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High prevalence of SARS-CoV-2 antibodies in pregnant women, after the second wave of infections in the inner city of Johannesburg, Gauteng Province, South Africa

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Abstract

Objectives

After South Africa's second wave of COVID-19, this study estimated the SARS-CoV-2 seroprevalence among pregnant women in inner-city Johannesburg, South Africa.

Methods

In this cross-sectional survey, 500 non-COVID-19-vaccinated pregnant women (aged ≥12 years) were enrolled, and demographic and clinical data were collected. Serum samples were tested using the Wantai SARS-CoV-2 spike Ab ELISA and Roche Elecsys® Anti-SARS-CoV-2 nucleocapsid antibody assays. Seropositivity was defined as SARS-CoV-2 antibodies on either (primary) or both (secondary) assays. Univariate Poisson regression assessed risk factors associated with seropositivity.

Results

Median age was 27.4 years and HIV prevalence 26.7%. SARS-CoV-2 seroprevalence was 64.0% (95% CI:59.6%–68.2%) on the primary and 54% (95%CI:49.5-58.4) on the secondary measure. Most (96.6%) SARS-CoV-2-seropositive women reported no symptoms. On the Roche assay, we detected lower seroprevalence among women living with HIV (WLHIV) compared to HIV uninfected women (48.9% vs 61.7%, p=0.018), and especially low levels among WLHIV with a CD4<350 cells/ml compared to women without immune suppression (22.2% vs 56.4%, prevalence rate ratio=0.4; 95%CI:0.2–0.9; p=0.046).

Conclusions

Pregnant women attending routine antenatal care had a high SARS-CoV-2 seroprevalence after the 2nd wave in South Africa, with most having had asymptomatic infections.

Seroprevalence surveys in pregnant women present a feasible method of monitoring the course of the pandemic over time.

Keywords: SARS-CoV-2 seroprevalence; Serosurveys; Antenatal; HIV; South Africa; COVID-19; pregnant women

Introduction

In South Africa, by the end of the second COVID-19 wave in early February 2021, driven largely by the Beta variant (501Y.V2), almost 1.5 million COVID-19 cases and 47,000 deaths were recorded (NICD, 2021). By end September 2021, as South Africa exited the third wave, dominated by the Delta variant, just over 2.9 million diagnosed cases and more than 87,000 COVID-19 related deaths were reported (NDoH, 2021). Of these, more than one-third of cases and a quarter of deaths were from the Gauteng Province, the most densely populated province in South Africa, with the City of Johannesburg accounting for almost one third of diagnosed cases (NDoH, 2021, NICD, 2021). However, there remain major gaps in knowledge about the epidemiological parameters of SARS-CoV-2 in South Africa and case ascertainment through reverse transcription-polymerase chain reaction (RT-PCR)-based case identification may substantially underestimate the extent of infections. Once-off and serial seroprevalence studies may address this gap as they may be a more accurate indicator of population-level attack rate than diagnostic testing and could contribute to more accurate estimates of population immunity acquired by natural infection or through vaccination.

Sentinel surveillance of infectious diseases among pregnant women has previously been used as an indicator of disease burden at a population level (Fairlie et al., 2020). Repeated serosurveys among pregnant women are used extensively to track the HIV epidemic in sub-Saharan Africa and elsewhere, and to derive population-based HIV prevalence estimates (Collaborators, 2017, Eaton et al., 2014, Sangal et al., 2018). Sentinel serosurveillance among pregnant women could potentially serve as a proxy for SARS-CoV-2 disease burden

in the broader community (Fairlie et al., 2020). Findings could be characterised by HIV status age, and other potential risk factors for infection. Surveys of pregnant women offer advantages over other methods such as household surveys, which are logistically challenging, costly, often have high refusal rates, may have a low yield, and are unable to give local estimates unless sample sizes are considerable (Gutreuter et al., 2019, Kirakoya-Samadoulougou et al., 2016, Marsh et al., 2011). Between the second and third waves of the COVID-19 pandemic in South Africa, this study aimed to estimate the SARS-CoV-2 seroprevalence among pregnant women in urban antenatal clinics in inner-city Johannesburg, Gauteng by conducting a cross-sectional serological survey. As a secondary objective, we also evaluated the performance of the Wantai SARS-CoV-2 Ab ELISA assay compared to the Roche Elecsys® Anti-SARS-CoV-2 assay for antibody detection.

Methods

Study setting

This cross-sectional seroprevalence survey was conducted in Hillbrow, a densely populated inner-city neighbourhood of Johannesburg with large numbers of both documented and undocumented migrants characterised by overcrowded high-rise buildings, large numbers of both documented and undocumented migrants, high levels of unemployment, high prevalence of TB and diseases linked to poor water quality and sanitation (Frith, 2011, Rees et al., 2017). The HIV prevalence among pregnant women is estimated at just over 30% in the City of Johannesburg (Woldesenbet et al., 2019). Two antenatal care facilities based in the inner city of Johannesburg, were included in the study: Shandukani Midwife Obstetric

Unit (MOU) and Esselen Street Clinic. These clinics are about 500m apart and provide antenatal services to the same community, majority of whom reside in the Hillbrow area.

Participants

Pregnant women attending antenatal care aged ≥12 years were eligible for inclusion in the study if they had no acute illness and had not previously participated in a COVID-19 vaccine trial. Field staff approached women individually in the order that they arrived at the clinic to assess each woman's willingness and eligibility to participate in the study. Women who refused participation were replaced by the next woman that came to the clinic. Willing and eligible women completed informed consent procedures and were enrolled over a 12-week period from 17 March to 9 June 2021, until the target sample size was achieved. .

Data Sources

Participants completed a brief interviewer-administered questionnaire covering basic demographic and health characteristics, a COVID-19 symptom screen, and history of prior SARS-CoV-2 infection. Any suspected cases of COVID-19 were referred for SARS-CoV-2 testing according to Department of Health guidelines. Participants were also asked whether they had received a vaccine for COVID-19, and to provide details of such vaccination. In South Africa, only health care workers were offered vaccinations from the 17th February 2021. Subsequently, vaccinations were introduced to the general population using an age-based phased approach starting with persons over the age of 60 years on the 17th May 2021.

Data on HIV and syphilis status, parity, gravidity, gestational age, and suburb of residence, collected as part of standard of care, were extracted directly from the patient-held

antenatal clinic card. HIV viral loads (VL) and CD4+ T cell counts for women living with HIV (WLHIV) were extracted from the National Health Laboratory Service LabTrak platform. A VL of ≥100 copies/ml was categorised as elevated, while a CD4+ T-cell count of <350 counts/ml was categorised as immune suppressed.

Serum samples were collected and sent to the National Institute for Communicable Diseases (NICD) for testing. Samples were tested in parallel using two different enzyme-linked immunosorbent assays (ELISAs), to increase the positive predictive value given the expected prevalence of this virus at the time of conducting the study (Amanat et al., 2020), namely, the Wantai SARS-CoV-2 Ab ELISA (Beijing Wantai Biological Pharmacy Enterprise Co. Ltd, Beijing, China) and the Roche Elecsys® Anti-SARS-CoV-2 (Roche Diagnostics, Rotkreuz, Switzerland) serology test kit. The former measures total antibodies (IgM, IgG and IgA) against the receptor binding domain (RBD) in the S1 subunit of the spike protein (anti-S) (FDA, 2020). The latter, however, detects total antibodies (IgM, IgG and IgA) against SARS-CoV-2 using a recombinant nucleocapsid (N) protein (anti-N) (Roche, 2020).

Sample size

The sample size was calculated for a 95% confidence interval, and an estimated 30% SARS-CoV-2 seropositivity rate. The 95% confidence interval for a sample of 500 women under these assumptions was 26-34%.

Statistical analysis

Descriptive statistics were used to characterise the study population. For continuous data with a non-normal distribution, medians and inter-quartile ranges (IQR) were computed. For categorical data, proportions with 95% confidence intervals (95% CI) were computed and

differences between groups were evaluated using either a χ^2 test or prevalence rate ratios with associated 95% CI. SARS-CoV-2 seropositivity was determined in two ways: 1) detection of SARS-CoV-2 antibodies on *either* Wantai or Roche Elecsys assay (primary measure); 2) detection of SARS-CoV-2 antibodies on *both* Wantai and Roche Elecsys assays (secondary measure). The proportion of women with SARS-CoV-2 antibody detection (as described above) and associated 95% CI were computed, stratified by HIV and immune status. As a secondary objective of this study, the Roche Elecsys assay was used as the reference to calculate the sensitivity, specificity, positive predictive value, and negative predictive value with associated 95% CI for the Wantai assay. While, both assays have previously shown good performance with regards to sensitivity, the Roche Elecsys anti-N assay showed a higher specificity and intraclass correlation coefficient, and thereby less variability, when compared to the Wantai assay in an evaluation of commercially available high-throughput SARS-CoV-2 serologic assays (Stone et al., 2022). In addition, the Roche Elecsys assay has shown better performance in another South African study where the Roche assay did not detect antibodies in 3% of samples tested while the Wantai assay did not detect antibodies in 9% (Wolter et al., 2022).

A Cohen's Kappa for interrater agreement between the two assays was computed (Byrt, 1996). The epidemic curve was derived from weekly incident COVID-19 cases reported to the NICD, for the City of Johannesburg's Region F, where the two study sites are situated.

Univariate Poisson regression, with robust standard errors, was used to assess the association of demographic and clinical factors with SARS-CoV-2 seropositivity. Results are presented as prevalence rate ratios (PRR) through exponentiation of coefficients with 95%

confidence intervals (95% CI). The same methods were used to assess factors associated with willingness to be vaccinated. For multivariable analyses, independent variables with a p-value ≤0.25 in univariate analyses were considered for inclusion in the model (Hosmer and Lemeshow, 2000). All data were captured directly onto the REDCap (Research Electronic Data Capture) data platform (Harris et al., 2009), hosted at the University of the Witwatersrand in South Africa. Analyses were performed using Stata Version 15.1 (Stata Corp, College Station, TX, USA).

Results

Participant characteristics and SARS-CoV-2 history

During the study period, 950 pregnant women were screened, 904 were eligible, 402 declined participation, 502 (55.6%) were enrolled, and 2 women were subsequently excluded from the study; no parent or legal guardian available to sign consent for a minor's participation and a duplicate enrolment (Figure 1). The median age of participants was 27.4 years (IQR: 23.6 – 32.1). The study sample comprised 50.6% South Africans and 46.2% Zimbabwean foreign nationals. More than half (52.4%) of the women were in their third trimester of pregnancy with a median gestational age of 27 weeks (IQR:21-34) at the time of enrolment. The prevalence of HIV among women enrolled was 26.7 (Table 1).

Since March 2020, 19 (3.8%) women reported symptoms suggestive of SARS-CoV-2 infection. Of these women, 11 (57.9%) were tested for SARS-CoV-2 at the time of those symptoms and two reported that they tested RT-PCR positive (18.2%). A further 50 women who did not report any COVID-19 related symptoms previously had a SARS-CoV-2 diagnostic

test, 4 (8.0%) of whom tested SARS-CoV-2 RT-PCR positive. No participants had been vaccinated prior to study participation.

Seroprevalence of SARS-CoV-2

SARS-CoV-2 seroprevalence on the primary measure using detection on *either* the Wantai or the Roche Elecsys assay, was 64.0% (95% CI: 59.6 – 68.2%) while seroprevalence on the secondary measure using detection of antibodies on *both* the Wantai and the Roche Elecsys assay was 54% (95% CI: 49.5 – 58.4%) (Table 2). The prevalence of SARS-CoV-2 antibodies among pregnant women was similar for the Wantai (59.6%; 95% CI: 55.2 – 63.9%) and Roche Elecsys (58.4%; 95% CI: 53.9 – 64.0%) assays, independently (Table 2). However, of the 208 women who were seronegative on the Roche Elecsys assay, 28 (13.5%) were seropositive on the Wantai assay; while of the 292 women who were seropositive on the Roche Elecsys assay, 22 (7.5%) were seronegative on the Wantai assay. The overall interrater agreement between the 2 tests was 90% with a Cohen's κ=0.79; indicating good interrater agreement (Byrt, 1996). Using the Roche Elecsys assay as the reference test, the Wantai assay's performance was high; with a sensitivity of 92.5% (95% CI: 88.8-95.2%), specificity of 86.5% (95% CI: 81.1-90.9%), positive predictive value of 90.6% (95% CI: 86.7-93.7%), and negative predictive value of 89.1% (95% CI: 84.0-93.0%) (Table 3). Among WLHIV compared to HIV uninfected women, sensitivity and negative predictive values were higher, while specificity and positive predictive values were lower (Table 3).

Of the 496 women with a known HIV status, 131 were living with HIV. Of these, 76 (58.0%) were seropositive using the primary measure and 61 (46.6%) using the secondary measure of seropositivity. Women who were HIV uninfected were significantly more likely to be

seropositive on the Roche Elecsys assay compared to WLHIV (61.7% and 48.9%, respectively; p=0.018). On the Wantai assay, 55.7% of HIV uninfected and 61.1% of WLHIV were seropositive (p=0.298). For the 57 WLHIV who had available CD4+ cell counts, those with a low count (<350 count/ml) were significantly less likely to be seropositive on the Roche Elecsys assay (prevalence rate ratio (PRR)=0.4; 95%CI: 0.2 – 0.9, p=0.046) compared to women with higher CD4+ counts. Of the 18 WLHIV with low CD4+ cell counts, 4 were seropositive on both assays, while an additional 3 were positive on the Wantai assay (Table 2). All 6 women who were SARS-CoV-2 positive on a previous RT-PCR test, were also seropositive, with the time since SARS-CoV-2 RT-PCR positivity ranging from 3 to 13 months (Table 2).

Figure 2 depicts the proportion of SARS-CoV-2 seropositive pregnant women per epidemiologic week at the two study sites, plotted against the backdrop of the SARS-CoV-2 epidemic curve in Johannesburg's Region F. Peaks and troughs are evident at similar time points for both clinics. However, a divergence in the seroprevalence was noted in the last week of the study possibly due to the small number of participants enrolled at that time. Seroprevalence was highest in April and May 2021 (65.9% and 64.4%, respectively) (Table 4), which coincided with the start of the third wave of infections in South Africa, characterised by the highly transmissible Delta variant.

Factors associated with seropositivity

In univariate analyses, we observed no clinical or demographic factors that were significantly associated with SARS-CoV-2 detection when using both the primary measure of

seropositivity (antibody detection on *either* the Wantai or the Roche assay) and the secondary measure (antibody detection on *both* the Wantai and the Roche assay) (Table 4).

Two candidate variables were eligible for inclusion in the multivariable model: study site and age categories. However, neither of these weakly associated variables became significant predictors of the outcome when assessed together in a multivariable model. In a separate multivariable model restricted to HIV infected women, based on the literature, we also included indicators for HIV viral load elevation and CD4 suppression as variables of clinical importance. Again, none of the variables assessed became significant predictors of the outcome when taken together in the multivariable model. Results from these multivariable analyses are not reported.

Only 3.4% (11/320) of the women who were seropositive, reported ever having symptoms suggestive of COVID infection.

Willingness to vaccinate

Only 296 (59.4%) women reported being willing to be vaccinated should vaccines become available, while the rest (40.6%) reported being unsure or unwilling to be vaccinated. More women (174/250, 69.6%) at the Esselen Street Clinic were willing to receive a COVID-19 vaccine than at Shandukani MOU (122/250, 48.8%, p<0.001) (Supplementary Table 1).

Discussion

Between March and June 2021, we enrolled 500 pregnant women attending two high-volume antenatal clinics in a densely populated inner-city area of Johannesburg with an antenatal HIV seroprevalence of about 30%. A high proportion, 64.0%, of pregnant women

had evidence of prior SARS-CoV-2 infection. Overall, 96.6% of seropositive women did not recall having any symptoms of SARS-CoV-2 infection between March 2020 and the time of enrolment.

This serosurvey was conducted between South Africa's second and third waves. Given that IgG antibodies most commonly become detectable at 1-3 weeks post-infection (Sethuraman et al., 2020), it is highly likely that the seroprevalence detected in this study is due to the cumulative infections that occurred in the first and second waves, with a small proportion likely attributable to newer infections experienced at the start of the third wave. Previous seroprevalence surveys in pregnant women in South Africa have reported rates of between 30.8% in Gauteng and 38% in the Western Cape after the first wave of infections (George et al., 2021, Hsiao et al., 2020). A study among blood donors in South Africa, conducted after the peak of the second wave, reported seroprevalences ranging from 32% to 63% among four provinces (Sykes et al., 2021). A cross-sectional study conducted in three communities in South Africa, during and after the second wave of infection, reported an increase in seropositivity from 26.9% in December 2020 to 47.2% in April 2021 (Wolter et al., 2022). These findings across all studies, do not take into consideration the potential for waning antibody levels or seroreversion over time (Meyer, 2021, Peluso et al., 2021). This sequential increase in the seroprevalence with subsequent waves of infection and high seroprevalence gives credence to the fact that South Africa has experienced a more pervasive epidemic than estimated when using laboratory-based RT-PCR or antigen-diagnosed cases who are mostly symptomatic (Hsiao et al., 2020), resulting in a substantial underestimate of the proportion of the population previously infected. Repeated seroprevalence studies are likely a more accurate indicator of infection prevalence than RT-

PCR/antigen-based case identification. Serial seroprevalence studies of pregnant women are easily implementable and could be built into routine blood testing during antenatal clinic visits as pregnant women have regular scheduled clinical appointments. These studies among pregnant women as a sentinel population will allow for timely, efficient, low-cost determination of prevalence of immunity, changes in immunity over time, durability of immune protection, vaccine uptake and vaccine penetrance over time.

In WLHIV, Hsaio *et al* (Hsiao et al., 2020) reported 42% seroprevalence after the peak of the first wave of the epidemic among women attending antenatal clinics in Cape Town Metropolitan (Metro) sub-districts. As expected, we found a higher seroprevalence of 58.0% among WLHIV in Johannesburg after two waves of infection. WLHIV with a low CD4 cell count (<350 count/ml) were less likely to be seropositive on the Roche Elecsys assay (PRR=0.39; 95%CI: 0.16 – 0.98) compared to women with higher CD4 counts, although this analysis was restricted to only 57 of the 131 WLHIV in whom CD4 cell counts were available. In addition to the four seropositive cases detected on both assays, the Wantai assay detected an additional three seropositive cases among WLHIV with a low CD4 cell count, most likely due to the Wantai assay measuring anti-S antibodies. Individuals living with HIV that are immunocompromised have been shown to be less likely to develop a SARS-CoV-2 antibody response (Meiring et al., 2022, Wolter et al., 2022), as well as have lower IgG concentrations and pseudovirus neutralising antibody titres than HIV-uninfected persons (Spinelli et al., 2021, Wolter et al., 2022). In addition, anti-N antibodies have been shown to wane faster than anti-S antibodies post infection (Choudhary et al., 2021, Lumley et al., 2021). Therefore, immunocompromised WLHIV were less likely to be seropositive, and were more likely to test positive on the anti-S assay than the anti-N assay. Using a combination of

the Roche Elecsys and Wantai assays in this study, produced a higher yield of SARS-CoV-2 seroprevalence. Nevertheless, it is possible that serology may underestimate previous infection among WLHIV, in particular those with advanced immunosuppression.

Studies evaluating clinical course and pregnancy outcomes in women infected with SARS-CoV-2 during pregnancy showed high rates of Intensive Care Unit admission, Caesarean section delivery, higher maternal mortality, and pre-term delivery (Budhram et al., 2021, Carrasco et al., 2021, Della Gatta et al., 2020). In our study we were unable to ascertain when SARS-CoV-2 infection occurred, except in those who reported a positive SARS-CoV-2 antigen test. It is reassuring that in our study population many women have some expected level of immunity while they are pregnant because of prior natural infection. Previous SARS-CoV-2 infection is expected to have some level of protection against repeat SARS-CoV-2 infection (although this may not hold for newer variants of concern). Similarly, one COVID-19 vaccine with a prior infection, results in robust immunity, comparable to two vaccines (Bertollini et al., 2021, Keeton et al., 2021, Shrestha et al., 2021).

During the study period, only 56% of pregnant women reported willingness to be vaccinated against COVID-19. Apart from the observed difference between clinic sites, we found no other demographic or clinical factors associated with willingness to be vaccinated. This study did not investigate reasons for vaccine hesitancy. However, other studies of SARS-CoV-2 vaccine hesitancy, conducted prior to vaccine availability to the public in South Africa, report variable levels of survey respondents indicating willingness to accept COVID-19 vaccination, ranging from 52% to 82% (Cooper et al., 2021, Skjefte et al., 2021). Factors potentially associated with willingness to be vaccinated included older age, urbanicity,

geographic location and female sex (Cooper et al., 2021, Skjefte et al., 2021). A common theme in these studies, was concern about COVID-19 vaccine safety and effectiveness, highlighting the need for communication and education strategies specific for populations of interest to encourage vaccination (Cooper et al., 2021). Willingness to vaccinate among women in this study, may have also been influenced by the lack of guidelines for vaccinations in pregnant women at the time. In end August 2021, after completion of our study, the South African Department of Health released a circular, recommending all pregnant and lactating women get vaccinated with either of the available vaccines.

Limitations

This study had several limitations. We experienced a high refusal rate among women attending routine antenatal visits resulting in a possible selection bias in women who enrolled, though very few enrolled women reported symptoms or prior positive SARS-CoV-2 tests. The study was conducted at two antenatal facilities within a limited geographical area, thus potentially limiting generalisability to other urban environments. The cross-sectional design of the survey leads to uncertainty around the timing of prior SARS-CoV-2 infection, potential recall bias of symptoms experienced up to one-year prior to study enrolment and the potential for an underestimation of SARS-CoV-2 infection due to waning antibody levels among women who were infected in the 1st wave. It is possible that the proportion of women experiencing symptomatic SARS-CoV-2 infection was underestimated, though another study conducted in South Africa at a similar time also reported low proportions (3.4%) of seropositive persons with symptoms (Wolter et al., 2022). This study only detected binding antibodies; we did not determine whether these were neutralizing antibodies with the ability to neutralize the SARS-CoV-2 virus. Lastly, we did not collect information on co-

morbidities and risk factors for SARS-CoV-2 disease such as diabetes, obesity, and socio-economic status which have been found to be associated with SARS-CoV-2 seropositivity in other studies in South Africa (George et al., 2021, Wolter et al., 2022) Given that almost 70% of the population of Johannesburg is overweight or obese (Nordisk, 2016), and many have a low socio-economic status (Rees et al., 2017), we postulate that we may have seen similar associations between these risk factors and seropositivity .

Conclusions

This easily accessible population of pregnant women attending routine antenatal care visits had a high SARS-CoV-2 seroprevalence, with most women experiencing asymptomatic disease. WLHIV and those with immune suppression were less likely to have anti-N antibodies and therefore less likely to have anti-N antibodies detected on the Roche assay. Seroprevalence surveys could present a feasible, low-cost method of monitoring the course of the pandemic, vaccine uptake and vaccine hesitancy in a population and should ideally be conducted after each wave of infection.

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Conflict of Interest

CC has received grant support from Sanofi Pasteur, Advanced Vaccine Initiative, and payment of travel costs from Parexel. NW and AvG have received grant support from Sanofi Pasteur and the Bill and Melinda Gates Foundation.

Ethical Approval Statement

Permission to conduct this study was obtained from the University of the Witwatersrand's Human Research Ethics Committee (no: 200913) and the Gauteng Province Department of Health. Informed consent (signed by participants ≥18 years or parent/legal guardian for minors) and assent for minors (< 18 years) was completed for each participant.

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Figure Captions

Figure 1. Flow diagram of the number of pregnant women screened, eligible and included in the study

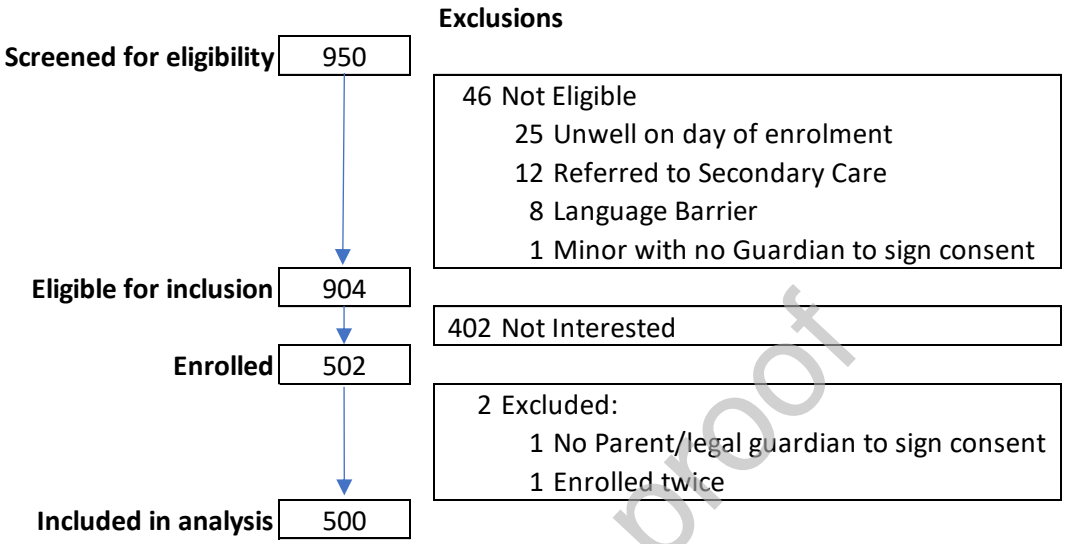
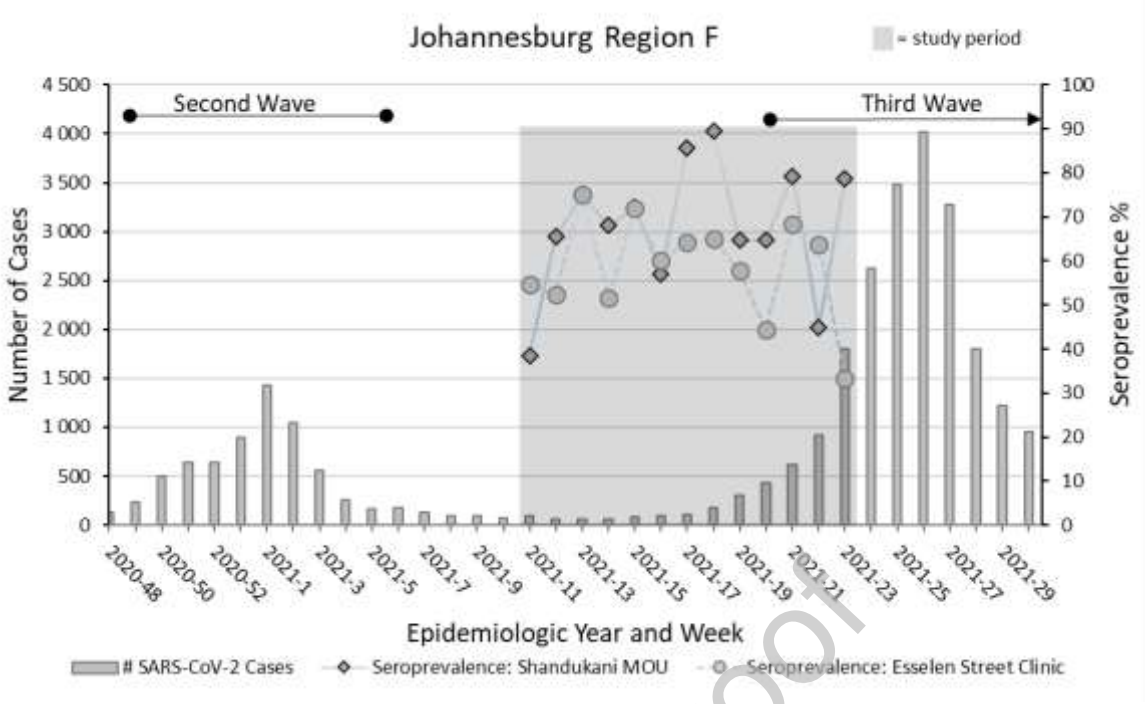


Figure 2. Proportion of SARS-CoV-2 seropositive pregnant women per epidemiologic week at Shandukani MOU and the Esselen Street Clinic over the study period (17 March 2021 to 9 June 2021), plotted against the backdrop of the SARS-CoV-2 epidemic curve in Johannesburg's Region F in South Africa between 22 November 2020 and 01 August 2021



Enrolments per week	2021-11	2021-12	2021-13	2021-14	2021-15	2021-16	2021-17	2021-18	2021-19	2021-20	2021-21	2021-22	2021-23
Shandukani Clinic	13	29	12	25	18	21	21	19	17	17	24	20	14
Esselen Clinic	11	21	24	31	25	15	14	20	26	27	19	11	6
Total	24	50	36	56	43	36	35	39	43	44	43	31	20