Seroprevalence of Anti-Sars-Cov-2 Antibodies among Patients Visiting Hospital-Based Sentinel Sites in the Rift Valley Region, Kenya

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DOI*:* https://dx.doi.org/10.4314/ajhs.v38i1.2

Abstract

Background: Accurate estimation of the spread of severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) is crucial for guiding public health prevention strategies. Underreporting from polymerase chain reaction (PCR) based testing, especially in low- and middle-income countries, often obscures the true extent of exposure and immunity to coronavirus disease 2019 (COVID-19). This study aimed to determine the seroprevalence and antibody levels against SARS-CoV-2 among patients visiting hospital-based sentinel sites in Kenya's Rift Valley region.

Method: A cross-sectional study was conducted at four hospital-based sentinel sites among patients with symptoms of acute respiratory infection. Patients aged six months or older visiting the sites between January 2022 and December 2022, presenting with symptoms of acute respiratory infection, were enrolled. Clinical and sociodemographic data were collected using a structured questionnaire. A quantitative enzyme-linked immunosorbent assay (ELISA) kit detected IgG antibodies against SARS-CoV-2 S protein in serum samples.

Results: Among 557 individuals enrolled in the study, the seroprevalence of SARS-CoV-2 antibodies was 97.8% (n=545). Vaccinated individuals demonstrated significantly higher odds of seropositivity compared to unvaccinated counterparts, with an odds ratio of 3.33 (95% CI: (0.4, 27.87). While seropositivity remained consistent across age groups, older adults exhibited the highest odds. Site-specific differences in enrolment and vaccination rates highlight potential access-related disparities.

Conclusion: Our findings indicate prior exposure to SARS-CoV-2 within the study region, underscoring the significant impact of natural infection and vaccination efforts in driving SARS-CoV-2 seroprevalence within the Rift Valley region. Despite the underestimation of infections and disparities in vaccination access, this study demonstrates the effectiveness of vaccination for conferring SARS-CoV-2 immunity. To ensure equitable vaccination coverage, targeted interventions should focus on increasing vaccine accessibility through mobile clinics and community-based vaccination campaigns while addressing vaccine hesitancy through targeted public health messaging and community engagement.

Keywords: *Seroprevalence; SARS-CoV-2; COVID-19; Vaccination; Antibodies*

[*Afr. J. Health Sci*. 2025;38(1): Article 2. https://doi.org/10.4314/ajhs.v38i1.2**]**

Introduction

Severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), responsible for coronavirus disease 2019 (COVID-19), resulted in significant morbidity and mortality globally [1]. The epidemic waves and the emergence of different SARS-CoV-2 variants have progressed at varying paces and volumes in different countries [2]. As of April 2024, more than 704 million confirmed cases and more than 7 million deaths worldwide had been reported. In Kenya, reported cases reached approximately 344,000 cases and 5,689 deaths [3]. COVID-19 surveillance often underestimates the true extent of exposure and immunity in the community because PCR testing misses asymptomatic and pre-symptomatic cases, as it's mainly used for symptomatic individuals [4,5]. Specifically, underreporting is worse in regions with limited healthcare access or testing capacity. Global seroprevalence studies have revealed substantial variations in SARS-CoV-2 exposure across populations. Evidence from multiple countries indicates that reported COVID-19 cases considerably underestimate the true extent of infection [6,7].

By September 2021, the global seroprevalence from infection or vaccination was estimated to be 59.2%, with notable variations between regions [6]. However, these studies may be subject to biases, such as selection bias due to non-random sampling, for instance, the selection of specific groups such as blood donors. COVID-19 vaccinations in Kenya commenced in March 2021, with priority being given to frontline workers and the elderly [8]. Vaccinations were progressively extended to the rest of the population, such that by July 2022, 23.4% of the population had received at least one dose of the COVID-19 vaccine [3]. Several seroprevalence studies have been conducted in Kenya to assess the burden of SARS-CoV-2 and the development of population immunity [9]. These studies have revealed diverse transmission patterns nationwide, with varying seroprevalence rates in different regions and sub-populations. A national sero-surveillance study of Kenyan blood donors reported an adjusted seroprevalence of 48.5% in early 2021, with higher rates observed in the capital city, Nairobi, compared to rural areas[10], underscoring the influence of factors like population density and healthcare access on transmission dynamics 10.

The advent of the Omicron variant led to a rapid increase in infections and a subsequent rise in seropositivity in various regions across the globe. For instance, a sero-surveillance study in Switzerland conducted post-Omicron reported an estimated seroprevalence of 93.8%, with 72.4% attributed to natural infection [11], while a similar study conducted in Kilifi and Nairobi in 2022 revealed a seroprevalence of 70% and 90%, respectively [12].

While Kenya has conducted seroprevalence studies, there is a paucity of data on SARS-CoV-2 sero-epidemiology in different regions. This study focused on high-altitude areas of Nakuru, Iten, and Nandi, where varying demographics, healthcare access, and socioeconomic factors could affect disease transmission and immunity. For instance, rural communities in these areas may face unique challenges in accessing healthcare and vaccination services, potentially impacting their susceptibility to infection and overall immune status. Additionally, understanding seroprevalence among patients presenting with acute respiratory infection (ARI) symptoms in sentinel sites, particularly after the emergence of the highly transmissible Omicron variant, provides important insights into the real burden of disease and population immunity.

Estimating the burden of SARS-CoV-2 infection in a population is crucial for public health planning, resource allocation, and understanding pandemic dynamics. Although neutralisation assays remain the gold standard for assessing functional immunity, enzyme-linked immunosorbent assays (ELISA) provide a robust and scalable alternative for population-level seroprevalence studies, reliably detecting binding antibodies that indicate past exposure

or vaccination. The study aimed to assess the overall seroprevalence of antibodies and the levels of antibodies against SARS-CoV-2 among patients at hospital-based sentinel sites in the Rift Valley, to understand the immune status of the region following several waves of SARS-CoV-2 infections.

Methodology

Study area

This study was conducted in four hospital-based sites established for acute respiratory infections(ARI) sentinel surveillance. They included Olenguruone Level 4 Hospital (Nakuru County), Molo Level 4 Hospital (Nakuru County), Iten County Referral Hospital (ICRH; Elgeyo Marakwet County) and Kapsabet County Referral Hospital (KCRH; Nandi County). Figure 1.

Sampling technique and participant enrolment

A hospital- and laboratory-based cross-sectional study design was employed, utilizing a consecutive sampling technique for participant enrolment. All eligible patients aged six months or older who presented with symptoms of acute respiratory infection at the sentinel sites between January 2022 and December 2022 were enrolled sequentially as they presented to the facilities. This ensured that the entire eligible patient population was captured.

Case definition

An acute respiratory infection (ARI) case was defined according to the World Health Organisation's (WHO) case definition as an individual with one or more of the following symptoms: fever, cough, sore throat, or respiratory distress within the last seven days[13].

Sample collection and processing

Blood samples were drawn from the patients into anticoagulant-free vacutainers and were allowed to clot by leaving them standing at room temperature for 2 hours. Then, centrifugation was done at 1500xg for 10 minutes to separate the serum. The serum samples were transported in a cold chain to the Sample Management and Receiving Facility (SMRF) at the KEMRI, where they were stored at -80°C until ready for processing.

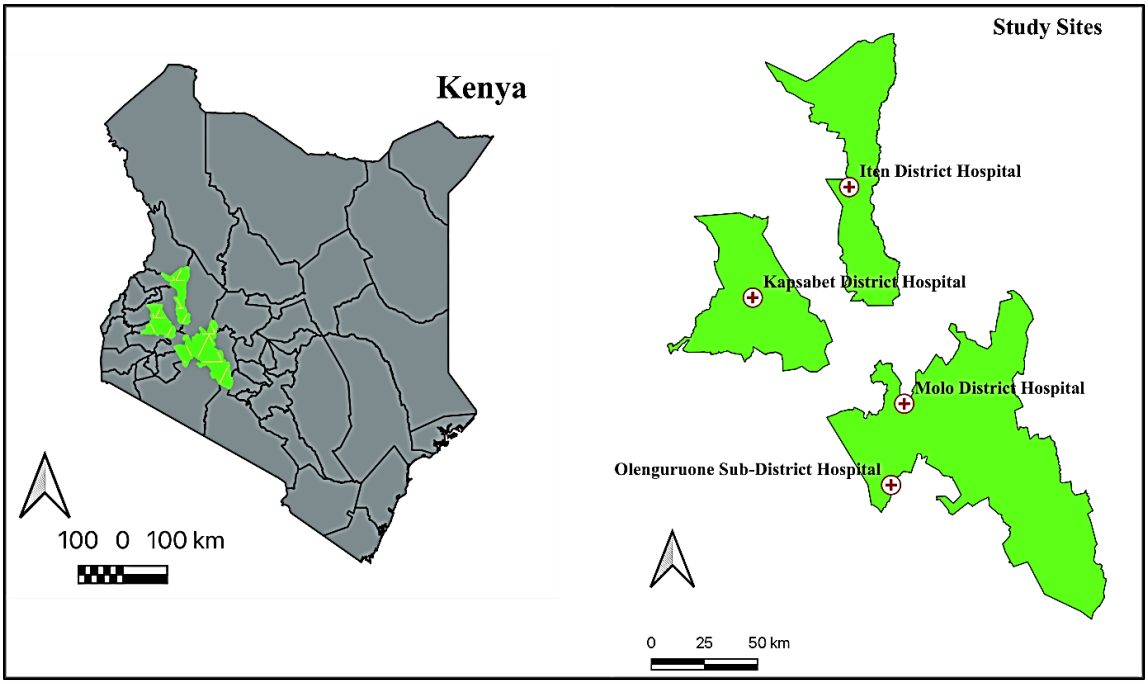

Figure 1
Map Showing Hospital-Based Sentinel Sites in Rift Valley, Kenya, January 2022-December 2022

Laboratory analysis

We used the Invitrogen Human SARS-CoV-2 spike IgG ELISA kit (Thermo Fisher Scientific, Waltham, USA) to determine the presence and quantify immunoglobulin G (IgG) antibodies against SARS-CoV-2 S protein in human serum. The assay utilises microtiter plate wells pre-coated with a trimerised Spike protein, which, due to its unique 3D structure, enhances the specificity of the assay by reducing the potential for cross-reactivity with antibodies generated against other coronaviruses. The test was done according to the manufacturer's protocols[14].

Briefly, the reagents and serum samples were brought to room temperature, and all the controls and buffers were reconstituted accordingly. For quality control of each assay run, manufacturer-provided positive and negative controls were systematically included. A two-fold serial dilution of the high positive control was used to prepare assay standards, with a range from 15000 units/ml to 234 units/ml. The wells were washed twice, and 90 µl of assay buffer was added, followed by 10 µl of standards, controls, or samples. The microtiter plate was covered with an adhesive strip and then incubated for 30 minutes at 37 °C. The plates were washed three times, after which 100 µl of HRP conjugate solution was added to each well and incubated for 15 minutes at 37 °C. The plates were washed three times, and 100 µl substrate solution was added and incubated at room temperature for 15 minutes. Finally, 100 µl of stop solution was added, and the optical density (OD) was measured at 450 nm on a microplate reader (Multiskan EX, Lab systems, Finland). The cut-off for positivity was determined by calculating the ratio of the patient sample's absorbance versus the calibrator's absorbance. The results were interpreted according to the manufacturer's instructions, where a ratio of <1.0 is considered negative, ≥1.0 to <1.3 is deemed indeterminate, and ≥1.3 is considered positive. Indeterminate results were retested. We utilised a linear regression model with OD values and corresponding antibody concentrations from serial dilutions to determine the Binding Antibody Units (BAU)/ml (BAU/ml) for the positive samples.

Data analysis

Socio-epidemiological data and laboratory results were merged in Microsoft Excel. Overall anti-SARS-CoV-2 IgG seroprevalence was calculated, with participants stratified by age: 0–18, 19–30, 31–40, 41–50, 51–60, and >61 years. Descriptive statistics were used to analyse data distribution across four facilities, accounting for demographic attributes such as age, gender, prior SARS-CoV-2 infections, and vaccination status. Binary logistic regression analysis was conducted to determine factors associated with SARS-CoV-2 seropositivity. The regression model included covariates such as prior infection history, vaccination status, and demographic characteristics. Statistical significance was defined as a p-value less than 0.05 for differences among variants. All analyses were performed using Stata software (version 18; StataCorp, 2023).

Ethical considerations and data collection

This study was conducted in accordance with the ethical standards outlined in the Declaration of Helsinki and received approval from the Kenya Medical Research Institute's (KEMRI) Scientific and Ethics Review Unit (SERU No: KEMRI/SERU/CVR/012/4126).

Informed written consent was obtained from all participants in English, Kiswahili, or the relevant local dialect. For adult respondents, consent was obtained directly, whereas for minors, written informed consent was provided by a parent or guardian. Additionally, children aged 13 to 17 years were asked to provide assent before participation, following parental consent. Socio-epidemiological data from each patient were recorded using a standardised questionnaire, including a unique identifier and demographics.

Results

This study included 557 participants (60.9% female, 39.1% male), mostly aged 19–30 or over 61. Seroprevalence was high at 97.8%, suggesting extensive prior SARS-CoV-2 exposure and the impact of vaccination coverage.

Vaccination coverage was 55.3% overall, with higher rates in males (58.3%) than in females (53.4%). Self-reported prior COVID-19 infection was low (7.0%), slightly higher in males (7.8%) than in females (6.5%). Table 1 provides the demographic summary.

ICRH enrolled the most participants (n=237), followed by Molo Level 4 Hospital (n=126), KCRH (n=103), and Olenguruone Level 4 Hospital (n=91) (Table 2). The distribution of study participants across the different sites by age group was heterogeneous, providing a good representation of persons of all ages in the population. ICRH primarily enrolled older adults (61+ years), while Molo Level 4 Hospital and KCRH mainly enrolled younger adults (19-30 years), and Olenguruone Level 4 Hospital predominantly enrolled middle-aged participants (41-50 years).

Vaccination coverage differed widely, ranging from 73.8% at Molo Level 4 Hospital to 31.9% at Olenguruone Level 4 Hospital (Table 3). At ICRH and Olenguruone Level 4 Hospital, there were about twice as many unvaccinated females as males, highlighting a gender disparity in vaccination coverage. This suggests differences in healthcare access, outreach, and health-seeking behaviour within each hospital's catchment area.

Table 1
Demographic Profiles of Study Participants by Gender, Age, Vaccination Status and Prior COVID-19 Infection, Rift Valley, Kenya, January 2022–December 2022

Demographic characteristics		Numbers (%)	Mean (Stdev)
	Male	218 (39.1)	
	Female	339 (60.9)	
	Total sample size	557	
Age groups	0-18	67 (12.0)	15.1 (3.4)
	19-30	127 (22.8)	25.2 (3.6)
	31-40	99 (17.8)	35.7 (2.9)
	41-50	96 (17.2)	46.1 (3.1)
	51-60	65 (11.7)	54.6 (2.6)
	61>	103 (18.5)	70.5 (7.9)
Vaccinated	Male	127(22.8)	
	Female	181(32.5)	
Prior COVID-19 infection	Female	22(4.0)	
	Male	17(3.1)	
Positivity	Negative	12 (2.2)	
	Positive	545 (97.8)	

Table 2
Distribution of Study Participants by Age Group and Health Facility in Rift Valley, Kenya, January 2022–December 2022

Age Groups	ICRH*	KCRH**	Molo	Olenguruone
0-18 (n=67)	28	14	15	10
19-30 (n=127)	52	25	35	15
31-40 (n=99)	32	24	23	20
41-50 (n=96)	40	14	16	26
51-60 (n=65)	32	7	16	10
61> (n=103)	53	19	21	10
All	237	103	126	91

*ICRH – Iten County Referral Hospital
**KCRH - Kapsabet County Referral Hospital

Self-reported previous COVID-19 infection rates were remarkably low across all health facilities (Table 4). No prior infections were reported among males at ICRH or among males and females at Olenguruone Level 4 Hospital. KCRH had slightly higher self-reported infection rates, but the overall low reporting contrasts with the observed high seroprevalence, indicating significant underreporting of past infections.

Table 3
Vaccination Coverage by Gender and Health Facility in Rift Valley, Kenya, January - December 2022.

	Vaccination			
	Female		Male	
	No (%)	Yes (%)	No (%)	Yes (%)
ICRH (n=237)	77(32.5)	74 (31.2)	41 (17.3)	45 (19)
KCRH (n=103)	21 (20.4)	29 (28.2)	15 (14.6)	38 (36.9)
Molo (n=126)	20 (15.9)	62 (49.2)	13 (10.3)	31 (24.6)
Olenguruone (n=91)	40 (44)	16 (17.6)	22 (24.2)	13 (14.3)

Table 4
Self-Reported Prior COVID-19 Infection Rates by Gender and Health Facility in Rift Valley, Kenya, January - December 2022

	Prior COVID-19 Infection			
	Female		Male	
	No (%)	Yes (%)	No (%)	Yes (%)
ICRH (n=237)	149 (62.9)	2 (0.8)	86 (36.3)	0 (0)
KCRH (n=103)	32 (31.1)	18 (17.5)	37 (35.9)	16 (15.5)
Molo (n=126)	80 (63.5)	2 (1.6)	43 (34.1)	1 (0.8)
Olenguruone (n=91)	56 (61.5)	0 (0)	35(38.5)	0 (0)

Table 5
Seropositivity of SARS-Cov-2 by Age Group and Health Facility in The Rift Valley, Kenya, January - December 2022

Facility	Age Groups	Negative	Positive	% Positivity prevalence
ICRH	0-18 (n=28)	1	27	96.4
	19-30(n=52)	0	52	100
	31-40(n=32)	0	32	100
	41-50(n=40)	0	40	100
	51-60(n=32)	1	31	96.9
	>61(n=53)	1	52	98.1
KCRH	0-18(n=14)	0	14	100
	19-30(n=25)	0	25	100
	31-40(n=24)	0	24	100
	41-50(14)	0	14	100
	51-60(n=7)	0	7	100
	61>(n=19)	0	19	100
Molo	0-18(n=15)	0	15	100
	19-30(n=35)	1	34	97.1
	31-40(n=23)	1	22	95.7
	41-50(n=16)	2	14	87.5
	51-60(n=16)	1	15	93.8
	61>(n=21)	2	19	90.5
Olenguruone	0-18(n=10)	0	10	100
	19-30(n=15)	0	15	100
	31-40(n=20)	1	19	95
	41-50(n=26)	1	25	96.2
	51-60(n=10)	0	10	100
	61>(n=10)	0	10	100

Seropositivity across age groups

Analysis of SARS-CoV-2 seroprevalence across different age groups revealed a high rate of seropositivity. Overall, 97.8% of participants across all age groups tested positive for SARS-CoV-2 antibodies. While the seroprevalence ranged from 96.9% to 99.2%, the observed differences between age groups were not statistically significant (Supplementary Table 1). The highest seropositivity rate (99.2%) was observed in the 19-30 age group, while the lowest rate (96.9%) was found in the 51-60 age group. Despite high seroprevalence across all age groups, one-way ANOVA revealed no significant differences in anti-S antibody titres between age groups, and this was confirmed by pairwise comparisons using Tukey's multiple comparisons test (Supplementary Figure 1). Analysis of seropositivity rates across individual study sites revealed some disparities (Table 5). KCRH exhibited 100% seropositivity across all age groups, while ICRH had a slightly lower rate (96.4%) among participants aged 0-18 years compared to the 100% rate at the other three sites.

Predictors of SARS-CoV-2 antibody seroprevalence

There was no significant gender difference in seropositivity, but vaccinated individuals had 3.33 times higher odds of SARS-CoV-2 antibodies. Prior infection also increased the odds of seropositivity. Most age groups showed similar rates, except those over 61, with significantly higher odds, likely due to prioritised vaccination. KCRH had the highest proportion of seropositive patients among the sites studied (Table 6).

Discussion

Understanding the true extent of SARS-CoV-2 exposure within a population is crucial for guiding public health strategies [7]. While routine testing identifies active infections, seroprevalence studies utilising anti-SARS-CoV-2 antibodies offer a broader picture of past exposure, including asymptomatic and undetected cases [15,16].

Table 6
Binary Logistic Regression for Factors Influencing SARS-Cov-2 Seropositivity

Predictors	Odds Ratio	95% CI	P-values
Gender			0.784
Male (Ref) vs Female	0.76	(0.10, 5.59)	
Age Group			
0 -18	Ref		
19-30	7.3448	(0.14, 386.33)	0.324
31-40	7.89	(0.04, 1868.54)	0.459
41-50	13.36	(0.01, 21352.24)	0.491
51-60	5.44	(0.00, 35775.08)	0.706
>61	26.62	(0.00, 5.04422E+06)	0.597
Age	0.95	(0.78, 1.17)	0.649
Elisa Quantification	1.0	(1.0, 1.0)	0.000
Prior SARS-CoV-2 Infection			
Yes vs No	3285.29	(0.00, 1.62E+248)	0.978
Vaccinated			
Yes vs No	3.33	(0.4, 27.87)	0.267
Health Facility			
ICRH	Ref		
KCRH	21939.78	(0.00, 6.49507E+175)	0.960
Molo	0.17	(0.01, 1.55)	0.116
Olenguruone	4.99	(0.08, 309.27)	0.445

Previous seroprevalence studies in Kenya, including nationwide surveys of blood donors, have offered insights into population-level immunity [10]. However, a significant knowledge gap remains, as these previous studies have largely excluded the demographically diverse Rift Valley region, characterised by varying healthcare access and unique socioeconomic conditions. This study provides sero-epidemiological insights into SARS-CoV-2 antibody prevalence and levels among symptomatic patients presenting at hospital-based sentinel sites in the Rift Valley region of Kenya.

A high seroprevalence of SARS-CoV-2 antibodies among patients who visited the hospital-based sentinel sites was observed, and this is consistent with similar studies worldwide, highlighting the widespread prevalence of SARS-CoV-2 [16]. Meta-analyses of standardised population-based studies show that the global seroprevalence of SARS-CoV-2 has risen considerably over time, with regional variation exceeding reported case numbers [6,17]. In a rural Ugandan cohort, seropositivity rose sharply following the Omicron wave, with most of the previously uninfected and unvaccinated individuals experiencing primary infection, while more than half of those with prior exposure showed evidence of probable reinfection. [18]. The high seroprevalence observed in this study may be partly explained by the timing of data collection following the Omicron wave, which was characterised by markedly increased transmissibility and substantial immune evasion, enabling infections even among previously immune individuals. Similarly, a study in Finland demonstrated a rapid increase in seroprevalence during the emergence of the Omicron variant, supporting this observation [19].

Despite the high seroprevalence, the study participants reported relatively low rates of prior COVID-19 infection, which likely reflects under-estimation, a common phenomenon in disease surveillance where many cases go undetected because they are asymptomatic or do not seek healthcare [20]. This is further compounded by limited testing resources and healthcare infrastructure in Africa [21,22]. Interestingly, further abroad, a study conducted in the USA revealed COVID-19 infections were likely three to twenty times higher than reported cases [23].

The high overall seropositivity rate suggests widespread exposure to SARS-CoV-2 across the study population, regardless of vaccination status. Consequently, this could have led to the elevated antibody titres, potentially masking differences induced by vaccination alone. It is therefore worth noting that hybrid immunity might generate robust antibody responses, further equalising antibody levels between groups[24]. If significant time has elapsed since infection or vaccination, antibody waning could contribute to the observed similarity in titres[25].

The study identified variations in vaccination coverage and self-reported infection rates across different study sites. While natural infection played a role in generating immunity among the study population, vaccination also likely contributed significantly. Contrasting patterns in vaccination status highlight potential inequalities in vaccine access, availability, and uptake. Studies have linked lower vaccination rates in some communities to vaccine hesitancy fueled by misinformation or distrust of public health authorities [26–28]. Olenguruone, the most rural of the sentinel sites, exhibited the lowest vaccine coverage, which reflects a broader pattern of vaccine inequity observed in other studies. Rural or remote communities frequently face logistical barriers, including distribution challenges, limited cold-chain storage capacity, or healthcare provider shortages, hindering access to essential vaccines [29,30].

Vaccination played a pivotal role, as vaccinated individuals had significantly higher odds of possessing antibodies against SARS-CoV-2. Evidence from a longitudinal study in the UK further underscores this effectiveness, showing that vaccination-induced antibody responses substantially reduce the risk of

subsequent COVID-19 infection[31], highlighting the effectiveness of vaccination in protecting populations [29]. Individuals over 61 years demonstrated significantly higher odds of SARS-CoV-2 seropositivity than other age groups, potentially highlighting the age-prioritised vaccination strategy, where older adults were among the first to receive vaccines [32].

Enrolment disparities between sites and distinct age-group preferences likely reflect differences in demographic engagement, service specialisation, and other access-related factors. Level 4 facilities, often located in rural areas, serve a localised population, while referral hospitals, typically found in urban centres, draw patients from a wider region and focus on providing specialised care. Access-related factors can create significant barriers for individuals from lower-income communities or specific age groups due to limited resources and transportation challenges [33,34]. Furthermore, the type of healthcare facility can influence enrolment patterns [35,36].

Conclusion

This study is subject to a few limitations. Recruiting only symptomatic individuals from hospital-based sentinel sites may introduce bias into the study findings, potentially skewing the seroprevalence estimates. Reliance on self-reported vaccination status and prior COVID-19 infection introduces the possibility of recall bias. ELISA, though valuable for detecting SARS-CoV-2 antibodies, may yield false positives due to cross-reactivity with other coronaviruses [37].

Additionally, the limited geographic scope in the Rift Valley restricts generalizability to other regions.

The high seroprevalence observed in this study starkly contrasts with reported COVID-19 cases, suggesting a significant underestimation of infections. Differences in vaccination rates and infection levels between study sites highlight potential disparities in vaccine access and uptake, indicating a need for targeted efforts to improve vaccination in rural and remote regions. Ensuring equitable vaccination and overcoming barriers to uptake are essential for wider vaccination coverage and mitigating future transmission.

Acknowledgements

We thank the study participants and sentinel site staff who supported this work. We also thank the county public health departments of the respective counties. This manuscript is published with the approval of the Director, KEMRI.

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Source of funding. This work is supported by a grant from Pfizer Global Medical Grants, Grant ID: 61773471. The funder had no role in the study design, implementation, decision to publish, or manuscript preparation.

Data availability statement. The data that support the findings of this study are available from the corresponding author, upon reasonable request and with approval from the KEMRI Data Governance Committee.

Conflicts of interest. The authors declare that they have no conflicts of interest.

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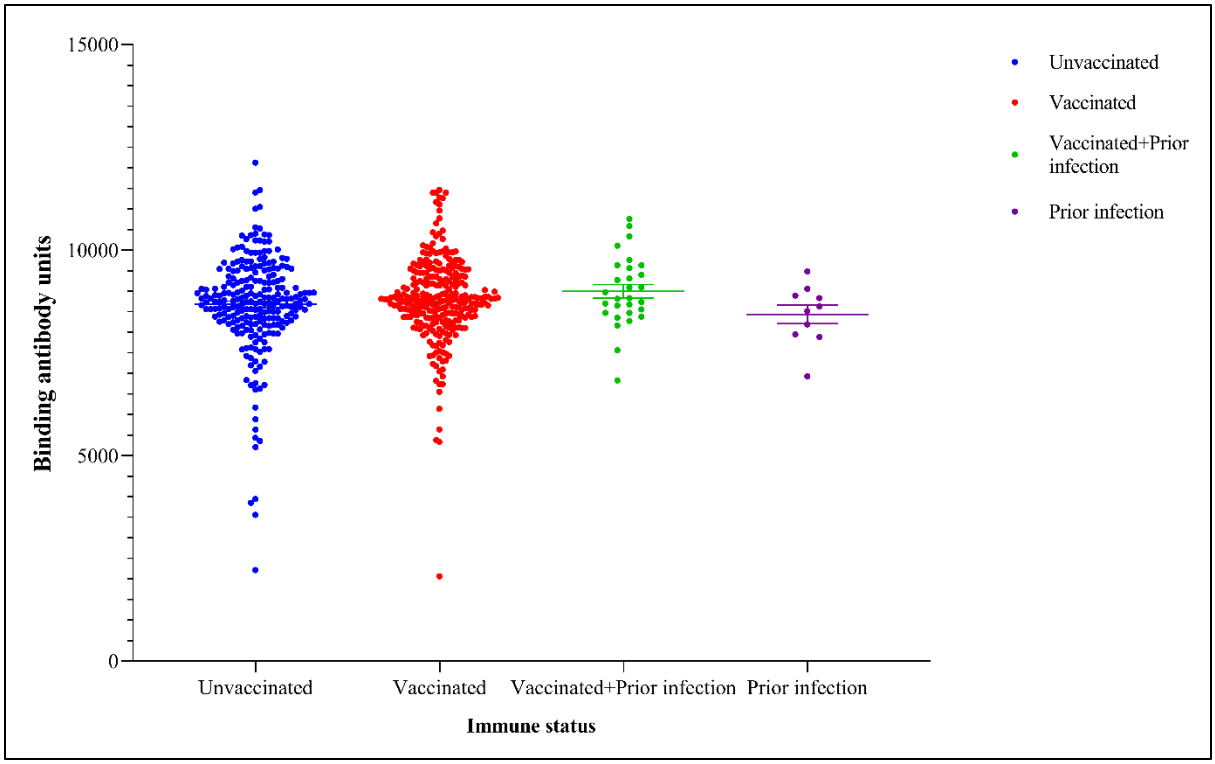
Supplementary Materials:

Supplementary Table 1

Age-Specific COVID-19 Seroprevalence in the Rift Valley, Kenya, January - December 2022. This Table Presents Data on the Prevalence of COVID-19 Antibodies among Different Age Groups in the Study.

Age Groups	Negative	Positive	Prevalence (%)
0-18 (n=67)	1	66	98.5
19-30 (n=127)	1	126	99.2
31-40 (n=99)	2	97	98
41-50 (n=96)	3	93	97
51-60 (n=65)	2	63	96.9
>61 (n=103)	3	100	97.1
All	12	545	97.8



Supplementary Figure 1:

A Graphical Comparison of Anti-S Antibody Titres (BAU/Ml) Across Different Vaccination and Infection Statuses.