

## Dynamics of SARS-CoV-2 exposure in Malawian infants between February 2020 and May 2021



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### ABSTRACT

**Background:** Very limited information is available on SARS-CoV-2 seroprevalence in infants in sub-Saharan countries.

**Objective:** In this study, we aimed to determine the rate and the temporal evolution of SARS CoV-2 seropositivity in breastfed Malawian infants.

**Study design:** Blood samples ( $n = 250$ ) from 158 infants, born to HIV-negative women and women living with HIV, collected from February 2020 to May 2021, were first tested using an Anti-IgG/A/M SARS CoV 2 ELISA assay against trimeric spike protein, and then, if positive, confirmed using a second ELISA assay detecting IgG against Receptor Binding Domain.

**Results:** The confirmed prevalence of anti-SARS CoV-2 antibodies was 31.0% (95% CI: 23.7%-38.3%) with no significant difference between HIV-exposed and HIV-unexposed infants (29.3% and 37.1% respectively,  $P = 0.410$ ). The presence of anti-SARS-CoV-2 IgG was not associated with maternal socioeconomic or demographic indices.

**Conclusions:** Our data underline the wide spread of the SARS-CoV-2 infection in the pediatric population in sub-Saharan Africa. Design of more specific serological tests for African samples and improvements in serosurveillance programs are needed for more rigorous monitoring of the dynamics of SARS-CoV-2 infection in Africa.

### 1. Background

The epidemiology of SARS-CoV-2 in sub-Saharan Africa is still poorly defined in terms of the number of infections and COVID-related deaths. At the end of January 2022, the African continent accounted for 2.4% of the global cases and 2.9% of the global deaths [1], with large variations across different regions. The lack of accountable data on SARS-CoV-2 seroprevalence in most of sub-Saharan Africa is a critical issue at a global level. Recent reviews of serological studies in African countries revealed that the pooled prevalence of anti-SARS-CoV-2 antibodies was around 20% with very high heterogeneity, ranging from 0 to 63% [2,3]. The different socio-demographic characteristics of the populations studied and the different efficiency in the surveillance systems between countries may be responsible for the marked differences in the seroprevalence rates. However, the reported data show a wide spread of SARS-CoV-2 in

Africa, despite a lower burden of morbidity and mortality compared to all other continents [4].

Most of the studies on SARS-CoV-2 seroprevalence in Africa have been performed in specific community groups such as blood donors, health workers, police forces and hospitalized patients [5–13]. Additional information on seroprevalence within particular groups could therefore be relevant. Only a few studies reported data from infants, who are a population poorly studied, mostly due to the lack of symptoms of SARS-CoV-2 infection in the young population [14]. Here we present data from Malawian infants (from 6 weeks of age to 12 months), whose blood was collected between February 2020 and May 2021, during a longitudinal study aimed to define the health status of HIV-exposed and -unexposed infants.

To date, no clear evidence of a higher incidence of SARS-CoV-2 infection among people living with HIV (PLWH) compared to the general

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population, has been observed in large population-based studies [15–18]. However, some studies have reported that the lack of HIV viral suppression seems to negatively impact the neutralizing immune response to SARS-CoV-2 variants [19].

In Malawi policies of SARS-CoV-2 infection containment have been activated from the end of March 2020 (restriction of public gatherings and events, closures of schools) [20]; nevertheless, at present less than 3% (about 550.000), over a population of 19 million, has been tested, resulting in a SARS-CoV-2 prevalence of about 12% [21].

**Objective:** In this context, the present study aims to determine the rate and the temporal trends of SARS-CoV-2 seropositivity among breastfed infants. We do believe that these data can help in defining the SARS-CoV-2 spread in a representative part of the population in Malawi, a country where the annual crude birth rate is 33,42 births per thousand population, and 0–4 years old infants represent 14.3% of the entire population [22].

## 2. Study design

### 2.1. Population characteristics

The present sub-study is nested in a larger study (started in January 2019, before the beginning of the SARS-CoV-2 pandemic, aimed to investigate the impact of maternal HIV infection and ART on exposed infants under Option B+ in Malawi and to assess the factors influencing maternal retention in care. The study also includes a cohort of HIV-negative women and their infants as a control group to compare the morbidity, mortality and immunological parameters of the infants.

The two cohorts of HIV-infected mothers and their infants (HIV-exposed uninfected, HEU) and HIV-negative women with their unexposed infants (HIV-unexposed uninfected, HUU) were followed with monthly visits from delivery until 12 months postpartum. All women (> 18 years of age) were enrolled during pregnancy, between 32 and 36 weeks of gestation, at three different antenatal Clinics: an urban site in Mandala, and two semi-urban sites in Chileka, and Machinjiri, all in the Blantyre area. HIV positive (or negative) test documentation was confirmed at enrollment when demographic, clinical, and socioeconomic information was collected. Birth weight and birth length for newborns were measured within 15 days of delivery.

### 2.2. Study samples

All the infants ( $n = 158$ , HEU=123, HUU =35) with samples collected after February 1, 2020, were included. Not all the infants had samples collected at the three time points (6 weeks, 6 and 12 months of age), and a total of 250 blood samples were available for this study. At 6 weeks blood was collected by locally trained people from the plantar surface of the infants' heel, and Dry Blood Spots (DBS) were prepared and stored at  $-20^{\circ}\text{C}$ . At 6 and 12 months, blood was collected from peripheral veins, and serum samples were stored at  $-80^{\circ}\text{C}$ . All samples were shipped in dry ice to the Laboratory of the National Center for Global Health of the Istituto Superiore di Sanità, where the DBS and serum samples were stored at  $-20^{\circ}\text{C}$  and  $-80^{\circ}\text{C}$  respectively, until processing.

### 2.3. Historical samples

A high prevalence of pre-existing serological cross-reactivity against SARS-CoV-2 has been observed in many studies conducted in sub-Saharan countries [23,24]. To exclude the cross-reactivity to SARS-CoV-2 antigens in the samples of the present study, a preliminary serological study on 95 African samples collected in the pre-pandemic era (2008–2009) and stored at  $-80^{\circ}$  in our facilities, was performed. Pre-pandemic negative control plasma samples were obtained from Malawian HEU infants enrolled in an observational study aimed to assess the safety and efficacy of antiretroviral therapy in Malawian pregnant women living

with HIV, (SMAC study, ethics approval n. 486, by the National Health Research Committee of Malawi) [25].

### 2.4. Enzyme-Linked immunosorbent assays for SARS-CoV-2

DBS elution was performed as previously described [26]. To minimize false-positive results, in this study the analysis of the 250 available samples (46 dried blood spots and 204 sera) was performed in 2 steps. In the first step, all samples were tested using the Human Anti-IgG/A/M SARS-CoV-2 ELISA assay, (The Binding Site Group Ltd, Birmingham, UK) against SARS-CoV-2 trimeric spike protein. Briefly, all serum samples were diluted 1:40 with sample buffer diluent and processed according to the manufacturer's instructions. The samples were considered positive when the result was  $\geq 1$  [calibrator coefficient  $\times$  (OD sample/OD calibrator cut-off)]. In the second step, all positive samples were re-run using the COVIDSeroIndex, Kantaro SARS-CoV-2 IgG Antibody RUO Kit (R&D Systems, Bio-Techne, Minneapolis, MN, USA), which targets the Receptor Binding Domain (RBD). According to the manufacturer's instructions, all samples with a cut-off index  $> 0.7$  (OD sample value/OD positive control value) were considered positive.

### 2.5. Statistical analysis

The SPSS software, version 27 (IBM Corp, 2017, Armonk, NY, USA) was used for statistical analyses. Results are presented as medians with interquartile range (IQR) and percentages. Demographic and socioeconomic differences between SARS-CoV-2 seropositive and seronegative infants were evaluated using the  $\chi^2$  test or the Fisher's exact test when appropriate for categorical variables, and by the Mann-Whitney U test for quantitative variables.

### 2.6. Ethical considerations

Ethical approval was obtained from the National Health Research Committee in Malawi (approval number #2085), and informed consent was obtained from all women attending the Ante-Natal Clinics.

## 3. Results

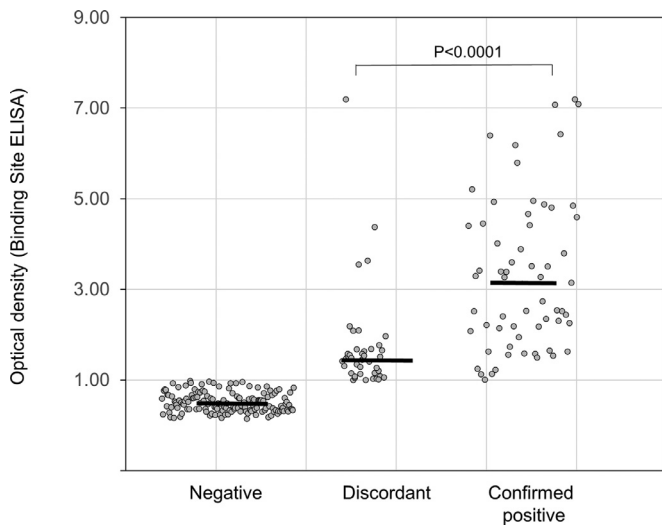
### 3.1. ELISA assays for the detection of anti-SARS-CoV-2 antibodies

Among the 95 historical samples analyzed with the assay for the detection of anti-IgG/A/M against the trimeric spike protein (The Binding Site Company), 8 (8.4%) tested positive. Considering all the historical samples as "true negative" to SARS-CoV-2, the specificity of this assay was 87.96%. The 8 positive samples were re-run in the second assay for IgG against the RBD protein, and all resulted negative.

Based on these premises, we used the same strategy for all the 250 study samples, first testing for anti-IgG/A/M against the trimeric spike protein (The Binding Site ELISA assay) and, if positive, re-testing with the second assay targeting RBD. One hundred and three samples (41.2%) were positive with the first ELISA assay. Of those, only 59.2% of samples (61/103) were confirmed positive with the second assay against the RBD protein, while the others (42/103, 40.8%) were negative. There was large variability in the OD values of the samples positive with the first assay, ranging from 1.00 to 7.20. However, the samples which were then confirmed positive with the second assay had a median OD of 3.15 (IQR: 2.01 - 4.53), significantly higher than the median OD of 1.46 (IQR: 1.14 - 1.67,  $p < 0.0001$ ), of the samples not confirmed in the second assay. The 147 negative samples a median OD of 0.48 (IQR 0.34 - 0.65) (Fig. 1).

### 3.2. Population characteristics

At enrollment, the median age of the 158 pregnant women was 30.3 (IQR: 24.0 - 33.0) years. Ninety-three mothers (58.9%) were living in a



**Fig. 1.** Optical density of the 250 samples from 158 infants tested by the Binding Site ELISA targeting trimeric SARS-CoV-2 spike. The samples are categorized as false positive or confirmed positive on the basis of the results obtained with the second ELISA assay (R&D Seroindex ELISA).

**Table 1**

Demographic characteristic of mothers at enrollment and neonatal profile at birth. Values are expressed as median and IQR or number and percentage.

Enrollment (36 weeks of pregnancy)	
N	158
Age (years)	30.3 (24.0 - 33.0)
BMI women	25.5 (23.9 - 28.4)
Educational levelNone/primary/secondary or higher	5/65/88 (3.2/41.1/55.7)
EmploymentUnemployed-housewife/employed	106/52 (67.1/32.9)
Residency:Urban/semirural/rural (%)	41/93/24 (25.9/58.9/15.2)
Electricity at home (yes/no,%)	75/83 (47.5/52.5)
Water at home (yes/no,%)	88/70 (55.7/44.3)
HIV status (positive/negative)	123/35 (77.8/22.1)
At delivery	
Vaginal delivery (n,%)	138 (87.3)
Neonatal weight (Kg)	3.6 (3.3 - 4.0)
Sex (M/F)	79/79 (50.0/50.0)

semirural environment, and almost half of the women had access to water ( $n = 88, 55.7\%$ ) and electricity ( $n = 75, 47.5\%$ ). Most of the women were HIV-positive ( $n = 124, 78.5\%$ ), all receiving antiretroviral therapy from the diagnosis of HIV infection (median 2.0 years, IQR: 0.0 - 7.0). Vaginal delivery was the most common delivery mode ( $n = 138, 87.3\%$ ). One hundred and six (67.1%) births occurred before February 2020, and only 52 pregnancies ended during the pandemic. All infants born from HIV+ women were negative for HIV infection (HEU infants). All the mothers exclusively breastfed their infants until month 6, when 95% of the infants were introduced to mixed feeding (maternal milk

**Table 2**

Prevalence of SARS-CoV-2 IgG in the 158 infants involved in the longitudinal study during the first three semesters of the COVID-19 pandemic, (from February 2020 to May 2021). The values are expressed as numbers, percentages and 95%CI.

Time of blood sampling	Prevalence of SARS CoV-2		Infants age at the time of analysis		
	N. positive/N. tested	% (95%CI)	6 weeks	6 months	12 months
Feb 2020 - May 2021	49/158	31.0% (23.7 - 38.3)	0/14	25/54	24/90
Feb - Jun 2020	17/64	26.6% (15.4 - 37.7)	0/12	7/20	10/32
July - Dec 2020	26/76	34.2% (23.3 - 45.1)	0/2	16/32	10/42
Jan - May 2021	6/18	33.3% (9.2 - 57.5)	0/0	2/2	4/16

and additional food) which was continued until 12 months in 98% of infants.

The study was affected by the global COVID pandemic, and a high rate of dropouts was observed. Of the 158 infants included in this study, 20 infants had completed the follow-up, having available samples from the three-time points (6 weeks, 6, and 12 months). Fifty-two infants had samples available from two-time points, and 86 infants had only 1 sample available. To determine the seroprevalence rate in the 158 infants, in the case of longitudinal determinations we considered for each infant the last negative sample tested or the first positive one.

**3.3. SARS-CoV-2 IGG seroprevalence in Malawian infants**

An overall prevalence of SARS-CoV-2 IgG antibody of 31.0% (95%CI: 23.7% to 38.3%) was found (49/158 infants tested), with no significant difference between the two populations of HEU and HUU infants (36/123, 29.3% and 13/35, 37.1%, respectively,  $P = 0.410$ ).

The longitudinal monitoring of the 158 infants allowed us to assess the temporal changes in COVID prevalence in Malawi; the study period was divided into three different subperiods, independent of infant's age: from February to June (first semester of 2020), from July to December (second semester of 2020) and from January to May 2021 (first semester of 2021). The results are reported in Table 2. In the first semester of 2020 among the 64 infants tested, the SARS-COV-2 seroprevalence was 26.6% (95% CI: 15.4 - 37.7). None of the 6-week-old infants ( $n = 12$ ) was positive for SARS CoV-2, while specific anti-SARS-CoV-2 antibodies were found with a similar proportion in 6 and 12-month-old infants (7/20, and 10/20, respectively). Interestingly, the first two cases of positivity to SARS-CoV-2 are dated February 2020. The IgG seroprevalence during the second period (July - December 2020), which included samples from 76 infants, mostly 6–12 months old, increased to 34.2%; (95%CI: 23.3 - 45.1). In the third period, 6 out of 18 (33.3%, 95%CI: 9.2 - 57.5) samples from infants aged 6 or 12 months were positive for SARS-CoV-2.

The seropositivity to SARS CoV-2 did not differ in the three geographical areas of Blantyre (Mandala, Chileka, and Machinjiri (31.0%, 22.8%, and 44.9%  $P = 0.547$ ).

No correlations were found when socio-demographic indicators were associated with the seropositivity in infants: the maternal factors, such as employment ( $p = 0.279$ ), educational level ( $p = 0.890$ ), or household water/electricity ( $p = 0.732$  and  $p = 1.00$ , respectively) were not associated to higher prevalence of SARS-CoV2 infection. Similarly, the area of residence (urban, semi-rural, or rural context,  $p = 0.500$ ) did not affect the rate of seropositivity (Table 3).

**4. Discussion**

In this study, performed in the temporal window between the beginning of the COVID pandemic in 2020 and the spring of 2021, we found seropositivity to SARS-CoV-2 IgG in 31% of Malawian infants attending three antenatal clinics in the city of Blantyre (in Mandala, Machinjiri, and Chileka). These data further confirm that the onset of pandemic in

**Table 3**

Correlations between infants seropositivity for SARS-CoV-2 and socio-demographic characteristics of their mothers. Values are expressed as absolute numbers and percentages. Chi-square test was used for statistical analysis.

	SARS-CoV-2 Ab Negative, n (%)	SARS-CoV-2 Ab Positive, n (%)	P values
<b>HIV status (n, %)</b>			
HUU infants	22 (62.9)	13 (37.1)	0.410
HEU infants	87 (70.0)	36 (29.3)	
<b>Employment</b>			
Housewife/unemployed	32 (29.9)	20 (39.2)	0.279
Employed	75 (70.1)	31 (60.8)	
<b>Educational status</b>			
None/Primary	47 (43.9)	23 (45.1)	0.890
Secondary or above	60 (56.1)	28 (54.9)	
<b>Residency</b>			
Urban	26 (24.3)	15 (29.4)	0.500
Semirural	64 (59.3)	29 (56.9)	
Rural	17 (15.9)	7 (13.7)	
<b>Electricity at home</b>			
No	56 (53.2)	27 (59.9)	1.000
Yes	51.0 (47.7)	24 (47.1)	
<b>Water at home</b>			
No	46 (43.0)	24 (47.1)	0.732
Yes	61 (57.0)	27 (52.9)	

the African continent started in the first months of 2020; the first COVID-19 case in Africa was officially reported in Egypt in February 2020 [27], but a recent retrospective study conducted in Congo suggested that the virus was present already in January 2020, before the first official case [28]. As correctly predicted by initial modeling studies, the strong economic relationships, which include large travel volume, between many African countries, including Malawi, and China could have facilitated the early exposition to SARS-CoV-2 [29].

In our study, only 52/158 pregnancies ended after February 2020. Although the first laboratory-confirmed case of COVID-19 in Malawi was reported on April 2, 2020, in the city of Lilongwe [30], our serological data strongly suggest that the virus was already circulating in February 2020, when 2 cases of positivity for SARS CoV-2 IgG were detected in infants samples. It is important to note that all infants in this study were living in the area of Blantyre, which is the second Malawian city for population density and is home to an international airport. Previous studies on COVID-19 diffusion have indicated in the larger cities the earlier cases of positivity due to the population density and their direct connectedness to other countries via international travel [31].

The global seroprevalence rate (49 out of 158 infants, 31.0%) that we found is consistent with other serosurveillance studies in African countries; Mandolo and colleagues found a dramatic increase in seroprevalence among blood donors (range: 18.5% to 64.9%) from different Malawian cities, during the period October 2020 - May-2021 [32]. The different seroprevalence rates can be explained by the different methodological approaches, while the increasing trend in seropositivity to SARS-CoV-2 is indicative of the global spread of COVID-19 infection observed in most African countries; in Kenya, SARS-CoV-2 antibodies prevalence in blood donors increased from 4.3% to 48.5% over 1 year [33], and positivity to SARS-CoV-2 raised from 10.9% in June 2020 to 54.7% in January 2021 in the rural population in Mali [34]. A recent surveillance study reported a seroprevalence as high as 48.0% in health workers in Ethiopian hospitals [35]. The main characteristic of these studies is that SARS-CoV-2 infections were identified by routine tests, implying that asymptomatic infections are very common in the African population.

In our study, considering infants of this age, serology should be interpreted as representative of the exposure of mother/infant dyads; maternal SARS-CoV-2 IgG are efficiently transferred across the placenta [36,37] and can be detected in the breastmilk of COVID-19 vaccinated

and infected women, and transferred to the infants through breastmilk [38,39]. From the temporal analysis of our data, we hypothesize that most of the COVID-19 exposures occurred after birth: none of the 6-week-old infants whose blood was analyzed had reactivity to SARS-CoV-2 antigens. Although from 6 months of age the infants start to produce their immunoglobulins, we cannot make assumptions on the maternal/infant origins of the anti-SARS-CoV-2 IgG that we found, considering the prolonged breastfeeding in our cohort.

Population-based studies have demonstrated that socioeconomic factors, such as household infrastructure, access to water and hygiene facilities [40,41], crowding [42] have a direct impact on COVID-19 infection rates and disease severity. In our cohort we did not find correlations between the presence of SARS-CoV-2 IgG and the demographic, geographic, or economic indices; the rate of seroprevalence remained quite stable over time, indicating a more limited exposure to SARS-CoV-2, probably related to the lower involvement in social activities of the mother/infant dyads. More importantly, no higher SARS-CoV-2 prevalence was observed in HIV+ mothers/HEU infants compared to HIV-negative dyads. Considering that the adult rate of HIV prevalence in Malawi is 8.9%, and the number of infants prenatally exposed to HIV is estimated to be about half a million, [43,44] these results are reassuring and support the results of previous studies showing that HIV infection/exposition does not increase susceptibility to COVID-19 infection [45,46]. It has to be underlined that in the present study all the women living with HIV were under long-term antiretroviral treatment with a good adherence profile, which are protective factors against adverse outcomes of SARS-CoV-2 infection [47].

Similar to other studies, we faced the problem of the aspecific cross-reactivity of African specimens in the ELISA assays. Although the Binding Site ELISA assay we used, targeting IgG, IgA IgM against SARS-CoV-2 Spike, has been successfully used in serosurveillance studies [48,49], in the analysis on pre-pandemic samples we found that 8.4% of results were false-positive. Limited specificity of commercially SARS-CoV-2 IgG ELISAs in the African population has been observed by many authors [23,24,50-53]; malaria or endemic pathogens have been indicated as the possible interfering molecules in the ELISA assays [53-55], but until now there is no consensus on the responsibility of the aspecific background reactivity.

In this study, the aspecific reactivity towards SARS-CoV-2 antigens has been offset through the use of a second ELISA test with a different target antigen, a strategy already suggested by others [56]. The first serological assay targets the trimeric recombinant spike protein which presents many immunogenic epitopes and strong immunoreactivity [57,58]. The second assay is based on the antibody detection of RBD. The Receptor Binding Domain is a small fragment of the spike protein and may have reduced assay sensitivity compared to a full-length protein [59], but it is a highly specific target antigen in serological diagnostic assays [59,60]. The first assay detected IgG, IgM, and IgA specific for SARS-CoV-2 infection, and the second was specific only for the IgG isotype; we, therefore, cannot exclude that some IgM/IgA specific responses in the first assay (possibly representing early infections) were lost in the second assay; however, in contrast with the typical serological responses, in which IgM response precedes the IgG mount, the onset of SARS-CoV-2 infection seems characterized by the appearance almost simultaneous of IgM and IgG antibodies [61,62], and both IgM and IgA responses are strictly correlated to IgG responses [63,64].

The use of two assays targeting different antigens could be considered one of the major limits of this study. However, in the lack of specific serological assays, our method seems to provide reliable results, as demonstrated by the performance obtained on pre-pandemic samples.

This study has other important limitations; the sample size is limited, and we did not have the corresponding maternal samples. However, the determination of maternal serological status would not add essential information, considering that the main objective of the study was to determine the spread of the infection. A considerable strength of this study was the evaluation of the infant population which is usually not



considered in COVID-19 epidemiological studies although being affected as well by COVID-19 disease.

In conclusion, our data suggest that SARS-CoV-2 was relatively common in the city of Blantyre as soon as February 2020. Despite a high seroprevalence rate, neither infants nor their mothers reported any symptoms, indicating that the infection has continued to spread undetected, potentially allowing new virus variants to emerge. The investigation of the real burden of COVID-19 infection in Africa should be a priority. There is a need for more sensitive and specific antibody assays for SARS-CoV-2 IgG detection, to allow the implementation of rigorous surveillance programs to track in real-time the pandemic diffusion in the African countries.

#### Authors' contribution

SB and MG were responsible for the design of the study and drafted the manuscript. SB was responsible for statistical analysis. CMG designed and supervised the laboratory procedures. SO supervised the implementation of the project. RM, TK and RL were responsible for data and sample collection at the clinical sites, RA was involved in the laboratory assays. MF and MA contributed to the data acquisition and to the interpretation of data. PS and MCM contributed to the critical revision of the manuscript for important intellectual content. All authors read and approved the final manuscript.

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#### Declaration of Competing Interest

The authors declare that there is no conflict of interest.

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