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SARS-CoV-2 seroprevalence in people living with HIV in South Sudan

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ABSTRACT

Objectives: The burden of SARS-CoV-2 infection in people living with HIV (PLHIV) in South Sudan is unknown.
Methods: We conducted a cross-sectional seroprevalence survey of SARS-CoV-2 immunoglobulin (Ig) G antibodies and other diseases of public health importance (strongyloidiasis, toxoplasmosis) in PLHIV in South Sudan during April 1, 2020-April 30, 2022. We used a multiplex SARS-CoV-2 immunoassay to detect IgG antibodies targeting the SARS-CoV-2 spike, receptor binding domain, and nucelocapsid (N) proteins, and antigens for other pathogens (*Strongyloides stercoralis and Toxoplasma gondii*).
Results: Among 3518 samples tested, seroprevalence of IgG antibodies to SARS-CoV-2 spike protein and receptor binding domain 591 and nucleocapsid ranged from 1.4% (95% confidence interval [CI]: 0.9-2.1%) in April-June 2020 to 53.3% (95% CI: 49.5-57.1%) in January-March 2022. The prevalence of *S. stercoralis* IgG ranged between 27.3% (95% CI: 23.4-31.5%) in October-December 2021 and 47.2% (95% CI: 37.8-56.8%) in July-September 2021, and, for *T. gondii* IgG, prevalence ranged from 15.5% (95% CI: 13.3-17.9%) in April-June 2020 to 36.2% (95% CI: 27.4-46.2%) July-September 2021.
Conclusions: By early 2022, PLHIV in South Sudan had high rates of SARS-CoV-2 seropositivity. Surveillance of diseases of global health concern in PLHIV is crucial to estimate population-level exposure and inform public health responses.

Introduction

SARS-CoV-2, the virus that causes COVID-19, emerged in December 2019, and the World Health Organization declared a pandemic in March 2020. By May 1, 2022, 512 million cases were reported worldwide, including over 8.8 million reported from countries in Africa [1]. Case identification and reporting in African countries was hindered by numerous challenges, including limited surveillance, contact tracing, and testing. By June 2021, the estimated pooled seroprevalence from infection or vaccination across Africa was 65.1% [2]. SARS-CoV-2 seroprevalence studies have characterized prevalence among populations, reflecting clinical and subclinical infection, geographic distribution, and potentially associated demographic factors; these studies have helped inform public health policies and mitigation measures [3–6].

Emerging data report higher risk of mortality in persons co-infected with HIV and SARS-CoV-2, especially in persons with lower clusters of differentiation (CD)4 T-cell counts, HIV viral load non-suppression, and lower COVID-19 vaccination response in people living with HIV (PLHIV) who have advanced immunodeficiency [7–10]. There are limited data in PLHIV that estimate the burden of SARS-CoV-2 infection and potential immunity in this population [11–14].

South Sudan, a country with a population of 11.2 million people, detected its first case of COVID-19 on April 5, 2020. South Sudan did not routinely report SARS-CoV-2 prevalence among the general population or sub-populations, such as PLHIV. From a public health perspective, the lack of integration of epidemiologic surveillance and programmatic data limited the understanding of the distribution and determinants of SARS-CoV-2 infection in PLHIV. A 2020 seroprevalence

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study of 2214 household participants using a single enzyme-linked immunosorbent assay measurement of immunoglobulin (Ig) G targeting the receptor binding domain (RBD) of the SARS-Co-V-2 spike (S) protein from dried blood spot (DBS) samples obtained between August 10 and September 11, 2020 in Juba, South Sudan found a crude seroprevalence of 22.3%; after accounting for waning antibody levels, age, and sex, the adjusted seroprevalence was 38.3% (95% credible interval 31.8-46.5%) [15]. No specific COVID-19 interventions targeted PLHIV as a vulnerable population. COVID-19 vaccination began in April 2021 and was made available to facilities where PLHIV had access; however, there was limited outreach to high-risk groups [16]. Initial COVID-19 vaccination availability included AstraZeneca, followed by J&J, and messenger RNA vaccines. The vaccination coverage of the overall population, however, was very low, with only 27.9% (3,123,255 individuals) of the country's population having received at least one vaccine dose as of May 2022. COVID-19 vaccine uptake in PLHIV was not routinely captured. South Sudan had an estimated 160,000 (130,000-210,000) PLHIV in 2022 [17]. By April 2022, 43,806 PLHIV were receiving antiretroviral therapy (ART) in South Sudan through the US President's Emergency Plan for AIDS Relief (PEPFAR) [18] (data reported as part of PEPFAR Monitoring, Evaluation, and Reporting). PLHIV in South Sudan also receive ART from developmental partners, such as the Global Fund and Medecins Sans Frontiers, among others.

We evaluated the seroprevalence of SARS-CoV-2 IgG antibodies and of other diseases of public health importance (strongyloidiasis, toxoplasmosis) in PLHIV accessing PEPFAR-supported viral load testing services in South Sudan over a 2-year period. We used a multiplex SARS-CoV-2 immunoassay to detect IgG antibodies targeting the SARS-CoV-2 S, RBD, and nucelocapsid (N) proteins and antigens for other pathogens (*Strongyloides stercoralis and Toxoplasma gondii*). We documented the demographic, epidemiologic, and clinical characteristics of PLHIV with evidence of IgG antibodies against SARS-CoV-2 or to the other pathogens. Findings may inform future COVID-19 response activities in South Sudan and provide useful information for HIV service delivery models that may serve a key role in mitigating the potential negative impact of COVID-19 and other co-infections on the health and well-being of PLHIV.

Table 1
SARS-CoV-2 seroprevalence in people living with HIV in South Sudan, April 1, 2020-April 30, 2022: patient demographics and clinical characteristics.

Characteristic	N (%)
Total	3518 (100.0)
State	
Central Equatoria State	1125 (32.0)
Eastern Equatoria State	637 (18.1)
Lakes States	421 (12.0)
Western Bahr el Ghazal	460 (13.1)
Western Equatoria State	875 (24.9)
Age (years)	
0-14	159 (4.5)
15-29	860 (24.4)
30-44	1827 (51.9)
45+	672 (19.1)
Gender	
Male	1071 (30.4)
Female	2447 (69.6)
Pregnancy and breastfeeding status	
Not pregnant or breastfeeding	2020 (82.6)
Pregnant and/or breastfeeding	427 (17.4)
Year of treatment initiation	
<2011	146 (4.2)
2011-2017	1303 (37.0)
2018-2022	1948 (55.4)
Unknown	121 (3.4)
Regimen class	
Integrase strand transfer inhibitor	3302 (93.9)
Non-nucleoside reverse transcriptase inhibitor	111 (3.2)
Protease inhibitor	52 (1.5)
Unknown	53 (1.5)
Viral load result (copies/mL)	
<1000	3008 (85.5)
≥1000	510 (14.5)
Sampling round	
1: April-June 2020	900 (22.8)
2: July-September 2020	385 (9.7)
3: October-December 2020†	0 (0.0)
4: January-March 2021	190 (4.8)
5: April-June 2021†	0 (0.0)
6: July-September 2021	436 (11.0)
7: October-December 2021	621 (15.7)
8: January-March 2022	676 (18.3)
9: April 2022	310 (7.8)

Methods

Study design

We conducted a cross-sectional SARS-CoV-2 serosurvey on PLHIV on ART from the five PEPFAR-supported states of South Sudan (Central Equatoria, Eastern Equatoria, Western Bahr el Ghazal, Western Equatoria, and Lakes), using HIV viral load DBS remnant samples collected as part of routine HIV care in nine survey rounds (Table 1) during April 1, 2020-April 30, 2022. We included samples from patients who had a complete identification number on the HIV viral load request forms (VLRFs) and could be linked to demographic and regimen data stored in South Sudan's viral load sample management system. Sample sizes were determined based on the number of documented PLHIV on PEPFAR-supported ART during the last quarter of 2020 and calculated to ensure a one-sided 95% upper exact (Clopper–Pearson) confidence interval of no greater than 2.00% for an estimated prevalence of 0.2% after adjusting for non-response. For each survey round, 1000 viral load samples were selected using stratified random sampling, with equal allocation of 200 samples per state. In the first two rounds, anticipating potential low retrieval rates of remnant viral load samples from several states (i.e. Eastern Equatoria, Lakes, and Western Bahr el Ghazal) with historic low rates of viral load testing, 500 additional samples were selected from each of the two largest states, Central Equatoria and Western Equatoria. When fewer than 200 samples were available, a take-all approach was used (Table S2). Selected samples collected during February-March 2020 were tested but excluded from final analysis because this period preceded the first known case of COVID-19 in South Sudan. Due to challenges related to sample storage and limited access to samples for retrieval and identification, none of the samples selected during survey rounds three (October-December 2020) and five (April-June 2021) were tested. In addition, we modified our sampling strategy for the final two survey rounds (January-March 2022 and April 2022) due to commodity limitations. Sampling weights were calculated as the inverse of the selection probability within each stratum. Sampling weights were then adjusted for non-response, accounting for specimens that were selected in the sample but were not subsequently tested (i.e. due to sample loss).

Data on facility geographical location, age, sex, date of ART initiation, current ART regimen (classified as integrase strand transfer inhibitor–based, non-nucleoside transfer inhibitor–based, protease inhibitor–based, or unknown), and pregnancy and breastfeeding status were obtained from VLRF entered in the viral load sample management.

HIV viral load samples were collected using standard venipuncture techniques, spotted onto DBS sample cards, and tested on the Abbott platform at the HIV reference laboratory located in the national public health laboratory in Juba, South Sudan. Remnant HIV viral load samples were stored at -80^0C with desiccant, monitored for humidity conditions, and shipped to the US Centers for Disease Control and Prevention (CDC) for testing.

Laboratory testing

Multiplex serologic testing using an optimized and validated multiplex bead assay (MBA) targeting SARS-CoV-2 RBD antigens RBD541

and RBD591, N, and full-length S proteins was performed to determine antibody seropositivity, along with testing for antibodies to pathogens that cause strongyloidiasis and toxoplasmosis (Figure S1). The specific testing used was developed by the Global Neglected Tropical Disease Team within the National Center for Emerging and Zoonotic Infectious Diseases, US CDC [19].

Antigen coupling to microspheres

Antigens were coupled to carboxylated MagPlex microsphere beads (Luminex Corporation, Austin, TX, USA) at a concentration of 6 μg for S protein, 6 μg for RBD591, 3 μg for N protein, 1.4 μg for the recombinant immunodiagnostic antigen for strongyloidiasis, NIE, and 2 μg for the recombinant *Toxoplasma gondii* surface antigen, SAG2A, per 12.5×10^6 beads. Coupled beads were added to buffer with a pH 7.2 for NIE, S, and N proteins and a pH of 5 for RBD591 protein and SAG2A [19], following previously published methods [20].

Multiplex bead assay

The CDC received DBS samples at ambient temperature, which were stored at −20°C until processing. All samples were tested for antibodies against SARS-CoV-2 (S protein, N protein, and RBD591), toxoplasmosis, and strongyloidiasis using an MBA, as described elsewhere [19,21].

Seropositivity cutoff determination

Samples were considered seropositive for SARS-CoV-2 IgG if both S and RBD591 median fluorescence intensity (MFI) values exceeded the corresponding positivity thresholds of 328 and 281, respectively; N protein seropositivity was determined separately using an MFI threshold of 389. S+ classification was based on positivity for S and RBD591. Antibodies against SARS-CoV-2 S and N proteins were ascertained using the CDC-established MBA cutoffs to measure trends in seroprevalence estimates for each round using the S and N antibody categories: S+N+, S+N−, and S+: S+N+ or S+N−, S−N−.

SARS-CoV-2 infection was defined as being IgG antibody positive for S and N proteins (S+N+). SARS-CoV-2 antibody–negative serostatus was defined as being negative for S protein and or RBD591 (S−). Antibody positive for S protein and negative for N protein (S+N−) reflected four potential interpretations (Figure S1): (i) vaccine-derived antibodies, (ii) antibody from infection with waning N antibodies, (iii) antibody from infection with failure to develop detectable N antibodies, or (iv) a combination of antibodies from infection and vaccination. We estimated the seroprevalence of the proportion of individuals who were S+N+ (infected), S+N− (vaccinated, not infected, infected with waned N antibody, or infected without N antibody development), and S+ overall (either S+N+ or S+N−).

Other pathogens were assessed using antigens against the waterborne/foodborne (*S. stercoralis*) and neglected tropical (*T. gondii*) diseases, strongyloidiasis and toxoplasmosis, respectively. Seropositivity was defined using the MFI threshold of 485 and 113 for *S. stercoralis* NIE and *T. gondii* SAG2, respectively (Table S1).

The results were not returned to individuals given that MBA has not been approved for diagnostic use for these pathogens. Data were sourced from the existing VLRF and linked to serologic test results using a study identifier.

Statistical analysis

All analyses were performed using Python 3.10.9 and R 4.3.0 and accounted for survey design (sample weighting and stratification). We estimated seroprevalence of SARS-CoV-2 antibodies for each survey round as the proportion of samples with detected SARS-CoV-2 IgG out of total samples tested per round. The mean MFI value of each target antibody was also calculated for each survey round. Pooled and state-level estimates and 95% confidence intervals (CIs) were calculated for all estimates per sampling round. State-level estimates and CI were weighted for non-response but did not incorporate design weights. For seroprevalence estimates, Wald CI were used, except when the estimated seroprevalence was exactly zero or one; in these instances, the Clopper−Pearson exact method was used. All CI incorporated a finite population correction. Seroprevalence and mean MFI estimates and corresponding CI were similarly calculated for antibodies against *S. stercoralis* and *T. gondii* proteins. Demographics and clinical characteristics of sampled patients were tabulated for each sampling round and over all rounds.

Results

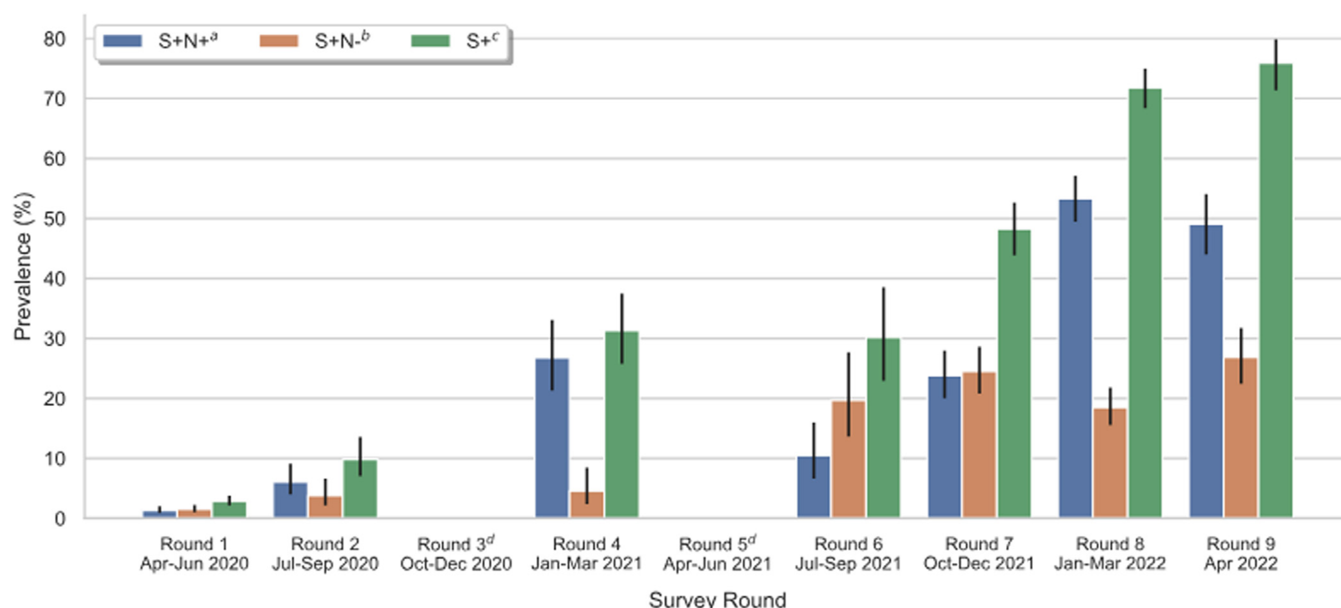
In this cross-sectional SARS-CoV-2 serosurvey, 3518 HIV viral load DBS remnant samples collected as part of routine HIV care during April 1, 2020-April 30, 2022 were tested using MBA for IgG antibodies against three SARS-CoV-2 proteins and one protein each from *T. gondii* and *S. stercoralis*. Sampling was conducted in eight 3-month rounds and one 1-month round (April 2022); the number of viral load results sampled in each round varied (round one: 2000, round two: 2000, round three: 900, round four: 1000, round five: 971, round six: 1000; round seven: 1005, round eight: 999, and round nine: 333). Overall, due to logistical challenges, 34.5% (3518 of 10,208) of the sampled specimens were included and tested (Table S2) and no sampled viral load specimens collected during round three and round five were tested. Across all rounds, 2477 (69.6%) samples were from females and 1827 (51.9%) were from patients aged 30-44 years. A total of 3302 (93.9%) samples were from PLHIV on integrase strand transfer inhibitor regimens; 3008 (85.5%) were virologically suppressed (viral load <1000 copies/mL); and 1948 (55.4%) were from PLHIV who initiated ART in 2018 or later (Tables 1, S3).

The seroprevalence of S+N+ IgG antibodies (infected) ranged across the five states from 1.4% (95% CI: 0.9-2.1%) in round one to 49.0% (95% CI: 44.0-54.1%) in April 2022 (round nine), peaking at 53.3% (95% CI: 49.5-57.1%) in January-March 2022 (round eight). S+N− seropositivity remained <5% until round six, when it rose to 19.7% (95% CI: 13.6-27.7%) and peaked at 26.9% (95% CI: 22.5-31.8%) in April 2022 (Figure 1). The seroprevalence of SARS-CoV-2 S+ IgG antibodies (potential combination of vaccination and/or infection) ranged from 2.9% (95% CI: 2.2-3.8%) in April-June 2020 to 75.9% (95% CI: 71.4-79.9%) in April 2022. The estimated mean MFI values for S, RBD, and N protein antibodies increased between April-June 2020 and April 2022 from 91 (95% CI: 64-119) to 7130 (95% CI: 6218-8042), from 117 (95% CI: 77-156) to 7079 (95% CI: 6118-8039), and from 126 (95% CI: 112-140) to 1625 (95% CI: 1351-1898), for S, RBD, and N protein, respectively (Figure 2). All samples tested from the pre-study period (February/March 2020) were SARS-CoV-2 IgG antibody–negative. Seroprevalence estimates within each state were similar to the overall estimates (Figure S2, Table S3).

The prevalence of IgG antibodies to *S. stercoralis* ranged between 27.3% (95% CI: 23.4-31.5%) in October-December 2021 and 47.2% (95% CI: 37.8-56.8%) in July-September 2021 and the prevalence of IgG antibodies to *T. gondii* ranged between 15.5% (95% CI: 13.3-17.9%) in April-June 2020 and 36.2% (95% CI: 27.4-46.2%) in July-September 2021 (Table S5).

Discussion

Considering the limited access to diagnostic testing, underreporting, and potential for asymptomatic cases, this SARS-CoV-2 IgG antibody seroprevalence study provides important data on the burden and spread of SARS-CoV-2 infection in PLHIV in South Sudan over time. Using the seroprevalence estimate from PLHIV who were S+N+ as a lower bound of previous SARS-CoV-2 infection and an upper bound that included the S+N− for PLHIV, with potentially waned N antibody response in the context of very low vaccination coverage in the states assessed, by the



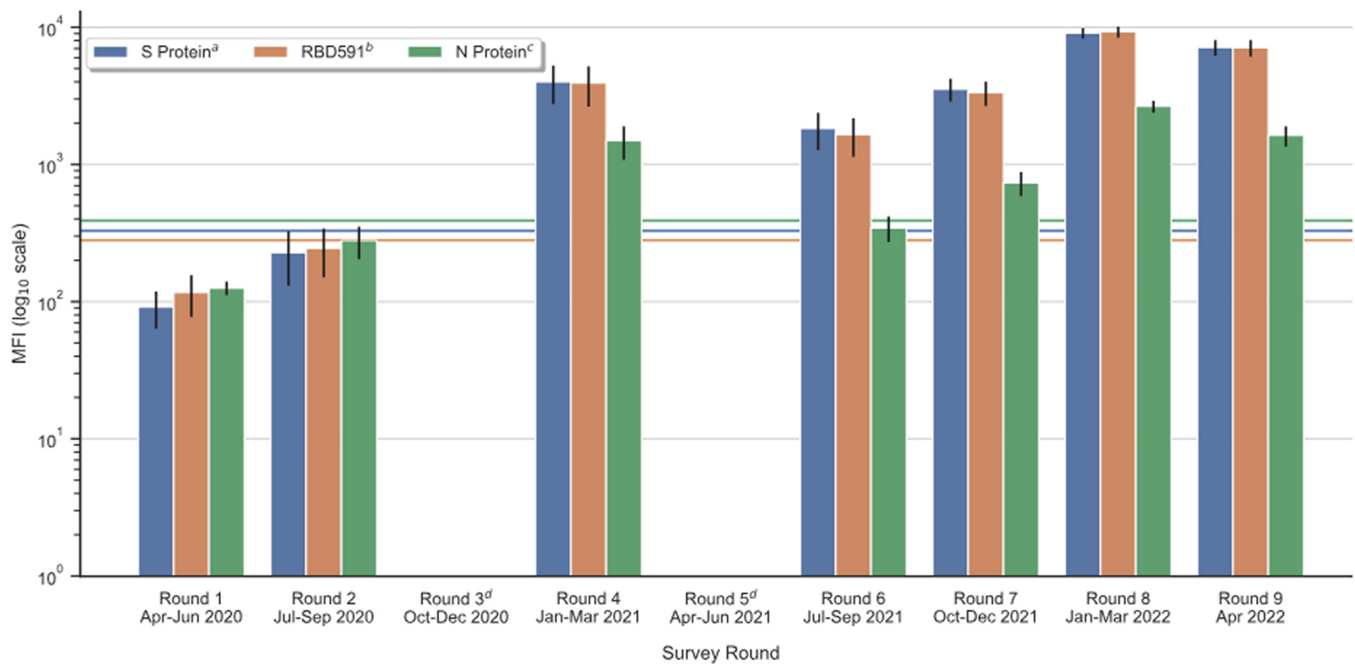
	Round 1 Apr-Jun 2020	Round 2 Jul-Sep 2020	Round 3[d] Oct-Dec 2020	Round 4 Jan-Mar 2021	Round 5[d] Apr-Jun 2021	Round 6 Jul-Sep 2021	Round 7 Oct-Dec 2021	Round 8 Jan-Mar 2022	Round 9 Apr 2022
S+N+[a]	1.4 (0.9 - 2.1)	6.1 (4.0 - 9.1)	--	26.8 (21.3 - 33.1)	--	10.4 (6.7 - 16.0)	23.8 (20.0 - 28.0)	53.3 (49.5 - 57.1)	49.0 (44.0 - 54.1)
S+N-[b]	1.5 (1.0 - 2.2)	3.8 (2.1 - 6.6)	--	4.6 (2.4 - 8.5)	--	19.7 (13.6 - 27.7)	24.5 (20.8 - 28.6)	18.5 (15.6 - 21.8)	26.9 (22.5 - 31.8)
S+[c]	2.9 (2.2 - 3.8)	9.9 (7.1 - 13.6)	--	31.3 (25.8 - 37.5)	--	30.2 (22.9 - 38.6)	48.3 (43.9 - 52.7)	71.8 (68.4 - 75.0)	75.9 (71.4 - 79.9)

Figure 1. SARS-CoV-2 seroprevalence. Proportion (95% confidence interval) of samples tested each round that were S+N+, S+N−, and S+ overall. Confidence intervals were calculated accounting for the survey design (weighting and stratification). MFI, median fluorescence intensity; N, nucleocapsid; RBD, receptor binding domain; S, spike.[a]S+N+: S protein antibody MFI value ≥328 AND RBD antibody MFI value ≥281 AND N protein antibody MFI value ≥389; [b]S+N−: S protein antibody MFI value ≥328 AND RBD antibody MFI value ≥281 AND N protein antibody MFI value < 389; [c]S+: S protein antibody MFI value ≥328 AND RBD antibody MFI value ≥281, irrespective of N protein antibody MFI value; [d]No samples were collected in October-December 2020 or April-June 2021.

end of the study period, more than three-quarters (75.9%) of PLHIV in the states surveyed may have had a SARS-CoV-2 infection.

Understanding the epidemiology of COVID-19 in PLHIV is an important aspect of overall pandemic control. PLHIV on ART interact with the medical system for routine HIV treatment monitoring via repeated HIV viral load testing over time; this study leveraged existing investments in clinical, laboratory, and data and posed little to no burden to patients and health care providers. This approach of testing routinely collected remnant samples using a repeat cross-sectional approach with epidemiologic, clinical, and ancillary information, such as vaccine coverage, hospitalizations, and death, may help provide an understanding of the burden and dynamics of SARS-CoV-2 in PLHIV and other diseases of public health interest. Diagnostic assays that detect neutralizing antibody in conjunction with IgG against SARS-CoV-2 antigens and differentiate vaccine from naturally acquired immunity may help refine future seroprevalence estimates and prevention and response activities [6].

COVID-19 surveillance in South Sudan was limited in geographical locations and populations, influenced by changes in policy, funding, infrastructure, human resources, and technical capacity over time [22]. This may have led to underreporting and limited case detection, with data primarily coming from sentinel surveillance, outbound traveler screening, point of entry screening, and testing of symptomatic individuals or case contacts [23]. With the low vaccine coverage in South Sudan, seroprevalence data help provide an estimate of cumulative incidence of past SARS-CoV-2 infection and the proportion of the PLHIV in the sampled locations that may potentially remain susceptible to infection or re-infection [24] and inform health care resource decisions, including the implementation of countermeasures such as vaccination, public health, and social measures. To the best of our knowledge, this is the first serosurvey for SARS-CoV-2 infection in PLHIV in South Sudan. The magnitude of SARS-CoV-2 seropositive PLHIV in this study reflect the potential for significant case contribution by this population, underscoring the need to strengthen the overall surveillance and inclusion of PLHIV. If seroprevalence rates serve as an indicator of partial immunity, it may identify sub-populations of PLHIV that may be more susceptible and require targeted interventions. Seropositivity, however, may not equate to future protection, and waning immunity and correlates of protection are not completely understood in the HIV-uninfected and PLHIV populations, especially with frequent emergence of SARS-CoV-2 variants [6]. Individuals who were S+N− before the time of South Sudan's COVID-19 vaccination inception in April 2021 likely reflected PLHIV with previous infection and waned N antibody response, whereas, after vaccination introduction, this group also included those with vaccination. The measurement of S+ antibodies in this population, therefore, may reflect those acquired by natural infection. Vaccination coverage information with MBA results may help provide key surveillance data to distinguish vaccine vs naturally acquired immunity to strategically direct interventions and resources. To do this, information systems enhancements to capture key parameters and integrate data, such as vaccine administration, COVID-19 case management, and epidemiologic data with surveillance data, may provide crucial information to understand SARS-CoV-2 co-infection and outcomes and inform future planning. In addition, other disease surveillance data, such as for malaria, neglected tropical diseases, parasitic diseases, and vaccine preventable diseases (VPDs), may strengthen multi-disease sectoral coordination and response, including public health interventions such as vaccination for VPDs and other control efforts.



	Round 1 Apr-Jun 2020	Round 2 Jul-Sep 2020	Round 3[d] Oct-Dec 2020	Round 4 Jan-Mar 2021	Round 5[d] Apr-Jun 2021	Round 6 Jul-Sep 2021	Round 7 Oct-Dec 2021	Round 8 Jan-Mar 2022	Round 9 Apr 2022
S protein[a]	91.2 (63.7 - 118.7)	227.7 (131.1 - 324.4)	--	4,001.6 (2,755.8 - 5,247.4)	--	1,830.3 (1,275.8 - 2,384.7)	3,544.5 (2,866.7 - 4,222.3)	9,060.2 (8,254.1 - 9,866.3)	7,130.0 (6,217.6 - 8,042.5)
RBD591[b]	116.5 (77.2 - 155.8)	245.7 (150.5 - 340.9)	--	3,914.5 (2,640.0 - 5,189.1)	--	1,653.6 (1,138.3 - 2,168.8)	3,345.5 (2,669.7 - 4,021.3)	9,257.9 (8,403.5 - 10,112.2)	7,078.6 (6,118.3 - 8,038.9)
N protein[c]	125.7 (111.7 - 139.7)	278.3 (204.6 - 352.0)	--	1,492.7 (1,082.9 - 1,902.5)	--	343.9 (271.3 - 416.4)	733.7 (587.0 - 880.4)	2,648.0 (2,379.1 - 2,916.8)	1,624.7 (1,351.2 - 1,898.2)

Figure 2. Estimated mean median fluorescence intensity values for antibodies to SARS-CoV-2 in people living with HIV on antiretroviral therapy in South Sudan by round, pooled across states (log-scale), April 2020-April 2022. Mean (95% confidence interval) median fluorescence intensity values for antibodies to SARS-CoV-2 in people living with HIV on antiretroviral therapy in South Sudan by round, pooled across states (log-scale), April 2020-April 2022. Confidence intervals were calculated accounting for the survey design (weighting and stratification). [a]S: spike; [b]RBD: receptor binding domain; [c]N: nucleocapsid; [d]No samples were collected in October-December 2020 or April-June 2021.

Early in the COVID-19 pandemic, antibody assays were used for seroprevalence studies to estimate the protective status of specific populations. False-positive rates using commercially available SARS-CoV-2 IgG using enzyme-linked immunosorbent assay targeting N protein, subdomains of the S protein, or the RBD in samples originating from sub-Saharan Africa have been reported, with high antibody titers arising from previous infections to other coronaviruses, malaria (acute or previous infection), and other infectious diseases [25]. Multiplex immunoassays have been used to assess antibody responses to SARS-CoV-2 antigens in different populations [26], and serologic assays to multiple antigenic targets (S, RBD, N) may provide useful information on infection and vaccination. Characteristics of multiplex assays include low sample volume requirement, the ability to process samples eluted from DBS, minimum labor costs, high sensitivity and specificity, reproducibility, and the opportunity to measure trends over time of multiple diseases simultaneously. It may provide an assessment of population immunity, previous disease exposure, and identify immunization coverage gaps or waning immunity for VPDs to inform vaccination policies toward the goal of disease elimination [27,28]. Opportunities to detect multiple diseases of public health importance may help foster program partnerships for synergistic public health impact. Investments in laboratory (human resources, infrastructure, commodities, biorepository), technical and epidemiologic capacity development, and enabling policies will help strengthen the ability to monitor and evaluate diseases and inform public health interventions across diseases. The lack of diagnostic tools has made it difficult to understand the burden of co-infections with HIV. Toxoplasmosis is the most common central nervous infection in PLHIV not receiving appropriate prophylaxis. In immunosuppressed PLHIV, the parasite can reactivate and cause disease when the CD4 count decreases to <100 cells/μL [29]. Similarly, strongyloidiasis may result in life-threatening dissemination of larvae in individuals, especially those who are immunocompromised [30]. Understanding the burden and implications of co-infections, including potentially latent ones that may reactivate, in PLHIV is crucial for effective control programs.

Social marginalization, stigma, and other structural determinants may disproportionately increase the exposure to HIV and SARS-CoV-2. Service disruptions and reduced access to HIV prevention, diagnosis, and treatment continuity may negatively influence PLHIV, leading to lower CD4 T-cell counts, HIV non-suppression, or other co-morbidities and create a cycle for increased risk of poor outcomes if infected with COVID-19. As PLHIV on ART age, chronic conditions, such as hypertension, cardiovascular disease, and diabetes, may become prevalent and play a role in COVID-19 severity. Syndemic approaches to prevent SARS-CoV-2 infection and its sequelae in PLHIV, such as targeting PLHIV for COVID-19 vaccination, immune preservation with early HIV diagnosis and treatment, and diagnosis and treatment of co-morbidities, are needed. A greater understanding of SARS-CoV-2 infection in PLHIV will help programs and policymakers make data-driven decisions to optimize services that support HIV and COVID-19 prevention, diagnosis, and management goals and protect the well-being of PLHIV.

Study limitations

This study has several limitations. First, the study population was limited to PLHIV on ART who accessed viral load testing services from PEPFAR-supported states; therefore, the results may not be generaliz-

able to all PLHIV. The demographic and clinical data used for this analysis did not capture COVID-19–related symptoms, hospitalizations, or deaths; COVID-19 vaccination status; CD4 T-cell count; or other clinical data, such as co-morbidities, limiting our ability to assess the survey results' associations with COVID-19 diagnosis, disease severity, vaccination, and immunologic or clinical status. The rates of non-response were much higher than anticipated primarily due to insufficient remnant sample and sample loss, among others, limiting our ability to assess state-level estimates and perform regression analyses for differences in demographics, ART regimen, year of initiation, or viral load status.

SARS-CoV-2 antibody kinetics in PLHIV are not fully understood, and given that antibody responses decline over time, individuals who were previously infected may have had waning SARS-CoV-2 antibodies or failed to mount an immune response and were seronegative, potentially underestimating the true extent of previous infection in this population. In addition, there remains the inability to differentiate natural vs vaccine-mediated immunity in those who were S+N−, who may have had a previous infection with waned N antibodies, been vaccinated without prior infection, or a combination of previous infection with waned N antibodies and vaccination.

Conclusion

Our study findings contribute to the understanding of SARS-CoV-2 seroprevalence in PLHIV on ART in the context of a very challenging, low-resource, and health care–constrained setting as found in South Sudan. It demonstrates the feasibility and utility of testing remnant HIV viral load samples to estimate IgG antibodies to SARS-CoV-2 and other diseases. SARS-CoV-2 surveillance in PLHIV, along with data integration on vaccination, immunologic, HIV virologic status, and other clinical and sociodemographic information, is crucial to gain insight into effective clinical and public health responses for this population.

Declarations of competing interest

The authors have no competing interests to declare.

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Ethical approval

The study received ethical approval from the Republic of South Sudan Ministry of Health Research Ethics Review Board (Protocol No. A.13/6th/12/2021). This activity was reviewed by the CDC, deemed not research, and was conducted consistent with applicable federal law and CDC policy.[§] The CDC staff did not have contact with patients nor access to personal identifying information.

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Author contributions

HMC conceptualized the study. HMC, RS, DM, and ED developed the methodology. AS and DM performed the testing. KM collected and curated the data. KM, HMC, RS, DM conducted the formal analysis. KM, HMC, and AS accessed and verified the data. HMC, KM, DKL, ED, and AS drafted the original manuscript draft. All authors critically reviewed, revised, and approved the final manuscript.

Data sharing

Other than SARS-CoV-2 antibody testing, no new primary data were collected for this study. Data from this study are owned by the South Sudan Ministry of Health and requests for additional use may be directed to Gregory Wani, gregorywani@yahoo.com.

Disclaimer

The findings and conclusions in this report are those of the authors and do not necessarily represent the official position of the funding agencies. Applicable federal law for ethical review include: 45 C.F.R. part 46.102(l)(2), 21 C.F.R. part 56; 42 U.S.C. §241(d); 5 U.S.C. §552a; 44 U.S.C. §3501 et seq. [§]See e.g., 45 C.F.R. part 46, 21 C.F.R. part 56; 42 U.S.C. §241(d); 5 U.S.C. §552a; 44 U.S.C. §3501 et seq.

Supplementary materials

Supplementary material associated with this article can be found, in the online version, at doi:10.1016/j.ijregi.2024.100421.

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