

Subclinical *Plasmodium* spp. Infections in a Community Setting in Bangui, Central African Republic

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Background: Malaria is a major public health problem in the Central African Republic (CAR). Data on malaria epidemiology are often derived from confirmed cases of symptomatic malaria using passive detection approaches, with very limited knowledge of the extent of subclinical and submicroscopic infections.

Methods: A community-based cross-sectional study was conducted in Bangui, the capital of the CAR, to assess the prevalence of subclinical malaria parasitaemia. Proportions of positive tests for malaria parasites were determined by combining the results of antigen-based malaria rapid diagnostic test (mRDT), thick blood smear microscopy, and polymerase chain reaction (PCR).

Results: A total of 638 participants (mean age, 26.44 years (range, [1–75] years) with a sex ratio (M/F) of 1.22) were tested for malaria using all three detection methods. Proportions of positives were 32.45% by PCR, 27.59% by mRDT, and 23.51% by Giemsa-based thick blood smear microscopy, representing the burden of subclinical malaria. In addition, a 9.56% prevalence of submicroscopic infections was observed. Subclinical malaria was more common in individuals aged 15–49 years, and microspatial heterogeneity in positivity was observed, with the majority of cases occurring in semi-urban areas by mRDT and microscopy, respectively. Approximately, 80% of microscopy-positive subjects had a low parasite density (<1000 parasites/μL whole blood). Although *P. falciparum* was the most common species (98.55%), the transmission of *P. ovale* appears to be well-established in the area, occurring either as mono-infection (1.45%) or co-infection (1.93%) with *P. falciparum*.

Conclusion: This study of community malaria in Bangui highlights the high burden of subclinical malaria in the community and provides essential baseline data to guide future research on malaria parasites in the CAR, particularly regarding the circulating parasite species. The high prevalence of community malaria demonstrates its persistence as a major public health challenge in the country, highlighting the need to intensify its ongoing control using new tools such as the upcoming malaria vaccine.

Plain Language Summary: Malaria is widespread in the Central African Republic, with prevalence data based mainly on highly flawed medical records. Moreover, little is known about the extent of, and factors associated with subclinical *Plasmodium* infections, which are essential for guiding ongoing antimalarial interventions in the community. Subclinical infection is defined as an infection with no noticeable clinical symptoms.

This study demonstrated that one out of three people living in Bangui is carrying malaria parasite without showing any symptoms. This high prevalence of low-density *Plasmodium* infections in the Bangui community, especially in 15–49 age group, raises concerns about the persistence of malaria in the community and the opportunity for its elimination in CAR.

The current findings underscore the need to intensify community-based interventions, including vaccination, against malaria, particularly in CAR and Bangui.

Keywords: malaria, subclinical parasitaemia, Bangui, Central African Republic

Introduction

Malaria remains a major public health problem, particularly in sub-Saharan Africa, with an estimated 233 million cases in 2022, accounting for about 94% of cases globally and estimated deaths to 580 000, representing 95% of deaths globally.¹ Central African Republic (CAR) as one of the 7 high incidence (334.7 for 1000 habitants) in African countries, despite intensive control efforts.² Malaria transmission remains worrisome in the CAR. Over the past 20 years, a number of initiatives have been undertaken in CAR, including the introduction in 2005 of artemisinin-based combination therapies as first-line treatment for confirmed cases, sulfadoxine-pyrimethamine for intermittent preventive treatment during pregnancy (IPTp-SP), distribution since 2010 of long-lasting insecticide-treated nets (LLINs), introduction in 2013 of rapid diagnostic tests (RDTs), home-based management of malaria (PECADOM), and introduction of integrated community case management (iCCM) and community health worker (CHW) programs; however, gaps remain in the fight against the disease. According to the World Health Organization World Malaria Report 2023, malaria is responsible for more than 70% of the fever cases from 2019 to 2022 in CAR¹. It is the leading cause of morbidity and mortality, accounting for 50–60% of hospital admissions in the country.³

Malaria prevalence in endemic populations is an important indicator of the effectiveness of control interventions.⁴ Most published estimates of malaria prevalence are based on cases of symptomatic infections, including fever and flu-like illnesses, and therefore, involve passive diagnosis.

Nevertheless, infection with malarial parasites can be asymptomatic or, more accurately, subclinical as subtle symptoms and long-term health effects may occur.⁵ Subclinical infection is defined as an infection with no noticeable clinical symptoms. Submicroscopic malaria is defined as low-density *Plasmodium* infection detected using molecular methods alone. Effective malaria control requires combating both symptomatic and unknown “silent” infections, which may serve as reservoirs for ongoing transmission, and subclinical infections remain a hurdle in malaria control.⁶ Infected individuals without symptoms are a source of persistent infection and contribute to the continued transmission of malaria.⁷ The identification and management of asymptomatic carriers has become a new and increasingly important challenge for malaria control programs. Prior studies have demonstrated that screening the treatment of asymptomatic carriers and insecticide spraying is the most cost-effective and important intervention strategy to implement to control and interrupt the malaria transmission.⁸ Intermittent preventive treatment (IPT), administration of a full course of an antimalarial treatment for a population at risk of specified time points regardless of the infection status of individuals, has been suggested as a method of treatment for asymptomatic individuals to reduce transmission of malaria.⁹ Therefore, asymptomatic individuals have the potential to develop gametocytes that serve of veritable as reservoir of disease transmission, thus using the drugs that may predominantly inhibit gametocyte development or accelerate its clearance.¹⁰ In addition, there are often submicroscopic infections that are difficult to diagnose using conventional methods (microscopy and/or rapid diagnostic tests), requiring the use of molecular techniques such as polymerase chain reaction (PCR) for a more accurate diagnosis. Complementary strategies targeting the parasite reservoir could therefore provide significant help, particularly those focusing on asymptomatic carriers.

Malaria prevalence reports in CAR are often based on symptomatic infections and do not reflect the true prevalence in the community. Importantly, no study on malaria in the community has been conducted in Bangui that has one of the highest malaria rates for a major city in the world, with 60% of people with fever testing positive for malaria,³ or in other regions of the CAR. Although women and children are the most vulnerable to malaria, asymptomatic infections in the general population, particularly in adults due to premunition, constitute a major reservoir associated with persistent transmission, especially in the context of changing mosquito behaviour.¹¹

It is essential to target both symptomatic and asymptomatic parasitaemic cases in communities and to treat them with appropriate antimalarial drugs.¹² Furthermore, in the context of malaria vaccine introduction, defining malaria profiles at

the community level would serve as a basis for future studies aimed at distinguishing malaria infections and investigating premunition levels.

This study aimed to determine the prevalence of subclinical and submicroscopic malaria in the population of Bangui, and the CAR's main city and capital has some of the highest malaria rates for a major city in the world with 60% of people with fever testing positive for malaria.³

Materials and Methods

Study Design, Period and Site

This was a nested study using baseline data from a survey to estimate the cumulative population immunity to COVID-19 conducted from July 12 to August 20, 2021.¹³ The COVID sampling method has no impact on our study, because population expected prevalence or proportion of 50% (the default value) is used to ensure the largest possible sample size. In addition, community sampling is not linked to COVID-19 symptom profile or any symptoms. Briefly, the study was conducted on the general population of Bangui, the capital city of the Central African Republic. Bangui is a city comprised eight administrative division. A total of 25 districts in Bangui were selected using a probability sampling method, based on the population size of each district. Eleven households were randomly selected at the district level, and at least three members from each household were invited to participate in community screening for malaria. Information regarding each participant's age, sex, and place of residence was collected using a structured questionnaire. Any record with incomplete data was excluded from the study. Body temperature and other clinical manifestations were not examined to identify truly asymptomatic individuals, for, strictly speaking asymptomatic carrier was defined as a person with no symptoms, with a body temperature $<37.5^{\circ}\text{C}$, and who reported no fever during the 2 days before blood collection.¹⁴ In the present study based on detection of parasite carriers within the community, through active surveillance, the term “subclinical infection or subclinical malaria” is used to define the malaria positive individuals.

For each participant, a 2-milliliter blood sample was collected in an EDTA tube (BD Vacutainer®). The samples were stored in a cooler at 4°C and transported the same day to the Laboratory at the Pasteur Institute in Bangui for biological analysis. Thick blood smears were prepared for laboratory microscopy examination, and filter paper blood dots were prepared for PCR detection of parasite infection status and species. These Dry Blood Spot (DBS) samples were prepared on 3MM Whatman® (Brentford, United Kingdom) filter papers cut into strips of approximately $2\text{ cm} \times 5\text{ cm}$, to collect blood from the finger prick, with three blood spots collected per card and two cards per participant.

Malaria Biological Diagnosis

Laboratory Screening for Malaria

ParaHIT® *f* ver.1.0 (Ref. 55IC104-25, Arkray) rapid test for *P. falciparum* malaria was performed to screen all the samples. It is an immunochromatographic test based on the detection of *Plasmodium falciparum*-specific histidine-rich protein II (PfHRP-II) parasite antigen.

Light microscopy-based estimation of malaria parasite density

Thick smears were prepared for all samples, whether positive or negative by RDT and the diagnostic methods to determine the parasite and density. Giemsa-stained slides were air-dried and viewed under a 100X oil immersion microscope. Two independent microscopists read the slides and a third microscopist examined the slides for quality control. The parasite load was calculated using the following formula: number of parasites/numbers of white blood cells \times exact white blood cell counts according to WHO.¹⁵ Parasitemia was categorised as low (<1000 parasites/ μL blood), moderate (1000–4999 parasites/ μL blood), high (5000–99,999 parasites/ μL blood), or hyperparasitemia ($\geq 100,000$ parasites/ μL blood).¹⁶

Plasmodium species detection by nested PCR

DNA was extracted using the Chelex-100 method on dried blood spot samples.¹⁷ The extracted DNA was used to identify *Plasmodium* species using the technique described by Singh et al.¹⁸ A standard nested-PCR protocol was used for the evaluation of genus- and species-specific *Plasmodium* DNA within the highly conserved regions of the small-subunit

(SSU) rRNA gene according to the protocol described by Snounou et al.¹⁹ Nested-PCR is a technique in molecular biology that involves performing a second round of amplification using a product obtained from the first round of PCR. The targeted 18S (SSU) rRNA gene common to all four *Plasmodium* species was amplified using a specific primer pair (PCR1). Whenever the genus-specific PCR1 revealed positive results, the species-specific nest primers were used to determine the *Plasmodium* species (Table 1).

Data Processing and Statistical Analyses

The data were entered into an Access 2016 database, and statistical analyses were performed using STATA version 14 software (Stata Corp, College Station, TX, USA). Data were analysed using descriptive statistics (means, standard deviations, and confidence intervals). Proportions of positive tests were determined, and the proportion of malaria cases was compared for each characteristic using the chi-squared test. *P-values* <0.05 were considered to indicate statistical significance.

Results

Demographic Characteristics of the Study Population

During the study period, 638 individuals from 7 districts were included for analysis. The mean age of the participants was 26.44 ± 14.69 years, range 1–75 years, and the sex ratio (M/F) was 1.22. Over three-quarters (502/638, 78.68%) of the participants were aged ≥15 years (Table 2).

Proportion of Subclinical Infections and Circulating Species

The overall prevalence of *Plasmodium* spp. was 32.45% (95% CI 31.29 –33.61%) by PCR, 27.59% (95% CI, 25.75–30.35%) by mRDT, and 23.51% (95% CI, 20.64%–24.82%), respectively. Of the PCR-positive samples, 98.55% (204/207) were *Plasmodium falciparum*, 3.38% (7/207) were *Plasmodium ovale* and 1.93% (4/207) were *P. falciparum* / *P. ovale* coinfections. The prevalence of *P. ovale* in the study population was estimated at 1.1% (7/638) (Figure 1).

There were no significant differences in PCR results according to sex, age, or location. Using the mRDT, we found that malaria prevalence was higher in the 15–49 age group (30%) (Table 3). Participants under five years of age had a prevalence of <5% using the mRDT. The 4th district of Bangui showed the highest prevalence of malaria, with 57.14% of cases by mRDT compared with the other districts, with prevalence rates ranging between 23 and 28% (Table 3). No significant differences in sex or age were observed in the microscopic results. Nevertheless, a highly significant difference was observed according to district, with the 6th and 8th districts showing 32.43 and 37.80%, respectively, whereas the 1st and 2nd district showed prevalence rates of 1.87 and 7.5%, respectively (Figure 2, Table 3). This study was not conducted in the 3rd district because of the lack of security in the area.

Table 1 Primer Sequences Used to Identify *Plasmodium* Species and PCR Product Sizes

	PCR	Primers Names	Primers Sequences	Size of PCR Products
<i>Plasmodium</i> genus	PCR1	rPLU5 rPLU6	5'-cctgttggtgccttaaaccttc-3' 5'-ttaaattgttgagcttaaacg-3'	-
<i>P. falciparum</i>	Nested	rFAL-F	5'-cttttgagagggtttgtactttgagtaa-3'	205 bp
		rFAL-R	5'-tattccatgctgtagtattcaacaaaa-3'	
<i>P. ovale</i>		rOVA-F	5'-ttttgaagaatacattaggatacaattaatg-3'	800 bp
		rOVA-R	5'-catcggttcctctaagaagctttaccct-3'	
<i>P. vivax</i>		rVIV-F	5'-acgcttctagcttaatccacataact-3'	120 pb
		rVIV-R	5'-atttactcaaagtaacaaggacttccaagc-3'	
<i>P. malariae</i>		rMAL-F	5'-ataacatagttgtacgttaagaataaccgc-3'	144 bp
		rMAL-R	5'-aaaattcccatgcataaaaaattatacaaa-3'	

Table 2 Baseline Characteristics of the Study Population, Bangui, Central African Republic 2021

Variables	Frequency	
	Number	%
Sex		
M	287	45
F	351	55
Age group (year)		
<5 years	21	3.029
5–14 years	115	18.03
15–49 years	444	69.59
≥50 years	58	9.09
District		
1st	107	16.77
2nd	40	6.27
4th	28	4.39
5th	139	21.79
6th	74	11.6
7th	123	19.28
8th	127	19.91

Proportion of Submicroscopic Infections, and Parasite Density

Around, 9.56% of the participants had submicroscopic infection. However, 79% of submicroscopic infections occurred in the 15–49 age group. The parasite density among the study participants ranged from 80 to 18,706 parasites/ μ L. The mean

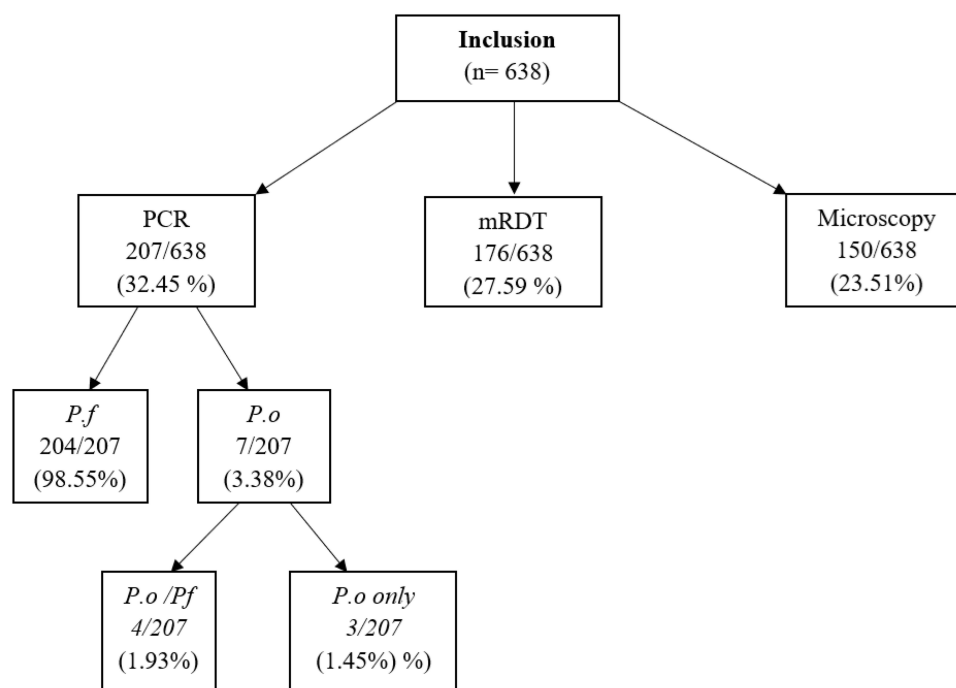


Figure 1 Outcomes of malaria in the study population in the study population. Prevalence using PCR, RDT and microscopy technical, Bangui, Central African Republic 2021.

Table 3 Characteristics of Study Population in Association With Malaria Diagnostic Tests, Bangui, Central African Republic 2021

Variables	N	PCR		P-value	mRDT		P-value	Microscopy		P-value
		Positives n (%)	Negatives n (%)		Positives n (%)	Negatives n (%)		Positives n (%)	Negatives n (%)	
Sex										
M	287	105(36.59)	182(63.41)	0.053	82(28.57)	205(71.43)	0.62	63(21.95)	224(78.05)	0.4
F	351	102(29.10)	249(70.90)		94(26.78)	257(73.22)		87(24.79)	264(75.21)	
Age group (years)				0.27			0.039			0.23
<5	21	3(14.29)	18(85.71)	0.16	1(4.76)	20(95.24)	0.033	1(4.76)	20(95.24)	0.00001
5–14	115	35(30.44)	80(69.56)		28(24.35)	87(75.65)		29(25.22)	86(74.78)	
15–49	444	151(34.01)	293(65.99)		134(30.2)	310(69.80)		106(23.87)	338(76.13)	
≥50	58	18(31.03)	40(68.97)		13(22.41)	45(77.59)		14(24.14)	44(75.86)	
Districts										
1st	107	40(37.38)	67(62.62)		29(27.10)	78(72.90)		2(1.87)	105(98.13)	
2nd	40	10(25)	30(75)		11(27.5)	29(72.5)		3(7.5)	37(92.5)	
4th	28	10(35.7)	18(64.3)		16(57.14)	12(42.86)		5(17.86)	23(82.14)	
5th	139	39(28.09)	100(71.91)		33(23.74)	106(76.26)		37(26.62)	102(73.38)	
6th	74	27(36.49)	47(63.51)		18(24.32)	56(75.68)		24(32.43)	50(67.57)	
7th	123	31(25.20)	92(74.80)		33(26.83)	90(73.17)		31(25.20)	92(74.8)	
8th	127	50(39.37)	77(60.63)		36(28.35)	91(71.65)		48(37.80)	79(62.2)	

Notes: Bold values represent the highest proportions with a statistically significant result.

of parasite densities was 909 parasites per μL (with a geometric mean of 357 parasites/ μL ; 95% CI 323–394). Approximately 80% of subjects who tested positive by microscopy had low parasite density, 12.67% had moderate parasite density, and 7.33% had high parasite density.

Discussion

This study is the first investigation of malaria prevalence in urban and semi-urban populations in the CAR. The results indicated a high prevalence of asymptomatic/subclinical malaria in CAR, with one in three individuals carrying the

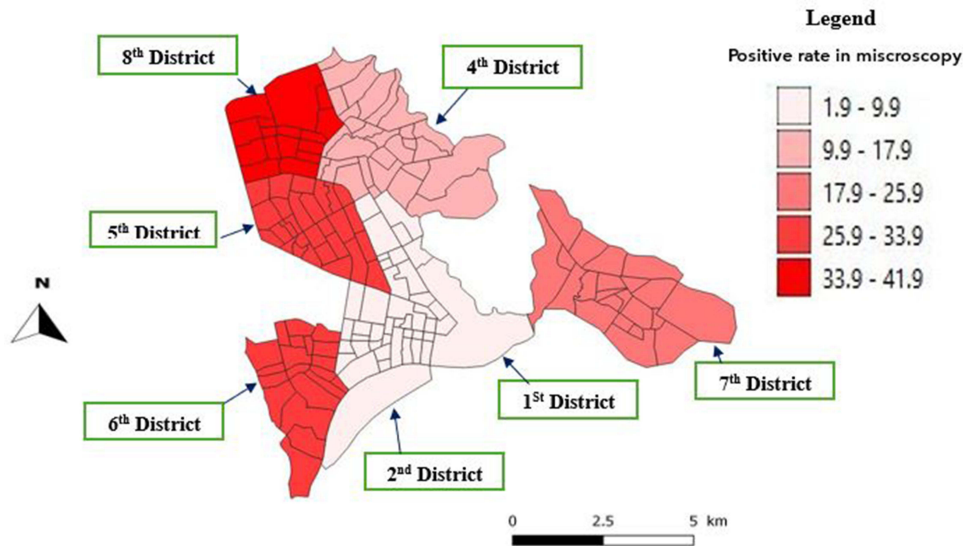


Figure 2 Mapping of the study area and malaria prevalence according to different districts with the microscopy results, Bangui, Central African Republic 2021.

disease and one in ten of those with high parasitaemia. Despite local efforts to reduce malaria transmission, it remains the most serious threat to public health in the Central African Republic (CAR). This is highlighted by the prevalence of malaria using microscopic techniques, which shows the existence of a human reservoir and the active circulation of the parasite in the population, particularly in Bangui. The prevalence of malarial parasitaemia in Bangui, as determined by light microscopy and RDT in our study, was slightly lower than that estimated at the national level. The PCR-positive proportion in our study is similar to that of previous findings in the country, estimated at 334.7 per 1000 inhabitants in the CAR.² In our previous studies, as in many others, malaria transmission intensities varied according to epidemiological facies, with prevalence often higher in rural than peri-urban and urban areas.²⁰ Given that our study area, Bangui, is an urban area and that 3/4 of the CAR population lives in rural areas, the burden of malaria in rural communities would therefore be higher. The WHO has indicated that the actual number of malaria cases in the CAR is grossly underestimated and may even be several times higher than reports suggest.²¹

The malaria proportion positive tests found in our study were higher than that of a community-based study carried out in several rural districts in other countries, including Tanzania, with 9%, 9%, and 29% malaria prevalence for microscopy, mRDT, and PCR, respectively.²² Several factors may explain the higher value of PCR compared to microscopy and mRDT, such as low parasitaemia undetectable by microscopy and mRDT because of the higher sensitivity of PCR (2–5 parasites/mL),²³ persistence of *Plasmodium* DNA which requires careful interpretation, and correlation with microscopy and clinical presentation.²⁴ The mRDT value can also be influenced by the persistence of PfHRP2 antigen from previous infections.²⁵ Factors such as transport and storage conditions and the inability of the parasite to express the *hrp2* target antigen can affect TDR performance.²⁶ Furthermore, a meta-analysis study of the risk ratio between RDT: PCR or RDT:microscopy prevalence, has shown that, on average, RDT detected 41% of PCR-positive infections and microscopy captured 87% of RDT-positive infections.²⁶ However, data for the comparison of RDT to PCR detection at high transmission intensity and in adults were sparse.²⁷

A similar study carried out in western Cameroon, a country bordering the CAR, showed a prevalence of 21% and 26% by mRDT and microscopy, respectively.²⁸ Another study carried out in Butajira, in the south-central region of Ethiopia, revealed a malaria prevalence of 21% using microscopy, which is similar to our findings.²⁹ In addition, a community-based study in rural areas in Nigeria showed higher prevalence rates of mRDT (56.8%) and 38.6% with microscopy.³⁰

Our study also revealed a significant proportion of submicroscopic infections (9.56%), which is comparable to that in a study carried out in three villages in Senegal, with values ranging from 2.89% to 12.5%.³¹ However, this was lower than that reported in a study conducted in Tanzania (18%).²² Parasite density being controlled by acquired immunity in infected host populations in high-transmission areas is more likely to have submicroscopic infections which are not detectable by microscopy and rRDT.⁵

Plasmodium falciparum infection was found in 98.55% and *P. ovale* in 3.38% only, and in coinfection, consistent with existing data showing that *P. falciparum* is responsible for 82–100% of all malaria cases.³² This result confirms the predominant circulation of *P. falciparum* in CAR and the low circulation of *P. ovale*, which is becoming endemic, as recent studies have shown that this species circulates, although at a rate of approximately 2%.³³

This study was conducted during the COVID-19 pandemic, which may have influenced our results. Empirical treatment with repurposed drugs such as chloroquine (CQ), hydroxychloroquine, ACT, and azithromycin, all anti-malarial drugs, was ongoing. Additionally, self-medication and traditional plant remedies are being used, which could have impacted the malaria prevalence at the time of our study.

Furthermore, the limited access to malaria diagnosis, treatment, and prevention during the global COVID-19 pandemic may have contributed to the increase in the prevalence of malaria in the community.³⁴

The burden of subclinical malaria was higher in age groups between 15 and 49 years (30%) than in other age groups in the current study, which is consistent with several studies on malaria at the community level, where adults appear to be more affected than the paediatric population.²⁹ This could be explained by the fact that paediatric malaria is often symptomatic and is, therefore, rapidly referred to health facilities. In addition, targeted malaria control interventions focusing on children under five years and pregnant women with free diagnosis and treatment could also explain the low number of children enrolled in our study and the low prevalence of malaria.

Overall, our results highlight the microspatial heterogeneity of malaria in the Bangui. Malaria prevalence was found to be higher in semi-urban boroughs (6th, 7th, and 8th) than in urban areas (1st, 2nd, 4th and 5th) which is consistent with a recent study on malaria conducted on a network of sentinel sites set up for influenza surveillance in CAR, including Bangui, where it showed a relatively high prevalence in semi-urban areas compared with urban areas.³³ Several other African studies have indicated that urban areas have a lower prevalence of malaria prevalence.¹⁸

Community-level malaria is an important indicator of the outcome of control programs. The identification of individuals with asymptomatic malaria via active surveillance using mRDT or PCR is an important component of malaria elimination efforts.³⁵ The information obtained from this study can be used to improve control and community-centred strategies. Asymptomatic, subclinical and submicroscopical infections behave like the submerged base of icebergs and are a hidden threat that hinders efforts aimed at control malaria.³⁶ Without treatment, they will continue to serve as a source of malaria reservoir and enhance transmission by transmitting the malaria parasites to a large number of mosquitoes, contributing to the persistence of malaria transmission within local populations and infecting children and pregnant women who are more vulnerable and may die of malaria.³⁷ Subclinical infections are important within a community as unknowing (asymptomatic) carriers of pathogens do not change their behaviour to prevent the spread of disease.³⁶ The treatment of the infective parasite reservoir of asymptomatic individuals/subclinical infection may be an important intervention strategy to interrupt the transmission. Future efforts to reduce malaria further will require moving beyond the treatment of clinical infections to targeting malaria transmission more broadly in the community.²⁷

Although the study was carried out during August and September, including the rainy season (May–October), this would not have had a significant impact on the results, as it has been shown that malaria transmission is perennial and therefore holoendemic in CAR.³³

This study had several limitations. To improve the findings, it would have been beneficial to include body temperature values for the participants, allowing for a more precise definition of the prevalence of asymptomatic parasitaemia in the area. Additionally, information on the community's perception of malaria and the use of preventive measures, such as MILDA and ITNs, could have been useful in determining factors associated with malaria parasite carriage in the area. It is worth noting that the mRDT Ag P.f/Pan could have provided more information on the current episode of malaria than the one we used (P.f), which also detects past infection.³⁸

Conclusion

Despite considerable efforts to mitigate malaria transmission in Bangui, our findings highlight that malaria remains a major public health challenge in this region. We identified a substantial human reservoir facilitating the active spread of the parasite, with one in every three residents affected, and a significant proportion suffering from high parasitaemia. Our findings also show that older children and adults are more likely to harbor subclinical malaria, contributing to the silent yet widespread burden of asymptomatic parasitaemia.

This study underscores the urgent need for sustained, targeted, and community-centred malaria control strategies in the CAR. In light of the promising development of the new malaria vaccine, integrating vaccination campaigns with strengthened community-based approaches is critical to amplify the impact. Special emphasis should be placed on improving household hygiene practices to reduce malaria reservoirs, particularly through education and community engagement initiatives. These combined efforts can address the human and environmental factors sustaining malaria transmission.

Our findings provide an essential baseline for future studies and interventions aimed at curtailing the pervasive impact of malaria on the CAR, paving the way for more effective and sustainable malaria control programs.

Abbreviations

CAR, Central African Republic; mRDT, malaria rapid diagnostic test; PCR, Polymerase Chain Reaction; WHO, World Health Organization; LLINs, Long-Lasting Insecticide-treated nets; PECADOM, home-based management of malaria; iCCM, Integrated Community case Management; CHW, community health workers; ACT, Artemisinin-based combination therapy; COVID-19, Coronavirus Disease 2019; EDTA, ethylenediaminetetraacetic acid; PfHRP-II, *Plasmodium falciparum*-specific histidine-rich protein II; DNA, deoxyribonucleic acid; rRNA, ribosomal Ribonucleic Acid; MILDA, long-acting insecticide-treated mosquito nets.

Ethics Approval and Consent to Participate

This study complies with the Declaration of Helsinki, authorized by the Ministry of Health of the Central African Republic (N° 603/MSPP/DIRCAB/CMRF-21) and by the Ethics and Scientific Committee of the University of Bangui (N°09/UB/FACSS/IPB/CES/20). Consent from each adult and the parents of the child was obtained before inclusion.

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Author Contributions

All authors made a significant contribution to the work reported, whether that is in the conception, study design, execution, acquisition of data, analysis and interpretation, or in all these areas; took part in drafting, revising or critically reviewing the article; gave final approval of the version to be published; have agreed on the journal to which the article has been submitted; and agree to be accountable for all aspects of the work.

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Disclosure

The authors declare that they have no competing interests. This paper has been uploaded to ResearchSquare as a preprint: <https://www.researchsquare.com/article/rs-4406334/v1>

References

1. WHO. *World Malaria Report 2023*. Geneva: World Health Organization; 2023.
2. World Health Organization, Global health observatory data, repository/world health statistics. Available from: <https://data.worldbank.org/indicator/SH.MLR.INCD.P3?locations=CF>. Accessed on 23, November 2023.
3. Politique nationale de lutte contre le paludisme de la RCA, Programme National de lutte contre le paludisme, ministère de la Santé et de la population, version octobre[National Malaria Control Policy in the Central African Republic, National Malaria Control Program, Ministry of Health and Population] 2016 French, 32 p.
4. Liu Z, Soe TN, Zhao Y, et al. Geographical heterogeneity in prevalence of subclinical malaria infections at sentinel endemic sites of Myanmar. *Parasit Vectors*. 2019;12(1):83. doi:10.1186/s13071-019-3330-1
5. Huang F, Takala-Harrison S, Liu H, et al. Prevalence of clinical and subclinical plasmodium falciparum and plasmodium vivax malaria in two remote rural communities on the Myanmar-China border. *Am J Trop Med Hyg*. 2017;97(5):1524–1531.
6. Ayanful-Torgby R, Sarpong E, Abagna HB, et al. Persistent Plasmodium falciparum infections enhance transmission-reducing immunity development. *Sci Rep*. 2021;11(1):21380. doi:10.1038/s41598-021-00973-5
7. Andolina C, Rek JC, Briggs J, et al. Sources of persistent malaria transmission in a setting with effective malaria control in eastern Uganda: a longitudinal, observational cohort study. *Lancet Infect Dis*. 2021;21(11):1568–1578. doi:10.1016/S1473-3099(21)00072-4
8. Kalula A, Mureithi E, Marijani T, Mbalawata I. Optimal control and cost-effectiveness analysis of age-structured malaria model with asymptomatic carrier and temperature variability. *J Biol Dyn*. 2023;17(1):2199766. doi:10.1080/17513758.2023.2199766
9. Lindblade KA, Steinhart L, Samuels A, Kachur SP, Slutsker L. The silent threat: asymptomatic parasitemia and malaria transmission. *Expert Rev Anti Infect Ther*. 2013;11:623–639.
10. Laishram DD, Sutton PL, Nanda N, et al. The complexities of malaria disease manifestations with a focus on asymptomatic malaria. *Malar J*. 2012;11(1):29. doi:10.1186/1475-2875-11-29
11. Sangbakembi-Ngounou C, Costantini C, Longo-Pendy NM, et al. Diurnal biting of malaria mosquitoes in the Central African Republic indicates residual transmission may be “out of control”. *Proc Natl Acad Sci*. 2022;119(21):e2104282119. doi:10.1073/pnas.2104282119
12. Debash H, Tesfaw G, Ebrahim H, et al. Symptomatic and asymptomatic malaria prevalence and its determinant factors in pastoral communities of Waghemira Zone, Northeast Ethiopia: a community-based cross-sectional study. *Health Sci Rep*. 2023;6(6):e1336. doi:10.1002/hsr2.1336
13. Manirakiza A, Malaka C, Longo JD, et al. Sero-prevalence of anti-SARS-CoV-2 antibodies among communities between July and August 2022 in Bangui, Central African Republic. *J Public Health Afr*. 2023;14(8):2315. doi:10.4081/jphia.2023.2315
14. Kambou SAE, Millogo KS, Sondo P, et al. Prevalence of asymptomatic parasitaemia among household members of children under seasonal malaria chemoprevention coverage and comparison of the performance of standard rapid diagnostic tests versus ultrasensitive RDT for the detection of asymptomatic parasitaemia in Nanoro, Burkina Faso. *Parasitol Res*. 2024;123(11):383. doi:10.1007/s00436-024-08380-1
15. WHO. *Basic Malaria Microscopy*. 2nd ed. ed. Geneva: World Health Organization; 2010.
16. Chipwaza B, Sumaye RD. High malaria parasitemia among outpatient febrile children in low endemic area, East-Central Tanzania in 2013. *BMC Res Notes*. 2020;13(1):251. doi:10.1186/s13104-020-05092-4

17. Walsh PS, Metzger DA, Higuchi R. Chelex 100 as a medium for simple extraction of DNA for PCR-based typing from forensic material. *Biotechniques*. 1991;10(4):506–513.
18. Singh B, Bobogare A, Cox-Singh J, Snounou G, Abdullah MS, Rahman HA. A genus- and species-specific nested polymerase chain reaction malaria detection assay for epidemiologic studies. *Am J Trop Med Hyg*. 1999;60(4):687–692. doi:10.4269/ajtmh.1999.60.687
19. Snounou G, Viriyakosol S, Zhu XP, et al. High sensitivity of detection of human malaria parasites by the use of nested polymerase chain reaction. *mol Biochem Parasitol*. 1993;61(2):315–320. doi:10.1016/0166-6851(93)90077-B
20. Maghendji-Nzondo S, Kouna LC, Mourembou G, et al. Malaria in urban, semi-urban and rural areas of southern of Gabon: comparison of the Pfmdr 1 and Pfcrt genotypes from symptomatic children. *Malar J*. 2016;15(1):420. doi:10.1186/s12936-016-1469-1
21. Korzeniewski K, Bylicka-Szczepanowska E, Lass A. Prevalence of asymptomatic malaria infections in seemingly healthy children, the Rural Dzanga Sangha Region, Central African Republic. *Int J Environ Res Public Health*. 2021;18(2):814. doi:10.3390/ijerph18020814
22. Rapp T, Amagai K, Sinai C, et al. Micro-heterogeneity of transmission shapes the submicroscopic malaria reservoir in coastal Tanzania. *J Infect Dis*. 2024;jiae276.
23. Berzosa P, de Lucio A, Romay-Barja M, et al. Comparison of three diagnostic methods (microscopy, RDT, and PCR) for the detection of malaria parasites in representative samples from Equatorial Guinea. *Malar J*. 2018;17(1):333. doi:10.1186/s12936-018-2481-4
24. Mayxay M, Pukrittayakamee S, Chotivanich K, Looareesuwan S, White NJ. Persistence of Plasmodium falciparum HRP-2 in successfully treated acute falciparum malaria. *Trans R Soc Trop Med Hyg*. 2001;95(2):179–182. doi:10.1016/S0035-9203(01)90156-7
25. Marti  ez-Vendrell X, Skjefte M, Sikka R, Gupta H. Factors affecting the performance of HRP2-based malaria rapid diagnostic tests. *Trop Med Infect Dis*. 2022;7(10):265. doi:10.3390/tropicalmed7100265
26. Si   A, EK, Solange Millogo K, Sondo P, Kabore B, Fifi Chantal Kouevi A, Bouda I. Prevalence of asymptomatic parasitaemia among household members of children under seasonal malaria chemoprevention coverage and comparison of the performance of standard rapid diagnostic tests versus ultrasensitive RDT for the detection of asymptomatic parasitaemia in Nanoro, Burkina Faso. *Parasitol Res*. 2024;123(11):383.
27. Wu L, van den Hoogen L, Slater H, et al. Comparison of diagnostics for the detection of asymptomatic Plasmodium falciparum infections to inform control and elimination strategies. *Nature*. 2015;528(7580):S86–S93. doi:10.1038/nature16039
28. Bamou R, Nematchoua-Weyou Z, Lontsi-Demano M, et al. Performance assessment of a widely used rapid diagnostic test CareStart   compared to microscopy for the detection of Plasmodium in asymptomatic patients in the Western region of Cameroon. *Heliyon*. 2021;7(2):e06271. doi:10.1016/j.heliyon.2021.e06271
29. Duguma T, Nuri A, Melaku Y. Prevalence of malaria and associated risk factors among the community of mizan-aman town and its catchment area in southwest Ethiopia. *J Parasitol Res*. 2022;2022:3503317. doi:10.1155/2022/3503317
30. Nwele DE, Onyali IO, Iwueze MO, Elom MO, Uguru OES. Malaria endemicity in the rural communities of Ebonyi State, Nigeria. *Korean J Parasitol*. 2022;60(3):173–179. doi:10.3347/kjp.2022.60.3.173
31. Niang M, Thiam LG, Sane R, et al. Substantial asymptomatic submicroscopic Plasmodium carriage during dry season in low transmission areas in Senegal: implications for malaria control and elimination. *PLoS One*. 2017;12(8):e0182189. doi:10.1371/journal.pone.0182189
32. Feufack-Donfack LB, Sarah-Matio EM, Abate LM, et al. Epidemiological and entomological studies of malaria transmission in Tibati, Adamawa region of Cameroon 6 years following the introduction of long-lasting insecticide nets. *Parasit Vectors*. 2021;14(1):247. Erratum in: Parasit Vectors. 2021;14(1):512. doi:10.1186/s13071-021-04745-y
33. Nzoumbou-Boko R, Pant  -Wockama CG, Ngoagoni C, et al. Molecular assessment of kelch13 non-synonymous mutations in Plasmodium falciparum isolates from Central African Republic (2017-2019). *Malar J*. 2020;19(1):191. doi:10.1186/s12936-020-03264-y
34. Bylicka-Szczepanowska E, Korzeniewski K. Asymptomatic malaria infections in the time of COVID-19 pandemic: experience from the Central African Republic. *Int J Environ Res Public Health*. 2022;19(6):3544. doi:10.3390/ijerph19063544
35. Chen I, Clarke SE, Gosling R, et al. “asymptomatic” malaria: a chronic and debilitating infection that should be treated. *PLoS Med*. 2016;13(1):e1001942. doi:10.1371/journal.pmed.1001942
36. Banegas S, Escobar D, Pinto A, et al. Asymptomatic malaria reservoirs in Honduras: a challenge for elimination. *Pathogens*. 2024;13(7):541. doi:10.3390/pathogens13070541
37. Smith T, Schellenberg JA, Hayes R. Attributable fraction estimates and case definitions for malaria in endemic areas. *Stat Med*. 1994;13(22):2345–2358. doi:10.1002/sim.4780132206
38. Grandesso F, Nabasumba C, Nyehangane D, et al. Performance and time to become negative after treatment of three malaria rapid diagnostic tests in low and high malaria transmission settings. *Malar J*. 2016;15(1):496. doi:10.1186/s12936-016-1529-6

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