Natural immunity and protection against variants in South African children through five COVID-19 waves: A prospective study

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ABSTRACT

Objectives: Children have been largely spared from serious disease through the COVID-19 pandemic despite a high exposure to SARS-CoV-2. Antibody responses to exposure and their role in protecting children from subsequent variant infection remain poorly understood.

Methods: This is a prospective cohort study of children in a South African community through ancestral/Beta/Delta/Omicron BA.1/BA.2 and BA.4/BA.5 SARS-CoV-2 waves (March 2020-October 2022). Health seeking behavior/illness was recorded and postwave serum samples measured for immunoglobulin (Ig) G to spike (S) (CoV2-S-IgG) by electrochemiluminescent immunosorbent assay. To estimate the protective CoV2-S-IgG threshold levels, logistic functions were fit to describe the correlation of CoV2-S-IgG measured before a wave and the probability for seroconversion/boosting thereafter.

Results: Despite little disease, 125 per 366 (34.2%) children (median age 6.7 years [interquartile range 5.99-7.4 years]) were seropositive after wave I, rising to 53.6%, 76.0%, and 96.2% and 99.2% after waves II (Beta), III (Delta), and IV and V (Omicron variants), respectively. CoV2-S-IgG induced by natural exposure protected against subsequent SARS-CoV-2 infection, with the greatest protection for Beta and least for Omicron. The levels of IgG specific for ancestral S antigen that provided a 50% protective threshold for the subsequent wave were lowest for the Beta and highest for the Omicron BA.1/BA.2 wave. In the multivariate analysis, maternal seropositivity (adjusted odds ratio = 2.57 [95% confidence interval: 1.72-3.82]) was strongly associated with child seropositivity.

Conclusion: Children responded robustly to successive waves of SARS-CoV-2, mounting IgG responses to S antigen that were protective against subsequent waves. In the absence of vaccination, almost all children were seropositive after wave V but none were hospitalized, suggesting that natural immunity alone may be sufficient to protect children in a pandemic setting.

Introduction

At the beginning of the SARS-CoV-2 pandemic, reports from China showed that children presented with milder symptoms than adults infected by SARS-CoV-2 [1,2]. Subsequent research in several different high prevalence settings and through different waves due to SARS-CoV-2 variants has confirmed that children often remain asymptomatic or develop mild disease [3–5]. The reasons for this have not been established, but several theories related to immunological differences between children and adults have been proposed. For example, Loske et al. showed that children had significantly higher basal expression of critical pattern recognition receptors in their airway epithelium and highly increased amounts of innate immune cells in their upper airways than adults and thus

have enhanced viral sensing and prevention of infection [6]. Other explanations have invoked immune system differences in thymic function, cross-reactive immunity to common coronaviruses, and differences in expression of the viral entry receptor angiotensin-converting enzyme 2 [7].

With exposure to each wave of the pandemic, differences have emerged between waves. For example, symptomatic pediatric clinical disease has been shown to vary by SARS-CoV-2 variant, with infection with Omicron associated with more severe croup and occurrence in older children; disease severity generally decreased through waves IV and V [8,9]. However current evidence indicates that, overall, most SARS-CoV-2 infections in children are asymptomatic, even in children residing in low- and middle-income countries (LMICs) [10] where respiratory viral infections are usually associated with more severe disease than children in high-income settings.

Relatively little is understood of natural immunity and the relationship between antibodies induced after exposure to SARS-CoV-2 and subsequent protection from infection/disease. We previously described development of natural and hybrid immunity in a cohort of South African mothers [11]. South Africa has experienced five well-defined SARS-CoV-2 waves of infection; the first was driven by the ancestral (Wuhan) strain, the second was dominated (>95%) by the Beta variant (B.1.351), the third was due to the Delta variant, and the fourth and fifth waves were due to the Omicron variants [12]. We showed that SARS-CoV-2 anti-spike (S) immunoglobulin (Ig) G induced by natural exposure protected mothers against subsequent SARS-CoV-2 infection, with the greatest protection for Beta and the least for Omicron [11]. Little is understood about the impact of natural infection on immunity to SARS-CoV-2 in children. Although natural infection with ancestral SARS-CoV-2 virus provides partial protection against re-infection with the same and closely related SARS-CoV-2 variants in adults [7,13], infection with Omicron, antigenically the most distant of the variants of concern to the ancestral wild type strain [14], has been associated with higher rates of re-infection [15,16]. In the current study, we extended our work to longitudinally investigate antibody protection in matched children through the five waves and the association with maternal infection. We investigated infection, illness, and serological responses to natural exposure to SARS-CoV-2 variants to derive estimates of levels of S-specific IgG associated with protection from subsequent infection after natural immunity and compared the thresholds of protection in children and mothers.

Methods

Children and matched mothers were followed up in an established South African birth cohort, the Drakenstein Child Health Study (DCHS) [17], through the COVID-19 pandemic from March 6, 2020 to October 4, 2022, spanning five waves. A convenience sample of sequential child participants (and their matched mothers) attending follow-up visits with blood sampling through all waves of the pandemic was studied. Serological responses to SARS-CoV2 were measured in five matched sera obtained after each of the waves, as defined by the SA National Institute of Communicable Diseases: wave I (ancestral strain) week 24-35 2020, wave II (Beta variant) week 48 2020-week 5 2021, wave III (Delta variant) week 19-37 2021, wave IV (Omicron variant BA.1/BA.2) week 45 2021-week 3 2022, and wave V week 16 2022-week 23 2022 (Omicron variant B.4/BA.5) [18].

The DCHS has established strong surveillance systems for illness [17] and the study team can be contacted at any time through a 24-hour study phone line and via community-based fieldworkers. Illness and any hospitalizations (all hospitalization occurs at a single public hospital serving the area) as well as intercurrent, mild, non-severe illness were monitored throughout the pandemic;

in addition, through this period, participants were seen at least every three months study staff. Additional study visits through each wave were initiated at primary care clinics, with serum samples obtained. Vaccination was unavailable to children younger than 13 years. The study was approved by the Human Research Ethics Committee, Faculty of Health Sciences University of Cape Town (HREC 401/2009). Mothers provided written informed consent, which was renewed annually.

Antibody measurements

Serum samples from children and unvaccinated mothers were tested for IgGs to S protein derived from ancestral SARS-CoV-2 (S-ancestral), Beta (S-Beta), Delta (S-Delta), and Omicron (S-Omicron) variants using an electrochemiluminescent immunosorbent assay on the Meso Scale discovery platform (MSD Rockville, MD, USA). The description and qualification of this quantitative binding assay has been described in detail [19]. The binding data generated in this assay are expressed in World Health Organization (WHO) international units because the assay is calibrated against the WHO international standard and the assay correlates well with functional measures of SARS-CoV-2 immunity [20]. The detection of S-ancestral IgG in this assay is highly specific (97.4%) and sensitive (90.3%) for exposure to SARS-CoV-2 and, hence, was used to define seropositivity (S-ancestral ≥1.09 WHO BAU/ml) and seroconversion after each wave. Geometric mean concentrations (95% confidence interval [CI]) of IgG levels for SARS-CoV2 antibodies were calculated. IgGs to S from different strains cross-react but higher titers are generated to the infecting strain; therefore, a ratio of S-variant IgG to S-ancestral IgG was calculated.

Statistical analysis

Data were analyzed using STATA 14.1 (STATA Corporation, College Station, TX, USA) and R (R core team 2021, version 4.1.2). Data were summarized as frequencies (percent) if categorical and median (interquartile range [IQR]) if continuous. The Wilcoxon rank-sum test (Mann-Whitney U test), Wilcoxon signed-rank test, and chi-square or Fisher's exact were used for crude comparisons, as appropriate. Seropositivity was measured longitudinally though each wave. A Kaplan–Meier plot was used to calculate the time in which participants became seropositive through the five waves; a participant was censored at the time of seropositivity.

Generalized estimating equations were used to identify the risk factors associated with seropositivity over the waves. A binomial distribution and logit link function, as well as robust standard errors to account for the presence of heteroscedasticity, were used in generating the generalized estimating equation models. The model was adjusted for wave, household, and child variables, as well as for maternal seropositivity.

To estimate threshold levels of antibodies induced by previous exposure, which may protect against subsequent SARS-CoV-2 infection, four-parameter logistic functions were fit to S IgG titers measured before and after the Beta, Delta, and Omicron waves. A detailed methodology is provided in the supplement. Similar to a logistic regression, the probability of seroconversion (defined as titers increasing by more than 1% postwave after the Beta, Delta, and Omicron waves) was estimated as a function of the amount of the antibody before a wave but using a more flexible link function using uninformative or weakly informative priors. This allowed estimation of infection attack rates in naïve (upper asymptote), the maximal protection achievable from naturally derived (lower asymptote), and an antibody threshold associated with protection against seroconversion (the inflection point of the curve where the probability of protection against seroconversion passes the 50% midpoint between the upper and lower asymptote). Sensitivity

analyses on the choice of % increase threshold (10% as opposed to 1%, and accounting for waning between samples) were also explored in the supplement. The software package R2Jags was used for Bayesian model fitting. The model code is available from the github repository: https://github.com/bquilty25/covid_seroconv.

Role of the funding source

The funders of the study had no role in study design, data collection, data analysis, data interpretation, or writing of the report. All authors had full access to all the data in the study and final responsibility for the decision to submit for publication.

Results

There were 366 children (median age 6.7 years [IQR 5.9-7.4 years]) and 339 matched mothers (median age 32.8 years [IQR 28.9-37.1 years]) included. The families were predominantly of low socioeconomic status living in crowded households with high cigarette smoke exposure (characteristics are summarized in Table S1). A total of 80 children were HIV-exposed but uninfected (21.9%) and one child was HIV-infected. Children were followed up for a median of 696 days (IQR 662-724), with blood sampling occurring between a median of 148 and 184 days after each wave. Although no vaccination was available for children, maternal vaccination against SARS-CoV2 was increasingly implemented as the pandemic progressed, predominantly from the wave III onward. The cumulative numbers of mothers vaccinated in each wave during this period was two (1%), 104 (30.7%), 216 (63.7%), and 231 (68.1%) in waves II, III, IV, and V, respectively [11].

There were no hospitalizations or severe illness in children. This was similar to the mothers, where there were only three COVID-19–related hospitalizations and no deaths [11]. Non-severe illness occurred in 26 (7.1%) children, two with ambulatory lower respiratory tract infection and 24 with upper respiratory illness. Despite this low morbidity, 125 (34.2%) children were seropositive after wave I, increasing through each wave to 99.2% after wave V (Table 1, Figure S1), indicating ongoing exposure. Only two (0.5%) children remained seronegative throughout all waves, whereas HIV exposure had no impact on the likelihood of seropositivity (Table S2). There was increasing concordance in seropositivity between children and mothers through the five waves, from 23% after wave I to 99% after wave V (Table S3). S IgG titers in children and mothers were comparable across waves and increased through each successive wave (Tables 1 and S5).

Although 33% of seronegative children seroconverted after wave II driven by the Beta variant, 52%, 89%, and 86% seroconverted after exposure to the Delta (wave III) and Omicron variants (waves IV and V), respectively, consistent with greater transmissibility of these variant of concerns (Table 1). A small number of children reverted to become seronegative after waves II, III, IV, and V (nine [2.5%], six [1.6%], four [1.1%], and one [0.3%], respectively).

To explore whether antibodies induced by natural infection prevented increases in S IgG in subsequent waves (a proxy for variant infection), we analyzed the changes in IgG after waves II, III, IV, and V in seropositive children (Table 2). Of the seropositive children, 17.6% (22 of 125) had increased S IgG after wave II (Beta variant) compared with 33% of seronegative children (Table 1). After wave III (Delta variant), the proportions responding were higher than those after the Beta wave but similar, irrespective of serostatus (55% of seropositive and 52% of seronegative children increased S IgG, respectively; Tables 1 and 2). The Omicron variant in wave IV resulted in the highest levels of response seen, with 89% (78 of 88) of seronegative and 69% (192 of 278) of seropositive children increasing their S IgG (Tables 1 and 2). Wave V was associated with a 49% increase in S IgG in those previously seroposi-

tive. A higher proportion of responders in the seronegative group than the seropositive group suggests protection from previous exposure, which occurred for the Beta and BA.1/BA.2 waves. Comparing sero-conversion to successive waves in the seronegative children revealed similar rates for the ancestral and Beta waves (33% and 34%) but progressively increasing rates for subsequent waves Delta (52%), BA.1/BA.2 78%, and BA.4/BA.5 (85.7%), suggesting increased infectivity of these variants.

Higher pre-wave antibody levels were associated with a lower probability of increased IgG after the subsequent wave (Table 2 and Figure 1), indicating a possible protective effect against infection with a subsequent variant. To explore the impact of infection induced pre-wave IgG in more detail, we estimated that the probability of increased titers in children with the lowest recorded S-ancestral IgG was 34.0% (95% credible interval [CrI]: 28.7-39.7%) during Beta, 58.4% (95% CrI: 52.7-63.8%) during Delta, 83.4% (95% CrI: 76.3-90.1%) during the Omicron BA.1/BA.2 wave, and 88.2% (95% CrI: 82.1-94.2%) in the Omicron BA.4/BA.5 wave (Table 3). In comparison, estimates for children with the very highest recorded antibodies titers were 7.1% (95% CrI: 2.2-14.6%), 22.0% (95% CrI: 7.5-41.7%), 11.9% (95% CrI: 0.5-45.4%), and 8.8% (95% CrI: 0.6-16.3%) for waves II, III, IV, and V, respectively. Substantially greater pre-wave S-ancestral IgG titers were required to provide protection against seroconversion before the Omicron waves compared with the Delta and Beta waves (Figure 1c, 1d, 1e; Table 3). These findings were also robust to the use of variant-specific titers despite a lower estimated threshold for Omicron (Table S4) because the ancestral and variant concentrations were highly correlated (Figure S2).

Based on the 50% reduction threshold, 64.8% (95% CrI: 50.4-72.8%), 18.4% (95% CrI: 9.7-42.9%), 11.5% (95% CrI: 3.6-28.8%), and 50.0% (95% CrI: 44.8-53.7%) of seropositive children had sufficient pre-wave antibodies to be protected against re-infection in the Beta, Delta, and two Omicron waves, respectively, (Table 3). These proportions were not dissimilar to those seen in unvaccinated mothers (Table 3) but the antibody levels providing the 50% reduction threshold differed between the variants and between children and mothers. Maternal thresholds were lower for Beta and Delta but higher for the Omicron variant waves (especially BA.1/BA.2), whereas the 50% reduction threshold increased for children and mothers from Beta to the BA.1/BA.2 waves before reducing for BA.4/BA.5 for children and mothers.

The multivariate analysis of factors associated with seropositivity in children indicated that age, maternal seropositivity, and wave were associated with seropositivity across all the waves in unadjusted analysis (Table S4). In the adjusted model, maternal seropositivity was strongly associated (adjusted odds ratio = 2.57, 95% CI 1.72-3.82) with child seropositivity, whereas a relatively low household income of 1000-5000 ZAR (US $60-300) was protective (adjusted odds ratio = 0.46, 95% CI: 0.26-0.82).

Discussion

More than 2 years since the pandemic started, many communities worldwide have been exposed to successive waves of SARS-CoV-2 infections. This exposure has altered their susceptibility to subsequent infection [21] and is likely responsible for the different disease profiles witnessed after the Omicron wave. In communities with previous widespread exposure and vaccination, Omicron infection has been relatively mild, whereas in communities where zero tolerance of COVID-19 has been pursued and, thus, relatively little disease-modifying population immunity has been acquired, the impact of Omicron has been more severe [22]. In this study of healthy unvaccinated children in a poor peri-urban area of South Africa, 34.2% were seropositive after the first wave of ancestral SARS-CoV-2, progressively increasing through each wave until all but II were seropositive. Although more mothers than chil-

Table 1

Anti-spike immunoglobulin G concentrations (GMC, 95% CI) in children after each wave of SARS-CoV-2. Children are stratified by their serostatus before the wave.

Seropositive[b] n (%)	After Wave I	After Wave II		After Wave III			After Wave IV			After Wave V			
	All (n = 366)	Seronegative pre-wave II (n = 241)	Seropositive Pre-wave II (n = 125)	All (n = 366)	Seronegative pre-wave III (n = 170)	Seropositive Pre-wave III (n = 196)	All (n = 366)	Seronegative pre-wave IV (n = 88)	Seropositive Pre-wave IV (n = 278)	All (n = 366)	Seronegative pre-wave V (n = 14)	Seropositive pre-wave V (n = 348)[a]	All (n = 362)[a]
	125 (34.2%)	80 (33.2%)	116 (92.8%)	196 (53.6%)	88 (51.8%)	190 (96.9%)	278 (76.0%)	78 (88.6%)	274 (98.6%)	352 (96.2%)	12 (85.7%)	347 (99.7%)	359 (99.2%)
GMCs (95% CI) in seropositive participants													
S-Ancestral	63.45 (43.45; 92.65)	28.13 (20.09; 39.40)	44.73 (34.47; 58.03)	37.01 (30.09; 45.53)	60.60 (45.17; 81.30)	59.09 (48.61; 71.84)	59.57 (50.67; 70.02)	41.77 (28.86; 60.45)	206.42 (173.29; 245.89)	144.88 (121.90; 172.19)	35.04 (15.26; 80.45)	200.84 (179.48; 224.75)	189.46 (168.72; 212.75)
S-Beta	27.93 (19.46; 40.08)	41.56 (27.81; 62.09)	23.69 (18.17; 30.89)	29.80 (23.72; 37.44)	46.44 (34.32; 62.84)	53.41 (44.35; 64.33)	51.10 (43.62; 59.86)	35.74 (24.85; 51.40)	183.25 (154.92; 216.77)	127.57 (107.79; 150.98)	52.33 (22.25; 123.06)	243.74 (218.21; 269.85)	231.52 (206.71; 256.41)
S-Delta	27.11 (17.98; 40.88)[c]	20.27 (14.54; 28.27)	22.42 (17.07; 29.44)	21.51 (17.45; 26.51)	69.66 (49.22; 98.60)	41.48 (34.00; 50.60)	48.88 (40.99; 58.28)	29.95 (20.20; 44.42)	143.22 (121.21; 169.23)	101.26 (85.45; 120.00)	25.80 (10.37; 64.17)	143.33 (128.27; 160.17)	135.35 (120.65; 151.84)
S-Omicron	12.21 (8.18; 18.24)[c]	8.89 (6.39; 12.36)	9.14 (7.07; 11.81)	9.03 (7.39; 11.04)	13.56 (10.40; 17.70)	13.38 (11.09; 16.15)	13.44 (11.53; 15.65)	58.53 (43.54; 78.50)	85.61 (70.10; 104.54)	78.69 (66.47; 93.16)	17.62 (7.62; 40.72)	89.31 (79.13; 100.79)	84.59 (74.79; 95.68)
S-Beta: S-ancestral	n/a	**1.48**	0.53	n/a	0.77	0.90	n/a	0.86	0.89	n/a	**1.49**	**1.21**	n/a
S-Delta: S-ancestral	n/a	0.72	0.50	n/a	**1.15**	0.70	n/a	0.72	0.69	n/a	0.74	0.71	n/a
S-Omicron: S-Ancestral	n/a	0.32	0.20	n/a	0.22	0.23	n/a	**1.40**	0.41	n/a	0.50	0.44	n/a

CI, confidence interval; GMC, geometric mean concentration; S, spike.

[a] Four children with missing serum samples after wave V.

[b] Seropositive defined as S antibodies to ancestral virus ≥1.09 World Health Organization BAU/ml.

[c] A total of 31 of 125 samples did not have sufficient serum.Notes: Two children remained seronegative across all waves.

Table 2

Before and after wave anti-spike IgG concentrations (GMC, 95% CI) in seropositive children after wave II (Beta), wave III (Delta), wave IV (Omicron), and wave V (Omicron). Children have been stratified into those whose IgG increased after the wave and those whose IgG did not increase.

	Changes in antibody titers between wave I and wave II (n = 125)		Changes in antibody titers between wave II and wave III (n = 196)		Changes in antibody titers between wave III and wave IV (n = 278)		Changes in antibody titers between wave IV and wave V (n = 348)	
	Wave I GMCs (95% CI)	Wave II GMCs (95% CI)	Wave II GMCs (95% CI)	Wave III GMCs (95% CI)	Wave III GMCs (95% CI)	Wave IV GMCs (95% CI)	Wave IV GMCs (95% CI)	Wave V GMCs (95% CI)
IgG increased	**n = 22**	**n = 22**	**n = 108**	**n = 108**	**n = 192**	**n = 192**	**n = 172**	**n = 172**
S-Ancestral	11.42 (4.33; 30.14)[a]	54.87 (24.56; 122.56)	23.25 (17.48; 30.91)[e]	76.01 (57.89; 99.79)	47.68 (39.81; 57.10)[j]	367.78 (311.21; 434.63)	48.53 (39.48; 59.65)[m]	242.65 (206.56; 285.03)
S-Beta	6.50 (2.51; 16.85)[b]	48.03 (20.51; 112.46)	16.65 (12.46; 22.25)[f]	61.66 (47.28; 80.42)	41.97 (35.14; 50.14)[j]	320.33 (271.25; 378.30)	44.49 (36.61; 54.07)[n]	295.26 (252.40; 345.39)
S-Delta	7.83 (2.53; 24.30)[c]	35.12 (16.77; 73.53)	14.73 (10.99; 19.77)[g]	52.59 (29.25; 70.45)	37.63 (31.01; 45.65)[k]	239.84 (202.91; 283.49)	35.97 (29.13; 44.42)[o]	179.49 (153.03; 210.52)
S-Omicron	3.35 (1.16; 9.64)[d]	15.86 (8.06; 31.20)	6.07 (4.63; 7.95)[h]	16.20 (12.38; 21.19)	11.10 (9.34; 13.19)[l]	179.38 (150.34; 214.04)	27.34 (22.44; 33.32)[p]	126.85 (106.47; 151.12)
IgG did not increase	**n = 103**	**n = 103**	**n = 88**	**n = 88**	**n = 86**	**n = 86**	**n = 176**	**n = 176**
S-Ancestral	91.52 (62.51; 133.98)[a]	29.15 (20.67; 41.12)	65.50 (50.51; 84.95)[e]	31.54 (22.45; 44.31)	97.91 (71.53; 134.03)[i]	43.16 (31.23; 59.64)	440.75 (377.48; 514.63)[m]	161.46 (136.80; 190.56)
S-Beta	38.12 (26.37; 55.12)[b]	14.66 (10.76; 19.97)	60.87 (44.75; 82.78)[f]	33.14 (23.90; 45.95)	79.28 (58.26; 107.88)[j]	41.25 (31.13; 54.67)	369.79 (313.23; 436.57)[n]	195.21 (165.46; 230.31)
S-Delta	36.06 (23.69; 54.89)[c]	14.61 (10.46; 20.40)	33.91 (25.84; 44.49)[g]	23.56 (17.39; 31.93)	87.66 (62.07; 123.80)[k]	34.98 (25.70; 47.59)	289.34 (247.63; 338.08)[o]	111.47 (95.01; 130.78)
S-Omicron	16.44 (10.89; 24.82)[d]	6.31 (4.71; 8.44)	14.59 (11.10; 19.18)[h]	8.53 (6.44; 11.31)	20.70 (15.41; 27.80)[l]	12.98 (9.61; 17.54)	235.66 (202.84; 273.80)[p]	61.57 (52.45; 72.28)

CI, confidence interval; GMCs, geometric mean concentration; Ig, immunoglobulin; S, spike protein; S-ancestral = S antibodies to ancestral virus; S-beta = S antibodies to beta variant; S-delta = S antibodies to delta variant.

[a] Wave I S-ancestral titers in those whose titers increased in wave II vs those whose titers declined or remained the same, P <0.001.

[b] Wave II S-beta titers in those whose titers increased in wave II vs those whose titers declined or remained the same, P <0.001.

[c] Wave I S-delta titers in those whose titers increased in wave II vs those whose titers declined or remained the same, P <0.001; n = 17 IgG increased titers & n = 74 IgG decreased titers.

[d] Wave I S-omicron titers in those whose titers increased in wave II vs those whose titers declined or remained the same, P <0.001; n = 17 IgG increased titers & n = 74 IgG decreased titers.

[e] Wave II S-ancestral titers in those whose titers increased in wave III vs those whose titers declined or remained the same, P <0.001.

[f] Wave II S-beta titers in those whose titers increased in wave III vs those whose titers declined or remained the same, P <0.001.

[g] Wave II S-delta titers in those whose titers increased in wave III vs those whose titers declined or remained the same, P <0.001.

[h] Wave II S-omicron titers in those whose titers increased in wave III vs those whose titers declined or remained the same, P <0.001.

[i] Wave III S-ancestral titers in those whose titers increased in wave IV vs those whose titers declined or remained the same, P <0.001.

[j] Wave III S-beta titers in those whose titers increased in wave IV vs those whose titers declined or remained the same, P <0.001.

[k] Wave III S-delta titers in those whose titers increased in wave IV vs those whose titers declined or remained the same, P <0.001.

[l] Wave III S-omicron titers in those whose titers increased in wave IV vs those whose titers declined or remained the same, P <0.001.

[m] Wave IV S-ancestral titers in those whose titers increased in wave V vs those whose titers declined or remained the same, P <0.001.

[n] Wave IV S-beta titers in those whose titers increased in wave V vs those whose titers declined or remained the same, P <0.001.

[o] Wave IV S-delta titers in those whose titers increased in wave V vs those whose titers declined or remained the same, P <0.001.

[p] Wave IV S-omicron titers in those whose titers increased in wave V vs those whose titers declined or remained the same, P <0.001.

dren seroconverted in wave I (51.9%), after wave V, all unvaccinated mothers (Table S5) and almost all children were seropositive. This rate of seropositivity in an unvaccinated population is, to the best of our knowledge, the highest reported and greatly exceeding population estimates for Africa of 65.7% [23]. Despite the high rates of successive exposure to SARS-CoV-2, no child became seriously ill or was hospitalized. This concurs with the recently published WHO analysis suggesting that Africa differentiates itself from other regions by its high number of asymptomatic (67%) infections [23] and a South African–based household infection study which estimated that 85.3% of infections were asymptomatic [24].

Previous exposure resulting in an antibody response to SARS-CoV-2 was associated with a reduced likelihood of infection. This finding is consistent with a reduced risk of re-infection in a household study conducted in South Africa [24] where a previous infection provided durable protection against re-infection throughout the study period, which included the Beta and Delta waves; the current study extends this observation through the Omicron waves.

In addition, seropositivity in a child was most strongly associated with maternal seropositivity, suggesting that household transmission may be a predominant means of transmission, especially in the early waves because schools were closed for long periods.

Substantially greater pre-wave S-ancestral IgG titers were required to provide protection against seroconversion before the Omicron waves than the Delta and Beta waves. The rates of infection (based on increased in antibody concentration) in the seronegative and seropositive children after wave IV due to Omicron BA.1/BA.2 wave were similar, and only those with very high levels of pre-existing antibodies had a reduced risk of infection, explaining the Omicron variants propensity for high rates of primary and re-infection [15]. Interestingly, the proportions of mothers and children who had sufficient pre-wave antibodies to be protected against re-infection in the Beta, Delta, and two Omicron waves were similar, but the absolute levels required differed. Maternal thresholds were lower for Beta (23.6 vs 70) and Delta (70 vs 118.7) but higher for the Omicron variant waves, especially BA.1/BA.2,
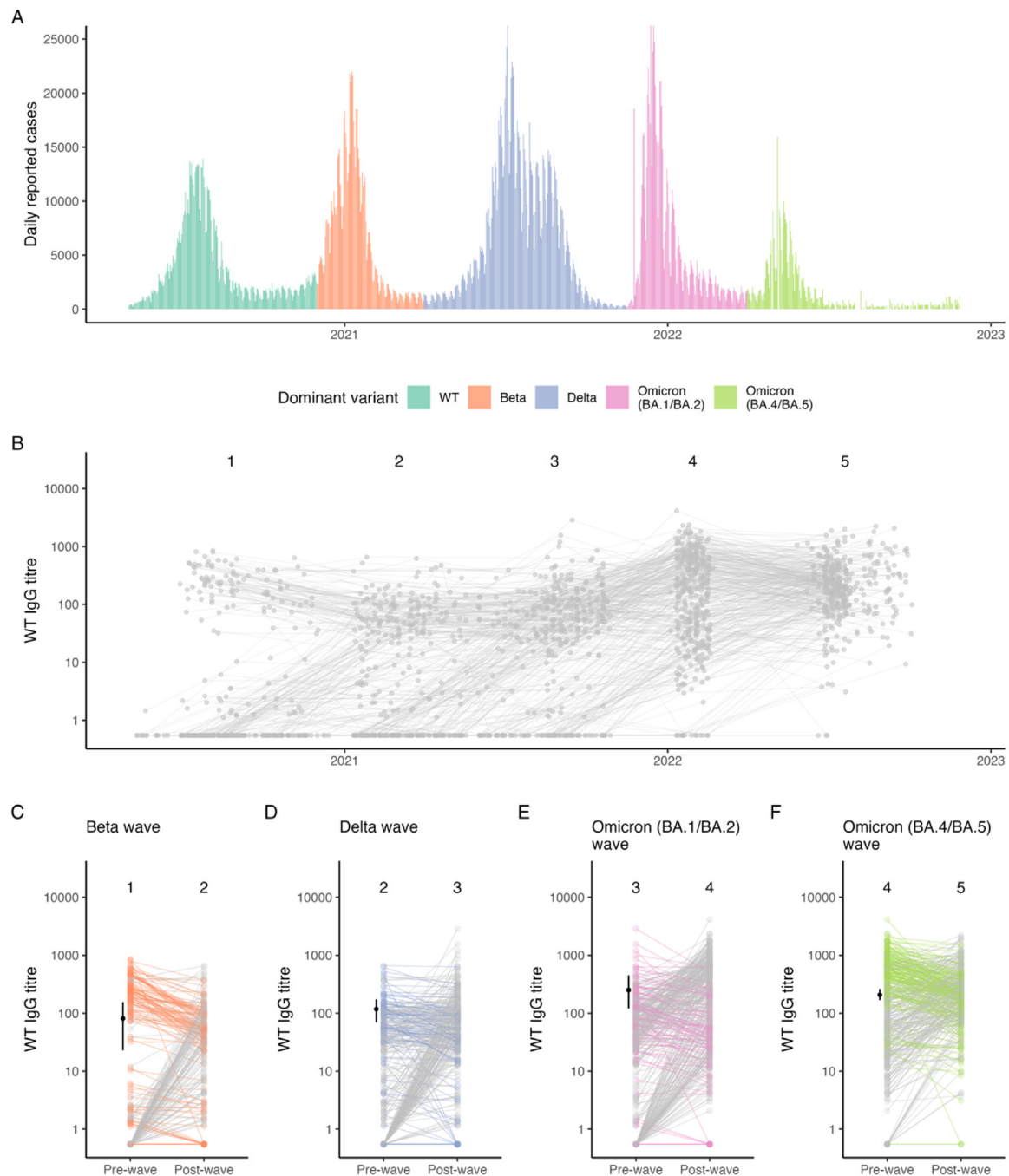
Figure 1. Progression of serostatus during the course of the COVID-19 pandemic in South Africa and estimated thresholds of protection against seroconversion. (a) Daily reported cases in South Africa from September 2020 to December 2022, colored by predominant circulating serotype, from https://covid19.who.int/WHO-COVID-19-global-data.csv. (b) Individual-level S-ancestral (WT) IgG titers over time. (c) Wave-specific change in WT IgG titers over the course of the Beta, Delta, Omicron, and Omicron variant waves, colored by whether antibody levels declined between samples, with estimated median and 95% credible interval threshold (dot and whisker) indicating 50% protection from seroconversion.

Ig, immunoglobulin; S, spike; WT, wild-type.

where the thresholds were 641 in mothers vs 257 in children (all values in BAU/ml). Although children may have required more antibody to the Beta and Delta waves than their mothers on account of being less antigen-experienced (and, thus, perhaps having less cross-reactive antibody), it is unclear why the 50% reduction threshold against BA.1/BA.2 should be so much higher for mothers than their children.

We were also able to demonstrate qualitative differences after exposure to variants between naïve and seropositive children, with naïve children mounting an IgG response dominated by the S antigen from the variant, whereas seropositive children responded with dominant ancestral IgG, irrespective of the variant they were exposed to, suggesting a degree of imprinting, as first described in adults by Röltgen et al. [25].

Our study has several limitations, including that infection to a variant was inferred from an increase in anti-S IgG. Because individuals were not tested for active infection unless symptomatic, we were unable to determine whether individuals were exposed during a wave unless seroconversion occurred, i.e. those who did not seroconvert during a wave may contain a mixture of those who

Table 3

Estimated levels of protection for minimal and maximal pre-wave S-ancestral antibody titers, 50% protection against infection (seroconversion) antibody titer threshold, and comparison of proportion of children and unvaccinated mothers with pre-wave titers above the threshold.

Wave	Age	Probability of increased titers at minimal pre-wave antibody levels (%, 95% CrI)	Probability of increased titers at maximal pre-wave antibody levels (%, 95% CrI)	50% reduction threshold (WHO BAU/ml, median, 95% CrI)	N	N increased	Proportion of seropositives with pre-wave antibody titers higher than threshold (median, 95% CrI	Count of seropositives with pre-wave antibody titers higher than threshold (median, 95% CrI)
II (Beta)	Mothers	50.7 (44.0, 58.5)	16.8 (1.9, 25.7)	23.6 (6.8, 65.8)	365	144	61.3% (41.4%, 77.0%)	117 (79, 147)
II (Beta)	Children	34.0 (28.7, 39.7)	7.1 (2.2, 14.6)	80.4 (23.2, 155.7)	366	102	64.8% (50.4%, 72.8%)	81 (63, 91)
III (Delta)	Mothers	64.5 (57.3, 72.4)	36.1 (15.1, 52.4)	70.0 (27.8, 327.3)	243	144	25.1% (4.7%, 50.9%)	43 (8, 87)
III (Delta)	Children	58.4 (52.7, 63.8)	22.0 (7.5, 41.7)	118.7 (59.7, 172.2)	366	199	18.4% (9.7%, 42.9%)	36 (19, 84)
IV (Omicron [BA.1/BA.2])	Mothers	77.0 (68.8, 83.8)	7.9 (1.3, 22.0)	641.6 (484.3, 882.7)	169	107	8.5% (4.7%, 11.3%)	9 (5, 12)
IV (Omicron [BA.1/BA.2])	Children	83.4 (76.3, 90.1)	11.9 (0.5, 45.4)	257.0 (121.6, 476.3)	366	270	11.5% (3.6%, 28.8%)	32 (10, 80)
V (Omicron [BA.4/BA.5])	Mothers	95.0 (80.8, 99.8)	6.1 (0.3, 16.9)	240.6 (133.9, 395.8)	321	106	56.9% (45.9%, 63.3%)	62 (50, 69)
V (Omicron [BA.4/BA.5])	Children	88.2 (82.1, 94.2)	8.8 (0.6, 16.3)	208.2 (165.5, 260.4)	362	185	50.0% (44.8%, 53.7%)	174 (156, 187)

CrI, credible interval.

were exposed and experienced an aborted infection due to sterilizing immunity and those who were unexposed. In addition, a degree of antibody waning may have taken place between pre-wave sampling and exposure in the subsequent wave, so the antibody levels at the time of exposure are likely to have been lower than when measured. We did not measure neutralizing antibody because we have previously shown excellent correlation between binding antibody, as measured in our laboratory and live virus or pseudo-virus neutralization [20]. We also did not have access to stored cells to evaluate cellular immune mechanism, although these may be more important for protecting against disease/serious disease manifestations rather than in prevention of infection. Our cohort consisted of children with a median age of 6.7 years, so the generalizability to a wider age range of children needs consideration; however, this cohort is representative of children living in resource-poor settings and LMICs. There was only one child living with HIV; however, 22% were HIV-exposed but uninfected, representing the predominant vulnerable childhood population affected by HIV, with strengthened HIV prevention programs. Although the overall levels of seroprevalence are higher than those reported for other African populations, those studies predate Omicron [23].

In summary, this study has shown very high seroprevalence to SARS-CoV-2 in a poor, peri-urban South African community, with minimal disease. Maternal and child infection were strongly associated, with progressively increasing seropositivity so that by wave V, almost everyone had been infected. Seropositivity via natural exposure to SARS-CoV-2 was associated with subsequent protection from variants, although, with Omicron, high levels of natural antibody were required to provide protection. The absence of significant morbidity in this cohort after the variant waves suggests that previous exposure has an important role in preventing disease if not infection, an important consideration now that SARS-CoV-2 appears to be endemic. A better understanding of the role of previous immunity and exposure may contribute to rational approaches to the use of COVID-19 vaccines, which may not be indicated for healthy children in LMIC settings.

Declaration of competing interest

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Author contributions

HJZ contributed to conceptualization, funding acquisition, methodology, supervision, and writing of original draft. RM, and LW performed data curation and formal analysis. MB and TB were involved in methodology and project administration. MJ and AH contributed to laboratory investigation and methodology. MPN was involved in conceptualization and methodology. BJQ and SF performed the formal analysis. DG contributed to conceptualization, formal analysis, methodology, supervision, and writing of original draft. All authors contributed to the final manuscript.

Data sharing

An anonymized, de-identified version of the data set can be made available upon request to allow all results to be reproduced.

Supplementary materials

Supplementary material associated with this article can be found, in the online version, at doi:10.1016/j.ijid.2024.107300.

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