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Seroprevalence of anti-SARS-CoV-2 IgG antibodies in Kenyan blood donors

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The spread of SARS-CoV-2 in Africa is poorly described. The first case of SARS-CoV-2 in Kenya was reported on March 12, 2020 and an overwhelming number of cases and deaths were expected but by July 31, 2020 there were only 20,636 cases and 341 deaths. However, the extent of SARS-CoV-2 exposure in the community remains unknown. We determined the prevalence of anti-SARS-CoV-2 IgG among blood donors in Kenya in April-June 2020. Crude seroprevalence was 5.6% (174/3098). Population-weighted, test-performance-adjusted national seroprevalence was 4.3% (95% CI 2.9–5.8%) and was highest in urban counties, Mombasa (8.0%), Nairobi (7.3%) and Kisumu (5.5%). SARS-CoV-2 exposure is more extensive than indicated by case-based surveillance and these results will help guide the pandemic response in Kenya, and across Africa.

Africa accounts for 17% of the global population (1) but by late July 2020 accounted for only 5% of the global COVID-19 cases and 3% of global COVID-19 deaths reported (2). This disparity has been attributed to limited capacity for diagnosis, timely implementation of stringent containment measures, a younger population structure and a predominance of asymptomatic and mild infections (3, 4). The first case of COVID-19 in Kenya was detected on March 12, 2020. Within one week the government instituted containment measures to limit the spread of the virus (5). By July 31, national surveillance recorded 20,636 cases and 341 deaths (6). This increase in cases is notably slower than the epidemic in Wuhan, Europe or the USA. Recently, it has been suggested that “the virus is spreading... ..with an attenuated outcome in Africa” but there are few data available to confirm or refute this assertion (7).

In countries affected early in the pandemic, serological surveillance was used to define cumulative incidence. For example, at the release of lockdown in Wuhan, 9.6% of staff resuming work were found to have anti-SARS-CoV-2 antibodies (8). At the end of the epidemic wave in Spain, seropositivity was 5.0% in a random population sample of 60,897 (9). As the epidemic curve declined in Geneva,

seroprevalence rose over three weeks from 4.8% to 10.9% (10). Currently, there are few estimates of SARS-CoV-2 seroprevalence in Africa in the literature (11).

Movement restrictions, in response to COVID-19, have limited the conduct of fieldwork for population-based serosurveys. Several countries have monitored seroprevalence in blood transfusion donors (12, 13) or expectant mothers attending ante-natal clinics (14). Here we report the results of a pragmatic national serosurvey using residual blood samples from transfusion donors across Kenya and a highly sensitive and specific assay for anti-SARS-CoV-2 spike immunoglobulin G (IgG).

We validated a widely-used enzyme linked-immunosorbent assay for SARS-CoV-2 IgG (15) with 910 serum samples from the pre-pandemic period and 174 sera from polymerase chain reaction (PCR) defined SARS-CoV-2 cases, and a well-characterized 5 sera panel from the National Institute of Biological Standards and Control (NIBSC) in the UK. For either receptor-binding domain (RBD) or whole spike, specificity was higher when using a ratio of the sample optical density (OD)/negative control OD than when using the raw sample OD plus 3 standard deviations to define seropositivity (table S1). By using OD ratios, both RBD and spike

ELISAs correctly classified 901 of 910 pre-pandemic samples as seronegative (table S1). However, the spike ELISA detected more seropositives (166 of 179 vs compared to 145 of 179 for RBD ELISA) among sera from SARS-CoV-2 PCR-positive individuals (fig. S2, A and B). Based on these data, we defined anti-SARS-CoV-2 IgG seropositivity as an OD ratio >2 and selected the spike ELISA for this study. The sensitivity and specificity, at this threshold, were 92.7% (95% CI 87.9-96.1%) and 99.0% (95% CI 98.1-99.5%), respectively (figs. S3, A and B, S5, and S6; and table S1). As previously noted (15), the RBD and whole spike ELISA responses were highly correlated (fig. S3C), with very little inter-assay variation (fig. S4).

A total of 3,174 blood transfusion samples were collected from four Kenya National Blood Transfusion Service (KNBTS) regional blood transfusion centers that are supported by several satellites and hospitals between April 30 and June 16, 2020, from individuals aged 15-66 years. Approximately half of the samples were drawn in Mombasa; the remainder were evenly distributed between Nairobi, Kisumu and Eldoret (Fig. 1 and table S2). We excluded 18 duplicate samples, 56 records missing data on age or collection date and two records from individuals aged ≥ 65 years. Policy in Kenya is to avoid blood donation from individuals >65 years, and we excluded these other data points as potentially unreliable. These exclusions left 3,098 samples for further analysis (Fig. 1).

Of the 3,098 samples, 174 were positive for anti-SARS-CoV-2 Spike IgG giving a crude seroprevalence of 5.6% (95% CI 4.8-6.5%). Crude seroprevalence varied by age ($P = 0.046$), ranging between 3.4-7.0% among adults 15-54 years; all 71 donors aged 55-64 years were seronegative (Table 1). Crude seroprevalence did not vary by sex ($P = 0.50$) but did vary geographically, from 1.9% in the Rift Valley region to 10.0% in the Western region ($P = 0.002$, Table 1).

Compared to the 2019 Kenya Population and Housing Census, our participants were more commonly male (82.0% in our study vs 49.3% in the census), had more persons aged 25-34 years (40.1% vs 27.3%) and more residents of coastal Counties (49.2% versus 9.1%, Table 2). We therefore adjusted the prevalence estimate for the demographics of the sample using post-stratification, and for the sensitivity and specificity of the test.

The Bayesian population-weighted and test-adjusted seroprevalence for Kenya was 4.3% (95% CI 2.9-5.8%, Table 1) and the posterior sensitivity and specificity estimates were 92.4% (95% CI 88.0-95.6%) and 98.9 (95% CI 98.2-99.5%), respectively. Seroprevalence was higher (4.2-5.2%) in the younger age groups (15-44 years) and declined in the older age groups (45-64 years) but was similar for both sexes. Seroprevalence was highest for those living in Mombasa, Nairobi and the Western region, although the number of observations for the Western region was small. The directly standardized

seroprevalence estimates are presented in table S3. Seroprevalence was also calculated for Counties that had at least 120 donors sampled. The three largest urban Counties of Mombasa, Nairobi, and Kisumu had SARS-CoV-2 seroprevalence of 8.0% (95% CI 5.5-11.1%), 7.3% (95% CI 4.2-11.4%) and 5.5% (95% CI 2.8-9.6%), respectively (table S4).

The frequency of blood donor sampling and crude seroprevalence estimates increased with time over the 7-week study period (Fig. 2). The median sample date was May 30, 2020 while the mid-point of the study was May 24, 2020. We did not adjust for sample date because the period of sampling varied for residents of different counties (Fig. 2C); instead we show the variation in crude prevalence over time (Fig. 2A).

Voluntary non-remunerated donors (VNRDs), who donate blood at community-based 'blood drives' comprised only 7.6% (236/3098) of our sample of donors; the remainder were family replacement donors (FRDs) who provide a unit of blood in compensation for a transfusion received by a sick relative. The two groups did not differ significantly by age ($P = 0.15$) or sex ($P = 0.51$, table S5). Crude seroprevalence was 8.5% (20/236) for VNRDs and 5.4% (154/2862) for FRDs. The median sample date for VNRDs (June 14, 2020) was two weeks later than that for FRDs (May 29, 2020).

Population exposure across Kenya, with a population-weighted test-adjusted seroprevalence of 4.3%, is considerably higher than was previously thought, based on the cases and deaths reported to date. Seroprevalence was particularly high in the three urban counties; Mombasa (8.0%), Nairobi (7.3%) and Kisumu (5.5%). Consistent with other studies, seroprevalence did not vary significantly by sex; (9, 10, 16) however, it peaked in 35-44-year-olds and was lowest for those ≥ 45 years, which is also consistent with existing reports where seroprevalence was found to be lower in older adults (9, 10).

SARS-CoV-2 seroprevalence in our study is comparable to estimates from large population-based serosurveys in China, Switzerland, Spain and the USA after the initial epidemic peak and following many tens of thousands of deaths (9, 10, 17, 18). Our results are also comparable to other surveys of blood donors in Brazil (13), Italy (12), and many parts of England (19). Kenya has an estimated population of 53 million in 2020 and 57% of the population is aged 15-64 years. If the transfusion donor seroprevalence of 4.3% was applied to all 15-64-year-olds it would suggest approximately 1.3 million infections. However, by the median sample date, May 30, 2020, only 2093 cases had been detected (of which approximately 90% were asymptomatic) and 71 deaths among all ages (6). Although it is difficult to extrapolate our data directly to the whole population, they do strongly suggest that the infection is more widespread in Kenya than the current PCR test results suggest and indicate a need for more systematic testing. The current PCR testing strategy targets symptomatic

individuals, health care workers, contacts of confirmed cases, international travelers, cross border truck drivers and residents of areas identified as hot spots.”

What are the potential explanations for the divergence in the ratio of observed cases or deaths to serologically defined infections inferred from transfusion donors in Kenya, compared to many high-income countries? (i) The seroprevalence could be over-estimated because of bias in the selection or behavior of blood transfusion donors. (ii) Cases could be under-ascertained by national public health surveillance though it seems unlikely that reporting of deaths and severe cases could be reduced by several orders of magnitude, and hospitals in Kenya were not overwhelmed by admissions with respiratory illness. (iii) The steep demographic age-pyramid results in a smaller vulnerable age group. In Kenya, only 3.9% of the population is aged 65 years or greater which is substantially less than, for example, 23.3% found in Italy; again, this would only explain a several-fold reduction in severe cases or deaths (4). (iv) There may be alternative mechanisms of immunity to SARS-CoV-2 including cell-mediated immunity (20, 21) perhaps as a result of HCoV-elicited immunity (22, 23). Despite our prior work showing HCoVs circulate in Kenya (24), we did not identify evidence of cross-reactive antibodies to endemic coronaviruses in our validation study.

Although blood donors are not representative of the Kenyan population as a whole, we adjusted for demographic bias in the sample structure by standardization against the age, sex, and regional distribution of the Kenyan population. A substantial proportion (43%) of the population of Kenya is outside the age-range (15-64 years) sampled in this study and the seroprevalence in children <15 years and adults >65 years is often lower (9, 10); our estimate for blood donors may be higher than the estimate for the population as a whole. Blood donors also differ from the general population in their risk of exposure to SARS-CoV-2. For instance, potential donors are excluded from giving blood if they have been ill during the last six months so the sample may underestimate the population prevalence of SARS-CoV-2 antibodies; on the other hand, people who are shielding at home are unlikely to be captured in our sample leading to an overestimate of seroprevalence. Our exploration of the two distinct populations of blood donors, FRDs and VNRDs, suggests variation in the seroprevalence by donor group but, of note, 92% (2862/3098) of our sample came from the group with lower seroprevalence and exclusion of VNRDs reduced the crude seroprevalence in our study little, from 5.6% to 5.4%. Against these considerations, other countries have relied on blood transfusion donors for an early estimate of seroprevalence but later estimates from random population samples have not been substantially different (25, 26).

A key strength of this study is the rigorous validation that included testing positive and negative control samples from

the target population, as well as reference plasma from the UK NIBSC as part of a WHO-coordinated effort on SARS-CoV-2 seroepidemiology. In addition, we adopted a conservative seropositivity threshold to optimize assay specificity and sensitivity for our setting.

The pandemic response in countries with limited health care capacity has been driven by the aggressive implementation of control measures to limit transmission. Unfortunately, this strategy has been accompanied by enormous collateral costs, particularly in Africa. Modeled estimates of the disruptions of essential medical services, such as immunization and antenatal care, suggest an additional ~253,500 child deaths and 12,200 maternal deaths over six months in low and middle-income countries (27). In the absence of social protection, the economic effects of lockdown are debilitating so it is important to obtain an early measure of the trajectory of the epidemic.

Our study provides a national and regional estimate of population exposure to SARS-CoV-2 in an African country. The 4.3% prevalence in blood transfusion donors is in sharp contrast with the reported COVID-19 cases and deaths and supports the impression that disease may be attenuated in Africa (7).

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SUPPLEMENTARY MATERIALS

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Materials and Methods

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Fig. 1. Participant flow diagram for SARS-CoV-2 seroprevalence study of blood donors in Kenya. Exclusion criteria for the selection of samples with complete data.

Fig. 2. Timeline of sampling for SARS-CoV-2 seroprevalence in blood donors in Kenya. Against the timeline of the sampling period, panel A shows the weekly crude seroprevalence and 95% confidence interval, panel B shows the daily frequency of samples collected and panel C shows the temporal distribution of samples by region. Proportion, counts and regional distribution of donors during the study period.

Table 1. Crude, population-weighted, and test performance-adjusted SARS-CoV-2 anti-spike protein IgG seroprevalence by participant characteristics and regions. Prevalence estimates calculated using multilevel regression and post-stratification (MLRP) to account for differences in the sample population and the national population, subsequently adjusted for assay sensitivity and specificity.

	All samples	Seropositive samples	Crude seroprevalence		Kenya population (2019 Census)	Bayesian population-weighted seroprevalence*		Bayesian population-weighted, test-adjusted seroprevalence*	
			%	(95% CI)		%	(95% CI)	%	(95% CI)
Age									
15 – 24 years	808	49	6.1	4.5 – 7.9	9,733,174	5.1	3.7 – 6.9	4.4	2.7 – 6.4
25 – 34 years	1242	66	5.3	4.1 – 6.7	7,424,967	4.9	3.6 – 6.4	4.2	2.8 – 6.0
35 – 44 years	714	50	7.0	5.2 – 9.1	4,909,191	5.9	4.3 – 8.1	5.2	3.3 – 7.7
45 – 54 years	263	9	3.4	1.6 – 6.4	3,094,771	3.8	1.9 – 6.0	3.0	1.1 – 5.4
55 – 64 years	71	0	0		1,988,062	3.4	0.7 – 6.2	2.9	0.7 – 5.7
Sex									
Male	2540	146	5.7	4.9 – 6.7	13,388,243	4.4	2.9 – 6.2	3.6	1.9 – 5.8
Female	558	28	5.0	3.4 – 7.2	13,761,922	5.5	4.4 – 6.8	4.8	3.5 – 6.4
Regions									
Central	105	7	6.7	2.7 – 13.2	3,452,213	5.6	2.9 – 10.0	4.9	1.9 – 9.7
Mombasa	550	51	9.3	7.0 – 12.0	792,072	8.3	6.1 – 10.9	7.8	5.4 – 10.8
Other Coast	973	39	4.0	2.9 – 5.4	1,671,097	3.7	2.6 – 5.1	2.9	1.6 – 4.6
Eastern / N. Eastern	242	11	4.5	2.3 – 8.0	5,176,080	4.3	2.5 – 7.0	3.5	1.4 – 6.6
Nairobi	235	21	8.9	5.6 – 13.3	3,002,314	7.6	4.9 – 11.2	7.1	4.2 – 11.2
Nyanza	442	30	6.8	4.6 – 9.5	3,363,813	6.0	4.2 – 8.4	5.2	3.1 – 7.9
Rift Valley	481	8	1.7	0.7 – 3.3	7,035,581	2.1	1.1 – 3.6	1.5	0.4 – 3.1
Western	70	7	10.0	4.1 – 19.5	2,656,995	7.0	3.5 – 13.1	6.3	2.5 – 13.1
Total	3,098	174	5.6	4.8 – 6.5	27,150,165	4.9	3.9 – 6.2	4.3	2.9 – 5.8

*Reweighted prevalence estimates based on demographic data from the 2019 Kenya Population and Housing Census.

Table 2. General characteristics of the study population compared to the national population of Kenya. N is the number of individuals in each stratum.

		Blood transfusion samples		Kenya National Census 2019	
		N	%	N	%
Age	15-24 years	808	26.1	9,733,174	35.8
	25-34 years	1,242	40.1	7,424,967	27.3
	35-44 years	714	23.0	4,909,191	18.1
	45-54 years	263	8.5	3,094,771	11.4
	55-64 years	71	2.3	1,988,062	7.3
Sex	Male	2540	82.0	13,388,243	49.3
	Female	558	18.0	13,761,922	50.7
Regions	Central	105	3.4	3,452,213	12.7
	Mombasa	550	17.8	792,072	2.9
	Other Coast	973	31.4	1,671,097	6.2
	Eastern / N. Eastern	242	7.8	5,176,080	19.1
	Nairobi	235	7.6	3,002,314	11.1
	Nyanza	442	14.3	3,363,813	12.4
	Rift Valley	481	15.5	7,035,581	25.9
	Western	70	2.3	2,656,995	9.8
Total	Kenya 15-64 years	3098		27,150,165	