

Original Research Article

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Seroreactivity to SARS-CoV-2 during Pre-Pandemic and Pandemic Periods of Covid-19 of Populations Living in a Malaria Endemic Area in Burkina Faso

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ABSTRACT

Malaria-endemic countries in Africa have recorded the lowest number of covid-19 cases and deaths compared to other countries. Pre-existing Naturally acquired Immunity to malaria was an hypothesis that was stated to explain this situation. In a context where malaria is endemic, we carried out this study in order to investigate the existence of antibody cross-reactivity between COVID-19 and malaria and to estimate the seroprevalence of COVID-19 during the third year of the pandemic in the plateau central Region in Burkina Faso. Samples collected in 2007 and positive for IgG against *Plasmodium falciparum* alpha-helical coiled coil proteins were used to look for a possible antibody cross-reactivity with SARS-CoV-2 antigens. Samples collected in 2022 were used to estimate the seroprevalence of COVID-19. A total of 628 individuals of both sexes, aged over 5 years were enrolled. The seroprevalence of anti-SARS-CoV-2 antibodies was measured using an ELISA test and an RDT. All the samples collected in 2007 showed no cross-reactivity between SARS-CoV-2 and *Plasmodium falciparum*. The seroprevalence during the third year of the pandemic was 100% and 86.88% respectively by ELISA and RDT. The proportions of IgG and IgM antibodies were respectively 86.56% and 2.81%. The results did not show antibody cross-reactivity between SARS-CoV2 and *Plasmodium falciparum* in the study population. High exposure to SARS-CoV-2 was found in the study area in 2022. Due to their high specificity, WANTAI ELISA and Right Sign RDT can be considered as good serological tools for COVID-19 serosurveillance in a context where malaria is endemic.

Keywords

Seroreactivity,
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Introduction

The number of COVID-19 deaths in Africa during the pandemic has been low compared to the others continents (Ioannidis and Contopoulos-Ioannidis, 2023). Indeed, the African continent has recorded the lowest rate of contamination and deaths in the world. Burkina Faso, a malaria endemic country recorded its first case of COVID-19 on March 9, 2020, making the country the sixth affected in Sub-Saharan Africa and the fourth in West Africa (Zio *et al.*, 2022). In April 2023, Burkina Faso had 22.453 confirmed cases of COVID-19 with 396 deaths (MSHP, 2023). Pre-existing immunity to SARS-CoV2 is one of the hypotheses that was stated to explain the less severe outcomes of COVID-19 patients in Africa. Several studies documented cross-reactive humoral immunity to SARS-CoV2 in African samples that predate the pandemic years (Pedersen *et al.*, 2022; Traore *et al.*, 2022; Iriemenam *et al.*, 2023; Borrega *et al.*, 2021; Ige *et al.*, 2021; Woodford *et al.*, 2021; Baker *et al.*, 2021; Steinhardt *et al.*, 2021; Emmerich *et al.*, 2021; Tso *et al.*, 2021). Pre-existing Naturally acquired Immunity to malaria that is wide spread in Africa was also mentioned to explain this situation. In Burkina Faso, to date, only one study assessed the pre-existing cross-reactivity to SARS-CoV2. The results of this study show a preexisting cross reactivity between SARS-CoV2 Spike protein and *Plasmodium* (Lapidus *et al.*, 2022). In the framework of the serological tools of COVID-19, several serological tools, ranging from Rapid Diagnostic tests to Immunoenzymatic assays have been used. These studies showed a variety of seroprevalence. Indeed, studies carried out nationally on the seroprevalence of COVID-19 in 2021 showed that most individuals had anti-SARS-CoV-2 antibodies. Seroprevalence was 55.7% in Bobo Dioulasso and 37.4% in Ouagadougou in 2021 (Struck *et al.*, 2022).

The aim of this study was to search for a cross-reactive antibody response between *Plasmodium falciparum* and SARS-CoV2 and to measure the seroprevalence level of COVID-19 during the third year of the pandemic in a malaria endemic setting in Burkina Faso.

Materials and Methods

Study Site and Population

The samples used in this study were collected at two distinct periods in the Central Plateau region, Burkina

Faso. Part of the samples tested was collected as part of an epidemiological survey on malaria conducted in August 2007 in four villages (Barkoundouba, Barkounbilen, Bassy and Zanga) in the Ziniaré health district (Mangano *et al.*, 2015; Ouédraogo *et al.*, 2019). The other part of the samples was collected in 2022 during a seroprevalence survey on Covid-19 in Méguet and Ziniaré in the Central Plateau region.

The laboratory analyzes were carried out at the Biomedical Research Laboratory at the Health Sciences Research Institute (IRSS) in Ouagadougou. The study population consisted of 628 male and female individuals, aged over 5 years. Three hundred and eight (308) blood samples were taken before the advent of COVID-19 in 2007 on the one hand and 320 blood samples were taken during the COVID-19 pandemic in 2022 on the other hand.

Study Design, Data and Samples Collection

This is a study composed of two cross-sectional surveys during which samples were collected in 2007 and 2022. During the investigations, blood samples were taken and the serum obtained after centrifugation was stored at -20°C and used for laboratory analyses.

Laboratory Analyses

Laboratory analyzes were carried out on serum stored in the laboratory. The search for anti-SARS-Cov2 antibodies was carried out at the Biomedical Research Laboratory (LaReBio) at the Health Sciences Research Institute (IRSS), using Rapid Diagnostic Tests (RDT) and the ELISA (Enzym-Linked Immunosorbent) technique assay.

Measurement of anti-*Plasmodium falciparum* antibodies

A measurement of the level of IgG antibodies directed against ninety-two (92) *Plasmodium falciparum* antigens with alpha-helical coiled coil motifs was carried out on samples collected in 2007. This measurement was carried out using the protein microarray technique. The test was carried out in several stages: immobilization of the peptides, screening of the samples, scanning and measurement of the fluorescence of the microchips and quantification of the fluorescence signal. The microarray assay used for the measurement of IgG antibodies to

Plasmodium falciparum was already described were (Ouédraogo *et al.*, 2019). Briefly, the following steps were performed.

Screening of samples

The slides on which the microchips were printed were incubated for one hour at room temperature with the blocking solution (2% BSA in 1X PBS), in order to saturate all non-specific binding sites. The slides were then washed with washing solution (PBS1X + 0.01% Tween 20), for three (3) minutes. Then, the slides were incubated with the plasma samples diluted 1: 100 for one hour at room temperature.

After the incubation time, the slides were washed with washing buffer, then were incubated with the secondary antibody (Goat anti-human IgG Alexa 647, Southern blot) at a concentration of 10 µg/ml for twenty (20) minutes. The slides were then washed with washing buffer and dried by centrifugation (3000 rpm/min for 1 minute). Each plasma sample was analyzed in duplicate.

Fluorescence scanning and measurement

Pretreated slides were scanned using a scanner (Perkin-Elmer, PRECISELY) which uses a laser-based scanning system. This system makes it possible to read the fluorescence signal emitted by each spot located inside the matrix. Images were generated using Scan Array TM software, according to the manufacturer's instructions.

All slides were scanned using constant machine settings: 90% laser power, 60% multiplied photo gain and 20% scanning resolution. The fluorescence reading was taken at a wavelength of 633 nm. The images were generated using the internal Scan Array software and saved in JPEG and TIFF formats. JPEG format was used for image viewing and TIFF format is used for fluorescence signal quantification.

Quantification of the fluorescence signal and processing of malaria serology data

Fluorescence quantification was performed using Pro Scan Array TM Express software according to the manufacturer's instructions. The signals obtained from the scanned slides were analyzed and translated into digital data. The data was stored in Excel format. The quantified fluorescence signals were analyzed using

Excel. The measurements obtained for each spot were corrected against the background noise of the negative control (PBS). This made it possible to identify signals above background noise. After subtracting the background, negative values and zero values were considered zero. The data were normalized before statistical analyzes were performed.

Detection of anti-SARS-CoV-2 antibodies

Each sample was analyzed with the Sandwich ELISA test and with the RightSign Rapid Diagnostic Test (RDT) for the detection of anti-SARS-CoV-2 antibodies. The Wantai kit was used to perform the ELISA test for quantification of total antibodies (IgM and IgG). This test did not allow us to distinguish between the different types of antibodies. On the other hand, the Right Sign RDT used allowed distinction according to the type of immunoglobulins (IgM, IgG). The reagents kits were used according to manufacturer's guidelines. The table 1 summarizes the characteristics of the serological reagents.

Data management and Statistical analysis

Data from 2007 was entered in duplicate using Epi info. Regarding the survey carried out in 2022, a questionnaire was designed with Microsoft-Excel 2016 for data collection. It was pre-tested and validated by the investigators before the start of collection. Data entry was done with Microsoft Excel and then exported to STATA (Version 17.0, College Station, TX:StataCorp).

A descriptive analysis was carried out to determine the sociodemographic characteristics of the study population and the seroprevalence of the different antibodies in this population. A p-value < 0.05 was considered a statistically significant difference in the comparison tests.

Ethical considerations

The samples used in this work were obtained during two sero-epidemiological studies carried out respectively in 2007 and 2022. These two investigations obtained approval from the ethics committee for biomedical research of the Ministry of Health of Burkina Faso (deliberation N°.2007-48; deliberation N°.2022-). Participants, parents or guardians of the children had given consent for further use of the samples for research purposes.

Results and Discussion

Sociodemographic characteristics of study population

The study population is composed by male and female of both sexes and over 5 years old. The characteristics of the study population are shown in table 2

Seroprevalence and intensity of IgG antibody response to *Plasmodium falciparum*

The seroprevalence of IgG antibodies to *Plasmodium falciparum* antigens in our study population from 2007 varies from 28.66% to 97.66%. The intensity level of the IgG response was also assessed and the results ranged from 99.7 to 6905 PMC (Photo Multiplier Count). Table 3 shows the results of the seroprevalence of anti-*Plasmodium falciparum* antibodies.

Overall results of anti-SARS-CoV2 seroprevalence

All samples collected in 2007 were seronegative for anti-SARS-CoV2 antibodies regardless of the test used, however in 2022 we had a seroprevalence of 100% with the ELISA test and 86.88% with the RDT. The table 4 show the seroreactivity of the samples according to time of collection.

Relationship between seroprevalence of anti-SARS-CoV2 antibodies and sex in 2022

The results of seroprevalence according to sex shows that there were as many women tested positive for the COVID-19 serological RDT as men with respective proportions of 87.65% and 86%. All the results are shown in Table 5.

Relationship between seroprevalence of anti-SARS-CoV2 antibodies and age

Table 6 shows the results of COVID-19 seroprevalence according to age in 2022. This seroprevalence was high in all age groups.

Frequency of immunoglobulin M and G

The results of the seroprevalence showed that 2.81% of the samples were positive for IgM, 86.56% for IgG. The

individuals tested positive for IgM and IgG simultaneously were 2.5%. The table 7 shows the frequency of immunoglobulin type.

The study aimed to evaluate the seroreactivity to SARS-Cov2 of populations living in a malaria endemic area during prépandemic and pandemic periods of Covid-19. The study objectives were to search for a possible serological cross reactivity between SARS-Cov2 and *Plasmodium falciparum* and to assess the Seroprevalence of Covid-19 during the third year of the pandemic in Burkina Faso.

Samples collected in 2007, time corresponding to a prepandemic period for Covid-19, were used for the search of a possible serological cross-reactivity between *Plasmodium falciparum* and SARS-Cov2. All the collected samples at that time were tested positive for anti-*Plasmodium falciparum* IgG.

Concerning the search for a possible serological cross-reactivity between SARS-Cov-2 and *Plasmodium falciparum*, the study results have not shown a cross reactivity between the two pathogens.

In comparison, other authors showed a cross-immunity between SARS-CoV2 and *Plasmodium* antigens depending on the type of the antigens (Traore *et al.*, 2022). Other authors have also found similar results to ours with an absence of cross-reaction between SARS-CoV-2 and *Plasmodium* antigens (Talla *et al.*, 2022).

This could be explained by the fact that the antigens in the kits we used are recombinant antigens of the specific binding domain (RBD) of SARS-CoV2 and not the S protein itself.

Regarding the seroprevalence in the study site in 2022, the results have shown a high seroprevalence of covid-19, ranging from 86.88% to 100%, respectively with RDT and ELISA test. These results show that during the third year of covid-19 pandemic in Burkina Faso, the majority of the study population were in contact with the virus and this allow them to acquire antibodies against the SARS-cov2 either through natural infection or vaccination.

The difference observed between the results of the WANTAI ELISA test (100%) and the RightSign RDT (86.5%) could be explained by the fact that the ELISA test is more sensitive and more specific than the RDT.

Table.1 Characteristics of the serological tests

Characteristics	WANTAI SARS-CoV2 Ab ELISA	Right Sign CoVID-19 IgG/M Rapid Test
Manufacturer	Beijing Wantai Biological pharmacy Enterprise (China)	BiotesT Hangzhou Biotest Biotech (China)
Antigen incorporated in the test	Receptor Binding Domain (RBD) of protein S	Receptor Binding Domain (RBD) of protein S
Principle	EnzymImmuno Assay	Immunochromatography
Antibodies detected	(IgM + IgG)	IgM or IgG
Sensibility	94.36%	90%
Specificity	100%	98.10%

Table.2 Sociodemographic characteristics of the study population

Sex	Status			<i>p-value</i> 0,239
	2007	2022	Total	
Male	130 (42, 21%)	150 (46.88%)	280 (44.69%)	0.001
Female	178 (57.79%)	170 (53.12%)	348 (55 .41)	
Total	308(100.00%°	320 (100.00%)	628 (100.00%)	
Age (years)				
5-14	82 (26.62%)	33 (10.31%)	115 (18.31%)	
15-24	74(24.03%)	84 (26.25%)	158 (25.16%)	
25-34	52 (16.88%)	85 (26.56%)	137 (21.82%)	
5-14	82 (26.62%)	33 (10.31%)	115 (18.31%)	
15-24	74(24.03%)	84 (26.25%)	158 (25.16%)	
25-34	52 (16.88%)	85 (26.56%)	137 (21.82%)	
35-44	57 (18.51%)	45 (14.06%)	102 (16.24%)	
45-54	21 (6.82%)	34 (10.62%)	55 (8.76%)	
55-64	13 (4.22%)	15 (4.69%)	28 (4.46%)	
≥ 65	9 (2.92%)	24 (7.50%)	33 (5.25%)	
Total	308 (100%)	320 (100%)	628 (100%)	

Table.3 Seroprévalence and intensity of IgG antibody response to *Plasmodium falciparum*

Characteristics	Minimum	Maximum
Seroprevalence	28.66%	97.66%
Geometricmean (PMC)	99.7	6905
Ratio	0.73	136

Table.4 Global seroreactivity to SARS-Cov2

year	RDT Right Sign			ELISA Wantai		
	Negative	Positive	Total	Negative	Positive	Total
2007	308 (100.00%)	0 (0. 00%)	308 (100%)	308 (100%)	0 (0.00%)	308 (100%)
2022	42 (13.12%)	278 (86.88%)	320 (100%)	0 (0.00%)	320 (100%)	320 (100%)
Total	350 (55.73%)	278 (44.27%)	628 (100%)	308 (49.04%)	320 (50.96%)	628 (100%)

Table.5 Seroprevalence of anti-SARS-CoV2 antibodies according to sex in 2022

Sex	TDR Right Sign			ELISA Wantai		
	Negative	Positive	Total	Negative	Positive	Total
Male	21 (14.00%)	129 (86.00%)	150 (100%)	0 (0.00%)	150 (100%)	150 (100%)
Female	21 (12.35%)	149 (87.65%)	170 (100.00%)	0 (0.00%)	170 (100%)	170 (100%)
Total	42 (100.00%)	278 (100%)	320 (100.00%)	0 (0.00%)	320 (100%)	320 (100%)

Table.6 Seroprevalence according to age

Age (years)	TDR Right Sign			ELISA Wantai		
	Negative	Positive	Total	Negative	Positive	Total
5-14	5 (15.15%)	28 (84.85%)	33 (100%)	0 (0.00%)	33 (100%)	33 (100%)
15-24	9 (10.71%)	75 (89.29%)	84 (100%)	0 (0.00%)	84 (100%)	84 (100%)
25-34	10 (11.76%)	75 (88.24%)	85 (100%)	0 (0.00%)	85 (100%)	85 (100%)
35-44	6 (13.33%)	39 (86.67%)	45 (100%)	0 (0.00%)	45 (100%)	45 (100%)
45-54	7 (20.59%)	27 (79.41%)	34 (100%)	0 (0.00%)	34 (100%)	34 (100%)
55-64	1 (6.67%)	14 (93.33%)	15 (100%)	0 (0.00%)	15 (100%)	15 (100%)
≥ 65	4 (16.67%)	20 (83.33%)	24 (100%)	0 (0.00%)	24 (100%)	24 (100%)
Total	42 (100%)	278 (100%)	320 (100%)	0 (0.00%)	320 (100%)	320 (100%)

Table.7 Seroprevalence according to Immunoglobulin type

Immunoglobulin type	RDT Right Sign		
	Negative	Positive	Total
IgM	311 (97.19%)	9 (2.81%)	320 (100%)
IgG	43 (13.44%)	277 (86.56%)	320 (100%)
IgM + IgG	312 (97.5)	8 (2.5%)	320 (100%)

The prevalence found in our study is higher than that found by [Struck et al., \(2022\)](#) during a study on SARS-CoV-2 seroprevalence in 2021 in the cities of Ouagadougou and Bobo Dioulasso with respective seroprevalences of 37.4% and 55.7% ([Struck et al., 2022](#)). Others studies also conducted before the ours show less seroprevalence compared to ours ([Ouedraogo et al., 2023](#); [Kabore et al., 2023](#); [Salam Sawadogo et al., 2023](#)).

This could be explained by the fact that our samples were collected in different localities and one year apart; which allowed a greater exposure time for our population unlike theirs. The frequency of COVID-19 seropositivity was high in all age groups. This could be explained by the fact that all age groups were exposed more or less in the same way to SARS-CoV-2. The study results are different from those found by other authors which found

higher seropositivity in the age groups of 10 to 19 and 20 to 44 in Fianarantsoa and Bobo Dioulasso ([Struck et al., 2022](#)).

It was also found that there were as many women testing positive for anti-SARS-CoV2 antibodies as men, unlike other studies which found higher seropositivity in women ([Gebhard et al., 2020](#)). The frequency of immunoglobulin type showed that IgG and IgM prevalence were respectively 86.53% and 2.81%. The concomitant presence of IgM and IgG was 2.5%. The results showed the IgG antibodies were the most prevalent, meaning that the majority of the study population were in contact with the virus during an old infection. IgM antibodies reveal a recent or current infection and 2.81% of individuals were having COVID-19 at the time of the survey. Several seroprevalence studies reported similar results with high IgG

seroprevalence. The frequency of IgM was higher among men than in women with a proportion of 3.33% in men compared to 2.35% in women. On the other hand, the frequency of IgG was higher in women with a proportion of 87.06% than in men with a proportion of 86%.

While more women had already been in contact with the virus. It was also noted in our results that the frequency of IgG was high in all age groups in general while that of IgM was only high in the 55 to 64 age group. These results show that at the time of the survey in 2022 almost all individuals had previous contact with SARS-CoV-2.

The assessment of the seroprevalence, of COVID-19 during the third year of the pandemic, showed a high prevalence, meaning a high exposure of the population to SARS-CoV-2 in the study area. Furthermore, the study did not show antibody cross-reactivity between *Plasmodium falciparum* alpha-helical coiled coil proteins and SARS-CoV-2 in the Central Plateau region in Burkina Faso. Wantai ELISA and RightSign RDT are highly specific due to the purity of the antigens used in their design. Therefore, these tests are suitable for serosurveillance of COVID-19 in malaria endemic areas.

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Authors' Contributions

OO, DK, IN, SBS, HGO: study design and implementation. SN, IS, CD, TS, AAZ, STS, TRC: Data collection and supervision. NS, CD: Laboratory testing of samples. OO: manuscript drafting. SD, YT, YEH, AZ, GC, IN, SBS, RS, HGO: Correction of the manuscript. All authors: Final Review and approval of the manuscript

Data Availability

The datasets generated during and/or analyzed during the current study are available from the corresponding author on reasonable request.

Declarations

Ethical Approval Not applicable.

Consent to Participate Not applicable.

Consent to Publish Not applicable.

Conflict of Interest The authors declare no competing interests.

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