2 (ACE2), or the nucleocapsid (NC) protein that encapsulates the viral genome. The factors that affect the performance of tests include the severity of infection, lack of seroconversion, the rate of waning of antibody titres, and the period to serological testing post infection.

In early 2020, the actual prevalence of COVID-19 in South Africa was uncertain – even though the data from routine PCR test based surveillance showed the devastating impact of the disease in terms of mortality, morbidity and economic impact. Seroprevalence studies conducted in the country were restricted to selected sites or provinces and population groups and did not provide a nationally representative estimation of infections across all areas of the country (Aurum Institute, 2020, Kleynhans *et al.*, 2021, Mutevedzi *et al.*, 2021, SANBS, 2021, Sykes *et al.*, 2021, Wolter, 2021). Data from the South African National Blood Service (SANBS) using blood donor specimens that were collected after wave 2 of the pandemic (January 2021), estimated that seroprevalence ranged from 8 to 15 times higher (31.8% to 62.5%) than the confirmed prevalence by PCR in four provinces (Sykes *et al.*, 2021). Data collected from the remaining five provinces in May 2021 estimated a seroprevalence ranging from 37.6% to 48.5%, with an overall national estimate of 47.4% (SANBS, 2021). The Health Care Utilization Study (HUTS) – a crosssectional community survey in three communities assessing severe respiratory illness and influenza-like illness, and another study conducted as part of the Prospective Household cohort study of Influenza, Respiratory Syncytial virus, and other respiratory pathogens community burden and Transmission dynamics in South Africa (PHIRST), conducted in two provinces, showed seroprevalence estimates ranging from 45% to 49% and 18% to 59% in different age groups and time of collection in the two studies respectively (Wolter, 2021, Kleynhans *et al.*, 2021). However, a study that used dried blood spots collected between November 2020 and January 2021 in the Gauteng Province, and, February and March 2021 in the North West province, reported seroprevalence estimates of less than 20% (Mutevedzi *et al.*, 2021). Similarly seroprevalence estimates of around 20% were reported by a study conducted in two selected mining groups in Gauteng and the Northern Cape provinces between November and December 2021 (Aurum Institute, 2021). It is important to note that there was variation in both the periods during which data was collected as well as the antibody tests used in these studies.

In addition to data from these localised studies, there was a need for a nationally representative study to determine the national prevalence of SARS-CoV-2 in South Africa to better characterise the pandemic in the country including community susceptibility, the proportion of COVID-19 cases that remained asymptomatic, and to inform interventions to manage the epidemic, including, vaccinating strategies. Therefore, the Human Sciences Research Council and its partners underook a nationally respresentative survey.

Objectives

The ***primary objectives*** of the survey were:

- to determine the extent of the COVID-19 virus infection in the general population with age-specific infection prevalence, as determined by seropositivity
- to determine the proportion of asymptomatic or subclinical COVID-19 infections.

The ***secondary objectives*** of the study were:

- to determine risk factors for COVID-19 virus infection
- to estimate the prevalence of COVID-19 antibodies in age and sex sub-groups
- to assess antibody levels quantitatively and neutralising antibody concentrations for future comparisons with correlates of protection for possible herd immunity estimations.

CHAPTER 2

Methods

2.1 Study design and sampling

This was a cross-sectional multi-stage stratified cluster population-based household seroprevalence survey implemented in all nine provinces. The survey targeted all the locality types (urban areas, rural formal and rural informal areas) within the selected geographic Small Area Layers (SALs) stratified by province.

The study design and sample size calculation were adapted from the WHO vaccination coverage cluster surveys: reference manual (WHO, 2018). The original design targeted 19 620 individuals from 436 SALs to realise an effective sample size of 13 734 individuals from 6 540 households or Visiting Points (VPs). This sample size was based on an assumption that the SARS-CoV-2 prevalence was 2% in South Africa in June 2020, with a margin of error of 2%, and a joint (household and individual) response rate of 70%. It was adequately powered to provide estimates by province, locality type and for four metros in the country (Cape Town, Johannesburg, eThekwini, Nelson Mandela Bay). However, after data collection from November 2020 to February 2021, the realised response rate was lower than anticipated (46%). The survey data collected until then (February 2021), the status of the epidemic, and survey field conditions were reviewed, and after consultations with the Survey Advisory Committee and the Funder, the sample size was revised for improved estimates in some population sub-groups. Considering the more realistic SARS-COV-2 prevalence of 20%, and a response rate of 64%, a sample size of 12 625 individuals who provided a blood specimen for antibody testing was projected to achieve precision levels of 1.5% or lower. Thus, during the second round of data collection, the study targeted an additional 200 SALs across all nine provinces to reach 6 930 respondents who would provide a blood specimen (from 9 990 individuals approached). The main rationale for the second round of data collection was to supplement the data that was collected during the first round, collecting more data in all the provinces, and increasing the numbers from the White and Indian population groups – which were underrepresented.

The survey was therefore conducted in two rounds. Round 1 took place between November 2020 and February 2021, and Round 2 between April and June 2021. They covered periods when the original SARS-CoV-2 (Wuhan D614G) virus strain and the Beta variant were circulating in the country. Each round consisted of two distinct phases: Phase 1 included community entry in which awareness of the study was shared with relevant stakeholders at the provincial, district and SAL levels; and Phase 2 included data collection at the household level. Overall, 15 households were randomly selected for inclusion across 647 SALs nationally.

It is important to note that data collection occurred before the implementation of wide-scale vaccination in the country. In South Africa, the vaccination rollout started on 17 February 2021 and initially focused on healthcare workers. This was followed by a phased approach for essential workers, those in congregate settings, and then by age group starting with the elderly. By the end of the data collection period (15 June 2021), 2 067 424 vaccinations had been administered (around 3.4% of the population).

2.2 Survey inclusion and exclusion criteria

Individuals meeting the following criteria were included in the survey:

- A *de facto* household member, that is, an individual who is a usual resident of the household. (A household is defined as a group of two or more people living together).
- Individuals aged 12 years and older.
- Individuals who had previously been tested for COVID-19 or who were persons under investigation (PUIs).
- Individuals who had tested positive for COVID-19 and were self-isolating.
- Individuals who had received the COVID-19 vaccination.
- Individuals who could provide informed consent (assent and parental or guardian consent were required for those younger than 18 years old).

Individuals meeting the following criteria were excluded from the survey:

- Household members below the age of 12 years.
- Individuals living in nursing or old-age homes, and those in hotels, as well as homeless people living on the streets, or in shelters.
- Individuals who were residing in or confined to institutions such as military barracks, prisons, hospitals, and hostels at boarding schools, colleges and universities.
- Individuals who were not mentally competent to give informed consent.

2.3 Survey operations

The survey implementation consisted of four main stages: (i) stakeholder engagement at the provincial and district level, as well as community entry at the SAL level to engage local gatekeepers such as ward councillors, police services, local clinics, community leadership, body corporates and tribal authorities, for their support of the study; (ii) data collection at household level, consisting of household and individual interviews, and collection of venous blood specimens; (iii) laboratory testing; and (iv) data analysis.

The individual interviews were based on a short questionnaire adapted from the WHO population-based age-stratified seroepidemiological investigation protocol for COVID-19 virus infection (WHO, 2020) – with additions from other questionnaires developed by the HSRC, the South African Medical Research Council (SAMRC) and the South African Population Research Infrastructure Network (SAPRIN) – and collected information on socio-demographic characteristics, health status and various risk factors for SARS-CoV-2 infection. The information included the history of SARS-CoV-2 infection, history of exposure to contacts, co-morbidities, and behavioural prevention practices such as hand washing and social distancing. Heads of households were asked to complete an initial questionnaire that was used for household enumeration. The data was collected electronically on the tablets and was sent to the study server hosted by Epicentre.

2.3.1 Safety procedures

Survey field staff were restricted to individuals below the age of 50 years without any co-morbidities or other major risk factors for SARS-CoV-2. They were screened and tested for COVID-19 at survey initiation, with additional regular screening according to a staff safety standard operating procedure (SOP) during the data collection period. The national guidelines were followed whenever anyone screened positive.

Each fieldworker was supplied with personal protective equipment (PPE), and with materials for blood draws and waste disposal. The PPE included surgical face masks, face visors, gloves and hand sanitisers. The masks and gloves were changed regularly and between different households. Visors were sanitised between different households. Handling and disposal of waste, (sharps and non-sharps) was in accordance with national regulations.

2.3.2 Data collection

Other fieldwork material included aerial photomaps showing the locations of the preselected households in each SAL, and directions to get there. When the fieldwork team arrived at a selected household, the supervisor approached the head of the household to introduce the study and the field team. Once the household head agreed to participate, he/she was required to complete a consent form. Thereafter, a general household questionnaire (see Appendix A1) was administered by a fieldworker, and then all members of the household aged 12 years and older – including the head of the household were invited to participate by completing interviewer administered individual questionnaires (see Appendix A2). Informed consent was obtained from those aged 18 years and older. Assent and parental/guardian consent was required for children aged 12 – 17 years to participate. A blood specimen was collected from each participating individual. Figure 1 shows the flow of the data collection process.

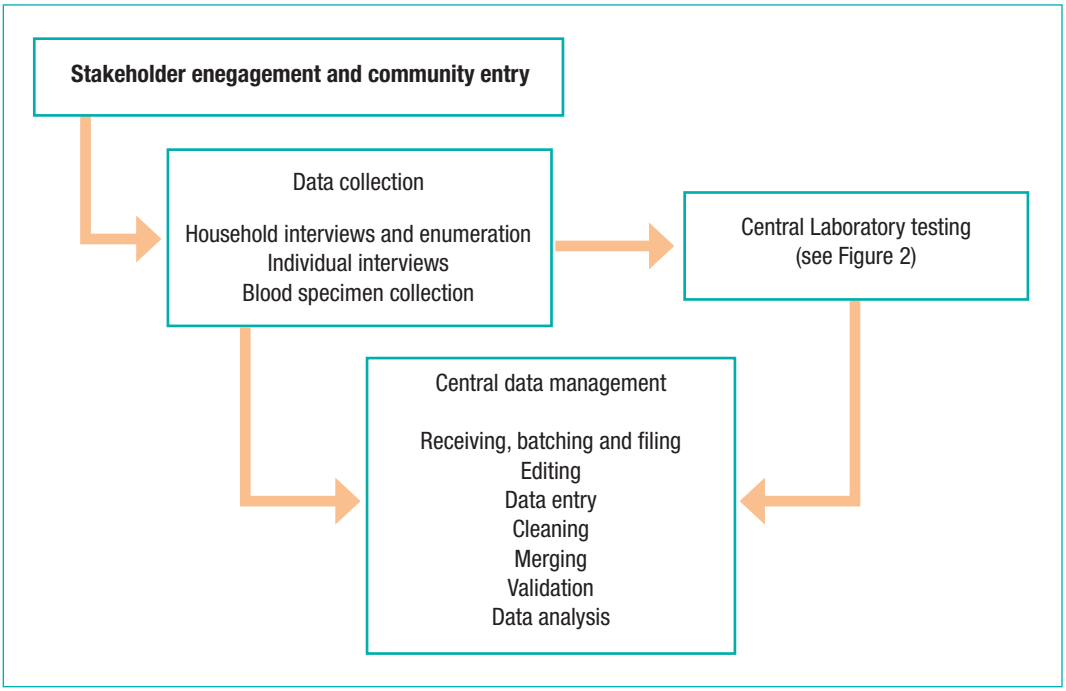

Figure 1: Survey flow diagram

Annexure B outlines the timelines for the data collection across provinces. In South Africa the first wave of the pandemic occurred between March 2020 – November 2020, and data collection for the survey started in November 2020 in all provinces except the North-West (where it started during the second week of December). Data collection continued in all provinces with intermittent breaks during waves 2 (December 2020 – March 2021) and 3 (May 2021 – October 2021).

2.3.3 Blood specimen collection and courier

Venous blood specimens (8.5 ml) were collected into Serum-separating tubes (SST). These were then stored and couriered under cold chain conditions to the testing laboratory at the Centre for HIV and STIs at the National Institute for Communicable Disease (NICD) within 24 hours of collection. The specimens were tracked using tracking sheets and waybills from the field to the laboratory.

On arrival at the laboratory, the managers matched the study barcodes on the SST tubes to tracking sheets before examining the quality of each specimen. The study barcode on each specimen was recorded and a laboratory barcode assigned. All the barcodes and the demographic information on the specimen tracking sheets were captured on the Laboratory Information Management System (LIMS).

2.3.4 Laboratory testing

Accurate and reliable serological assays are essential for epidemiological surveillance. Systematic reviews highlighted the various challenges of the assays regardless of format (Kritsotakis, 2020, Lisboa Bastos et al., 2020). Head-to-head comparisons of chemiluminescent immunoassays (CMIAs) highlighted the challenges and the need for refinement of assays, especially to improve sensitivity (Houlihan & Beale, 2020, National SARS-CoV-Serology Assay Evaluation Group, 2020). Studies have also demonstrated differences in the S- and NC-based assays, and results can vary significantly when reporting point prevalence in the same cohort when using NC assays alone (Bolotin *et al.*, 2021, Di Germanio *et al.*, 2021b, Kahre *et al.*, 2021, Kerr *et al.*, 2021, Lumley *et al.*, 2021, Muecksch et al., 2021, Sasisekharan *et al.*, 2021, Ward *et al.*, 2020, Fenwick *et al.*, 2021). The underestimation of prevalence in the NC-based platforms ranged between 10.9% – 31%, and up to 45% in the post-acute phase when compared to the acute phase.

Given the complexity of the dynamics of antibody responses and the role of imperfect tests, recommended approaches to improve accuracy of estimates include, Bayesian corrections or inference of seroprevalence, adjustments based on follow-up of cohorts, correction of prevalence using reported sensitivity and specificity of the assay, or use of additional reference testing (Bobrovitz *et al.*, 2021, Bolotin *et al.*, 2021, Buss *et al.*, 2021, Larremore *et al.*, 2021, Sempos & Tian, 2021). The testing approach adopted in the present study based on accumulating evidence of the performance of the assays was to use in addition to the Abbott NC-based assay (Abbott Architect anti-SARS-CoV-2 immunoglobulin class G (IgG) chemiluminescent microparticle immunoassay (CMIA), Abbott Ireland, Diagnostics Division, Finisklin Business Park, Sligo, Ireland), the Euroimmun Anti-SARS-CoV-2 ELISA (IgG), and an S-based based assay (EuroImmun; EUROIMMUN Medizinische Labordiagnostika AG, Lübeck, Germany) that was locally validated beyond the acute phase (>50 days). (Figure 2). The assay was shown to have a sensitivity ranging from 80% in asymptomatic disease to 91.7% in severe disease (Gededzha *et al.*, 2021). The assay has been recommended for surveillance, although it did not perform optimally in long-term follow-up with 40% underestimation at nine months, and a decline to 81% seropositivity after a median period of approximately 9 months post infection (Perez-Saez *et al.*, 2021; Kahre *et al.*, 2021). The results presented in this report are based on the Euroimmun assay. 10% of the samples positive on the Abbott assay were tested using the Zhejian OG IgG/IgM rapid test as part of quality assurance.

Neutralising assay testing

A subset of specimens positive on the Abbott assay was selected for analysis using a pseudotyped neutralisation assay. Two assays were then performed on these specimens, sequentially:

- An ELISA binding assay using a full-length spike from the original D614G strain. Only specimens that were positive on ELISA were then tested with the neutralisation assay (Wibmer *et al.*, 2021).
- Neutralisation assay using SARS-CoV-2 wild type D614G (the original Wuhan D614G virus) and Beta spike pseudotyped lentiviral particles.

To determine the neutralisation potency of the SARS-CoV-2 specific monoclonal antibodies present in the specimens, we performed the neutralisation assay using the 293T/ACE2. MF cell line. This cell line was optimised for this assay by a modification resulting in the overexpression of human ACE2, the receptor for SARS-CoV-2. Neutralisation titres were defined as the plasma dilution at which viral entry was reduced by 50% (ID50) (Sholukh *et al.*, 2020).

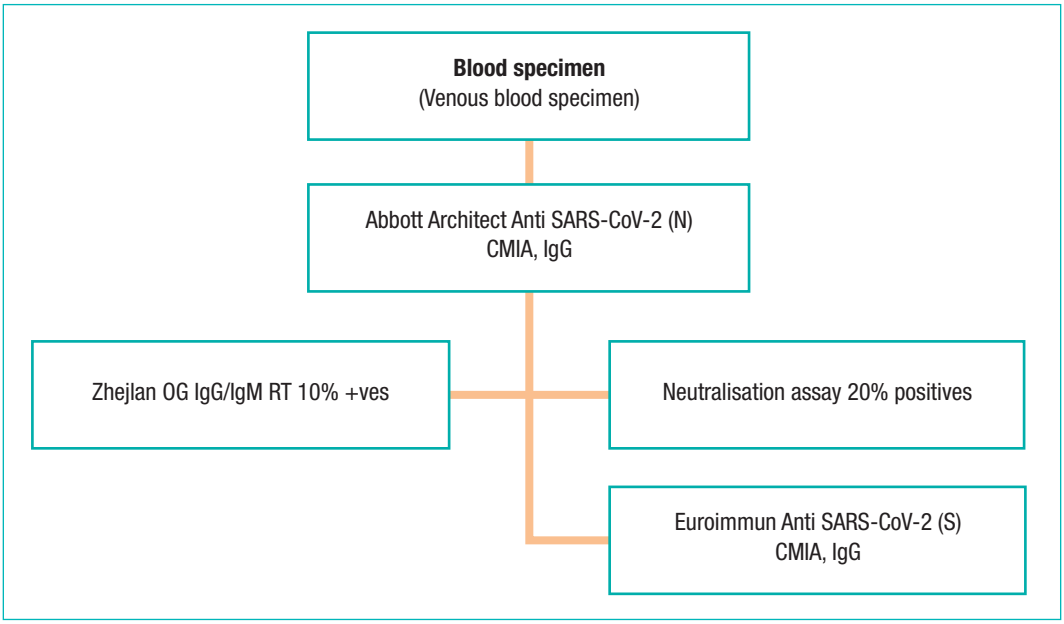

Figure 2: Survey laboratory testing algorithm

CMIA – chemiluminescent microparticle immunoassay; IgG – Immunoglobulin G; IgM – Immunoglobulin M; OG – Orient Gene; RT - Rapid Test

2.4 Ethical considerations

Ethical approval of the survey protocol was provided by the HSRC's Research Ethics Committee, which is accredited by the South African National Health Research Ethics Council (NHREC) and has Federal Wide Assurance (FWA) with the US Office of Human Research Protections.

Social distancing was maintained during interactions between survey staff and between survey staff, stakeholders and respondents. Other national safety protocols such as using face masks, frequent use of sanitisers for cleaning hands, and the use of PPE (gloves, visors and gowns), were implemented to ensure safety. Any symptomatic respondents were referred for medical advice and care at their nearest health facility, and were also counselled on preventive behaviours and actions as per the national guidelines.

2.5 Data management and weighting

The data was captured using the RedCap software programme on Personal Digital Assistants (PDAs). The Asynchronous transfer occurred via the General Packet Radio Service (GPRS), Wi-Fi, 3G, or USB cable to the study server hosted by Epicentre. The data was then transferred into Stata version 15.0 for analysis. Laboratory results were merged with the questionnaire data on completion of the survey.

The data was weighted before analysis to correct for unequal sampling probabilities and disproportionate allocation of SALs by race, province, and locality type, and ensure the required minimum representation. The VP sampling weight was calculated and corrected for non-response. The final VP sampling weight was computed as the product of the SAL sampling weight and the VP sampling weight. Individual sampling weights were computed considering participation within households and were further adjusted for testing non-response. The final individual weights were the product of the SAL sampling weight, the VP sampling weight and the individual weight. The final individual weights were benchmarked against 2020 mid-year population estimates by age, race, sex, and province. (Statistics South Africa (StatsSA, 2020).

2.6 Statistical analysis

Weighted percentages were computed and categorical variables were compared using Pearson χ^2 tests, and $p<0.05$ indicated statistical significance. Bivariate and multivariate logistics regression analyses were used to identify socio-demographic, behavioural, and health status factors associated with SARS-CoV-2 seropositivity. Crude odds ratios (ORs), adjusted odds ratio (aORs) with 95% confidence intervals (CIs) and $p<0.05$ were used to determine the direction and strength of associations, and statistical significance. The analyses were conducted in Stata version 15.0. Prevalence estimates are presented as weighted percentages. Results of the analysis of the neutralisation assay testing are presented as unweighted percentages of the samples that were selected and successfully tested for this aspect. MS Excel was used to analyse the raw data, and Graphpad prism for cohort analyses.

Results

3.1 Generalisability of the survey results

Table 1 shows a comparison of the socio-demographic characteristics of the survey sample to the 2020 mid-year population estimates provided by StatsSA (StatsSA, 2020). The sample was benchmarked to the 2020 mid-year population to ensure the sample was generalisable to the population of South Africa of the survey age range (12 years and older). The weighted survey sample closely matched the mid-year estimates in terms of sex, age, locality type, and province with the percentages well within a 5% difference.

Table 1: Demographic characteristics of the NCAS 2021 survey sample compared to the mid-year population estimates (12 years and older) for South Africa, 2021

Variable	Weighted Sample		Mid-Year Population 2020	
	n	**%**	***N***	**%**
Total	**45 841 248**	**100**	**45 870 066**	**100**
*Sex				
Male	22 214 403	48.5	22 163 895	48.3
Female	23 626 845	51.5	23 706 171	51.7
*Age group (years)				
12 – 17	6 215 375	13.6	6 231 054	13.6
18 – 35	19 661 011	42.9	18 756 280	40.9
36 – 49	10 886 155	23.8	10 673 670	23.3
50+	9 073 748	19.8	10 209 062	22.3
Locality type				
Urban area	31 012 732	67.7	31 054 035	67.7
Rural formal areas	12 813 947	28.0	12 843 618	28.0
Rural informal areas	2 014 568	4.4	2 018 283	4.4
Province				
Western Cape	4 818 419	10.5	5 634 980	12.3
Eastern Cape	4 851 909	10.6	4 965 784	10.8
Northern Cape	854 213	1.9	992 874	2.2
Free State	2 245 568	4.9	2 268 479	4.9
KwaZulu-Natal	8 659 935	18.9	8 517 369	18.6
North-West	3 257 475	7.1	3 141 633	6.8
Gauteng	13 010 425	28.4	12 500 883	27.3
Mpumalanga	3 630 568	7.9	3 573 775	7.8
Limpopo	4 512 736	9.8	4 274 288	9.3

**Totals do not add to the overall total as some people did not indicate their age or sex.*

3.2 Response rates

A total of 10 109 VPs were approached to participate in the survey (Table 2). Of the 10 109 VPs approached, 5 580 agreed to participate, giving a household response rate of 55%. Of all the VPs approached, 43.1% refused to take part in the survey. Of the valid VPs 1.6% were empty. Eastern Cape and Western Cape provinces had high participation rates, while Gauteng had the lowest participation rate.

Table 2: Visiting point response rates by province characteristics, NCAS, South Africa, 2021

Province	Total VPs visited	Households Interviewed		Households Refused		Absent/ Missing/ Other	
	N	**%**	**95% CI**	**%**	**95% CI**	**%**	**95% CI**
Western Cape	1 707	62.0	59.7 – 64.3	37.6	35.3 – 39.9	0.5	0.2 – 0.9
Eastern Cape	1 097	74.4	71.7 – 76.9	25.4	22.9 – 28.1	0.2	0.0 – 0.7
Northern Cape	620	52.9	49.0 – 56.8	44.7	40.8 – 48.6	2.4	1.5 – 4.0
Free State	617	57.5	53.6 – 61.4	42.1	38.3 – 46.1	0.3	0.1 – 1.3
KwaZulu-Natal	2 194	52.8	50.7 – 54.9	46.2	44.1 – 48.3	1.0	0.7 – 1.5
North-West	571	52.2	48.1 – 56.3	39.8	35.8 – 43.8	8.1	6.1 – 10.6
Gauteng	2 006	44.3	42.1 – 46.5	54.3	52.1 – 56.5	1.4	1.0 – 2.0
Mpumalanga	647	53.9	50.1 – 57.8	45.1	41.3 – 49.0	0.9	0.4 – 2.0
Limpopo	650	51.4	47.5 – 55.2	43.4	39.6 – 47.2	5.2	3.8 – 7.2
Total	**10 109**	**55.2**	**54.3 – 56.2**	**43.1**	**42.2 – 44.1**	**1.6**	**1.4 – 1.9**

A total of 16 646 individuals were eligible to participate in the study, of whom 90.8% agreed to be interviewed (Table 3). 81.9% of the individuals that participated in the survey completed the interview and provided blood specimens. The participation rates were higher among females when compared to males. Participation also varied by race, with Coloureds having the highest response rate (97.0%), followed by Black Africans (90.5%).

Table 3: Respondents aged 12 years and older who were eligible, interviewed and provided a blood specimen compared with those who were interviewed but refused to provide a blood specimen, NCAS, South Africa, 2021

Variables	Total	Interviewed		Interview refused		Interviewed and tested		Interviewed not tested	
	N	***n***	**%**	***n***	**%**	***n***	**%**	***n***	**%**
Total	**16 646**	**15 115**	**90.8**	**1531**	**9.2**	**13 640**	**81.9**	**1475**	**8.9**
Age group (years)									
12 – 14	867	741	85.5	126	14.5	660	76.1	81	9.3
15 – 19	1 606	1 392	86.7	214	13.3	1 286	80.1	106	6.6
20 – 24	1 634	1 429	87.5	205	12.5	1 316	80.5	113	6.9
25 – 29	1 616	1 438	89.0	178	11.0	1 327	82.1	111	6.9
30 – 34	1 489	1 344	90.3	145	9.7	1 204	80.9	140	9.4
35 – 39	1 561	1 437	92.1	124	7.9	1 285	82.3	152	9.7
40 – 44	1 262	1 177	93.3	85	6.7	1 062	84.2	115	9.1
45 – 49	1 292	1 204	93.2	88	6.8	1 071	82.9	133	10.3
50 – 54	1 210	1 146	94.7	64	5.3	1 042	86.1	104	8.6
55 – 59	1 157	1 087	93.9	70	6.1	979	84.6	108	9.3
60 – 64	1 049	992	94.6	57	5.4	893	85.1	99	9.4
65 – 69	719	672	93.5	47	6.5	593	82.5	79	11.0
70 – 74	531	503	94.7	28	5.3	450	84.7	53	10.0
75 – 79	300	279	93.0	21	7.0	240	80.0	39	13.0
80+	297	272	91.6	25	8.4	233	78.5	39	13.1
Race									
African	10 364	9 380	90.5	984	9.5	8 484	81.9	896	8.6
White	1 104	980	88.8	124	11.2	827	74.9	153	13.9
Coloured	3 979	3 859	97.0	120	3.0	3 681	92.5	178	4.5
Indian	1 091	836	76.6	255	23.4	591	54.2	245	22.5
Sex									
Male	7 153	6 326	88.4	827	11.6	5 731	80.1	595	8.3
Female	9 443	8 788	93.1	655	6.9	7 912	83.8	876	9.3
Total	16 596	15 114	91.1	1482	8.9	13 643	82.2	1471	8.9
Province									
Western Cape	3 580	3 461	96.7	119	3.3	3 367	94.1	94	2.6
Eastern Cape	2 857	2 847	99.6	10	0.4	2 717	95.1	130	4.6
Northern Cape	1 021	958	93.8	63	6.2	909	89.0	49	4.8
Free State	1 149	1 095	95.3	54	4.7	1 076	93.6	19	1.7
Kwazulu-Natal	3 174	2 760	87.0	414	13.0	2 319	73.1	441	13.9
North-West	752	630	83.8	122	16.2	548	72.9	82	10.9
Gauteng	2 367	1 933	81.7	434	18.3	1 501	63.4	432	18.3
Mpumalanga	854	749	87.7	105	12.3	630	73.8	119	13.9
Limpopo	892	682	76.5	210	23.5	573	64.2	109	12.2

3.3 SARS-CoV-2 seroprevalence

Overall, the SARS-CoV-2 seroprevalence estimates using the Euroimmun assay across all nine provinces (*n*=2 783) was 19.6% (95% CI 17.9 – 21.3). This translated to an estimated 8 675 265 people exposed to the virus across South Africa by June 2021. Free State province had the highest seroprevalence followed by Eastern Cape and Western Cape provinces. The lowest seroprevalence was found in Mpumalanga and Limpopo provinces (Figure 3).

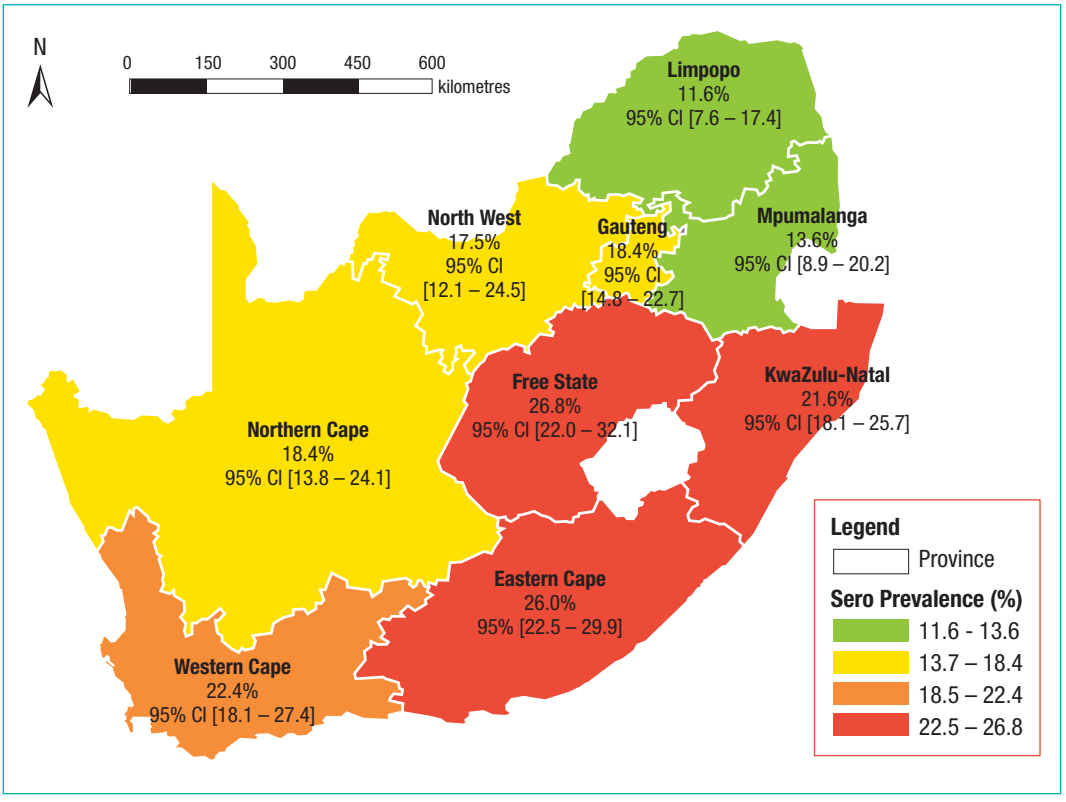

Figure 3: SARS-CoV-2 seroprevalence by province among people 12 years and older, NCAS, South Africa, 2021

Table 4 shows the estimated number of people infected with the SARS-CoV-2 virus in the different provinces. Gauteng and KwaZulu-Natal provinces had the highest estimated number of infected people while the Northern Cape had the lowest number.

Table 4: Estimated number of people infected with the SARS-CoV-2 virus by province among people 12 years and older, NCAS, South Africa, June 2021

Province	Estimated number of people exposed	Lower estimate	Higher estimate
National	8 675 265	7 508 393	9 842 137
Gauteng	2 282 219	1 645 574	2 918 864
Kwazulu-Natal	1 825 287	1 338 912	2 311 663
Eastern Cape	1 222 662	806 440	1 638 884
Western Cape	1 078 349	561 128	1 595 569
Free State	580 486	300 072	860 899
North West	555 870	282 316	829 424
Limpopo	503 325	266 492	740 157
Mpumalanga	480 308	223 122	737 495
Northern Cape	146 759	89 695	203 824

Among metropolitan areas (*n*= 4 999), Mangaung had the highest SARS-CoV-2 seroprevalence followed by Nelson Mandela Bay and the City of Cape Town (Figure 4). SARS-CoV-2 seroprevalence was lower in Ekurhuleni and eThekwini.

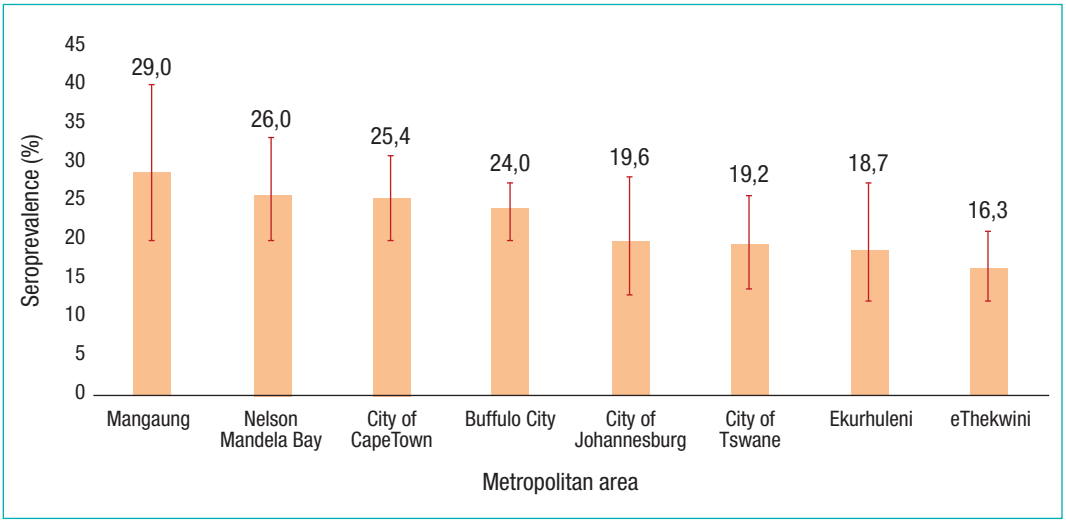

Figure 4: SARS-CoV-2 seroprevalence by metropolitan areas among people 12 years and older, NCAS, South Africa, June 2021

Females had a higher SARS-CoV-2 seroprevalence compared to males in all age groups (Figure 5). Among females, seroprevalence was significantly higher among those aged 12 to 17 years (p=0.020), while there was no significant difference in seroprevalence by age among males.

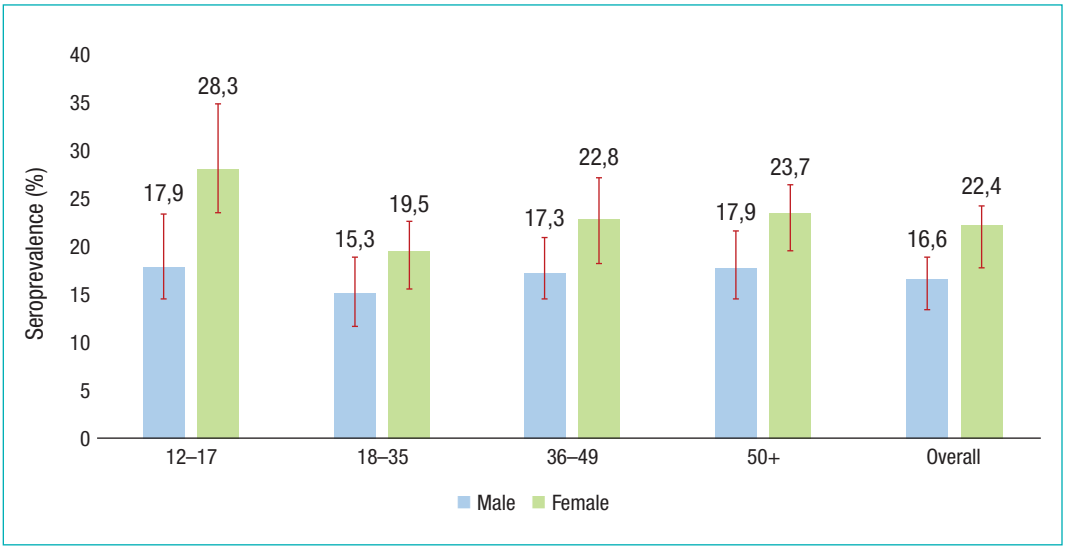

Figure 5: SARS-COV-2 seroprevalence by age and sex among the people 12 years and older, NCAS, South Africa, June 2021

Table 5 shows that an estimated 8.7 million people aged 12 years and older had been exposed to and infected by the SARS-CoV-2 virus in South Africa by the end of the data collection for the survey. This included 5.1 million females and 3.2 million people aged 18 to 35 years old.

Table 5: Estimated number of people exposed to SARS-CoV-2 infection by age and sex among people 12 years and older, NCAS, South Africa, June 2021

Province	Estimated number of people exposed	Lower estimate	Higher estimate
National	8 675 265	7 508 393	9 842 137
Sex			
Male	3 558 415	2 976 704	4 140 126
Female	5 116 849	4 381 584	5 852 114
Age group			
Younger than 18 years	1 390 809	1 060 450	1 721 168
18 – 35 years	3 277 975	2 703 139	3 852 811
36 – 49 years	2 128 032	1 746 983	2 509 080
50+ years	1 878 447	1 592 974	2 163 920

Figure 6 shows SARS-CoV-2 seroprevalence by sex across locality types. Overall, rural formal areas had the highest overall seroprevalence, with females living in rural formal areas recording the highest estimate at 28.9%.

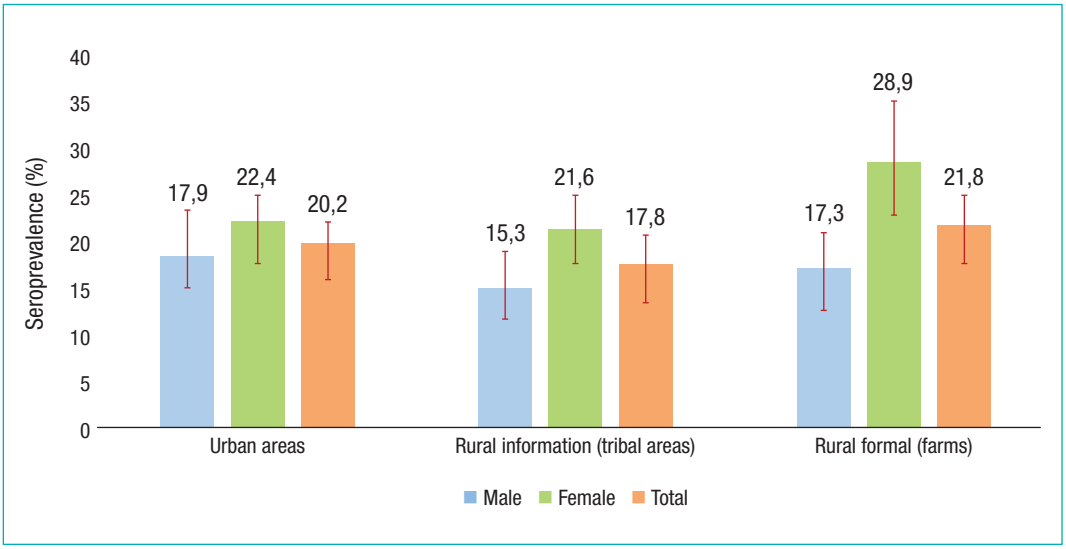

Figure 6: SARS-CoV-2 seroprevalence by sex in each locality type among people 12 years and older, NCAS, South Africa, June 2021

3.4 SARS-CoV-2 seroprevalence and associated factors

Bivariate logistic regression models showed a statistically significant association between SARS-CoV-2 seropositivity and sex and age, with higher estimates in females and those 12-17 years old. (Table 6). There were no significant associations between SARS-CoV-2 seropositivity and other socio-demographic characteristics.

Table 6: SARS-CoV-2 seroprevalence and association with socio-and demographic characteristics among people 12 years and older, NCAS, South Africa, June 2021

Variables	SARS-CoV-2 seroprevalence				Bivariate models			
Sex	*N*	%	95% CI	*p*-values	OR	95% CI		*p*-value
Male	5 534	16.6	14.6 – 18.7	<0.001*	1			
Female	7 678	22.4	20.4 – 24.5		1.5	1.2	1.7	<0.001*
Age group (years)								
12 – 17	1 363	23.2	19.2 – 27.8	0.011*	1			
18 – 35	4 494	17.3	15.0 – 19.9		0.7	0.5	0.9	0.005*
36 – 49	3 060	20.1	17.6 – 22.8		0.8	0.6	1.1	0.159
50+	4 294	21.3	19.1 – 23.7		0.9	0.7	1.2	0.430
Employment status								
Unemployed	7 154	18.9	17.0 – 20.9	0.684	1			
Employed	4 394	19.5	17.1 – 22.0		1.0	0.9	1.2	0.684
Household size (number of people)								
1	418	14.5	9.1 – 22.5	0.548	1			
2 – 3	878	14.8	11.3 – 19.1		1.02	0.54	1.91	0.951
4+	1 029	17.6	14.3 – 21.4		1.3	0.7	2.2	0.426
Locality type								
Urban	9 097	20.2	18.0 – 22.5	0.211	1			
Rural informal (tribal areas)	2 816	17.8	15.1 – 20.8		0.9	0.7	1.1	0.208
Rural (farms)	1 301	21.8	18.7 – 25.3		1.1	0.9	1.4	0.415

**Statistically significant*

Table 7 shows that there was no significant association between SARS-CoV-2 seropositivity with symptoms or other socio-behavioural factors.

Table 7: SARS-CoV-2 seroprevalence and association with having symptoms and socio-behavioural factors among people 12 years and older, NCAS, South Africa, June 2021

Variables	SARS-CoV-2 seroprevalence				Bivariate models			
	N	%	95% CI	*p*-value	OR	95% CI		*p*-value
Any symptoms# in the past 14 day								
No	12 482	19.5	17.7 – 21.3	0.596	1			
Yes	732	21.2	15.6 – 28.1		1.1	0.8	1.6	0.596
Any symptoms# in the past 3 months								
No	12 475	19.4	17.6 – 21.2	0.212	1			
Yes	739	23	17.7 – 29.2		1.2	0.9	1.7	0.213
Left your province/village/suburb/township in the past 7days								
No	3 313	18.5	15.9 – 21.4	0.204	1			
Yes	2 475	21.1	18.2 – 24.3		1.2	0.9	1.5	0.204
Attended an event or gathering								
No	4 386	18.8	16.5 – 21.3	0.153	1			
Yes	1 395	22.3	18.3 – 26.9		1.2	0.9	1.7	0.154
Close contact with people outside your home in the past 7 days								
No	1 480	18	14.6 – 22.0	0.303	1			
Yes	4 320	20.3	18.0 – 22.8		1.2	0.9	1.5	0.304
Number of people you were in close contact with away from home								
1 – 3 people	597	20.4	14.9 – 27.1	0.426	1			
4 – 7 people	768	14.1	10.4 – 18.7		0.6	0.4	1.1	0.079
8 – 10 people	758	18.1	13.6 – 23.7		0.9	0.5	1.4	0.571
11 – 15 people	637	20.1	14.0 – 27.9		1.0	0.6	1.7	0.954
16 – 20 people	743	22.5	17.7 – 28.3		1.1	0.7	1.9	0.619
more than 20 people	2 282	20.1	16.8 – 23.8		1.0	0.6	1.6	0.946
Contact with a person with suspected/confirmed COVID-19 infection								
No	9 320	19.2	17.3 – 21.3	0.162	1			
Yes	547	25.5	19.3 – 33.0		1.4	1.0	2.1	0.058
Do not know	2 858	19.4	16.6 – 22.5		1.0	0.8	1.2	0.923
COVID-19 contact setting with suspected/positive case								
Health Care Setting	25	26.1	11.0 – 50.2	0.394	1			
Family Setting	320	26.3	18.7 – 35.6		1.0	0.3	3.1	0.988
Workplace Setting	125	30.2	16.9 – 47.8		1.2	0.3	4.6	0.764
Public Transport setting	13	7.4	1.3 – 33.0		0.2	0.0	1.7	0.145
In a retail store	9	10.0	1.8 – 40.0		0.3	0.0	2.5	0.272
Other (Specify)	59	15.2	6.1 – 33.0		0.5	0.1	2.2	0.358
Tested for the COVID-19 virus								
No	11 155	19.1	17.3 – 20.9	0.054	1			
Yes	1 513	23.2	19.1 – 28.0		1.3	1.0	1.7	0.054

 # symptoms refers to one or more of the COVID-19 infection related symptoms:- fever ≥38°C, chills, fatigue, muscle ache (myalgia), sore throat, cough, runny nose (rhinorrea),shortness of breath (dyspnea), wheezing, chest pain, other respiratory symptoms, headache, nausea/vomiting, abdominal pain

Table 8 shows that SARS-CoV-2 seroprevalence was significantly higher among those who reported having hypertension OR 1.4 (95% CI 1.1 – 1.7, p=0.003), with no significant associations with other comorbidities.

Table 8: SARS-CoV-2 seroprevalence and association with a medical history and comorbidities among people 12 years, NCAS, South Africa, June 2021

Variables	SARS-CoV-2 seroprevalence				Bivariate models			
	N	%	95% CI	*p*-value	OR	95% CI		*p*-value
Tuberculosis								
No	13 141	19.6	17.9 – 21.3	0.834	1			
Yes	73	21.0	10.4 – 37.7		1.1	0.5	2.5	0.834
Moderate to severe asthma								
No	13 036	19.6	17.9 – 21.4	0.473	1			
Yes	178	16.0	8.7 – 27.5		0.8	0.4	1.5	0.474
Other chronic lung diseases								
No	13 187	19.6	17.9 – 21.3	0.542	1			
Yes	27	26.2	9.5 – 54.6		1.5	0.4	5.0	0.545
Hypertension/high blood pressure								
No	11 224	19.1	17.3 – 20.9	0.003*	1			
Yes	1 990	24.6	21.0 – 28.6		1.4	1.1	1.7	0.003*
Diabetes								
No	12 412	19.4	17.7 – 21.3	0.13	1			
Yes	802	23.5	18.6 – 29.2		1.3	0.9	1.7	0.131
Cancer (that is not in full remission)								
No	13 183	19.6	17.9 – 21.3	0.778	1			
Yes	31	16.9	5.4 – 41.9		0.8	0.2	3.0	0.778
HIV								
No	12 660	19.5	17.7 – 21.3	0.477	1			
Yes	554	21.3	16.7 – 26.8		1.1	0.8	1.5	0.477
Cardiovascular conditions								
No	13 057	19.5	17.8 – 21.2	0.099	1			
Yes	157	33.0	17.0 – 54.3		2.0	0.9	4.9	0.107
Other chronic conditions#								
No	13 193	19.6	17.9 – 21.3	0.865	1			
Yes	21	17.9	5.7 – 43.8		0.9	0.2	3.2	0.865

*#Lung and kidney conditions; *Statistically significant – All health conditions based on self-report*

The final multivariate logistic regression model shows that increased odds of SARS-CoV-2 seropositivity were significantly associated with being female [aOR=1.44 (95% CI 1.23 – 1.70), p<0.001] when compared to being male. Those who had hypertension had significantly higher odds of SARS-CoV-2 seropositivity [aOR=1.28 (95% CI 1.00 – 1.64), p=0.048] when compared to those who did not have hypertension. Decreased odds of SARS-CoV-2 seropositivity were significantly associated with individuals aged 18 – 35 years old [aOR=0.69 (95% CI 0.53 – 0.90), p=0.007] compared to those aged 12 – 17 years old.

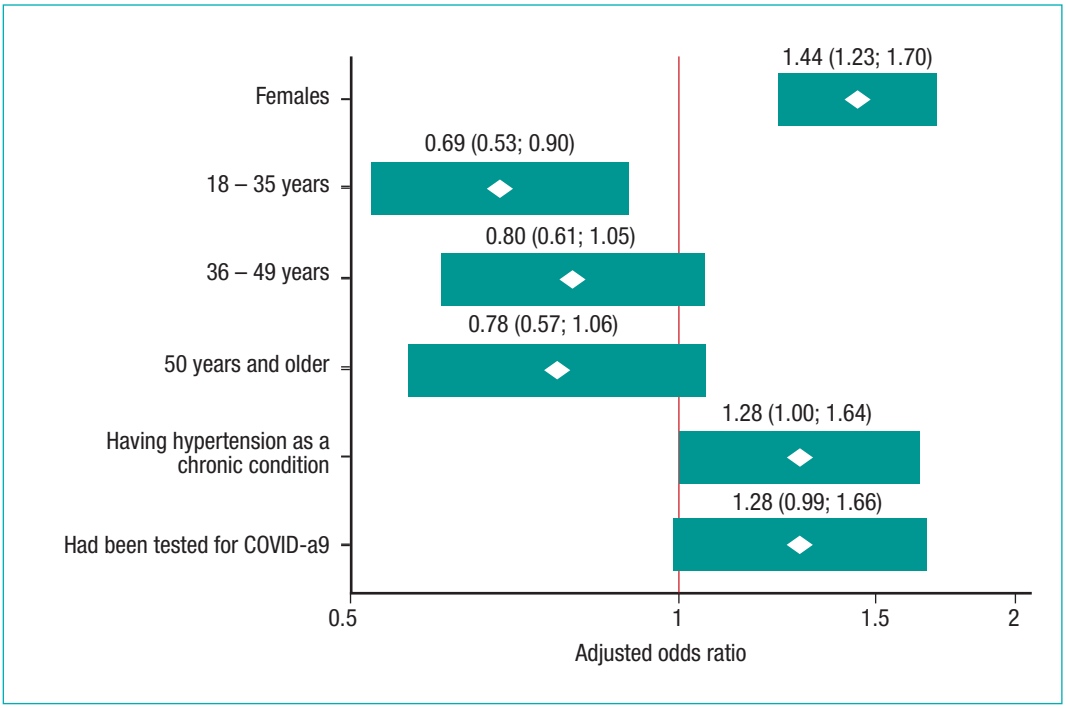

Figure 7: Multivariate logistic regression model of factors associated with SARS-CoV-2 seropositivity among people 12 years and older, NCAS, South Africa, June 2021

3.5 Neutralising antibody assay testing results

Neutralising antibody assay testing was conducted on a portion of samples that tested positive on both the Abbott assay and an NICD in-house IgG binding assay. This was based on the rationale that only samples which contain spike binding antibodies have the capacity to neutralise the virus. A total of 754 samples were positive on the Abbott ELISA and on the in-house ELISA. These specimens were collected between November 2020 and February 2021, and although the timing of specimen collection varied from province to province, the collection was generally during the second wave of infections in South Africa, and most (55%) were collected in November 2020.

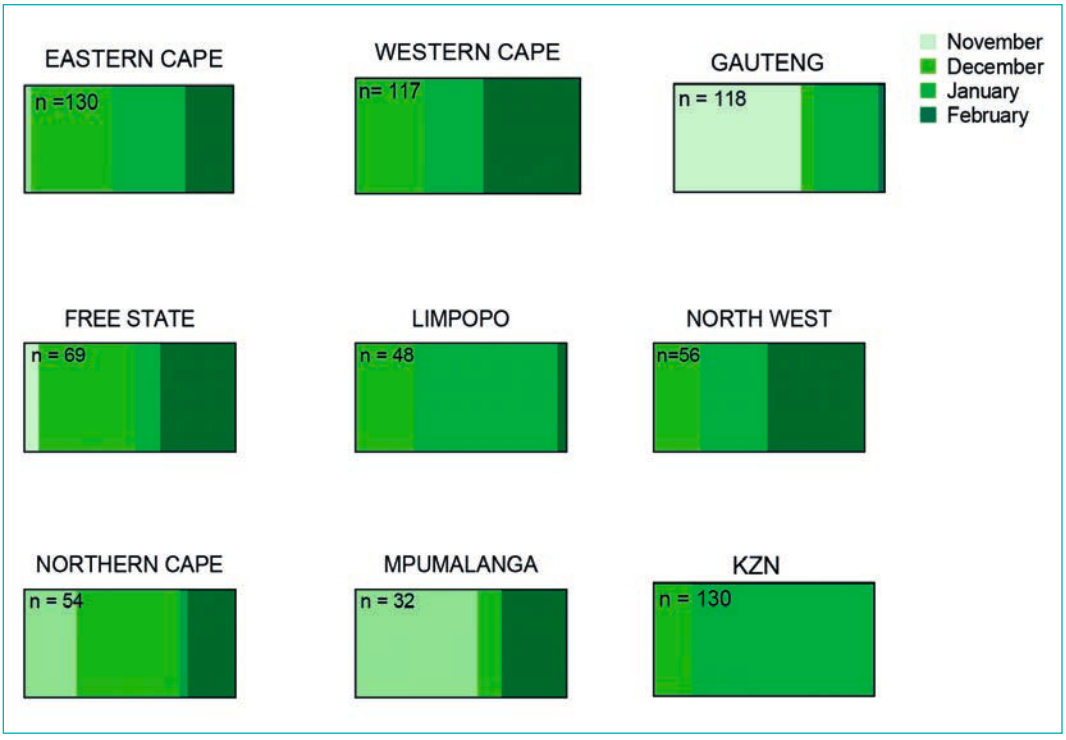

Figure 8: Timing of the collection of specimens tested for neutralisation across provinces November 2020 to February 2021 NCAS, South Africa.

3.5.1 Overall neutralisation profiles

Forty-five percent (45%) of these samples resulted in cross-neutralisation of both SARS-CoV-2 variants tested (Figure 9). This corresponds to the date of collection during or after South Africa's second wave for the majority of these samples. Twenty-eight percent (28%) of samples from all provinces neutralised the D614G strain only, a phenotype indicative of infection by the ancestral strain as opposed to the Beta variant. A subset of the samples (a total of 18% as shown in Figure 9) neutralised the Beta variant while failing to neutralise the ancestral D614G variant. This was expected as studies had shown that donors infected with the Beta variant exhibited a three-fold drop in their ability to neutralise the ancestral D614G strain (Moyo-Gwete et al, 2021).

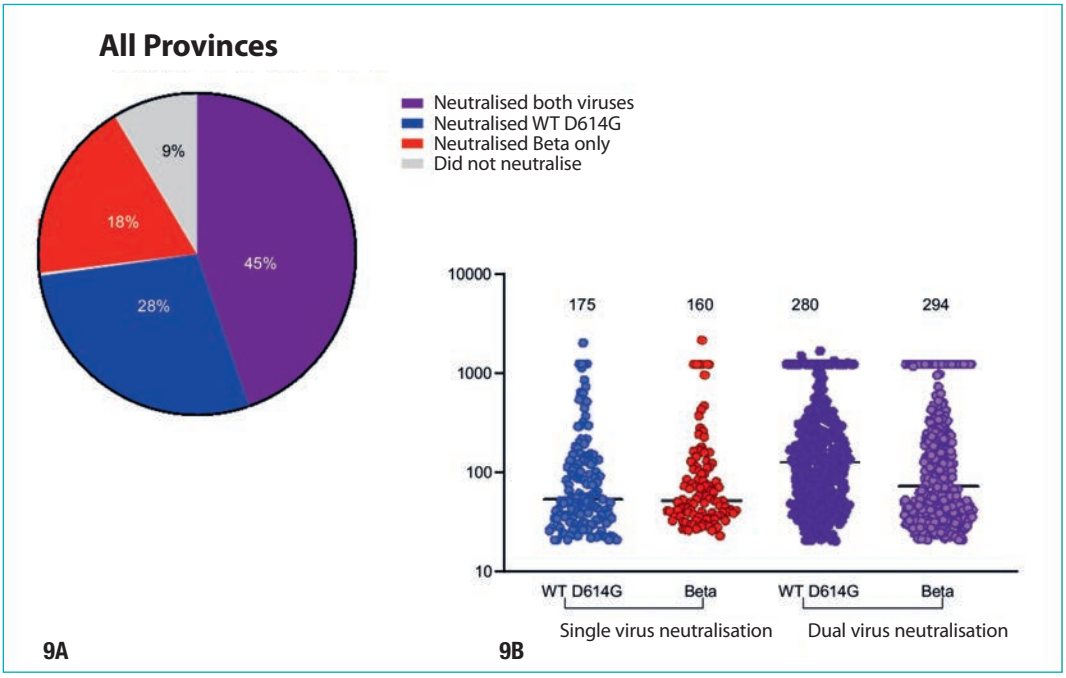

Figure 9: Overall neutralisation profile for all 9 provinces, people 12 years and older, South Africa November 2020 to February 2021: A). The pie chart indicates the ratio of samples that neutralised both variants, Beta only, the ancestral strain only, and the total samples that did not neutralise either variant. B) The dot plot shows a comparison between samples that neutralised both strains and samples that neutralised only one strain in terms of geometric mean titre against either D614G or Beta (GMT, shown on top of each graph)

Nine percent (9%) of all samples failed to neutralise either virus (Figure 9A), with each province having between 4% and 11% of such samples (Figure 10). Possible reasons for this include waning antibody concentrations over time – dependant on the time gap between infection and sample collection. Freeze-thaw cycles during sample storage and transport may also be responsible for a drop in plasma antibody concentrations. The dot plot in Figure 9B further supports these findings as samples that neutralised both viruses, that is, samples collected later during the second wave, show a higher geometric mean titre (GMT) compared to samples that only neutralised one strain. A breakdown of the individual neutralisation profiles for each province (shown in Figure 10) provided a more nuanced understanding of the nature of SARS-CoV-2 infections in South Africa during wave 2.

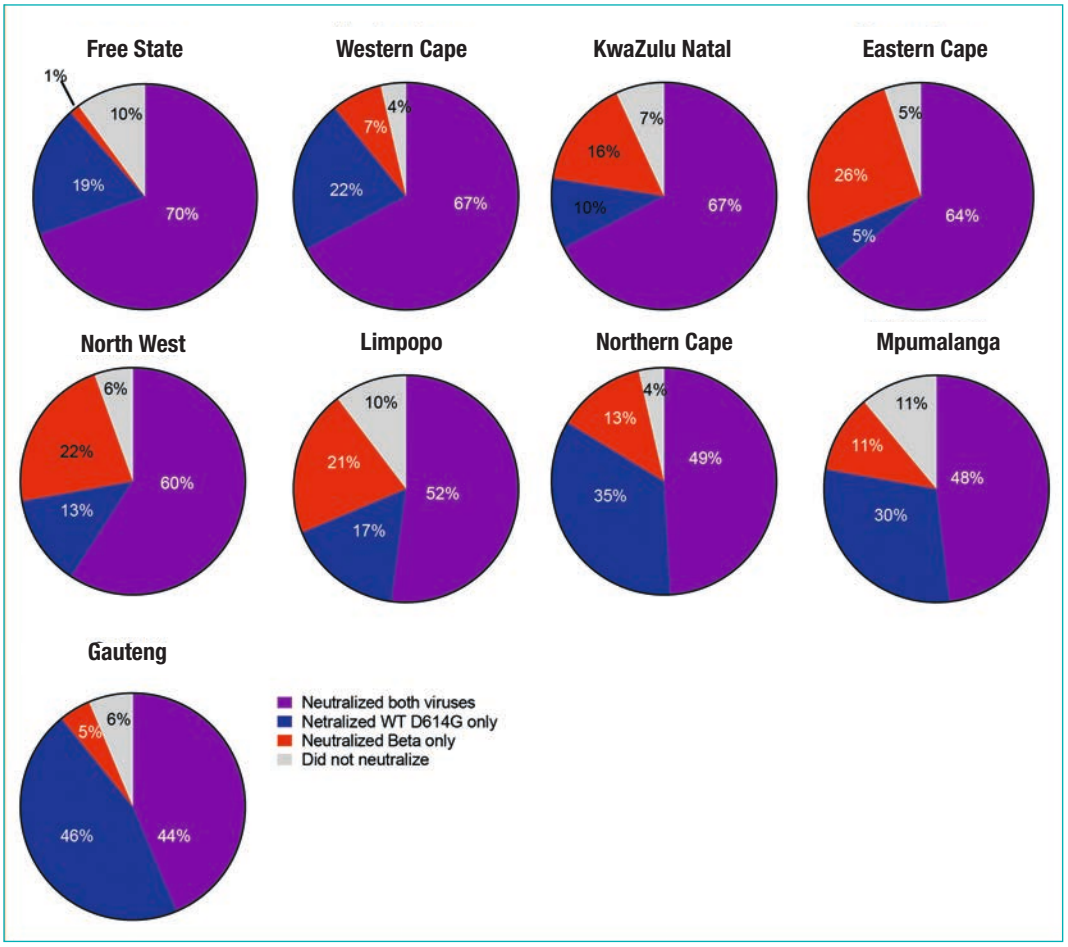

Figure 10: Neutralisation profiles for individual provinces, people 12 years and older, South Africa November 2020 to February 2021. Each pie chart shows the number of samples in each province that neutralised both variants, Beta only, the wild type D614G only, or neither variant.

At 46%, Gauteng province notably had the highest percentage of samples that only neutralised the ancestral D614G strain, while only 44% of samples from this province neutralised both strains. This indicates that Gauteng had the highest subset of donors infected with the ancestral D614G strain. This result is not surprising given that 62% of the Gauteng samples tested were collected in November 2020 and that the spread of the Beta variant to Gauteng occurred after this variant became dominant in the coastal provinces of Eastern Cape, Western Cape and KwaZulu-Natal. The graphs in Figure 11 show differences in the ability to neutralise the two strains for Gauteng and the Eastern Cape. The two provinces are representative of the two phenotypes observed based on sample collection timing, with the Eastern Cape samples being collected later into the second wave when compared to Gauteng.

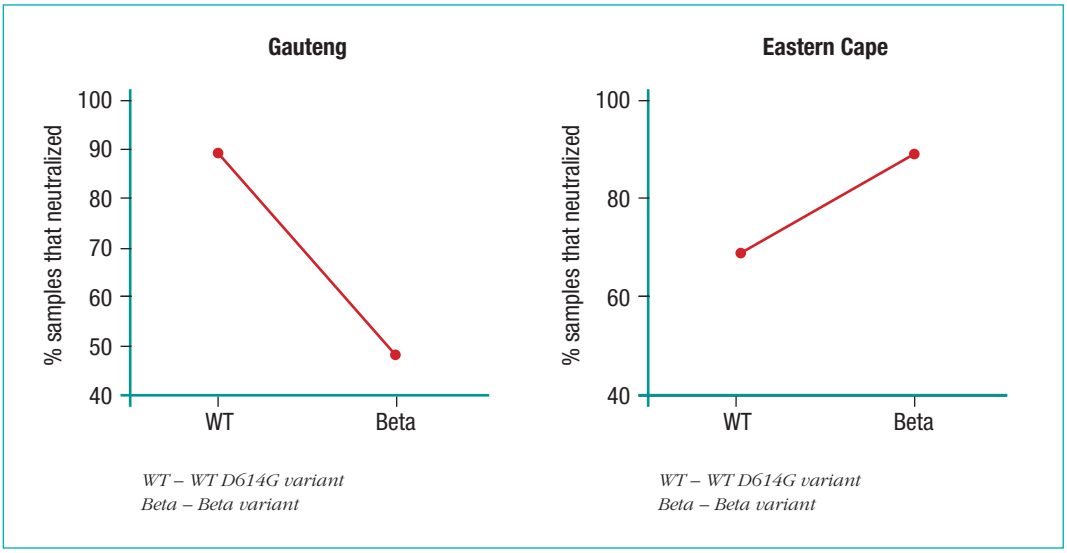

Figure 11: Phenotypic differences in Beta neutralisation potency among provinces, people 12 years and older, South Africa, November 2020 to February 2021.

This phenotype for Gauteng was also noted for provinces like Mpumalanga and the Northern Cape, where a higher percentage of samples were collected during November and December 2020 as opposed to early 2021, when the Beta variant was more dominant. The two provinces also had a later introduction of the Beta variant in 2020. In the Western Cape, 74% of specimens could neutralise the Beta variant (67% neutralized both variants and 7% the Beta variant only)– which is reflective of the majority of infections in the second wave being caused by the Beta variant just before the collection of most of these specimens.

KwaZulu-Natal samples were mostly collected in January 2021, and this is reflected in the neutralisation profile, with as much as 67% of the samples neutralising both variants, and a notable 16% neutralising the Beta variant only (Figure 10). A similar phenotype is observed for provinces whose samples were mostly collected in early 2021, that is the North West, Limpopo, Eastern Cape (Figure 10), and Free State Provinces.

At 70%, the Free State had the highest number of samples that neutralised both variants, although just one percent neutralised the Beta variant only. The fact that 19% of samples from the Free State only neutralised the ancestral D614G strain indicates that some second wave infections were due to the ancestral D614G strain in this province (Figure 10).

Neutralisation potency

Neutralization potency of samples against the ancestral D614G variant decreased with time, as opposed to that of samples against the Beta variant, which increased with time as the second wave of infections progressed and circulation of the Beta variant increased (Figure 12).

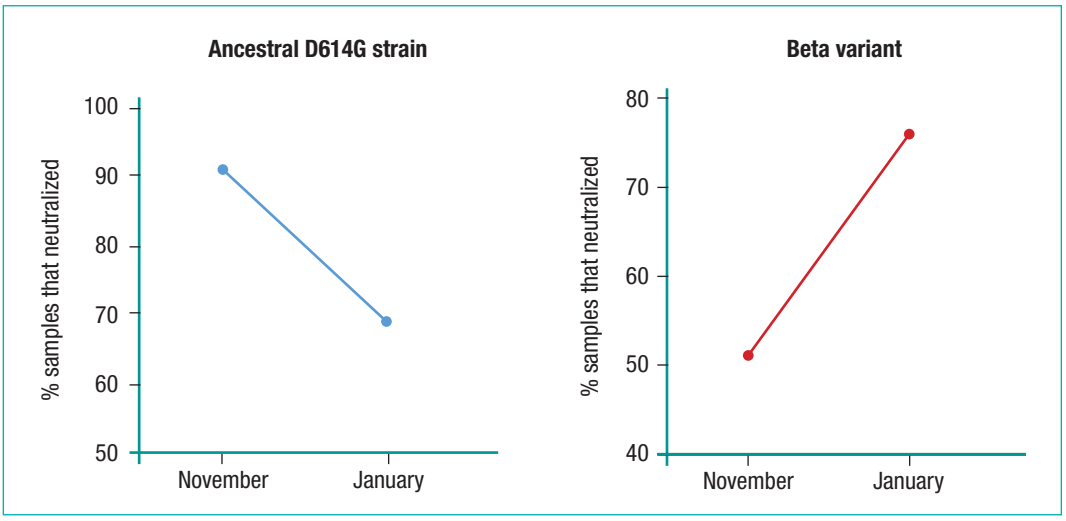

Figure 12: Differences in neutralization potency over time: people 12 years and older, South Africa, November 2020 to February 2021.

Neutralisation potency was generally low for most of the samples against both SARS-CoV-2 virus strains, with titres >1:1 000 being rare – regardless of which variant was tested. The potency of each of the two viral strains differed substantially over the four months during which samples were collected. (Figure 13). Overall neutralisation potency for the ancestral strain reduced over time, whereas that of the Beta variant increased over time – consistent with the change in the dominant variant during late 2020 and early 2021 (Figure 13).

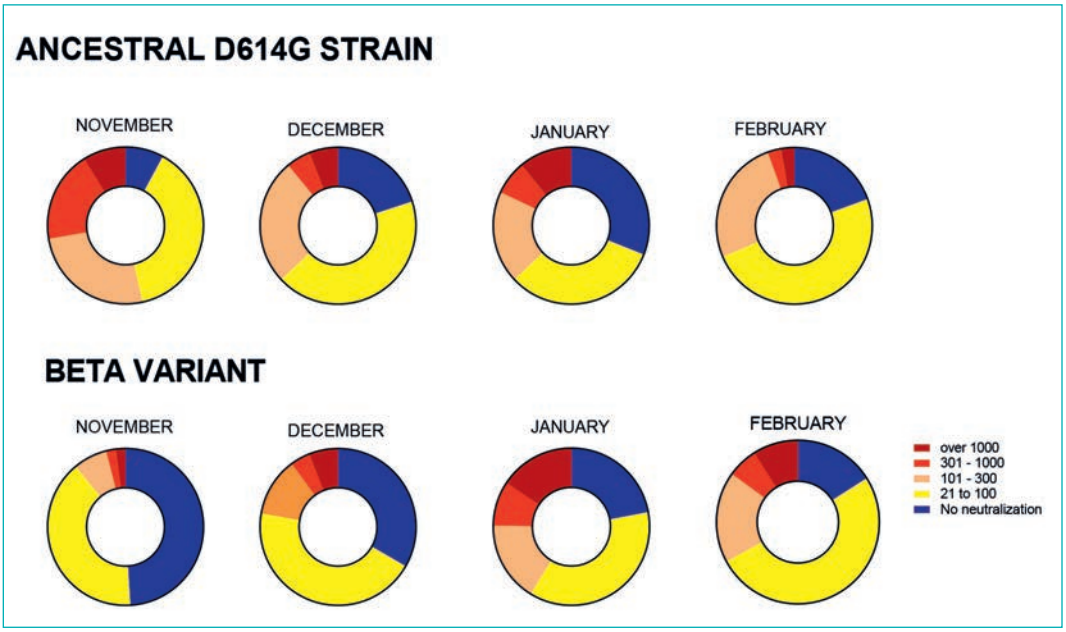

Figure 13: Summary of neutralisation potency per month: people 12 years and older, South Africa, November 2020 to February 2021 Neutralisation potency of NCAS samples against the ancestral D614G strain (top row) and the Beta variant (bottom row) over time for the nine provinces varied over time. The darker the colour, the higher the neutralisation potency

Discussion

This nationally representative population-based serosurvey of South Africans 12 years and older, estimated SARS-CoV-2 seroprevalence over the period November 2020 to June 2021 at 19.6% (95% CI 17.9 – 21.3). This translates to an estimated 8 675 265 people being exposed to and infected by the virus across South Africa by 15 June 2021 (the end of the survey sampling period). This is 5 times higher than the reported cumulative number of SARS-CoV-2 cases for all ages (1 675 013) on 2 June 2021[1] when allowing for the period between infection and the development of IgG antibody responses. The survey timeline covers the period when the original SARS-CoV-2 (WT D614G) virus strain and the more transmissible Beta variant were circulating in the country and was mostly before the circulation of the even more transmissible Delta strain. These findings highlight the extent of infections and transmission, and the under- ascertainment of PCR-derived estimates. The estimates could be even higher given the limitations of assays and seroreversion in some people (Chen *et al.*, 2021, Kritsotakis, 2020, Lisboa Bastos *et al.*, 2020, Perez-Saez *et al.*, 2021). Furthermore, given the timing of the survey in relation to vaccination rollout and vaccination coverage in the country at the time of the survey, it is unlikely that a significant proportion of the Euroimmun assay antibody responses in this survey would be attributed to vaccination.

The survey found substantial geographical variability in seroprevalence by province, with seroprevalence higher in the Free State, Eastern Cape and Western Cape provinces. Seroprevalence was lower in Mpumalanga and Limpopo provinces. Seroprevalence estimates also varied between metropolitan areas and were higher in Mangaung, Nelson Mandela and the City of Cape Town. SARS-CoV-2 seropositivity was also higher in rural formal/farm areas – especially among females – when compared to the urban and rural informal areas. Our findings are comparable to those reported from a cross-sectional study undertaken in Gauteng province between November 2020 and January 2021 that reported a 19.1% overall seroprevalence, with substantial variation across subdistricts in the province (Mutevedzi *et al.*, 2022). This Gauteng study however also included children younger than 12 years old. Preliminary results of a COVID-19 healthcare utilisation and seroprevalence survey (HUTS study) conducted in three communities in Pietermaritzburg in the KwaZulu-Natal Province, Klerksdorp in the North West Province, and Mitchell's Plain in the Western Cape Province, reported a higher overall prevalence of 35.8% for the period November 2020 to January 2021 (Aitken *et al.*, 2021). Seroprevalence from blood donor samples collected over the period January 2021 to May 2021 was estimated at 47.4% also with variation across provinces (SANBS, 2021). In Kenya crude seroprevalence from blood donors over the period April 2020 to September 2020 was estimated at 9.4% (Adetifa *et al.*, 2021). A cross sectional survey in 6 districts in Zambia (4-27 July 2020) reported a lower seropositivity of 2.1% attributed by the survey investigators to stringent physical and social distancing measures implemented in the country (Mulenga *et al.*, 2021). A systematic review and meta-analysis of studies from January 2020 to December 2020 found lower seroprevalence estimates from national studies than regional and local studies, and suggested that marginalized and high-risk groups were disproportionately affected (Bobrovitz *et al.*, 2021). Variation in seroprevalence estimates over geographic areas and time are influenced by several factors including variability in exposure to the virus attributable to social and living conditions that impact transmission, transmissibility of circulating strains, changes in antibody levels over time post infection including seroreversion, and variability in the performance of diagnostic tests.

1 https://www.nicd.ac.za/latest-confirmed-cases-of-covid-19-in-south-africa-2-june-2021

In the final multivariate logistics regression model of our analysis, increased odds of seropositivity were associated with females and those aged 12 to 17 years old. By early July 2021, PCR based testing also showed more cumulative cases in females which was attributed to their high representation in high risk occupations and differences in care seeking practices betweem men and women (NICDc, 2021). Women also generally fulfil caring roles in the home – this would have included caring for those with COVID-19 in the home, thus also increasing their risk of infection. Our data also contributed to evidence highlighting infections in adolescents. Among other factors, the risks for adolescents are attributed to the fact that adolescents tend to have more contacts than adults, and that adults were more likely to adhere to masking and social distancing because they felt vulnerable (Rumain *et al.*, 2021). Our findings suggest that adolescents were more likely than adults to contract and spread the virus because they may have been mingling in large groups, including at school and in social gatherings like attending parties, with poor adherence to prevention guidelines. Since young people are more likely to be asymptomatic or have mild symptoms compared to adults, this posed significant implications for transmission of infections from these settings to household members of adolescents. The survey also found that many people with hypertension were infected, a notable finding since reports have shown that hypertension significantly increases the risk of severe clinical outcomes and hospitalisation in patients with COVID-19 infection (Ran *et al.*, 2020, WCDOH, 2021).

Neutralisation studies showed that 45% of samples neutralised both virus strains. Across all provinces between 4 and 11% of samples were unable to neutralize either strain of the circulating virus. This highlighted the urgent need for vaccinations. Overall neutralization potency for the ancestral strain reduced over time, whereas that of the Beta strain increased over time – consistent with the change in the dominant strain during late 2020 and early 2021. Neutralization potency was generally low since this was a general population survey, with generally well people who were not hospitalized and therefore had low viral loads following infection.

Limitations and strengths of the study

This was the first nationally representative survey of its nature in South Africa. It was conducted before widespread vaccination against SARS-CoV-2, and thus some presents a national baseline assessment of infections by age, sex and locality type. The survey also provided information about neutralization activity in specimens from infected people who were not hospitalised and had mild infections. However, there were some limitations. The survey collected data over a prolonged period in a rapidly changing pandemic, and therefore the overall estimates should be interpreted with this in mind. The data however, provide a national picture of estimate of exposure to the virus across all provinces and locality types by June 2021. (Estimates over each of the two survey rounds are shown in Annexure D). The household response was low (55%). However, where households agreed to participate, the individual response was high with 90.8% of eligible individuals interviewed and 81.9% interviewed and tested.

The associations presented in this report should not be regarded as causal since this was cross-sectional study that cannot determine causality. The study also excluded children younger than 12 years, whose infections are largely mild and/or asymptomatic, hence the estimates do not apply to the entire population. Finally, these estimates should be interpreted considering the limitations and differences of antibody assays for SARS-CoV-2 (Føns & Krogfelt, 2021, Peluso *et al.*, 2021, Vanshylla *et al.*, 2021). The survey estimates may be an underestimate given the reported lower sensitivity of assays over time post infection, the varying (and unknown) periods between infection and blood specimen collection, and the possibility of seroreversion (Peluso *et al.*, 2021).

Conclusion

This study provided nationally representative estimates of the prevalence of SARS-CoV-2 antibodies in South Africa over the period November 2020 to June 2021 for people 12 years and older. An estimated 8 675 265 people aged 12 years and older had been exposed to and infected by the virus by June 2021 – and estimate that is approximately 5 times the cumulative number of infections based on PCR testing for people of all ages at this time. The survey was completed before widespread vaccinations against the virus and indicated the level of community susceptibility at the time. The survey also identified provinces, metropolitan areas, and localities predominantly affected by the pandemic during a period that coincided with the second SARS-CoV-2 wave in the country.

Women were significantly more likely to be infected indicating their vulnerability and highlighting the need for additional support for women given factors that likely increase their risk. People aged 18-35 years old were less likely to be infected than young people (12-17 years old) indicating a need for youth focused strategies which were limited at the time of the survey. The findings also highlighted the risk of infections in rural areas, in particular on farms where some living conditions may have accelerated the spread of the virus. Hence preventive measures and vaccination should always be rolled out equitably to all areas of the country. The survey also showed that less than half of the samples tested neutralised both the original and the then more transmissible Beta strain of the SARS-CoV-2 virus, which indicated the importance and the urgency of vaccination roll out.

Finally, there is a need for ongoing surveillance that includes population-based serosurveys to provide updated and comprehensive information about infections and immune responses in different populations across the country as the pandemic evolves. Future population-based surveys of the prevalence of SARS-CoV-2 antibodies should also assess vaccine-induced antibodies to monitor the dynamics of the pandemic and evaluate the effectiveness of the vaccination campaign in South Africa.

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Appendix A1:
Household Questionnaire

(Questionnaires developed and adapted from other sources by the HSRC, remain a property of the HSRC, and use is subject to acknowledging the HSRC)

Household barcode number	Barcode

A	GEOGRAPHIC AND INTERVIEW PARTICULARS								
Province									
Small area layer									
Visiting point number									

Address:

_____

_____

B	INTERVIEW DETAILS						
	Year		Month		Day		Response code
First visit	2	0					
Second visit	2	0					
						Final response code	

Response code

1 = Interview completed
2 = Not a valid visiting point
3 = No one living here (unoccupied)
4 = Refusal by household head
5 = Refusal by other resident
6 = Partly completed/appointment made
7 = No one at home
8 = No one eligible to complete questionnaire
9 = Incapacitated
10 = Other

INTERVIEW STARTING TIME:			:		

INTERVIEWER: NAME AND EMPLOYEE NUMBER ……..							

REFUSAL PARTICULARS			
No.	**Questions and filters**	**Coding categories**	
1.	At what point did the respondents refuse to take part in the survey?	At the gate or door ..... 1 After explanation of the survey ..... 2 After identifying the respondent ..... 3 During the household interview ..... 4 Other 9	
2.	What was the reason for the refusal?	Too busy to grant interview ..... 1 Not available now ..... 2 Too late in the evening ..... 3 Don't participate in surveys ..... 4 Object to topic of the survey ..... 5 Object to provide information of household members ..... 6 Do not allow strangers on property ..... 7 Enumerated in the recent population census ..... 8 Other 9	
2a.	How old were you at your last birthday? (in years)?	In years	
2b.	Sex of person refusing	Female ..... 1 Male 2	
2c.	Race of person refusing	Black African ..... 1 White ..... 2 Coloured ..... 3 Indian/Asian 4	

AVAILABILITY OF SERVICES			
No.	**Questions and filters**	**Coding categories**	
3.	What is the main source of drinking water for members of your household?	Piped water (tap) in dwelling ..... 1 Piped water (tap) in site/yard ..... 2 Bottled water ..... 3 Water carrier/tanker ..... 4 Rain-water tank ..... 5 Borehole / well / spring ..... 6 Dam/river/stream ..... 7 Public / communal tap ..... 8 Other 9	
4.	What kind of toilet facilities does your household have?	Flush toilet (own ..... 1 Flush toilet (shared ..... 2 Bucket latrine ..... 3 Pit latrine with ventilation ..... 4 Pit latrine without ventilation ..... 5 No facility/bush/field ..... 6 Other 7	
5.	What is this household's main source of energy for cooking purposes?	Electricity ..... 1 Coal ..... 2 Wood ..... 3 Gas ..... 4 Paraffin ..... 5 Animal dung ..... 6 Other 7	
6.	How many rooms does your dwelling consist of?	Rooms ..... ☐☐	
7.	How many rooms in your dwelling are used for sleeping? NOTE: A room may also have another purpose besides as a bedroom.	Rooms for sleeping ..... ☐☐	

HOUSEHOLD INCOME			
No.	**Questions and filters**	**Coding categories**	
8.	Did you receive any income from any source in the last month?	Yes ..... 1 No 2	
9.	What is the main source of income in this household during the last month?	Formal salary/earnings (taxable) ..... 1 Formal salary/earnings (no tax) ..... 2 Contributions by adult family members/relatives ..... 3 Contributions by younger family members/relatives (<18 yrs) ..... 4 Government pensions / grants ..... 5 Grants/donations by private welfare organisations ..... 6 Other 9	
10.	The cost of living is a concern for many families. Can you tell me what this household can afford? Which option best describes your household situation?	Not enough money for basic things ..... 1 Money for food/clothes, but short on other things ..... 2 Have most of the important things but few luxury goods ..... 3 Have money for holidays and luxury goods 4	

The following questions deal with the people who usually live in this household and who slept here last night. Please list the persons who usually live in your household and guests of the household who stayed here last night, starting with the head of the household.

HOUSEHOLD MEMBERS AND THEIR CHARACTERISTICS										
Person no.	**Relationship to head of household**	**Sex**	**Residence**		**Age**	**Population group / race**	**Language**	**Grants**		**Individual Form number**
	What is the relationship of this person to the head of the household?*	Is this person male or female?	Does this person usually live here?	Did this person stay here last night?	How old is this person? (How old at last birthday) (Write 00 if under 1 year).	African=1 White=2 Coloured=3 Indian=4	Language spoken most often at home**	Does this person receive any grants?	Type of grant? ***	Link the person to the individual form
(1)	(2)	(3)	(4)	(5)	(6)	(7)	(8)	(9)	(10)	(11)
		M F	YES NO	YES NO	IN YEARS			YES NO		
1		1 2	1 2	1 2				1 2 Go to next person		
2		1 2	1 2	1 2				1 2 Go to next person		
3		1 2	1 2	1 2				1 2 Go to next person		
4		1 2	1 2	1 2				1 2 Go to next person		
5		1 2	1 2	1 2				1 2 Go to next person		
6		1 2	1 2	1 2				1 2 Go to next person		
7		1 2	1 2	1 2				1 2 Go to next person		

HOUSEHOLD MEMBERS AND THEIR CHARACTERISTICS										
Person no.	**Relationship to head of household**	**Sex**	**Residence**		**Age**	**Population group / race**	**Language**	**Grants**		**Individual Form number**
	What is the relationship of this person to the head of the household?*	Is this person male or female?	Does this person usually live here?	Did this person stay here last night?	How old is this person? (How old at last birthday) (Write 00 if under 1 year).	African=1 White=2 Coloured=3 Indian=4	Language spoken most often at home**	Does this person receive any grants?	Type of grant? ***	Link the person to the individual form
(1)	(2)	(3)	(4)	(5)	(6)	(7)	(8)	(9)	(10)	(11)
		M F	YES NO	YES NO	IN YEARS			YES NO		
8		1 2	1 2	1 2				1 2 Go to next person		
9		1 2	1 2	1 2				1 2 Go to next person		
10		1 2	1 2	1 2				1 2 Go to next person		
11		1 2	1 2	1 2				1 2 Go to next person		
12		1 2	1 2	1 2				1 2 Go to next person		
13		1 2	1 2	1 2				1 2 Go to next person		
14		1 2	1 2	1 2				1 2 Go to next person		

HOUSEHOLD MEMBERS AND THEIR CHARACTERISTICS										
Person no.	**Relationship to head of household**	**Sex**	**Residence**		**Age**	**Population group / race**	**Language**	**Grants**		**Individual Form number**
	What is the relationship of this person to the head of the household?*	Is this person male or female?	Does this person usually live here?	Did this person stay here last night?	How old is this person? (How old at last birthday) (Write 00 if under 1 year).	African=1 White=2 Coloured=3 Indian=4	Language spoken most often at home**	Does this person receive any grants?	Type of grant? ***	Link the person to the individual form
(1)	(2)	(3)	(4)	(5)	(6)	(7)	(8)	(9)	(10)	(11)
		M F	YES NO	YES NO	IN YEARS			YES NO		
15		1 2	1 2	1 2				1 2 Go to next person		
16		1 2	1 2	1 2				1 2 Go to next person		
17		1 2	1 2	1 2				1 2 Go to next person		
18		1 2	1 2	1 2				1 2 Go to next person		

* Codes for q.2 Relationship to head of household:		** Codes for q.8 Language		*** Codes for q.10 (Grants)
01 = Head	08 = Brother / sister	01 = Afrikaans	08 = Sepedi	1= Old age
02 = Wife / husband / Partner	09 = Niece/nephew	02 = English	09 = Setswana	2 = Disability
03 = Son / daughter	10 = Other relative	03 = Isindebele	10 = Tshivenda	3 = War veterans
04 = Son-in-law / Daughter-in-law	11 = Adopted/foster/ Stepchild	04 = Isiswati	11 = Xitsonga	4 = Care dependency
05 = Grandchild	12 = Not related	05 = Isixhosa	12 = Other african	5 = Foster grant
06 = Parent	98 = Don't know	06 = Isizulu	13 = Other european / asian	6 = Child support
07 = Parent-in-law		07 = Sesotho		7 = Grant-in-aid
				9 = Don't know

Appendix A2:
Individual Questionnaire

Household barcode number	Barcode
Individual Questionnaire number	Barcode

A	**GEOGRAPHIC AND INTERVIEW PARTICULARS**								
Province									
Small area layer									
Visiting point number									
Person number of respondent									

B	**INTERVIEW DETAILS**						
	Year		Month		Day		Response code
First visit	2						
Second visit	2						
Third visit	2						
					Final response code		

Response code

1 = Interview completed and sample taken
2 = Interview completed but sample not taken
3 = Appointment made for interview and/or sample
4 = Selected respondent not at home
5 = Refusal by head of household
6 = Refusal by respondent
7 = Other

INTERVIEW STARTING TIME:			:		

INTERVIEWER: NAME AND EMPLOYEE NUMBER ……							

C	**DEMOGRAPHIC PARTICULARS**

1	**How old were you at your last birthday? (in years)**		

2	**Are you a male, female or other?**	Female	Male	Other
		1	2	3

3	To which population group do you belong?			
	Black African	White	Coloured	Indian/Asian
	1	2	3	4

4	What is the highest educational level that you have obtained?	
	None	0
	Primary	1
	Secondary	2
	Matric	3
	Tertiary	4

5	How would you describe your present employment situation?	
	Employed – full time (fixed salary per month)	1
	Employed – informal sector/ part time (non-fixed salary per month)	2
	Unemployed	3
	Home Duties (not looking for work)	4
	Full-time Student	5
	Retired	6
	Self Employed	7
	Other (specify any lockdown related circumstance not captured above)	8

6	What is your current marital status? (Marital status referring to legal, traditional or common-law)	
	Married	1
	Never married	2
	Divorced / separated	3
	Widower / Widow	4

D	MEDICAL HISTORY		
7a	Are you currently being treated for a chronic condition?	Yes	No
		1	2
			Go to Q7e
b	Are you comfortable sharing what you are suffering from?	Yes	No
		1	2

c	If Yes, please specify If No, ***Go to 7d*** ☐ Tuberculosis ☐ Moderate to severe asthma ☐ Other chronic lung disease ☐ HIV ☐ Hypertension ☐ Diabetes ☐ Chronic liver disease ☐ Chronic kidney disease ☐ Heart failure or ischaemic heart disease ☐ Other cardiovascular conditions ☐ Cancer (that is not in full remission) ☐ Other (please specify)		
d	Have you ever had TB in the past?	Yes	No
		1	2
e	Have you ever any lung conditions that left damage (scarring) in your lungs?	Yes	No
		1	2
f	Are you currently using immunosuppressant drugs especially if you are suffering from any of the below conditions?	1	2
g	If Yes, for what condition? If No, ***Go to 8a*** ☐ I had organ transplants ☐ I have liver disease ☐ I have Rheumatoid arthritis ☐ I have ankylosing spondylosis ☐ I have systemic lupus erythematosus (SLE) ☐ I have sarcoidosis ☐ I have psoriasis Other (please specify)		

E	**HEALTH RISK BEHAVIOUR**

8a	Do you currently smoke tobacco?	Yes	No
		1	2
			Go to Q9a
b	If yes, how often do you smoke?	Daily	Less than daily
		1	2

F	SYMPTOM HISTORY

9	Recently there is a new disease that has started afflicting people. It is called Coronavirus or COVID-19. Since 5th of March have you experienced any of the following symptoms?	Yes, past 14 days	Yes, past 3 months	No
a	Fever ≥38°C	1	2	3
b	Chills	1	2	3
c	Fatigue	1	2	3
d	Muscle ache (myalgia)	1	2	3
e	Sore throat	1	2	3
f	Cough	1	2	3
g	Runny nose (rhinorrea)	1	2	3
h	Shortness of breath (dyspnea)	1	2	3
i	Wheezing	1	2	3
j	Chest pain	1	2	3
k	Other respiratory symptoms	1	2	3
l	Headache	1	2	3
m	Nausea/vomiting	1	2	3
n	Abdominal pain	1	2	3
o	Diarrhoea	1	2	3
				NO to all Go to Q11a

10a	Did any of these symptoms require you to seek medical attention?	Yes	No	N/A
		1	2	3
b	Did any of these symptoms require you to miss work or school?	1	2	3
c	Did any of these symptoms require you to be hospitalised?	1	2	3

11a	Have you had contact with anyone with suspected or confirmed COVID-19 virus infection?	Yes	No	Unknown
		1	2	3
			Go to Q12a	

b	**If Yes, dates of last contact (DD/MM/YYYY)**	D	D	M	M	Y	Y	Y	Y
c	Was contact in a healthcare setting?	Yes				No			
		1				2			
d	Was contact in a family setting?	1				2			

e	Was contact in a work setting?	1	2
f	Was contact in a public transport setting?	1	2
g	Was contact in a retail store?	1	2
h	Where you screened for COVID-19 after this contact?	1	2
i	Did you self-isolate/quarantine after the contact?	1	2
			Go to Q12a

j	For how long did you self-isolate / quarantine? (in days)		

k	If you did not self-isolate / quarantine how many people did you have contact with?		

l	Have you been screened for the Coronavirus as part of the nationally screening and testing programme?	Yes	No
		1	2

m	Have YOU personally been tested for the Coronavirus?	Yes	No
		1	2
			Go to Q12a

n	Are you willing to tell me the test result you received?	Yes	No	Never received result
		1	2	3
			Go to Q11p	

o	What was the result of that COVID-19 test?	Positive	Negative	Indeterminate
		1	2	3

p	If the test was positive have you recovered from the infection?	Yes	No
		1	2

q	If the test was negative did you experience any COVID-19 symptoms after the test?	Yes	No
		1	2

r	Did you self-isolate/quarantine after the test?	Yes	No
		1	2
			Go to Q12a

s	For how long did you self-isolate / quarantine? (in days)		

t	If the test was negative and you developed symptoms where you tested again?	Yes	No
		1	2

u	What was the result of that COVID-19 test?	Positive	Negative	Indeterminate
		1	2	3

v	Have you been vaccinated for COVID-19? (this includes participating in a vaccine trial for covid19)	Yes	No
		1	2
			Go to Q12a

w	When were you vaccinated (DD/MM/YYYY)	D	D	M	M	Y	Y	Y	Y

G	**ACCESS TO PREVENTION FOR COVID-19 INFECTION**

12a	Had adequate access to water at home for hand hygiene	Yes	No
		1	2
b	Over the past seven days, have you had adequate access to soap at home for hand hygiene?	1	2
c	Over the past seven days, have you had access to a cloth mask when you had to leave the house?	1	2
d	Over the past seven days, have you had access to a hand sanitizer when you could not access water and soap?	1	2
e	Do you know a place in your community or close to you, where you can be tested for Covid-19?	1	2

H	**SELF-PERCEIVED RISK**

13	How do you rate your PERSONAL RISK of contracting the Coronavirus (COVID-19)?	Very high risk	High risk	Moderate risk	Low risk	Very low risk
		1	2	3	4	5

14	Why do you believe that you are at the SELECTED LEVEL of risk? (Select all that's applicable)	
a	I have other health conditions	1
b	Because of my work	2
c	Because of my age	3
d	Because of where I live	4
e	Because I do not have access to water and soap at home	5
f	Because I do not have a mask	6
g	Because it is difficult to self-isolate where I stay	7
h	Because my place of work is not equipped to deal with COVID-19	8

i	Because I use public transport	9
j	Other (specify)	10

I	**TRAVEL AND MOVEMENT/ PHYSICAL DISTANCING**		
15a	Over the past 14 days, have you left your home? This might have been to get food or water, or for medical care, or to help friends and neighbours, or to go to work or go to school?	Yes 1	No 2 ***Go to Q15f***
b	Over the past seven days, have you come into close contact with people outside your home where it was not possible to practice social distancing (places such as a lift or a queue at the shop or public transport)?	Yes 1	No 2
c	Over the past seven days, have you left your province/ village/ suburb/township/ area?	1	2

d	The last time you were away from home, how many people did you come into close contact with? (Within 2 metres). If you are not sure, please make your best guess.	1-3	4-7	8-10
		11-15	16-20	more than 20

e	Have you attended an event or gathering such as a funeral or food parcel distribution in the last 14 days?	Yes 1	No 2

f	In the last 14 days did anyone visit your home and either left immediately or spent a night?	Yes	No	Don't know
		1	2	3
g	How many such visitors have you had in the last 14 days? If you are not sure, please make your best guess			

THANK YOU VERY MUCH FOR AGREEING TO PARTICIPATE AND ASSIST US IN THIS IMPORTANT RESEARCH PROJECT

Appendix B:
Data collection timeline and epidemic timelines by province

MONTH	WEEK	EPIDEMIC WAVES	VACCINATION ROLL OUT	VACCINATION ROLL OUT	WESTERN CAPE	EASTERN CAPE	NORTHERN CAPE	FREE STATE	KWAZULU NATAL	NORTH WEST	GAUTENG	MPUMALANGA	LIMPOPO
Nov-20	1	Wave 1	No vaccinations	No vaccinations									
Nov-20	2												
Nov-20	3												
Nov-20	4												
Dec-20	1	Wave 2											
Dec-20	2												
Dec-20	3												
Dec-20	4												
Jan-21	1												
Jan-21	2												
Jan-21	3												
Jan-21	4												
Jan-21	5												
Feb-21	1												
Feb-21	2												
Feb-21	3		Phase 1	Front line and health care workers									
Feb-21	4												
Mar-21	1												
Mar-21	2												
Mar-21	3												
Mar-21	4												

MONTH	WEEK	EPIDEMIC WAVES	VACCINATION ROLL OUT	VACCINATION ROLL OUT	WESTERN CAPE	EASTERN CAPE	NORTHERN CAPE	FREE STATE	KWAZULU NATAL	NORTH WEST	GAUTENG	MPUMALANGA	LIMPOPO
Apr-21	1												
Apr-21	2												
Apr-21	3												
Apr-21	4												
May-21	1												
May-21	2												
May-21	3		Phase 2	People aged 60 years and older									
May-21	4												
Jun-21	1												
Jun-21	2				Last week of data collection in all provinces								
Jun-21	3			Teachers and support staff									
Jun-21	4												
Jul-21	1			People aged 50-59 years AND Essential workers (police force, congregant settings)									
Jul-21	2												
Jul-21	3	Wave 3		People aged 35-49 years AND Frontline social workers									
Jul-21	4			Media workers and journalists									
Aug-21	1												
Aug-21	2												
Aug-21	3												
Aug-21	4		Phase 3	People aged 18-34 years									
Aug-21	5												
Sep-21	1												
Sep-21	2												
Sep-21	3												
Sep-21	4												

Legend

Data collection period

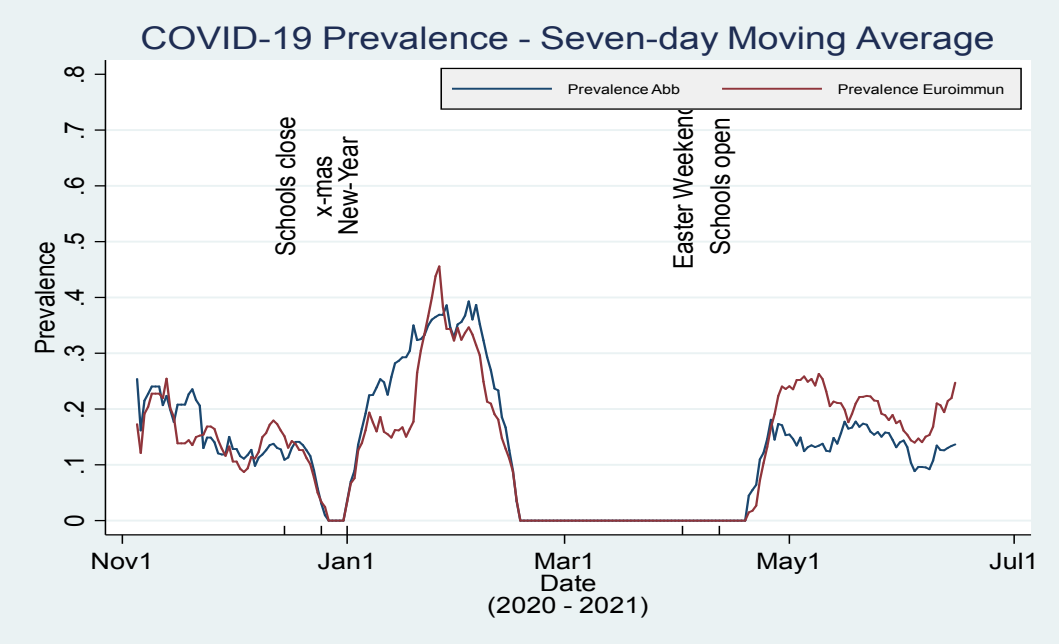
None data collection period

Appendix C:
Abbott assay compared to Euroimmun assay

		Abbott assay			Euroimmun assay		
category	**N**	**weighted prevalence (%)**	**lower estimate**	**upper estimate**	**weighted prevalence (%)**	**lower estimate**	**upper estimate**
National	13309	16.6	15.2	18.1	19.6	17.9	21.3
Sex							
Male	5572	15.5	13.5	17.7	16.6	14.6	18.7
Female	7737	17.6	16.0	19.4	22.4	20.4	24.5
Age group							
Less than 18	1379	16.2	13.2	19.8	23.2	19.2	27.8
18-35	4535	15.9	13.9	18.1	17.3	15.0	19.9
36-49	3074	15.5	13.3	18.1	20.1	17.6	22.8
50+	4321	19.7	17.6	22	21.3	19.1	23.7
Province							
Mpumalanga	610	11.8	7.7	17.6	13.6	8.9	20.2
Limpopo	567	13.0	8.9	18.5	11.6	7.6	17.4
North West	533	14.3	9.7	20.5	17.5	12.1	24.5
Gauteng	1468	15.2	12.2	18.8	18.4	14.8	22.7
Western Cape	3336	15.9	13.1	19.1	22.4	18.1	27.4
Free State	1042	16.2	12.1	21.4	26.8	22	32.1
Northern Cape	841	18.3	14.0	23.6	18.4	13.8	24.1
KwaZulu-Natal	2279	20.8	17.9	24.1	21.6	18.1	25.7
Eastern Cape	2635	22.0	17.9	26.9	26.0	22.5	29.9

Appendix D:
Annexure D: Estimates by survey round

	Round 1			Round 2			Overall		
	n	Prevalence (%)	Estimated number of people exposed	*n*	Prevalence (%)	Estimated number of people exposed	*n*	Prevalence (%)	Estimated number of people exposed
Sex									
Male	2 232	15.5	1382671	3,302	17.3	2175743	5,534	16.6	3558415
Female	3,227	20.1	1861090	4,451	23.9	3255760	7,678	22.4	5116849
Total	5,459	17.8	3243761	7,753	20.8	5431503	13,212	19.6	8675264



Changes in prevalence over the survey period

Abb - Abbott assay; Euroimmun - Euroimmun assay