






SARS-CoV-2 seroprevalence among blood donors in Uganda: 2019–2022

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Abstract

Background: The true burden of COVID-19 in low- and middle-income countries remains poorly characterized, especially in Africa. Even prior to the availability of SARS-CoV-2 vaccines, countries in Africa had lower numbers of reported COVID-19 related hospitalizations and deaths than other regions globally.

Methods: Ugandan blood donors were evaluated between October 2019 and April 2022 for IgG antibodies to SARS-CoV-2 nucleocapsid (N), spike (S), and five variants of the S protein using multiplexed electrochemiluminescence immunoassays (MesoScale Diagnostics, Rockville, MD). Seropositivity for N and S was assigned using manufacturer-provided cutoffs and trends in seroprevalence were estimated by quarter. Statistically significant associations between N and S antibody seropositivity and donor characteristics in November–December 2021 were assessed by chi-square tests.

Results: A total of 5393 blood unit samples from donors were evaluated. N and S seropositivity increased throughout the pandemic to 82.6% in

Abbreviations: BAU, Binding antibody units; COVID-19, Coronavirus Disease 2019; EDTA, Ethylene diamine tetraacetic acid; HCW, Health care workers; HBV, Hepatitis B virus; HCV, Hepatitis C virus; HIV, Human Immunodeficiency Virus; IgG, Immunoglobulin G; JHU IRB, Johns Hopkins University School of Medicine Institutional Review Board; LOD, Limit of detection; MERIT, Mirasol Evaluation of Reduction in Infections Trial; N, Nucleocapsid; RBD, Receptor binding domain; SARS-CoV-2, Severe acute respiratory syndrome coronavirus 2; S, Spike protein; SSA, Sub-Saharan Africa; TTI, Transfusion transmitted infections; UV, Ultraviolet; UBTS, The Ugandan Blood Transfusion Services; UNCST, Uganda National Council for Science and Technology; WHO, World Health Organization.

For affiliations refer to page 1362

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January–April 2022. Among seropositive individuals, N and S antibody levels increased ≥ 9 -fold over the study period. In November–December 2021, seropositivity to N and S antibody was higher among repeat donors (61.3%) compared with new donors (55.1%; $p = .043$) and among donors from Kampala (capital city of Uganda) compared with rural regions ($p = .007$). Seropositivity to S antibody was significantly lower among HIV-seropositive individuals (58.8% vs. 84.9%; $p = .009$).

Conclusions: Despite previously reported low numbers of COVID-19 cases and related deaths in Uganda, high SARS-CoV-2 seroprevalence and increasing antibody levels among blood donors indicated that the country experienced high levels of infection over the course of the pandemic.

KEYWORDS

blood donors, COVID-19, epidemiology, SARS-CoV-2, serological testing, Uganda

1 | INTRODUCTION

The burden of COVID-19 in low- and middle-income countries remains poorly characterized. Even prior to the availability of SARS-CoV-2 vaccines, some countries in Africa reported lower numbers of COVID-19-related hospitalizations and deaths than other regions globally.¹ Only 5% of the total recorded COVID-19 cases and 3% of the COVID-19 related deaths have originated in Africa, despite comprising 17.2% of the world's population.² One postulated reason for the low number of cases and deaths compared with other regions is age; sub-Saharan Africa (SSA) has a young population, whereas advanced age is a major risk factor for severe COVID-19, including death.^{3,4} However, access to COVID-19 testing, test hesitancy and deficient reporting mechanisms could underestimate cases in SSA. In an analysis by the World Health Organization (WHO), the true number of infections of SARS-CoV-2 was sevenfold higher than that which was reported, and two in three COVID-19 related deaths were not being registered.⁵ This reflects a wider problem of deficient or incomplete systems to register deaths in low- and middle-income countries, particularly in Africa.⁶

Serosurveillance (i.e., antibody testing of groups or populations) can be used to estimate the burden of disease. It is particularly advantageous when conducted early in an outbreak, to track infection incidence and guide public health interventions (e.g., vaccination), by identifying those groups of people who remain most at risk of infection. Examples of SARS-CoV-2 serosurveillance studies in Uganda are rare.^{7,8} One study, (November 2020 to January 2021), evaluated 753 health care workers (HCW) at 26 health facilities in South-Central Uganda along with 227 participants who had reported COVID-19 like symptoms in a phone-based survey (May–August 2020).⁷ A

quarter (26.7%) of the HCW and 15.6% of the phone-based survey participants were SARS-CoV-2 seropositive, with no difference by sex, age, or occupation. Another study leveraged a pre-existing malaria study in two districts in Eastern Uganda, where 441 participants were tested at four time points between 2020 and 2022.⁸ By the end of the Delta wave (i.e., before widespread vaccination), nearly 70% were seropositive. By the end of the observation period, 96% of participants were seropositive. However, there are limited data available representing a broader population in Uganda and throughout SSA.

Blood donors offer an invaluable resource to study epidemic dynamics, providing access to large numbers of individuals, across a diverse geography with routine capture of demographic information.^{9–12} We took advantage of a large repository of blood donor samples to better characterize the COVID-19 epidemic in Uganda through serial cross-sectional assessment of seropositivity from first reporting of SARS-CoV-2 through April 2022.

2 | STUDY DESIGN AND METHODS

2.1 | Participants and sample collection

Samples were collected as a part of the Mirasol Evaluation of Reduction in Infections Trial (MERIT), a randomized clinical trial located in Uganda investigating the effect of a riboflavin plus UV light pathogen reduction technology on the incidence of transfusion transmitted infections (TTIs).¹³ The Ugandan Blood Transfusion Services (UBTS), which is responsible for all blood collections in Uganda,^{14,15} collects approximately 300,000 units of blood a year. UBTS supplies fresh nonleukocyte-reduced whole blood units for MERIT and only collects blood from

voluntary nonremunerated donors. At the time of donation, individuals complete a donor health questionnaire on their overall health and history of any recent illness or fever, and a blood specimen is collected in a diversion pouch for routine testing. ABO and Rhesus (Rh) D blood groups are determined using the NEO (Immucor). All donors are screened for serological evidence of the four major TTIs using an ARCHITECT (Abbott) platform: human immunodeficiency virus (HIV), hepatitis B virus (HBV), hepatitis C virus (HCV), and *Treponema pallidum* (syphilis). As part of the MERIT, additional blood is collected in ethylenediamine tetraacetic acid (EDTA) tubes from the diversion pouch for further testing.¹³ MERIT samples were collected from October 2019 to April 2022 in Kampala, Uganda, separated into plasma and red cell pellets, and stored at -80°C until testing. In November–December 2021, additional samples were collected from blood donors to assess SARS-CoV-2 seroprevalence from a broader geographic range of four districts in Uganda (Kampala, Jinja, Masaka, and Hoima). These additional samples are separate from the MERIT collections and referred to as the second cohort of blood donors.

Blood donor information, including age, sex, new versus repeat donor status, blood group typing, and TTI results, were extracted from the UBTS donor database and subsequently de-identified. Given the de-identification, repeat donors may have multiple records in the MERIT data and/or may have samples in both the MERIT data and the second cohort.

2.2 | Ethical approvals

The MERIT trial protocol was approved by the Joint Clinical Research Center Institutional Review Board in Uganda, the Uganda National Council for Science and Technology (UNCST), the Ugandan National Drug Authority, the Johns Hopkins University School of Medicine Institutional Review Board (JHU IRB), and the US Department of Defense Human Research Protection Office. Collection of the second cohort (November–December 2021) was approved by the Uganda Cancer Institute Research Ethics Board, the UNCST, and the JHU IRB.

2.3 | SARS-CoV-2 binding antibody detection

Multiplexed sandwich immunoassays using MULTI-ARRAY electrochemiluminescence detection technology (MesoScale Diagnostics, Gaithersburg, MD) were used to evaluate IgG binding antibodies to ancestral SARS-CoV-2 nucleocapsid (N), spike protein (S), and S1 receptor

binding domain (RBD). In addition, five variants of the S protein (B.1.1.7[Alpha], B.1.351[Beta], P.1[Gamma], B.1.617.2[Delta], and BA.1[Omicron]) were used. The assays were run following the manufacturer's instructions and binding antibody levels were measured independently of one another in arbitrary units per milliliter (AU/mL). Analytes were considered “detectable” if the sample had a signal greater than 2.5 standard deviations of the plate-specific blank. Values below the lower limit of detection (LOD) or above the upper LOD were extrapolated from the standard curve by the manufacturer software. Manufacturer-provided cutoffs, which were validated in a United States population, were used to define samples as positive for N and S antibodies. Conversion to WHO binding antibody units (BAU/mL) was performed using the manufacturer-provided conversion. Cutoffs to assign positivity and WHO BAU conversion recommendations were not available for the S variants.

2.4 | SARS-CoV-2 neutralizing antibody titers

A microneutralization assay was used to perform and quantify neutralizing antibody titers against 100 fifty percent tissue culture infectious doses, as previously described.^{16,17} The neutralizing antibodies were determined against SARS-CoV-2/USA/DC-HP00007/2020, EPI_ISL_434688, which was obtained from surveillance performed in the Johns Hopkins Hospital Network.¹⁸ All positive samples by MSD for S and/or N from October to December 2019 were evaluated for neutralizing antibodies along with six samples from 2020 that were positive for both N and S by the MSD assay.

2.5 | Vaccination data

National vaccination data were obtained from the WHO COVID-19 vaccination dashboard.¹⁹ Vaccination data were not available for blood donors.

2.6 | Statistical analyses

Available donor characteristics and N and S antibody levels were summarized separately for MERIT donors and the second cohort. Data analysis was conducted and figures were generated using R version 4.2.1.²⁰ For MERIT donors, data were pooled by quarter to provide reliable estimates. The primary outcome for this study was the quarterly seroprevalence of both N and S antibodies (i.e., positive for both) among Ugandan blood donors from October–December 2019 to January–April 2022. With the

TABLE 1 Characteristics of Ugandan blood donors, October 2019–April 2022.

	MERIT cohort n (%)	Second cohort n (%)
Total	3517 (100.0)	1876 (100.0)
Timepoint		
October–December, 2019	104 (3.0)	-
January–March, 2020	303 (8.6)	-
April–June, 2020	48 (1.3)	-
July–September, 2020	166 (4.7)	-
October–December, 2020	359 (10.2)	-
January–March, 2021	456 (13.0)	-
April–June, 2021	528 (15.0)	-
July–September, 2021	375 (10.7)	-
October–December, 2021	511 (14.5)	1876 (100.0)
January–April, 2022	667 (18.9)	-
Age, years		
16–19	449 (12.8)	129 (6.9)
20–24	821 (23.3)	447 (23.8)
25–29	867 (24.7)	495 (26.4)
30–34	578 (16.4)	288 (15.4)
35–39	352 (10.0)	211 (11.2)
≥40	448 (12.7)	306 (16.3)
Missing	2 (0.1)	-
Sex		
Female	968 (27.5)	474 (25.3)
Male	2547 (72.4)	1402 (74.7)
Missing	2 (0.1)	-
Donation site		
Kampala	3517 (100.0)	1245 (66.4)
Hoima	-	174 (9.3)
Jinja	-	119 (6.3)
Masaka	-	338 (18.0)
Donor type		
First time	1096 (31.2)	1525 (81.3)
Repeat	139 (4.0)	351 (18.7)
Missing	2282 (64.9)	-
Blood group		
O	1692 (48.1)	952 (50.7)
A	912 (25.9)	441 (23.5)
B	767 (21.8)	406 (21.6)
AB	136 (3.9)	72 (3.8)
Missing	10 (0.3)	5 (0.3)
RhD antigen		
Negative	91 (2.6)	46 (2.5)
Positive	3421 (97.3)	1826 (97.3)
Missing	5 (0.1)	4 (0.2)
HIV^a		
Negative	3490 (99.2)	1851 (98.7)
Positive	23 (0.7)	17 (0.9)
Missing	4 (0.1)	8 (0.4)

(Continues)

TABLE 1 (Continued)

	MERIT cohort n (%)	Second cohort n (%)
HBV^a		
Negative	3461 (98.4)	1823 (97.2)
Positive	48 (1.4)	44 (2.3)
Missing	8 (0.2)	9 (0.5)
HCV^a		
Negative	3475 (98.8)	1855 (98.9)
Positive	37 (1.1)	13 (0.7)
Missing	5 (0.1)	8 (0.4)
Syphilis^a		
Negative	3436 (97.7)	1790 (95.4)
Positive	78 (2.2)	78 (4.2)
Missing	3 (0.1)	8 (0.4)
Any TTI^b		
Negative	3324 (94.5)	1721 (91.7)
Positive	178 (5.1)	146 (7.8)
Missing	15 (0.4)	9 (0.5)

^aAll infections were assessed by serology. HIV status was determined by antigen/antibody. HBV status was determined by the presence of HBV surface antigen. HCV status was determined by presence of HCV antibodies. Syphilis infection was determined by *Treponema pallidum* hemagglutination (TPHA) test.

^bBlood unit samples that are positive for one or more of HIV, HBV, HCV, or syphilis are considered positive for any transfusion transmitted infection (TTI).

introduction and distribution of SARS-CoV-2 S protein-specific vaccines in early 2021, S and N seroprevalence were also evaluated separately. Clopper–Pearson confidence intervals (CIs) were calculated. Monthly summaries of the proportion of the Ugandan population who had received at least one dose of COVID-19 vaccine were summarized from WHO data. N and S antibody levels were plotted against each other and the distribution of antibody levels among seropositive donors was examined by year of collection to assess changes in antibody levels over time. Antibody levels for S variants were examined by year.

Within the second cohort (November–December 2021), the prevalence of N and S antibodies individually and combined were examined by donor characteristics. Associations were assessed by chi-square tests and a two-tailed *p* value <.05 was considered statistically significant.

3 | RESULTS

3.1 | Population characteristics

Among MERIT donors, serology and donor data were available for 3517 blood unit samples collected between

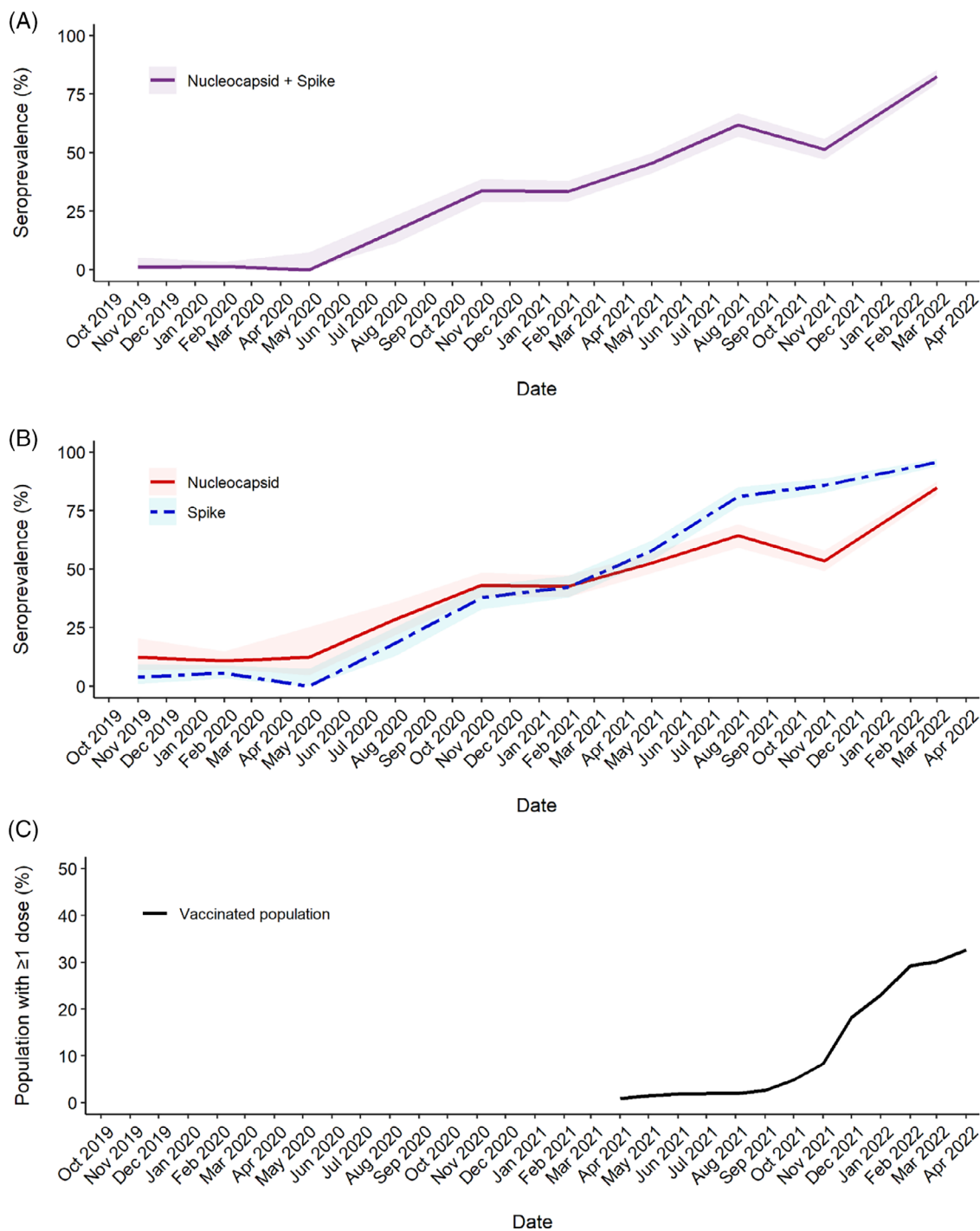


FIGURE 1 Temporal trends in SARS-CoV-2 seroprevalence among Ugandan blood donors and COVID-19 vaccination in Uganda. Proportion of donated whole blood unit samples positive from the MERIT donors ($n = 3517$) for both nucleocapsid and spike antibodies (A), proportion of donated whole blood unit samples positive for either nucleocapsid or spike antibodies individually (B), and the proportion of Ugandans who had received ≥ 1 dose of COVID-19 vaccine (C). Manufacturer-provided cutoffs were used to determine positivity for SARS-CoV-2 seroprevalence. The shaded regions of panels A and B reflect Clopper-Pearson confidence intervals. [Color figure can be viewed at [wileyonlinelibrary.com](https://onlinelibrary.wiley.com)]

October 2019 and April 2022, 48.0% of whom were 20–29 years of age and 72.4% were male (Table 1). At least one TTI was detected in 5.1% of the samples. The second cohort (November–December 2021) constituted an

additional 1876 unit samples with both serology and donor data; 50.2% of donors were 20–29 years of age and 74.7% were male. At least one TTI was detected in 7.8% of the samples.

3.2 | SARS-CoV-2 seroprevalence and COVID-19 vaccination

From October to December 2019, N and S seropositivity by the MSD assay was 1.0%. However, all of these MSD positive samples were negative by the live-virus neutralization assay, while all MSD positive samples evaluated from 2020 had detectable neutralizing antibodies. Throughout the pandemic, N and S seropositive continued to increase and reached 82.6% in January–April 2022 (Figure 1A). Three distinct peaks in seropositivity were seen in October–November 2020 (33.9%), July–September 2021 (61.9%), and January–April 2022 (82.6%). Separately, N seropositivity increased from 12.5% in October–December 2019 to 84.9% in January–April 2022; S seropositivity increased from 3.8% in October–December 2019 to 95.8% in January–April 2022 (Figure 1B). The proportion of the Ugandan population that had received ≥ 1 COVID-19 vaccination remained at $< 2.0\%$ until

August 2021, after which vaccination coverage increased to 39.9% in April 2022 (Figure 1C).

3.3 | Nucleocapsid and spike antibody levels

Among MERIT donors, SARS-CoV-2 N and S antibodies levels increased over the study period, with donors transitioning from low-positive levels of N and S antibodies in 2019, to high-positive levels of antibodies in 2022 (Figure 2A). Among donors who were seropositive (i.e., antibody levels were above the manufacturer-provided cutoffs), median N antibody levels increased from 21.6 BAU/mL in October–December 2019 to 204.2 BAU/mL in January–April 2022 (Figure 2B) and median S antibody levels increased from 46.7 BAU/mL in October–December 2019 to 905.8 BAU/mL in January–April 2022 (Figure 2C). Median S-variant antibody levels increased over time (Figure S1).

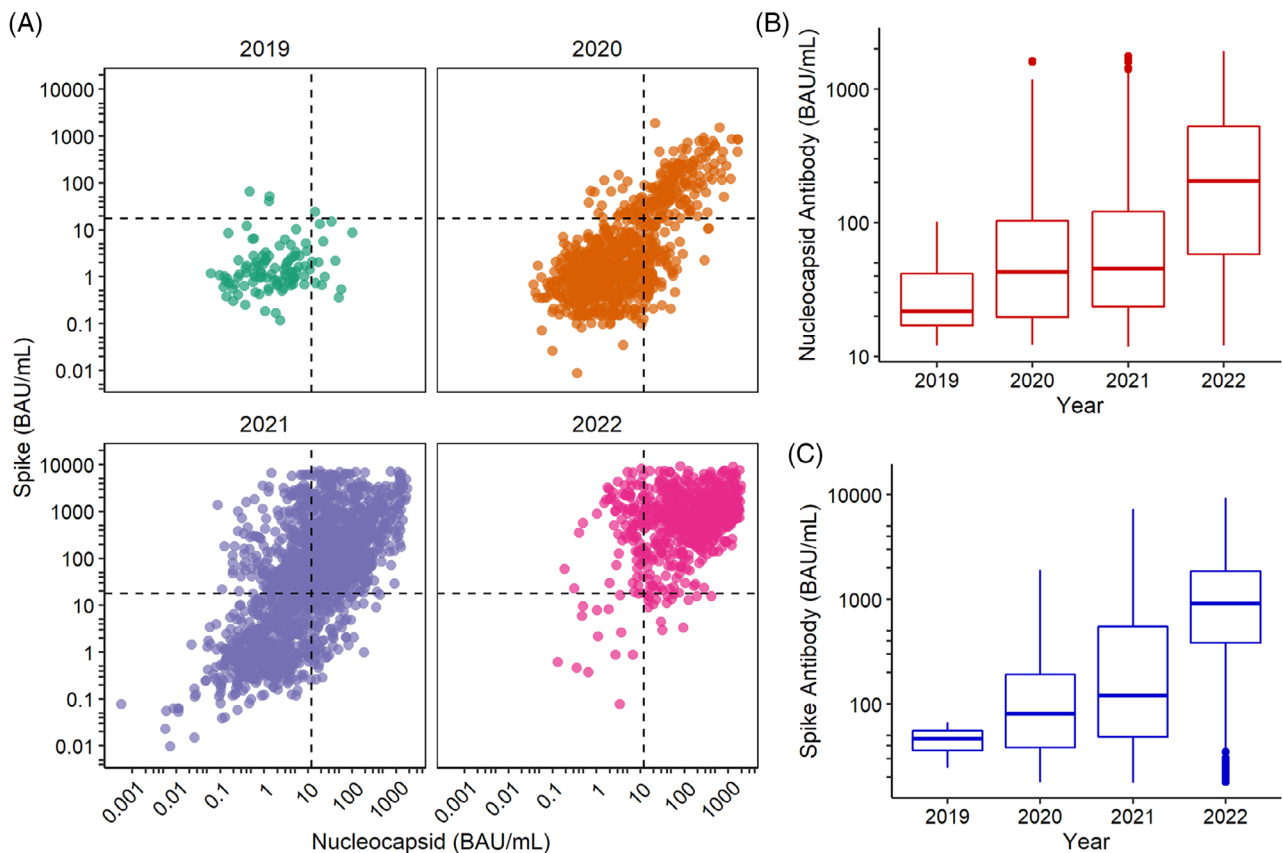


FIGURE 2 Nucleocapsid and spike binding antibody levels among Ugandan blood donors by year of donation. Panel A shows the measured nucleocapsid and spike antibody levels for each year of collection among MERIT donors ($n = 3517$). Samples are considered seropositive for nucleocapsid if antibody levels are ≥ 11.8 BAU/mL (vertical dashed line) and seropositive for spike if antibody levels are ≥ 17.7 BAU/mL (horizontal dashed line). Panel B shows the distribution of nucleocapsid antibody levels among samples that were above the cutoff for seropositivity. Panel C shows the distribution of spike antibody levels among samples that were above the cutoff for seropositivity. BAU/mL = Binding antibody units per milliliter. [Color figure can be viewed at [wileyonlinelibrary.com](https://onlinelibrary.wiley.com/terms-and-conditions)]

TABLE 2 Factors associated with SARS-CoV-2 seropositivity among Ugandan blood donors in the second cohort ($n = 1876$), November–December 2021.

	N	Seroprevalence ^a					
		Nucleocapsid and spike		Nucleocapsid		Spike	
		%	<i>p</i>	%	<i>p</i>	%	<i>p</i>
Age, years			0.299		0.235		0.492
16–19	129	58.1		60.5		83.7	
20–24	447	57.7		60.4		86.8	
25–29	495	53.5		57.2		83.6	
30–34	288	56.3		61.1		81.6	
35–39	211	52.1		56.9		85.3	
≥40	306	60.8		65.7		85.6	
Sex			0.410		0.957		0.268
Female	474	58.0		60.3		86.3	
Male	1402	55.7		60.1		84.0	
Donor type			0.043		0.166		<0.001
First time	1525	55.1		59.3		83.1	
Repeat	351	61.3		63.5		90.9	
Region			0.007		0.118		<0.001
Kampala	1245	58.8		61.8		87.5	
Hoima	174	47.7		55.2		77.6	
Jinja	119	47.9		52.9		83.2	
Masaka	338	54.4		59.2		78.1	
Blood group			0.522		0.412		0.282
O	441	54.7		58.2		86.8	
A	72	57.4		61.9		88.9	
B	406	57.9		62.1		84.2	
AB	952	61.1		62.5		83.4	
RhD Antigen			0.904		0.513		0.025
Negative	46	54.3		54.3		71.7	
Positive	1826	56.4		60.2		84.9	
HIV ^b			0.131		0.720		0.009
Negative	1851	56.5		60.2		84.9	
Positive	17	35.3		52.9		58.8	
HBV ^b			0.481		0.344		0.247
Negative	1823	56.5		60.0		84.8	
Positive	44	50.0		68.2		77.3	
HCV ^b			0.508		0.697		0.701
Negative	1855	56.2		60.1		84.6	
Positive	13	69.2		69.2		92.3	
Syphilis ^b			1.000		1.000		0.634
Negative	1790	56.3		60.1		84.5	
Positive	78	56.4		60.3		87.2	

TABLE 2 (Continued)

	N	Seroprevalence ^a					
		Nucleocapsid and spike		Nucleocapsid		Spike	
		%	<i>p</i>	%	<i>p</i>	%	<i>p</i>
Any TTI ^c			0.631		0.517		0.623
Negative	1721	56.5		59.9		84.8	
Positive	146	54.1		63.0		82.9	

^aSeroprevalence was assessed by the proportion of samples with antibody levels above the manufacturer-provided cutoffs for nucleocapsid and spike.

^bAll infections were assessed by serology. HIV status was determined by antibody/antigen positivity. HBV status was determined by the presence of HBV surface antigen. HCV status was determined by the presence of HCV antibodies. Syphilis infection was determined by *Treponema pallidum* hemagglutination (TPHA) test.

^cBlood unit samples that are positive for one or more of HIV, HBV, HCV, or syphilis are considered positive for any transfusion transmitted infection (TTI).

3.4 | Correlates of SARS-CoV-2 seropositivity

In the second cohort ($n = 1876$), N and S seropositivity was higher among repeat donors (61.3%) compared with new donors (55.1%; $p = .043$) and among donors from Kampala (58.8%) compared with Hoima (47.7%), Jinja (47.9%), and Masaka (54.4%; $p = .007$) (Table 2). N seropositivity alone did not associate significantly with any observed characteristics. S seropositivity was higher among repeat donors (90.9%) compared with new donors (83.1%; $p < .001$) and among donors from Kampala (87.5%) compared with Jinja (83.2%), Masaka (78.1%), and Hoima (77.6%; $p < .001$). S seropositivity was significantly lower among HIV-seropositive individuals (58.8% vs. 84.9%; $p = .009$).

4 | DISCUSSION

This blood donor serosurvey affords insight into the SARS-CoV-2 epidemic in Uganda. The findings demonstrate an incremental increase in seroprevalence over the study period with notable peaks corresponding to the successive waves of the major SARS-CoV-2 variants, such as Delta and Omicron. By the end of the observation period, almost 90% of participants were SARS-CoV-2 seropositive. In addition, the signal of positivity for both N and S protein antibodies increased dramatically, possibly indicating multiple infections or increasing titers following vaccination. If extrapolated, the collective findings question prior suggestions that Africa had been relatively spared from SARS-CoV-2²¹; rather the low numbers of cases likely reflect the challenges surrounding testing (i.e. access and hesitancy), reporting, and differences in clinical penetrance given the comparatively young population that acquired infection.

As of January 16, 2022, 170,279 cases and 3630 COVID-19-associated deaths have been reported in Uganda.²² By comparison, Argentina, a country of similar population size to Uganda, albeit an upper middle-income country, reported

10,004,679 cases and 130,249 COVID-19-associated deaths in the same time.²² The young population in Uganda (median age is 16.7 years vs. 31.5 years in Argentina)²³ could help to explain the low number of COVID-19 cases and deaths, as has been observed elsewhere in SSA.²⁴ But our finding of near universal exposure to SARS-CoV-2 among blood donors supports suboptimal testing and reporting being central to the skewed epidemiology of COVID-19 in Uganda and possibly other parts of SSA. Findings from a large modeling study (January 1, 2020, to December 31, 2021) estimated that only 1.4% of SARS-CoV-2 infections in the African region were reported and only a third (35.3%) of an estimated 439,500 COVID-19 deaths were attributed correctly.²⁵ Further, infections may be asymptomatic or mild, particularly in younger individuals, whereby those cases are unlikely to be tested. There also has been less published on COVID-19 in Africa: in a large meta-analysis (i.e. representing 95 studies and 29,776,306 individuals) to quantify the percentage of asymptomatic infection among tested and confirmed populations, only one study from Africa was included.²⁶ Early in the pandemic, serosurveys in SSA were largely cross-sectional and conducted of small, targeted populations, demonstrating low to moderate SARS-CoV-2 seroprevalence.^{27,28} With time, very large meta-analyses have been published, thus demonstrating an increase in SARS-CoV-2 seroprevalence but also highlighting the regional and temporal heterogeneity in seroprevalence, collectively supporting gross underreporting of infections, particularly in the African region.^{29–31}

The dual testing of S and N antibodies in our study enabled tracking of the epidemic along with some distinction of natural infection versus vaccination. The overwhelming majority of vaccines (including those that were used in Uganda) elicit antibodies against the S protein. Therefore, SARS-CoV-2 N antibodies, which wane more quickly than S antibodies (i.e., 61.1% will serorevert within 6 months after mild infection), likely reflect natural exposure.^{32,33} Similarly, the presence of S antibodies prior to the introduction of S-based vaccines is ascribed to natural exposure. According to WHO, the first

reported case of SARS-CoV-2 in Uganda was in March 2020, and community transmission remained low until August, 2020.³⁴ This is reflected in the low levels of N and S seropositivity seen in Ugandan blood donors until July–September 2020, when seropositivity began to increase. COVID-19 vaccines became available in Uganda in March, 2021.³⁵ Following mRNA vaccination, S antibodies typically peak within weeks of vaccination and decline approximately 6 months after the last dose.³⁶ In our study, S seropositivity began to surpass N in April–June 2021, which likely reflects vaccine uptake and/or breakthrough infections. SARS-CoV-2 seropositivity increased rapidly during the Delta and Omicron waves, as reflected by the proportion of donors with high levels of N and S antibodies. Interestingly, between these two waves of infections, S seropositivity did not decline, likely due to the increases in vaccination. However, in 2022, there was evidence of both high levels of S and N antibodies, suggesting recent infection with or without vaccination; many of those cases likely represent re-infection following introduction of the highly transmissible Omicron variant.³⁷ Of note, HIV positive individuals had a significantly lower proportion of S antibodies. The interplay between HIV and COVID-19 is complex, yet susceptibility does not appear to be increased in people living with HIV (PLWH) after controlling for confounders.³⁸ Our study observation merits attention, particularly to understand whether rates of vaccination were influenced by HIV status.

Our study has limitations. Donor populations are not entirely representative of the general population: blood donors are a healthier, more affluent and more educated subset of the population, conferring lower risk of many infections.^{39,40} Further, the median age of the Ugandan population is younger than the donors examined in this study (16.7 years vs. 28.0 years). Specific to this study, although highly populous areas were sampled, there is still geographic underrepresentation. Three quarters of blood donors were male, further highlighting underrepresentation of women and potentially other demographic subgroups. Second, given that the study was not originally designed to assess COVID-19, clinical histories and vaccination status were lacking. Third, the platform used to test for antibodies in this study was not validated in a Ugandan population and thus the cutoffs may lack specificity, as demonstrated in the low false positive results in 2019 that was confirmed by the absence of neutralizing antibodies. However, the data demonstrate a remarkable rise in SARS-CoV-2 antibodies during the pandemic. Additionally, cross-reactivity of the SARS and SARS-CoV-2 N antigen against other human coronaviruses has been shown,^{41–45} and may explain detectable levels of SARS-CoV-2 N antibodies in the 2019 and early 2020 samples. Finally, waning of N antibodies, which occurs at

a rate approximately double that of S antibodies, could also lead to an underestimation of seroprevalence.⁴⁶

In conclusion, a high SARS-CoV-2 seroprevalence was observed among Ugandan blood donors, contrasting with the relatively low numbers of contemporaneous COVID-19 cases that had been reported. Possible explanations for the discordance between observed and reported cases include differences in clinical penetrance (i.e., a higher proportion of asymptomatic and/or mild infection), suboptimal capacity for testing, or reluctance to test (e.g., because of potential stigma, implications of a positive test in regard to quarantine measures, and the associated financial burden) and reporting. These data suggest that severity of disease varies by population attributes, and the findings underscore the overarching need to address pervasive, structural challenges pertaining to laboratory infrastructure, test hesitancy, and reporting.⁴⁷

AUTHOR CONTRIBUTIONS

The following roles were by the individual co-authors: study conception and design (Aaron A. R. Tobian, Evan M. Bloch, Dorothy Kyeyune, Ronnie Kasirye, Irene Lubega, and Oliver Laeyendecker), sample collection, processing and testing (Dorothy Kyeyune, Henry Ddungu, Swetha Ashokkumar, Feben Habtehyimer, Owen Baker, Ronnie Kasirye, Ezra Musisi, Heather A. Hume, Reinaldo E. Fernandez, Hellen Wambongo Musana, Aggrey Dhabangi, Jaiprasath Sachithanandham, Andrew Pekosz, Susan L. Stramer, and Denise Whitby), study coordination (Ruchee Shrestha, Mahnaz Motevalli, Joseph Ouma, Priscilla Eroju, and Telsa de Lange), data analysis and interpretation (Eshan U. Patel, Jodie L. White, Mary Kate Grabowski, Khan Moses, Steven J. Reynolds, Andrew D. Redd, Oliver Laeyendecker, Mary Glenn Fowler, Philippa Musoke, Susan L. Stramer, Denise Whitby, Peter A. Zimmerman, Jeffrey McCullough, Raymond Goodrich, Thomas C. Quinn, and Paul M. Ness), writing first draft of manuscript (Evan M. Bloch, Jodie L. White, and Aaron A.R. Tobian), and review and editing (all co-authors).

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REFERENCES

1. Estimating excess mortality due to the COVID-19 pandemic: a systematic analysis of COVID-19-related mortality, 2020–21. *Lancet*. 2022;399:1513–36.
2. Bamgboye EL, Omiye JA, Afolaranmi OJ, Davids MR, Tannor EK, Wade S, et al. COVID-19 pandemic: is Africa different? *J Natl Med Assoc*. 2021;113:324–35.
3. Grasselli G, Greco M, Zanella A, Albano G, Antonelli M, Bellani G, et al. Risk factors associated with mortality among patients with COVID-19 in intensive care units in Lombardy, Italy. *JAMA Intern Med*. 2020;180:1345–55.
4. Zhang Y, Luo W, Li Q, Wang X, Chen J, Song Q, et al. Risk factors for death among the first 80 543 coronavirus disease 2019 (COVID-19) cases in China: relationships between age, underlying disease, case severity, and region. *Clin Infect Dis*. 2022; 74:630–8.
5. Burki TK. Undetected COVID-19 cases in Africa. *Lancet Respir Med*. 2021;9:e121.
6. BBC. *Measuring Africa's data gap: the cost of not counting the dead [monograph on the internet]*. 2021 <https://www.bbc.com/news/world-africa-55674139>
7. Ssuuna C, Galiwango RM, Kankaka EN, Kagaayi J, Ndyababo A, Kigozi G, et al. Severe acute respiratory syndrome Coronavirus-2 seroprevalence in south-Central Uganda, during 2019–2021. *BMC Infect Dis*. 2022;22:174.
8. Briggs J, Takahashi S, Nayebare P, Cuu G, Rek J, Zedi M, et al. Reconstructing the SARS-CoV-2 epidemic in eastern Uganda through longitudinal serosurveillance in a malaria cohort. medRxiv. 2022; 2022.09.20.22280170.
9. Uyoga S, Adetifa IMO, Karanja HK, Nyagwange J, Tuju J, Wanjiku P, et al. Seroprevalence of anti-SARS-CoV-2 IgG antibodies in Kenyan blood donors. *Science*. 2021;371:79–82.
10. Uyoga S, Adetifa IMO, Otiende M, Yegon C, Agweyu A, Warimwe GM, et al. Prevalence of SARS-CoV-2 antibodies

- from a National Serosurveillance of Kenyan blood donors, January-march 2021. *JAMA*. 2021;326:1436–8.
11. Mandolo J, Msefula J, Henrion MYR, Brown C, Moyo B, Samon A, et al. SARS-CoV-2 exposure in Malawian blood donors: an analysis of seroprevalence and variant dynamics between January 2020 and July 2021. *BMC Med*. 2021;19:303.
 12. Sykes W, Mhlanga L, Swanevelder R, Glatt TN, Grebe E, Coleman C, et al. Prevalence of anti-SARS-CoV-2 antibodies among blood donors in Northern Cape, KwaZulu-Natal, Eastern Cape, and Free State provinces of South Africa in January 2021. *Res Sq*. 2021.
 13. Kasirye R, Hume HA, Bloch EM, Lubega I, Kyeyune D, Shrestha R, et al. The Mirasol evaluation of reduction in infections trial (MERIT): study protocol for a randomized controlled clinical trial. *Trials*. 2022;23:257.
 14. Hume HA, Ddungu H, Angom R, Baluku H, Kajumbula H, Kyeyune-Byabazaire D, et al. Platelet transfusion therapy in sub-Saharan Africa: bacterial contamination, recipient characteristics, and acute transfusion reactions. *Transfusion*. 2016;56:1951–9.
 15. Murphy KJ, Conroy AL, Ddungu H, Shrestha R, Kyeyune-Byabazaire D, Petersen MR, et al. Malaria parasitemia among blood donors in Uganda. *Transfusion*. 2020;60:955–64.
 16. Klein SL, Pekosz A, Park HS, Ursin RL, Shapiro JR, Benner SE, et al. Sex, age, and hospitalization drive antibody responses in a COVID-19 convalescent plasma donor population. *J Clin Invest*. 2020;130:6141–50.
 17. Patel EU, Bloch EM, Clarke W, Hsieh YH, Boon D, Eby Y, et al. Comparative performance of five commercially available serologic assays to detect antibodies to SARS-CoV-2 and identify individuals with high neutralizing titers. *J Clin Microbiol*. 2021;59(2):e02257–20. <https://doi.org/10.1128/JCM.02257-20>.
 18. Gniazdowski V, Paul Morris C, Wohl S, Mehoke T, Ramakrishnan S, Thielen P, et al. Repeated coronavirus disease 2019 molecular testing: correlation of severe acute respiratory syndrome coronavirus 2 culture with molecular assays and cycle thresholds. *Clin Infect Dis*. 2021;73:e860–e9.
 19. WHO. *COVID-19 vaccines: World Health Organization dedicated COVID-19 vaccination dashboard [monograph on the internet]*. 2022. Accessed December 5, 2022 <https://www.who.int/emergencies/diseases/novel-coronavirus-2019/covid-19-vaccines>
 20. *The R Project for Statistical Computing [monograph on the internet]*. 2023 <https://www.r-project.org/>
 21. Nordling L. *The pandemic appears to have spared Africa so far. Scientists are struggling to explain why [monograph on the internet]*. 2020 <https://www.science.org/content/article/pandemic-appears-have-spared-africa-so-far-scientists-are-struggling-explain-why>
 22. JHU. *Coronavirus COVID-19 global cases by the Center for Systems Science and Engineering at Johns Hopkins [monograph on the internet]*. The Center for Systems Science and Engineering (CSSE) at JHU, Johns Hopkins University & Medicine; 2022. <https://coronavirus.jhu.edu/map.html>
 23. World Economics. *Uganda's median age [monograph on the internet]*. 2023 Accessed January 7, 2023. <https://www.worldeconomics.com/Demographics/Median-Age/Uganda.aspx>
 24. Usuf E, Roca A. Seroprevalence surveys in sub-Saharan Africa: what do they tell us? *Lancet Glob Health*. 2021;9:e724–e5.
 25. Cabore JW, Karamagi HC, Kipruto HK, Mungatu JK, Asamani JA, Droti B, et al. COVID-19 in the 47 countries of the WHO African region: a modelling analysis of past trends and future patterns. *Lancet Glob Health*. 2022;10:e1099–e114.
 26. Ma Q, Liu J, Liu Q, Kang L, Liu R, Jing W, et al. Global percentage of asymptomatic SARS-CoV-2 infections among the tested population and individuals with confirmed COVID-19 diagnosis: a systematic review and meta-analysis. *JAMA Netw Open*. 2021;4:e2137257-e.
 27. Shaw JA, Meiring M, Cummins T, Chegou NN, Claassen C, Du Plessis N, et al. Higher SARS-CoV-2 seroprevalence in workers with lower socioeconomic status in Cape Town, South Africa. *PLoS One*. 2021;16:e0247852.
 28. Chibwana M, Jere K, Kamng'ona R, Mandolo J, Katunga-Phiri V, Tembo D, et al. High SARS-CoV-2 seroprevalence in health care workers but relatively low numbers of deaths in urban Malawi [version 2; peer review: 2 approved]. *Wellcome Open Res*. 2020;5.
 29. Chisale MRO, Ramazan S, Mwale SE, Kumwenda P, Chipeta M, Kaminga AC, et al. Seroprevalence of anti-SARS-CoV-2 antibodies in Africa: a systematic review and meta-analysis. *Rev Med Virol*. 2022;32:e2271.
 30. Lewis HC, Ware H, Whelan M, Subissi L, Li Z, Ma X, et al. SARS-CoV-2 infection in Africa: a systematic review and meta-analysis of standardised seroprevalence studies, from January 2020 to December 2021. *BMJ Glob Health*. 2022;7(8):e008793. <https://doi.org/10.1136/bmjgh-2022-008793>.
 31. Bergeri I, Whelan MG, Ware H, Subissi L, Nardone A, Lewis HC, et al. Global SARS-CoV-2 seroprevalence from January 2020 to April 2022: a systematic review and meta-analysis of standardized population-based studies. *PLoS Med*. 2022;19:e1004107.
 32. Terpos E, Stellas D, Rosati M, Sergeantanis TN, Hu X, Politou M, et al. SARS-CoV-2 antibody kinetics eight months from COVID-19 onset: persistence of spike antibodies but loss of neutralizing antibodies in 24% of convalescent plasma donors. *Eur J Intern Med*. 2021;89:87–96.
 33. Van Elslande J, Oyaert M, Lorent N, Vande Weygaerde Y, Van Pottelbergh G, Godderis L, et al. Lower persistence of anti-nucleocapsid compared to anti-spike antibodies up to one year after SARS-CoV-2 infection. *Diagn Microbiol Infect Dis*. 2022; 103:115659.
 34. WHO. *WHO health emergency dashboard: Uganda [monograph on the internet]*. 2022. Accessed November 22, 2022 <https://covid19.who.int/region/afro/country/ug>
 35. WHO. *Uganda receives 864,000 doses of COVID-19 vaccines [monograph on the internet]*. 2021 <https://www.afro.who.int/news/uganda-receives-864000-doses-covid-19-vaccines>
 36. Collier A-rY YJ, McMahan K, Liu J, Chandrashekar A, Maron JS, Atyeo C, et al. Differential kinetics of immune responses elicited by Covid-19 vaccines. *N Engl J Med*. 2021;385:2010–2.
 37. Bbosa N, Ssemwanga D, Namagembe H, Kiiza R, Kiconco J, Kayiwa J, et al. Rapid replacement of SARS-CoV-2 variants by Delta and subsequent arrival of omicron, Uganda, 2021. *Emerg Infect Dis*. 2022;28:1021–5.
 38. del Amo J, Polo R, Moreno S, Jarrín I, Hernán MA. SARS-CoV-2 infection and coronavirus disease 2019 severity in persons with HIV on antiretroviral treatment. *Aids*. 2022;36(2):161–168. <https://doi.org/10.1097/QAD.0000000000003132>.
 39. Patel EU, Bloch EM, Grabowski MK, Goel R, Lokhandwala PM, Bruncker PAR, et al. Sociodemographic and behavioral characteristics associated with blood

- donation in the United States: a population-based study. *Transfusion*. 2019;59:2899–907.
40. Busch MP, Bloch EM, Kleinman S. Prevention of transfusion-transmitted infections. *Blood*. 2019;133:1854–64.
 41. Bates TA, Weinstein JB, Farley S, Leier HC, Messer WB, Tafesse FG. Cross-reactivity of SARS-CoV structural protein antibodies against SARS-CoV-2. *Cell Rep*. 2021;34:108737.
 42. Che XY, Qiu LW, Liao ZY, Wang YD, Wen K, Pan YX, et al. Antigenic cross-reactivity between severe acute respiratory syndrome-associated coronavirus and human coronaviruses 229E and OC43. *J Infect Dis*. 2005;191:2033–7.
 43. Dobaño C, Santano R, Jiménez A, Vidal M, Chi J, Rodrigo Melero N, et al. Immunogenicity and crossreactivity of antibodies to the nucleocapsid protein of SARS-CoV-2: utility and limitations in seroprevalence and immunity studies. *Transl Res*. 2021;232:60–74.
 44. Patrick DM, Petric M, Skowronski DM, Guasparini R, Booth TF, Krajden M, et al. An outbreak of human coronavirus OC43 infection and serological cross-reactivity with SARS coronavirus. *Can J Infect Dis Med Microbiol*. 2006;17:330–6.
 45. Tso FY, Lidenge SJ, Peña PB, Clegg AA, Ngowi JR, Mwaiselage J, et al. High prevalence of pre-existing serological cross-reactivity against severe acute respiratory syndrome coronavirus-2 (SARS-CoV-2) in sub-Saharan Africa. *Int J Infect Dis*. 2021;102:577–83.
 46. Olmstead AD, Nikiforuk AM, Schwartz S, Márquez AC, Valadbeigy T, Flores E, et al. Characterizing longitudinal antibody responses in recovered individuals following COVID-19 infection and single-dose vaccination: A prospective cohort study. *Viruses*. 2022;14(11):2416. <https://doi.org/10.3390/v14112416>
 47. Wilson ML, Fleming KA, Kuti MA, Looi LM, Lago N, Ru K. Access to pathology and laboratory medicine services: a crucial gap. *The Lancet*. 2018;391:1927–38.

SUPPORTING INFORMATION

Additional supporting information can be found online in the Supporting Information section at the end of this article.

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