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About the Author

Dr. van Gemert is an epidemiologist and postdoctoral research fellow in the Vanuatu Health Program, Port Vila, Vanuatu, and with the Burnet Institute, Melbourne, Victoria, Australia. Her primary research interest is surveillance of infectious disease in resource-poor settings.

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Address for correspondence: Caroline van Gemert, The Burnet Institute, 85 Commercial Rd, Melbourne, VIC 3004, Australia; email: caroline.vangemert@burnet.edu.au

SARS-CoV-2 Seroprevalence after Third Wave of Infections, South Africa

Jackie Kleynhans, Stefano Tempia, Nicole Wolter, Anne von Gottberg, Jinal N. Bhiman, Amelia Buys, Jocelyn Moyes, Meredith L. McMorrow, Kathleen Kahn, F. Xavier Gómez-Olivé, Stephen Tollman, Neil A. Martinson, Floidy Wafawanaka, Limakatso Lebina, Jacques D. du Toit, Waasila Jassat, Mzimasi Neti, Marieke Brauer, Cheryl Cohen, for the PHIRST-C Group[1]

Author affiliations: National Institute for Communicable Diseases of the National Health Laboratory Service, Johannesburg, South Africa (J. Kleynhans, S. Tempia, N. Wolter, A. von Gottberg, J.N. Bhiman, A. Buys, J. Moyes, W. Jassat, M. Neti, C. Cohen); University of the Witwatersrand, Johannesburg (J. Kleynhans, S. Tempia, N. Wolter, A. von Gottberg, J.N. Bhiman, J. Moyes, C. Cohen); US Centers for Disease Control and Prevention, Atlanta, Georgia, USA (S. Tempia, M.L. McMorrow); US Centers for Disease Control and Prevention, Pretoria, South Africa (M.L. McMorrow); MRC/Wits Rural Public Health and Health Transitions Research Unit (Agincourt), University of the Witwatersrand, Johannesburg (K. Kahn, F.X. Gómez-Olivé, S. Tollman, F. Wafawanaka, J. du Toit); Johns Hopkins University Center for Tuberculosis Research, Baltimore, Maryland, USA (N.A. Martinson); Perinatal HIV Research Unit, University of the Witwatersrand, Johannesburg (N.A. Martinson, L. Lebina); Africa Health Research Institute, Durban, South Africa (L. Lebina); Ampath Pathology, Pretoria (M. Brauer)

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By November 2021, after the third wave of severe acute respiratory syndrome coronavirus 2 infections in South Africa, seroprevalence was 60% in a rural community and 70% in an urban community. High seroprevalence before the Omicron variant emerged may have contributed to reduced illness severity observed in the fourth wave.

South Africa has experienced 4 waves of severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) infections, the fourth dominated by the Omicron variant of concern (*1*). Data on the proportion of the population with serologic evidence of previous infection at the time of Omicron emergence are important to contextualize the observed rapid increases and subsequent quick decline in case numbers (*1*), as well as the lower severity compared with previous variants (*2*).

[1]Additional members of the PHIRST-C group who contributed to this article are listed at the end of this article.

We previously described the seroprevalence of SARS-CoV-2 in the PHIRST-C (Prospective Household Study of SARSCoV-2, Influenza, and Respiratory Syncytial Virus Community Burden, Transmission Dynamics, and Viral Interaction) cohort in a rural and an urban community at 5 timepoints during July 2020–March 2021 (*3*). By using the same methods (Appendix, https://wwwnc.cdc.gov/EID/article/28/5/22-0278-App1.pdf), we report seroprevalence at 4 additional timepoints through November 27, 2021, spanning the third, Delta-dominated wave (Appendix Figure 1), ending the week Omicron was identified (*4*). We tested serum samples by using the Roche Elecsys Anti-SARS-CoV-2 assay (Roche Diagnostics, https://www.roche.com); we considered a cutoff index ≥1.0 an indication of prior infection. The immunoassay detects nucleocapsid (N) antibodies; thus, it does not detect postvaccination antibody responses. We obtained seroprevalence 95% credible intervals (CrIs) by using Bayesian inference with 10,000 posterior draws (*5*). We estimated the age- and sex-adjusted number of infections and age-adjusted diagnosed cases, hospitalizations, deaths, case-to-infection ratio (CIR), hospitalization-to-infection ratio (HIR), and in-hospital and excess death fatality-to-infection ratio (FIR), as described previously (*3*) (Appendix). Third-wave infections were defined as participants who had a paired blood draw (BD) from the fifth timepoint of the previous study (BD5) (collected March 22–April 11, 2021) and from the ninth timepoint of this study (BD9) (collected November 15–27, 2021) and who were seronegative at BD5 and seropositive at BD9 or seropositive at BD5 but had a ≥2-fold higher cutoff index in BD9 (because 38 possible reinfections occurred after BD5 [Appendix]). We obtained vaccination status through reviewing vaccine cards that participants kept at home. The study was approved by the University of the Witwatersrand Human Research Ethics Committee (reference no. 150808); the US Centers for Disease Control and Prevention relied on local clearance (IRB approval no. 6840).

Overall, pre–third wave (BD5) SARS-CoV-2 seroprevalence adjusted for assay sensitivity and specificity was 26% (95% CrI 22%–29%) in the rural and 41% (95% CrI 37%–45%) in the urban community. After the third wave (BD9), overall seroprevalence increased to 60% (95% CrI 56%–64%) in the rural community and 70% (95% CrI 66%–74%) in the urban community (Figure; Appendix Table 1). In both communities, the largest increase in seroprevalence was seen in children 13–18 years of age, who also had the highest seroprevalence of all ages after the third wave: 80% (95% CrI 70%–88%) in the rural community (a 49% increase) and 83% (95% CrI 73%–90%) in the urban community (a 19% increase).

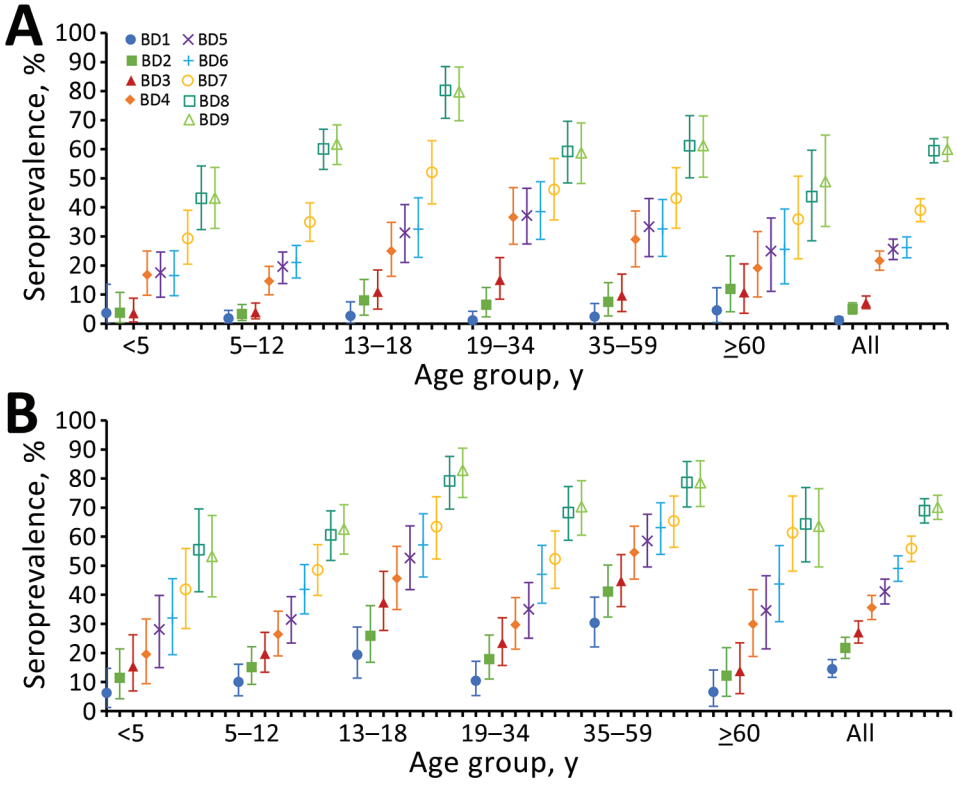

Figure. Severe acute respiratory syndrome coronavirus 2 seroprevalence at each blood collection, by age group, in a rural community (A) and urban community (B), South Africa, March 2020–November 2021. Baseline blood draw (BD1) collected July 20–September 17, 2020; second draw (BD2), September 21 – October 10, 2020; third draw (BD3), November 23–December 12, 2020; fourth draw (BD4), January 25–February 20, 2021; fifth draw (BD5), March 22–April 11, 2021; sixth draw (BD6), May 20–June 9, 2021; seventh draw (BD7), July 19–August 5, 2021; eighth draw (BD8), September 13–25, 2021; ninth draw (BD9), November 15–27, 2021. Error bars represent 95% credible intervals. Seroprevalence estimates adjusted for sensitivity and specificity of assay.

During the third wave of infections, the incidence at the rural site was 39% (95% CrI 24%–55%), resulting in a CIR of 3% (95% CI 2%–5%). HIR was 0.5% (95% CI 0.3%–0.7%) and in-hospital FIR was 0.1% (95% CI 0.1%–0.2%); excess deaths FIR was 0.5% (95% CI 0.4%–0.8%) (Figure; Appendix Figure 2).

In the urban community, the incidence during the third wave was 40% (95% CrI 26%–54%). CIR was a 5% (95% CI 4%–8%), and HIR was 2% (95% CI 2%–4%). In-hospital FIR was 0.4% (95% CI 0.3%–0.6%) and excess deaths FIR was 0.6% (95% CI 0.4%–0.9%) (Figure; Appendix Figure 2).

HIR and FIR were similar between wave 2 and 3 (Appendix Figure 3). SARS-CoV-2 vaccines became available in South Africa in February 2021, after the second wave. By the end of wave 3, only 8% (49/609) of participants were fully vaccinated (1 dose of Johnson & Johnson/Janssen or 2 doses of Pfizer-BioNTech) in the rural community and 19% (97/512) in the urban community (Appendix Table 2). Considering the overall low vaccination coverage in these communities during the study period, the similar HIR and FIR in wave 2 and 3 were likely driven by a combination of natural immunity and potentially a moderate effect attributable to vaccination.

Taken together, by the end of November 2021, just before the emergence of Omicron, the combined proportion of persons who had serologic evidence of previous infection (at any timepoint), were fully vaccinated, or both was 62% (389/631) at the rural community and 72% (411/568) at the urban community (Appendix Table 3).

After the third wave of infections in South Africa, we observed a ≥60% overall seroprevalence attributable to SARS-CoV-2 infection, ranging from 43% in rural community children <5 years of age to 83% in urban community children 13–18 years of age (Figure). CIR, HIR, and FIRs were similar between the second and third waves. Similar to our data, results from a study in Gauteng Province found seroprevalence of 56%–80% attributable to natural infection before the emergence of Omicron (*6*). The high seroprevalence before Omicron emergence may have contributed to reduced illness severity observed in the fourth wave (*2*).

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The investigators welcome enquiries about possible collaborations and requests for access to the dataset. Data will be shared after approval of a proposal and with a signed data access agreement. Investigators interested in more details about this study, or in accessing these resources, should contact the corresponding author.

About the Author

Ms. Kleynhans is an epidemiologist in the Centre for Respiratory Diseases and Meningitis, National Institute for Communicable Diseases. Her primary research interests include the epidemiology of respiratory diseases like influenza and COVID-19, vaccine impact studies, and modelling of infectious disease transmission dynamics.

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Address for correspondence: Jackie Kleynhans, Centre for Respiratory Diseases and Meningitis, National Institute for Communicable Diseases of the National Health Laboratory Service, 1 Modderfontein Rd, Sandringham, 2192, Johannesburg, South Africa; email: jackiel@nicd.ac.za

Angiostrongylus cantonensis in a Red Ruffed Lemur at a Zoo, Louisiana, USA

Jessica Rizor, Ryan A. Yanez, Tuddow Thaiwong, Matti Kiupel

Authors affiliation: Michigan State University Veterinary Diagnostic Laboratory, Lansing, Michigan, USA

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A red ruffed lemur (*Varecia rubra*) from a zoo in Louisiana, USA, was euthanized for worsening paresis. Brain and spinal cord histology identified eosinophilic meningoencephalomyelitis with intralesional adult *Angiostrongylus* sp. nematodes. PCR and sequencing confirmed *A. cantonensis* infection, indicating this parasite constitutes an emerging zoonosis in the southeastern United States.

Angiostrongylus cantonensis is a parasitic metastrongyloid nematode that has a neurotropic larval stage and is endemic throughout Southeast Asia and the Pacific Islands. The rat (*Rattus* spp.) is the main definitive host and a variety of gastropods serve as intermediate hosts. In rats, infections cause no brain damage and only some pulmonary disease in severe infections. However, in aberrant hosts, including humans and nonhuman primates, larvae cause severe eosinophilic meningoencephalitis. Clinical signs are associated with migration of the larvae and the immune response to dead or dying nematodes (*1*).

In 1987, *A. cantonensis* nematodes were detected in rats in New Orleans, Louisiana, USA (*2*); in 1995, a human case of eosinophilic meningitis was reported in North America in a child from New Orleans (*3*). *A. cantonensis* nematodes have now become endemic in the southeastern United States, as evidenced by reports of infection in a child in Texas (*4*); a horse from Mississippi (*5*); captive Geoffroy's tamarins (*Saguinus geoffroyi*) in Alabama (*6*); and several animals in Florida, including a white-handed gibbon (*Hylobates lar*), an orangutan (*Pongo pygmaeus*), a white-throated capuchin monkey (*Cebus capucinus*), a red ruffed lemur (*Varecia rubra*), and a nine-banded armadillo (*Dasypus novemcinctus*) (*7*,*8*). Ingestion of infected gastropods and paratenic hosts or unwashed contaminated vegetables are proposed routes of infection for aberrant hosts.

The International Union for Conservation of Nature lists red ruffed lemurs (*Varecia rubra*) as critically endangered (*9*). In June 2021, a 9-year-old male red ruffed lemur from a zoo in Louisiana was humanely euthanized because of hind limb paresis and a right head tilt that worsened over an 8-day period. The lemur was housed in a troop of 5 adult lemurs in an outdoor exhibit. Various snail species are common in the enclosure, but no other lemurs were clinically affected.

A necropsy performed at the Michigan State University Veterinary Diagnostic Laboratory (Lansing, Michigan, USA) identified no gross lesions. The laboratory formalin-fixed and processed the brain, the entire spinal cord, and all major organs for histopathology. Histopathologic examination revealed multiple transverse and longitudinal sections of adult nematodes within the subarachnoid space and neuropil of the cerebellum and brainstem. Nematodes were ≈50–70 μm in diameter and had a 3–4-μm thick smooth, eosinophilic cuticle and prominent lateral cords. Adult nematodes had coelomyarian musculature, and the pseudocoelom contained a reproductive tract and an intestinal tract lined by multinucleated cells with flocculent eosinophilic to brown material in the lumen (Figure). Nematodes were surrounded by hemorrhage and small numbers of eosinophils, neutrophils, macrophages, and glial cells. Several cerebellar folia were effaced by invading nematodes, hemorrhage, and inflammation. The cerebellar meninges were expanded by numerous eosinophils, fewer neutrophils, foamy macrophages, multinucleated giant cells, and lymphocytes. A representative section of thoracic spinal cord contained an identical single adult nematode in the subdural space. Another adult nematode had regionally effaced the dorsal horn in a section of lumbar spinal cord. The affected spinal cord had regional rarefaction of both gray and white

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SARS-CoV-2 Seroprevalence after Third Wave of Infections, South Africa

Appendix

Supplementary Methods

Study Population

PHIRST-C (A Prospective Household study of SARS-CoV-2, Influenza, and Respiratory Syncytial virus community burden, Transmission dynamics and viral interaction in South Africa) is a continuation from PHIRST (A Prospective Household observational cohort study of Influenza, Respiratory Syncytial virus and other respiratory pathogens community burden and Transmission dynamics in South Africa) (*1*). PHIRST-C is conducted in two communities with established influenza-like illness and pneumonia surveillance sites.

South Africa consists of nine provinces, which are divided into 52 districts, which form the second level of administration. Districts are further divided into municipalities. The rural site is located in the Bushbuckridge Municipality, Ehlanzeni District, Mpumalanga Province and forms part of a health and sociodemographic surveillance site (HDSS) at the Medical Research Council (MRC)/University of Witwatersrand Rural Public Health and Health Transitions Research Unit, Agincourt. During PHIRST, two of the 29 villages within the HDSS were selected according to convenience (proximity and burden of other studies within the site) each year between 2016–2018. A random selection of ≈50 households with a household size of 3 or more were approached for enrolment. The urban site is located in the Jouberton Township, Matlosana Municipality, Dr Kenneth Kaunda District, North West Province. A list of 450 global positioning system (GPS) coordinates were generated using Google Earth. Study staff approached the nearest house within 30 m of the coordinate point for enrolment. Approximately 50 households were enrolled each year during 2016–2018. At both sites, households were eligible if they consisted of 3 or more household members (sharing at least four meals a week).

All household members were approached for inclusion in the study, and households were eligible for inclusion if >80% of members consented to be enrolled.

For PHIRST-C, all households who participated in PHIRST from the urban site, and households from the 2017 and 2018 cohort in the rural site were approached for enrolment. To supplement the sample size, additional households at each site were approached using the same methods as for PHIRST. Villages in the rural site were restricted to those four used in 2017 and 2018 of PHIRST. Informed consent was obtained from participating adults or a parent/guardian for children <18 years of age. In addition to parent/guardian consent, assent was also obtained from children aged 7–17 years.

We collected baseline data and blood (blood draw [BD] 1) at enrollment (July 20–September 17, 2020) and every 2 months thereafter: BD2, September 21–October 10; BD3, November 23–December 12, 2020; BD4, January 25–February 20, 2021; BD5, March 22–April 11, 2021; BD6, May 20 – June 9, 2021; BD7, July 19 – August 5, 2021; BD8, September 13 – 25, 2021; BD9, November 15 – 27, 2021.

The Bushbuckridge Municipality within the Ehlanzeni District is considered as predominantly rural, with main industries being agriculture and tourism (*2*). The Ehlanzeni District has a population of 1,828,738, with sixty percent of the district's population being >18 years of age. The City of Matlosana Municipality is considered to be 88.2% urban, and is located in the Dr Kenneth Kaunda District, which has a population of 797,715 (*3*). Sixty-five percent of the district population is aged >18 years.

Calculation of Case-to-infection Ratio (CIR), Hospitalization-to-infection Ratio (HIR), and Fatality-to-infection Ratio (FIR) by Wave of Infection

We calculated the age- and sex-adjusted total number of infections, age-adjusted diagnosed cases, hospitalizations, deaths, CIR (number of infections compared to diagnosed cases), HIR and in-hospital and excess death FIR as described in below equations. We defined the third wave as March 22, 2021 (week 12) to November 14, 2021 (week 45), starting with BD5, and ending the week before BD9 started. Due to differences between the sex ratios of our cohort and the district population, the infection estimates were also adjusted for sex. Data sources used in these calculations are described in the next section. Age- and sex- standardized estimates for the selected endpoints for each wave were obtained as follows:

$$Infections = \frac{\sum_i (s_i \times SAp_i)}{\sum_i (SAp_i)} \times 100{,}000$$

Where s_i is the seroprevalence in the cohort in the respective community for age and sex group i and SAp_i is the South African population for age and sex group i. Calculated for wave 3 as the number of individuals seronegative at BD5 and seropositive at BD9. Furthermore, we also included possible re-infections where the individual was already seropositive at BD5, but had a ≥2-fold higher cutoff index (COI) in BD9 compared to BD5. We therefore included infections and re-infections that occurred during the third wave. Estimates only included participants with a blood draw 5 and 9 pair, and adjusted for sensitivity and specificity of test (*4*).

The ≥2-fold increase in COI cutoff to define possible re-infections was not externally validated, but based on PCR-confirmed infections from twice-weekly nasal swab collections between March 22, 2021 (BD5) to July 19, 2021 (BD7) in the same cohort (C. Cohen et al., unpub. data, https://doi.org/10.1101/2021.07.20.21260855). Increases in COI from BD5 to BD7 ranged from 1 to 93 fold. Of the 30 individuals who had a rRT-PCR-confirmed infection between BD5 and BD7, 16 (53%, sensitivity) had a ≥2-fold increase from BD5 to BD7, and of the 312 individuals that did not have a rRT-PCR infection, 301 (96%, specificity) did not have a ≥2-fold increase. We were therefore more likely to have underestimated re-infections.

$$Diagnosed\ cases = \frac{\sum_i \left((c_i \div p_{i(d)}) \times p_{i(SA)} \right)}{\sum_i \left(p_{i(SA)} \right)} \times 100{,}000$$

Where c_i is the number of diagnosed cases (RT-PCR and antigen-based tests) from the respective district reported to the NMCSS during wave 3 (March 22, to November 14, 2021) in age group i, $p_{i(d)}$ is the district population for age group i and $p_{i(SA)}$ is the South African population for age group i.

$$Hospitalizations = \frac{\sum_i \left((h_i \div p_{i(d)}) \times p_{i(SA)} \right)}{\sum_i \left(p_{i(SA)} \right)} \times 100{,}000$$

Where h_i is the number of hospitalizations from the respective district reported to COVID-19 Sentinel Hospital Surveillance (DATCOV) during wave 3 (March 22, to November 14, 2021) (*5*) in age group i, $p_{i(d)}$ is the district population for age group i and $p_{i(SA)}$ is the South African population for age group i.

$$In-hospital\ deaths = \frac{\sum_i \left((d_i \div p_{i(d)}) \times p_{i(SA)} \right)}{\sum_i \left(p_{i(SA)} \right)} \times 100{,}000$$

Where d_i is the number of in-hospital deaths from the respective districts reported to DATCOV during wave 3 (March 22, to November 14, 2021) (*5*) in age group *i*, $p_{i(d)}$ is the district population for age group *i* and $p_{i(SA)}$ is the South African population for age group *i*.

$$Excess\ deaths = \frac{(ExD\ x\ 0.85)\ \times\ p_{(SA)}}{p_{(SA)}} \times 100{,}000$$

Where *ExD* is the rate of provincial excess deaths adjusted to the South African population reported by South African Medical Research Council during wave 3 (March 22, to November 14, 2021) (*6*), and $p_{(SA)}$ is the total South African population. According to estimates only 85% of excess deaths are attributable to COVID-19 (*6*).

For infections, hospitalizations, in-hospital (minimum) and excess (maximum) deaths, 95% CIs were calculated using the Clopper-Pearson method with a Poisson distribution.

$$Case-to-infection\ ratio\ (CIR) = \frac{Diagnosed\ cases}{Infections}\ x\ 100$$

$$Hospitalization-to-infection\ ratio\ (HIR) = \frac{Hospitalizations}{Infections}\ x\ 100$$

$$Fatality\ -to-infection\ ratio\ (FIR) = \frac{Deaths*}{Infections}\ x\ 100$$

Confidence intervals for infection ratios were calculated as ratios from the 95% confidence intervals of infection, hospitalization and death rates.

Data Sources

Population Denominators (*7*)

Population numbers for each district, by age, were obtained from the StatsSA 2021 mid-year population estimates.

Notifiable Medical Conditions Surveillance System (NMCSS) (*8*)

Reverse transcription PCR (RT-PCR) testing in South Africa to detect SARS-CoV-2 RNA started on 28 January 2020. Rapid SARS-CoV-2 antigen testing was implemented in

November 2020. All laboratories in the private and public sector performing SARS-CoV-2 tests automatically feed testing data from their lab information systems to the NMCSS, from where daily and weekly reporting on national, provincial and district SARS-CoV-2 infections are performed. No serology tests are used for this reporting. Results from antigen tests may be underestimated in the NMCSS as not all antigen test results are captured on lab information systems which feed into NMCSS. The number of cases reported to the NMCSS in the Ehlanzeni District (rural community) and Dr Kenneth Kaunda District (urban community) was used as an estimate of reported, diagnosed SARS-CoV-2 infections.

COVID-19 National Hospital Surveillance (DATCOV) (*5*)

DATCOV is a national hospital surveillance system to which all hospitals where COVID-19 admissions occurred in the public and private sector in South Africa report. The case definition includes any person admitted with a positive RT-PCR test result for SARS-CoV-2; and may include individuals for whom the main cause of hospitalization was not SARS-CoV-2. In-hospital outcome was available for all patients. The total number of hospitalizations and in-hospital deaths in each district reported to DATCOV between March 22, to November 14, 2021 was used to calculate in the HIR and minimum FIR, respectively.

South African Medical Research Council (SAMRC) Report on Weekly Deaths (*6*)

The Burden of Disease Unit at the SAMRC produces a weekly report on excess deaths in South Africa. Deaths in South Africa are registered on the Department of Home Affair's National Population Register and includes all citizens with a South African identification number. The number of excess deaths were defined as the number of all-cause deaths in that week minus the number of deaths expected for the week based on 2014–2019 trends. Excess death estimates are adjusted for incomplete reporting, and death estimates in provinces are age-standardized to the national population. Eighty-five percent of the reported excess deaths were used to calculate the maximum FIR as suggested by SAMRC.

Seroreversions

Seven individuals were seronegative at BD5, and with at least one seropositive result during BD6 and BD8, and who were seronegative again at BD9. The maximum COIs for these individuals were low, ranging from 1.09 to 34.5. This fits with an initial low antibody response and subsequent antibody titer waning. In addition, there were five individuals who were

seropositive at BD5, whose antibody titers waned below detection during BD6 to BD8, but who were seropositive again at BD9. Two of these individuals had a lower COI in BD9 than in BD5, and were not included as possible re-infections during wave 3.

Limitations

Our study is limited by a small sample size, reducing the power for accurate seroprevalence estimates in small age strata, and inclusion of only 2 geographic sites, including only households with ≥3 people, with high unemployment in selected households compared to the community unemployment rate (*9*) and therefore may not be representative of other districts and provinces in South Africa. Our definition of re-infections has not been validated elsewhere and is based on a small number of infections that occurred between BD5 and BD7, and did not consider different variants. Based on data from the same cohort, these reinfections occurred in only a small portion (3%) of the cohort (C. Cohen et al., unpub. data, https://doi.org/10.1101/2021.07.20.21260855) and would have had a negligible influence on the infection ratios. CIR, HIR, and FIR formed part of an ecologic analysis, which is inherently prone to biases. Transmission dynamics within our cohort may not be similar to the transmission dynamics within the district. Twenty percent (260/1,277) of persons did not have a BD 5+9 pair, and bias could have been introduced if the seroprevalence were different for those without a BD 5+9 blood pair. Since we did not have complete serologic results for draws between BD5 and BD9 for all individuals with a BD 5+9 pair, we did not consider individuals who may have seroreverted during wave 3 as infections or re-infections. This may have led to an underestimation of infections, and overestimation of the CIR, HIR and FIR.

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Appendix Table 1. Individuals seropositive for SARS-CoV-2 antibodies with adjusted seroprevalence and 95% credible intervals by blood draw, site and age, July 2020 – November 2021, South Africa*

Site	Age group, y	Seropositive/Tested (Seroprevalence*, 95% CrI)								
		BD1	BD2	BD3	BD4	BD5	BD6	BD7	BD8	BD9
Rural	<5	0/25 (4, 0–14)	1/49 (4, 0–14)	2/78 (4, 1–9)	14/87 (17, 10–25)	15/89 (18, 10–26)	14/88 (17, 10–25)	26/90 (29, 20–39)	33/77 (43, 32–54)	36/84 (43, 33–54)
	5–12	2/148 (2, 0–5)	5/169 (3, 0–5)	7/195 (4, 2–7)	29/202 (15, 10–20)	39/201 (20, 15–25)	42/202 (21, 16–27)	70/202 (35, 28–42)	120/200 (60, 53–67)	122/198 (62, 55–68)
	13–18	1/69 (3, 0–7)	5/72 (8, 0–7)	8/80 (11, 5–18)	20/82 (25, 16–35)	24/78 (31, 22–41)	24/75 (32, 23–43)	40/77 (52, 41–63)	59/73 (80, 71–88)	57/71 (80, 70–88)
	19–34	0/87 (1, 0–4)	5/88 (7, 0–4)	13/91 (15, 8–23)	33/91 (37, 27–47)	35/95 (37, 28–47)	34/89 (39, 29–49)	40/87 (46, 36–57)	48/81 (59, 48–70)	47/80 (59, 48–69)
	35–59	1/76 (2, 0–7)	5/77 (8, 0–7)	7/80 (10, 4–17)	23/81 (29, 20–39)	27/82 (33, 24–44)	27/84 (33, 23–43)	35/82 (43, 33–54)	49/80 (61, 50–72)	49/80 (61, 50–71)
	≥60	1/40 (5, 0–12)	4/39 (12, 0–12)	4/44 (11, 4–21)	8/45 (19, 9–32)	10/42 (25, 14–39)	10/41 (26, 14–39)	14/40 (36, 22–51)	16/37 (44, 28–60)	18/37 (49, 33–65)
	All	5/445 (1, 0–2)	25/494 (5, 0–2)	41/568 (7, 5–9)	127/588 (22, 18–25)	150/587 (26, 22–29)	151/579 (26, 23–30)	225/578 (39, 35–43)	325/548 (59, 55–64)	329/550 (60, 56–64)
Urban	<5	2/45 (6, 1–15)	5/50 (11, 4–21)	7/50 (15, 7–26)	8/44 (20, 9–32)	13/48 (28, 16–41)	14/45 (32, 19–46)	19/46 (42, 28–56)	25/45 (56, 41–70)	24/45 (53, 39–67)
	5–12	11/116 (10, 5–16)	17/116 (15, 9–22)	24/125 (20, 13–27)	32/123 (26, 19–34)	38/122 (32, 24–40)	52/125 (42, 33–50)	60/124 (49, 40–57)	75/124 (61, 52–69)	77/123 (63, 54–71)
	13–18	14/75 (19, 11–29)	19/75 (26, 17–36)	30/81 (37, 28–48)	35/77 (46, 35–57)	41/78 (53, 42–64)	44/77 (57, 46–68)	47/74 (63, 52–74)	59/74 (79, 69–88)	60/72 (83, 73–90)
	19–34	10/102 (10, 5–17)	17/98 (18, 11–26)	23/100 (23, 16–32)	29/99 (30, 21–39)	34/98 (35, 26–45)	44/94 (47, 37–57)	50/96 (52, 42–62)	65/95 (68, 59–77)	62/88 (70, 61–79)
	35–59	33/110 (30, 22–39)	45/110 (41, 32–50)	52/117 (45, 36–54)	61/112 (55, 45–64)	65/111 (59, 49–67)	70/111 (63, 54–72)	70/107 (65, 56–74)	82/104 (79, 70–86)	82/104 (79, 70–86)
	≥60	3/57 (7, 2–14)	6/55 (12, 5–22)	7/56 (14, 6–23)	16/55 (30, 19–42)	18/53 (35, 23–48)	23/53 (44, 31–57)	32/52 (61, 48–74)	33/51 (64, 51–77)	30/47 (64, 50–76)
	All	73/505 (14, 12–18)	109/504 (22, 18–25)	143/529 (27, 23–31)	181/510 (36, 31–40)	209/510 (41, 37–45)	247/505 (49, 45–53)	278/499 (56, 51–60)	339/493 (69, 65–73)	335/479 (70, 66–74)

*Adjusted for assay sensitivity and specificity. Blood draw (BD) 1, July 20–September 17, 2020; BD2, September 21–October 10, 2020; BD3, November 23–December 12, 2020; BD4, January 25–February 20, 2021; and BD5, March 22–April 11, 2021; BD6, May 20 – June 9, 2021; BD7, July 19 – August 5, 2021; BD8, September 13 – 25, 2021; BD9, November 15 – 27, 2021.

Appendix Table 2. Number and percentage of PHIRST-C participants with known vaccination status (N) vaccinated for SARS-CoV-2 by BD9 (November 15 – 27, 2021) in the rural and urban community, South Africa*

		Rural, No. (%)				Urban, No. (%)		
Age group, y	N	Not vaccinated	Partially vaccinated	Fully vaccinated	N	Not vaccinated	Partially vaccinated	Fully vaccinated
<5	93	93 (100)	0 (0)	0 (0)	49	49 (100)	0 (0)	0 (0)
5–12	205	205 (100)	0 (0)	0 (0)	125	125 (100)	0 (0)	0 (0)
13–18	83	82 (99)	1 (1)	0 (0)	78	72 (92)	3 (4)	3 (4)
19–34	101	91 (90)	2 (2)	8 (8)	96	72 (75)	8 (8)	16 (17)
35–59	85	51 (60)	9 (11)	25 (29)	111	59 (53)	5 (5)	47 (42)
≥60	42	16 (38)	10 (24)	16 (38)	53	16 (30)	6 (11)	31 (58)
All	609	538 (88)	22 (4)	49 (8)	512	393 (77)	22 (4)	97 (19)

*Partially vaccinated: one dose of Pfizer-BioNTech; Fully vaccinated: one dose of Johnson & Johnson or two doses of Pfizer-BioNTech; at time of blood collection

Appendix Table 3. Number and percentage of PHIRST-C participants with either serologic evidence of previous infection (at any draw 20 July, 2020 - November 27, 2021) or fully vaccinated by BD9 (November 15 – 27, 2021) or both in the rural and urban community, South Africa*

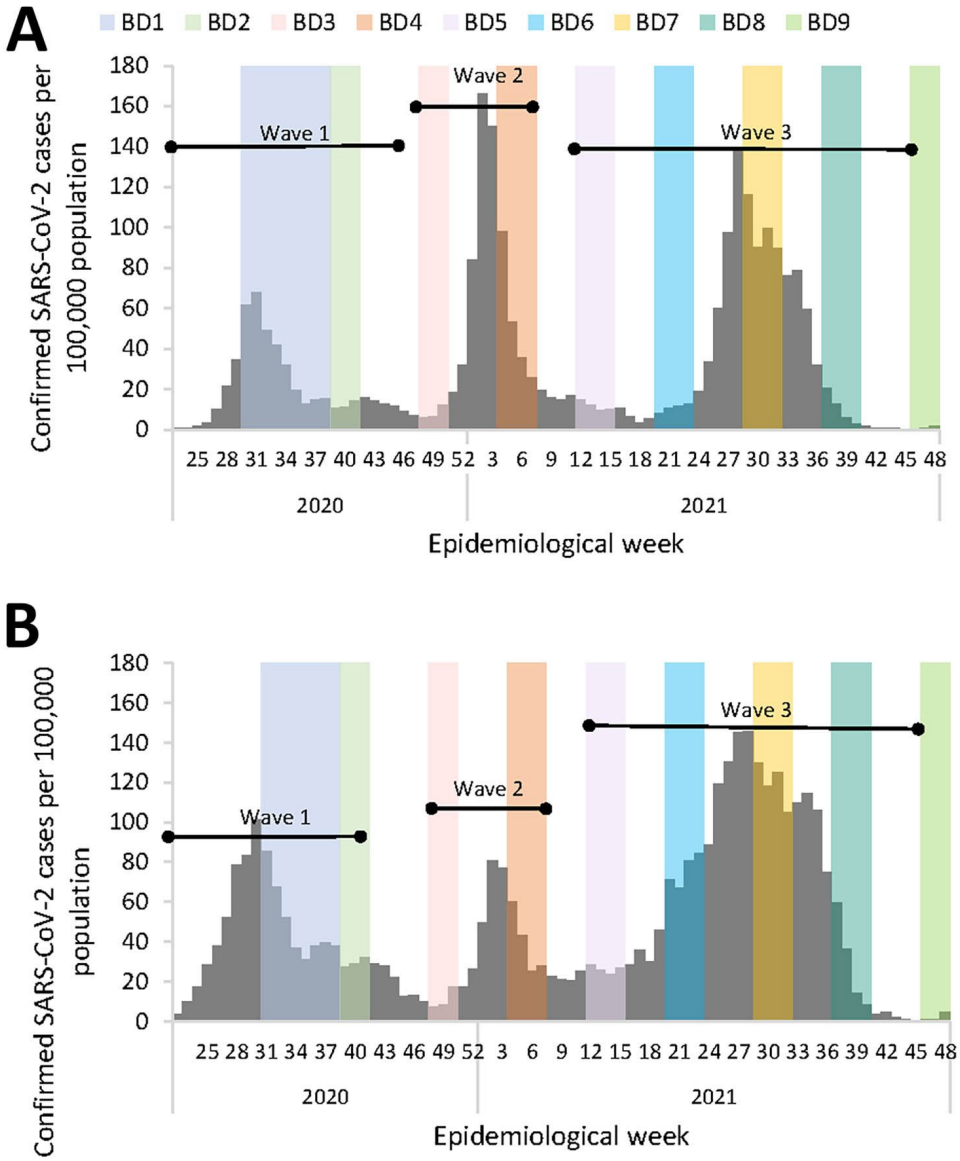
	n/N (%)	
Age group, y	Rural	Urban
<5	45/95 (47)	29/55 (53)
5–12	130/208 (63)	82/132 (62)
13–18	62/85 (73)	68/82 (83)
19–34	59/107 (55)	79/119 (66)
35–59	66/90 (73)	106/122 (87)
≥60	27/46 (59)	47/58 (81)
All	389/631 (62)	411/568 (72)

*Fully vaccinated: one dose of Johnson & Johnson or two doses of Pfizer-BioNTech; at time of blood collection

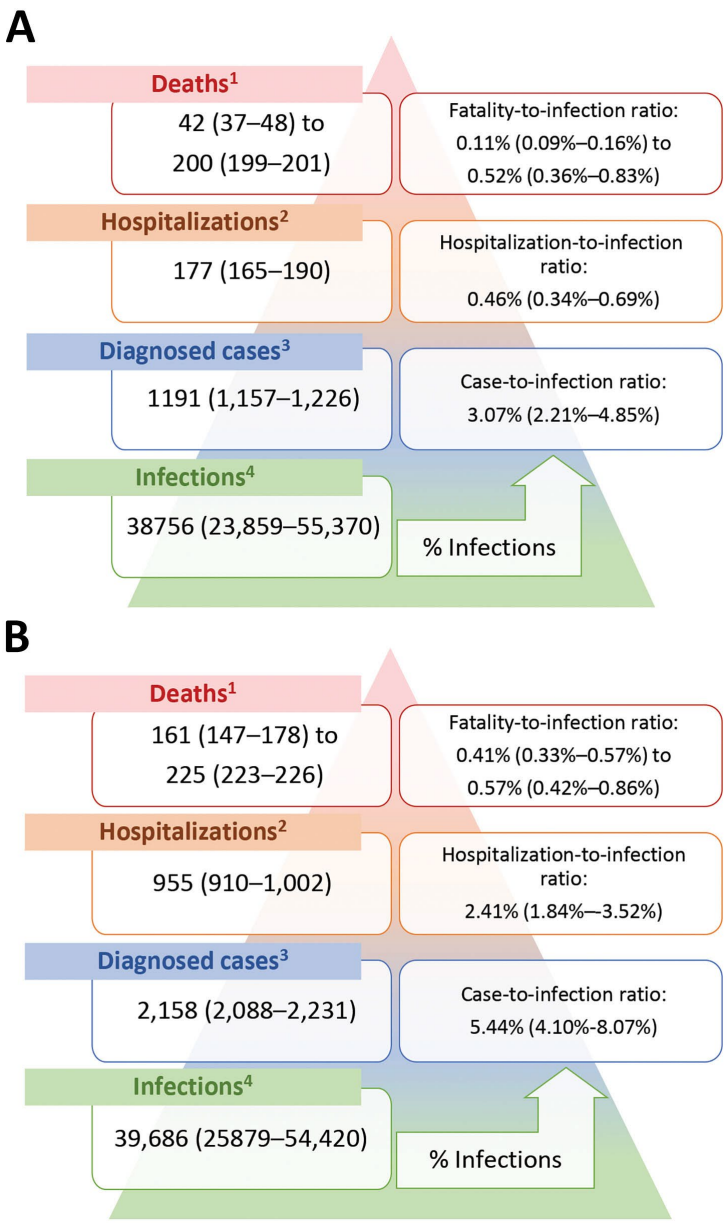
Appendix Table 4. The PHIRST-C Group

Name	Affiliation
Amelia Buys	Centre for Respiratory Diseases and Meningitis, National Institute for Communicable Diseases of the National Health Laboratory Service, Johannesburg, South Africa.
Anne von Gottberg	Centre for Respiratory Diseases and Meningitis, National Institute for Communicable Diseases of the National Health Laboratory Service, Johannesburg, South Africa. School of Pathology, Faculty of Health Sciences, University of the Witwatersrand, Johannesburg, South Africa.
Cheryl Cohen	Centre for Respiratory Diseases and Meningitis, National Institute for Communicable Diseases of the National Health Laboratory Service, Johannesburg, South Africa. School of Public Health, Faculty of Health Sciences, University of the Witwatersrand, Johannesburg, South Africa.
F. Xavier Gómez-Olivé	MRC/Wits Rural Public Health and Health Transitions Research Unit (Agincourt), Faculty of Health Sciences, School of Public Health, University of the Witwatersrand, Johannesburg, South Africa.
Floidy Wafawanaka	MRC/Wits Rural Public Health and Health Transitions Research Unit (Agincourt), Faculty of Health Sciences, School of Public Health, University of the Witwatersrand, Johannesburg, South Africa.
Jackie Kleynhans	Centre for Respiratory Diseases and Meningitis, National Institute for Communicable Diseases of the National Health Laboratory Service, Johannesburg, South Africa. School of Public Health, Faculty of Health Sciences, University of the Witwatersrand, Johannesburg, South Africa.
Jacques D. du Toit	MRC/Wits Rural Public Health and Health Transitions Research Unit (Agincourt), Faculty of Health Sciences, School of Public Health, University of the Witwatersrand, Johannesburg, South Africa.
Jinal N. Bhiman	Centre for Respiratory Diseases and Meningitis, National Institute for Communicable Diseases of the National Health Laboratory Service, Johannesburg, South Africa. School of Pathology, Faculty of Health Sciences, University of the Witwatersrand, Johannesburg, South Africa.
Jocelyn Moyes	Centre for Respiratory Diseases and Meningitis, National Institute for Communicable Diseases of the National Health Laboratory Service, Johannesburg, South Africa. School of Public Health, Faculty of Health Sciences, University of the Witwatersrand, Johannesburg, South Africa.
Kathleen Kahn	MRC/Wits Rural Public Health and Health Transitions Research Unit (Agincourt), Faculty of Health Sciences, School of Public Health, University of the Witwatersrand, Johannesburg, South Africa.
Kgaugelo Patricia Kgasago	Perinatal HIV Research Unit (PHRU), University of the Witwatersrand, Johannesburg, South Africa.
Limakatso Lebina	Perinatal HIV Research Unit (PHRU), University of the Witwatersrand, Johannesburg, South Africa. Africa Health Research Institute, Durban, South Africa. Africa Health Research Institute, KwaZulu-Natal, South Africa.
Linda de Gouveia	Centre for Respiratory Diseases and Meningitis, National Institute for Communicable Diseases of the National Health Laboratory Service, Johannesburg, South Africa.

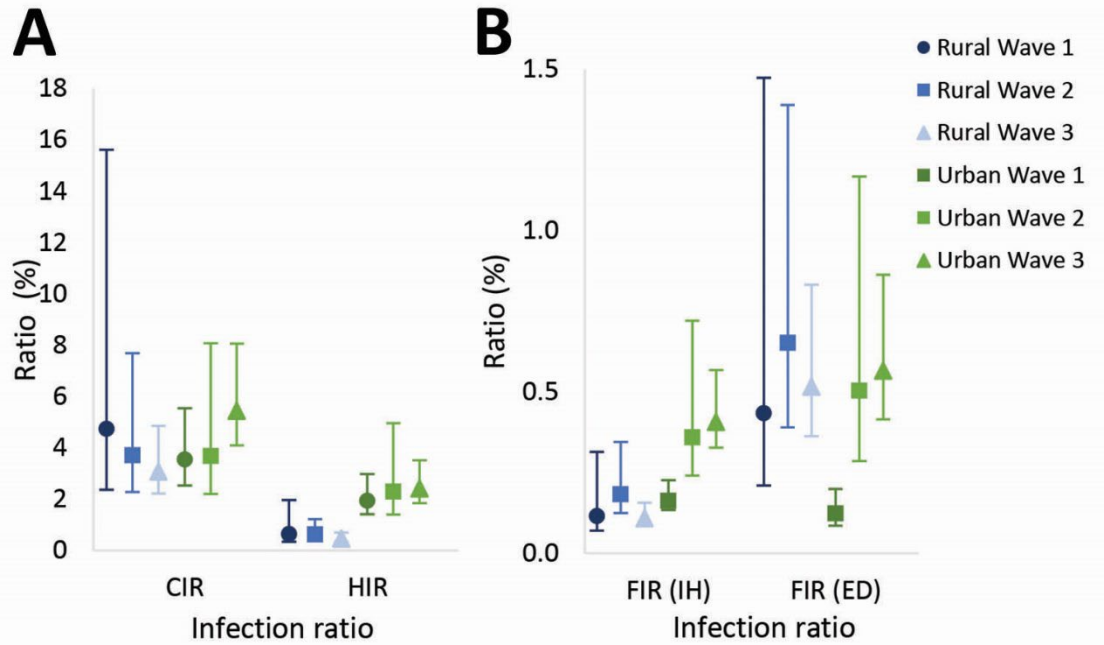
Name	Affiliation
Maimuna Carrim	Centre for Respiratory Diseases and Meningitis, National Institute for Communicable Diseases of the National Health Laboratory Service, Johannesburg, South Africa. School of Pathology, Faculty of Health Sciences, University of the Witwatersrand, Johannesburg, South Africa.
Meredith L. McMorrow	Influenza Division, Centers for Disease Control and Prevention, Atlanta, Georgia, United States of America. Influenza Program, Centers for Disease Control and Prevention, Pretoria, South Africa.
Mignon du Plessis	Centre for Respiratory Diseases and Meningitis, National Institute for Communicable Diseases of the National Health Laboratory Service, Johannesburg, South Africa. School of Pathology, Faculty of Health Sciences, University of the Witwatersrand, Johannesburg, South Africa.
Neil A. Martinson	Perinatal HIV Research Unit (PHRU), University of the Witwatersrand, Johannesburg, South Africa. Johns Hopkins University Center for TB Research, Baltimore, Maryland, United States of America.
Nicole Wolter	Centre for Respiratory Diseases and Meningitis, National Institute for Communicable Diseases of the National Health Laboratory Service, Johannesburg, South Africa. School of Pathology, Faculty of Health Sciences, University of the Witwatersrand, Johannesburg, South Africa.
Retshidisitswe Kotane	Centre for Respiratory Diseases and Meningitis, National Institute for Communicable Diseases of the National Health Laboratory Service, Johannesburg, South Africa. School of Pathology, Faculty of Health Sciences, University of the Witwatersrand, Johannesburg, South Africa.
Stefano Tempia	Influenza Division, Centers for Disease Control and Prevention, Atlanta, Georgia, United States of America. Influenza Program, Centers for Disease Control and Prevention, Pretoria, South Africa. School of Public Health, Faculty of Health Sciences, University of the Witwatersrand, Johannesburg, South Africa. MassGenics, Atlanta, Georgia, United States of America.
Stephen Tollman	MRC/Wits Rural Public Health and Health Transitions Research Unit (Agincourt), School of Public Health, Faculty of Health Science, University of the Witwatersrand.
Tumelo Moloantoa	Perinatal HIV Research Unit (PHRU), University of the Witwatersrand, Johannesburg, South Africa.



Appendix Figure 1. Timing of blood collection and district SARS-CoV-2 weekly incidence in a) rural community and b) urban community, March 2020 – November 2021, South Africa. November 23 – December 12, 2020; fourth draw (BD4) January, 25 – February 20, 2021; fifth draw (BD5) March 22 – April 11, 2021; sixth draw (BD6) May 20 – June 9, 2021; seventh draw (BD7) July 19 – August 5, 2021; eighth draw (BD8) September 13 – 25, 2021; ninth draw (BD9) November 15 – 27, 2021. Vertical lines represent 95% credible interval. Wave 1 (March 1 – November 21, 2020), 2 (November 22, 2020 – March 21, 2021) and 3 (March 22 – November 14, 2021) lines indicate period used for analysis. Laboratory-confirmed SARS-CoV-2 infections (reverse transcription polymerase chain reaction and antigen tests) as reported to the Notifiable Medical Conditions Surveillance System (NMCSS).



Appendix Figure 2. South African age-standardized SARS-CoV-2 infection, diagnosis, hospitalization and deaths per 100,000 population during wave 3 in the a) rural and b) urban community, March – November, 2021, South Africa. Standardized to South Africa mid-year population estimate for 2021. Wave 3: March 22 – November 14, 2021. [1]Minimum estimate: in-hospitalization deaths in districts based on COVID-19 Sentinel Hospital Surveillance report (DATCOV), maximum estimate: provincial excess deaths reported (South African Medical Research Council). [2]Hospitalizations in the district based on COVID-19 Sentinel Hospital Surveillance (DATCOV). [3]DiagnosedSARS-CoV-2 cases reported from districts from national SARS-CoV-2 surveillance (NMCSS). [1]Based on becoming seropositive or 2-fold increase in cutoff index (COI) between draw 5 and 9. [1]Values in bracket refer to 95% credible interval for infections and 95% confidence interval for all other estimates.



Appendix Figure 3. SARS-CoV-2 a) case-to-infection (CIR), hospitalization-to-infection (HIR) and b) in-hospital (IH) fatality-to-infection (FIR) and excess (ED) fatality-to-infection (FIR) ratios in a rural and urban community during the first, second and third wave of infections, March 2020 - November 2021, South Africa.