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High prevalence of asymptomatic *Plasmodium falciparum* malaria in Makenene, a locality in the forest-savannah transition zone, Centre Region of Cameroon

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ABSTRACT

Malaria transmission and prevalence is still not well documented across Cameroon particularly in medium-sized cities or localities representing high transit zone. Different risk factors could be associated with persistence malaria transmission such as population movement from high to low transmission settings. A cross-sectional community-based study was carried out to determine malaria prevalence and risk factors in Makenene, a small city in a forest-savannah which is a crossroads between different parts of the country where travellers usually stop-over day and night to rest. Using malaria diagnostic test (mRDTs from SD-BIOLINE) and microscopy (thin and thick blood smears), 406 participants from 237 households were tested for malaria infection. The prevalence of malaria was high irrespective of the detection method: mRDT (41.87%) or microscopy (38.42%). At household level, 46.41% of households had at least one case of malaria with an average of 1.41 infected individuals per household. Parasite density was also high with the majority of infected individuals (64.74%) bearing more than 500 parasites/µl. Only *Plasmodium falciparum* was found. The chances of being infected with malaria parasites was almost the same for all participants irrespective of the sleeping behavior, bednet usage, house type and environmental factors. The study supports high malaria transmission in the locality and the need for additional studies on vectors bionomics and transmission patterns.

1. Introduction

Malaria remains a high public health problem that affects half of the world's population. The recent WHO report estimates its incidence to 241 million cases in 2020, with about 627,000 deaths (WHO, 2021). Compared to 2019, there is a slight increase in the incidence, i.e. 5.6% in 2019 to 5.9% in 2020, mainly due to the disruption observed in health services during the COVID-19 pandemic (WHO, 2021). The burden of malaria is still higher in children under five years-old and pregnant women, but people of all ages are at risk of infection (Imboumy-Limoukou et al., 2020). The African Region continues to carry disproportionately the highest share of the global malaria burden with more than 95% of cases and deaths found in sub-Saharan Africa where *Plasmodium falciparum* is the main parasite agent (WHO, 2021). Some countries like Nigeria (26.8%), the Democratic Republic of the Congo (12.0%), Uganda (5.4%), Mozambique (4.2%), Angola (3.4%) and Burkina Faso (3.4%) accounted for 55% of all cases (WHO, 2021).

In Cameroon, malaria remains the main cause of hospital consultations with more than 5 million cases per year (Minsanté-PNLP, 2019) and represent 2.9% of all the world cases (WHO, 2021). The whole country is exposed to the risk of infection (Antonio-Nkondjio et al., 2019) with differences observed in the intensity of transmission between settings

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varying from 0.1 infective bites per person per night in Sahelian zone to 5.5 infective bites per person per night in the forest zone (Atangana et al., 2010). For several years, the country has been deploying multifaceted means to control malaria with a view to its elimination. Actions are being carried out by setting up vector control operations and rapid patient care by the administration of effective artemisinin-based combination molecules for malaria-positive cases (Minsanté-PNLP, 2019). In order to limit the expansion of drug resistance, a good diagnosis has to be done prior to the treatment, to identify and avoid treating malaria-free patients (Okiro & Snow, 2010; WHO, 2010). Identifying *Plasmodium* species is key for case management. This could be done through the use of different techniques such as microscopic examination of blood slides (Tchuinkam et al., 2015), and by immunochromatography using rapid diagnostic tests (RDTs) (Bamou et al., 2021; Wilson, 2012). These RDTs detect circulating antigens such as *P. falciparum*-specific histidine-rich protein 2 (PfHRP2), or histidine-rich protein 3 (PfHRP3) as well as lactate dehydrogenase (pLDH) or aldolase enzymes as in the pan-*Plasmodium* test (Moody, 2002). However, in asymptomatic malaria cases with low parasite densities, RDTs often fail to detect parasites due to low antigen concentrations (Mouatcho & Goldring, 2013; Bousema et al., 2014; Bamou et al., 2021). These asymptomatic malaria cases remain a challenge for malaria control programmes, as they influence transmission dynamics. The deletions in the parasite's PfHRP2 and PfHRP3 (PfHRP2/3) genes also render parasites undetectable by RDTs whose reaction mechanisms are based on histidine-rich protein 2 (HRP2). For this reason, monitoring malaria prevalence solely through RDTs could pose a threat to disease control programmes (Verma et al., 2018; Gendrot et al., 2019).

Although the scaling-up of malaria control strategies including mass distribution of long-lasting insecticide-treated nets (LLINs) across the continent contributed to a significant decrease in malaria morbidity and mortality (WHO, 2021), the effectiveness of these measures in Cameroon is threatened by the rapid expansion of insecticide resistance in vector populations (Etang et al., 2016; Antonio-Nkondjio et al., 2017), changes in vector feeding, biting and resting behaviour (Bamou et al., 2018), and probably the difference in malaria endemicity across the country due to the diversity of the ecological zones. The deep equatorial forest region is characterised by hyperendemic malaria (Antonio-Nkondjio et al., 2006; Bamou et al., 2018), the savannah zone - by a hyperendemic malaria (Atangana et al., 2010) and the Sahelian zone - by a hypoendemic malaria (Tabue et al., 2017; Antonio-Nkondjio et al., 2017). In addition, within each zone, some peculiarities exist which through human-induced or landscape variations such as highland areas (Tchuinkam et al., 2015), damps, other industrial infrastructures (Tabue et al., 2017) influence transmission pattern.

The present study assessed malaria prevalence in the locality of Makenene, a locality where regarding malaria research, only the level of knowledge, attitudes, and practices of the population with regard to malaria prevention was assessed (Djoufounna et al., 2022). Despite that study, malaria prevalence and intensity remain unknown. In addition, the locality is situated in a forest-savannah transition zone and is comprising a cosmopolitan population including natives and internally displaced people (IDPs) due to the socio-political crisis, as well as temporary travellers from different parts of the country. The study is intended to provide information that may be useful for a better management of malaria across the country.

2. Materials and methods

2.1. Study site

The study was conducted from September to October 2021 in the locality of Makenene, Cameroon. The city is located at an average altitude of 580 m in the Mbam and Inoubou Division of the Center Region of Cameroon. It is bordered to the north by the Noun River and to the south by the coastal region forest (between 3°20′N and 6°N) and to the west and east by the rivers Makenene and Nde (between 9°40′E and 13°E) (Letouzey, 1985). The climate is equatorial humid and characterized by two dry seasons and two rainy seasons of unequal length (with rainfall up to 721 mm/year): a long dry season (mid-October to March), a short dry season (June to mid-August), a long rainy season (mid-August to October), and a short rainy season (March to June). Makenene is located in the forest-savannah limit and its population estimated at 35,000 people is made up of residents and internally-displaced people (IDPs) from North-West and South-West parts of the country (Letouzey, 1985; PCD, 2011; DIPAMAK, 2018). The plant cover is made up of *c.*60% of savannah or shrub and *c.*40% of secondary forest. The main activity is agriculture to which are added small business, animal husbandry and urban transport by motorbikes among others. The city is full of some basic infrastructures including health centers, schools and public services (Letouzey, 1985; DIPAMAK, 2018).

2.2. Study population and sample size

The target population consisted of children, adolescents and adults of both genders living in Makenene households (HH). Different quarters/clusters or neighborhood in the locality were visited and only those where consent from the head of the household (HoH) was obtained were enrolled in the study. HH were selected randomly in the central and peripheric areas, but their number vary with quarters/clusters. In each household, a blood sample was taken from all members wishing to participate in the study and having given their consent. Since the prevalence of malaria in the locality and its surroundings was unknown, the sample size was estimated based on the population size of the locality using the "Yamane" formula (Tepping, 1968) as follows: $n = N/1 + N(e)^2$, where n is the size of sampling sought, N is the population size, and e is the level of precision (± 5% in this case). According to the last census, the locality has 35,000 inhabitants (DIPAMAK, 2018); the minimum sample size for this study was therefore estimated at 395 participants and we enrolled 406 participants in total.

2.3. Study design and data collection

This was a cross-sectional community-based study carried out from September to October 2021.This period corresponds to the end of rainy season and the most favorable time for the development of *Anopheles* mosquitoes. Investigations were carried out by a team composed of well-trained researchers from the Vector Borne Diseases Laboratory of the Research Unit of Biology and Applied Ecology (VBID-RUBAE) of the University of Dschang, Cameroon, two laboratory technicians from the District Medical Center (DMC) of Makenene with experience in community-based investigations and one community health worker. The community health worker was in charge of facilitating the integration of the research team in the community.

After explaining the purpose of the study to the participants, their consents and/or assents of children or family members under 18 years to participate to the study were collected. Some demographic and dwellings characteristics were collected using a pre-tested questionnaire (Supplementary file S1). Venous blood samples were collected from the participants and were stored in 5 ml EDTA tubes.

2.4. Field malaria diagnosis

Using a capillary pipette (5 μl) provided by the manufacturer, a tiny portion of blood was immediately used in the field to perform malaria rapid diagnostic tests (mRDTs; WHO, 2017). The mRDT is the immunochromatography form of the test which assists in the diagnostic of malaria by detecting specific antigens (proteins) produced by malaria parasites in the blood of an infected person. The mRDT used in this study was SD Bioline Malaria Ag P.f/Pan (05FK60) which has a sensitivity and a specificity of 95% and 99.5% respectively (Standard Diagnostics Inc., 65, Gyeonggi-do, Republic of Korea). This test can detect *P. falciparum* and other human-infecting plasmodia (*Plasmodium ovale*, *Plasmodium*

vivax and *Plasmodium malariae)* (SD Bioline Malaria Ag P.f/Pan, Standard Diagnostics Inc.).

The result was read 15 min after adding 4 drops of a "diluent" provided in the kit as recommended by the manufacturer. The presence of a single-colored band ("C" control line) in the result window indicated a negative result. When two colored bands ("P.f" test line and "C" control line) or three colored bands ("P.f", "Pan" test lines and "C" control line) appeared in the result window, whichever band appeared first, the result was considered positive respectively for *P. falciparum* or for mixed infection of *P. falciparum*, with either *P. vivax*, *P. malariae* or *P. ovale*. The test was considered positive even if the "P.f" and/or "Pan" bands were faint, and invalid if the control band ("C" control line) was not visible in the result window after performing the test. All this was done following the manufacturer guidelines.

2.5. Laboratory malaria diagnosis

Thick and thin blood films were performed by an expert in microscopy. Thick smears were stained for 15 min with a 10% Giemsa solution following a standard protocol (WHO, 2011). The presence and quantity (density) of parasites were checked during thick smears observation. Parasite density (parasites/μl of blood) was determined by the number of parasites after counting a total of 200 white blood cells. A slide was declared negative when in 100 fields scanned and for 1000 leukocytes counted, no parasite was found. Thin smears (dried and fixed with methanol, then stained with May-Grunewald Giemsa) were processed according to a standard protocol as well (WHO, 2011) on all positive slides with the double band P.f/Pan and on some other positive slides to confirm the presence of *Plasmodium* spp. in the blood. All slides were read by two experts (a laboratory technician of the DMC of Makenene and one scientist from VBID-URBEA, University of Dschang, Cameroon) for the correctness of the observation. Confirmation was done blindly on 50% of positive slides and 10% of randomly chosen negative slides by a senior technician from the *Institut de Recherche de Yaoundé - Organisation de Coordination pour la lutte contre les Endémies en Afrique Centrale* (IRY-OCEAC).

2.6. Statistical analyses

Data were coded and entered in an Excel 2016 database (Supplementary file S2), cross-checked and then analyzed in R software (R version 4.1.3, 2022-03-10). Percentages of the different variables were calculated as well as the geometric means (for parasite density or parasitaemia) with standard deviation where appropriate. Independent chi-square (χ^2) and Fisher's exact tests were used to compare proportions between categorical variables (gender, age groups), while Mann-Whitney-Wilcoxon and Kruskal-Wallis tests were used to compare parasite density distributions between categorical variables (gender, age groups). Univariate and multivariate logistic regression models were used to determine the potential risk factors of malaria prevalence. The performance of the mRTD used was determined through the calculation of sensitivity (SE (%) = [No. of true positives/(No. of true positives + No. of false negatives)] × 100) and specificity (SP (%) = [No. of true negatives/(No. of true negatives + No. of false positives)] × 100) following Florkowski (2008) and WHO (2017) with microscopy as the gold standard (reference) method. Results were considered significant when $P < 0.05$.

3. Results

3.1. Sociodemographic data for participants

Out of a total of 406 participants from 237 households, 248 (61.1%) and 158 (38.9%) were female and male, respectively, with a mean of 2.32 ± 1.73 individuals sampled per household (range: 1–8). The age of participants ranged from 6 months to 80 years with a mean of 22.35 ± 17.66 years-old. The adult age group of 25–64 years-old was the most represented (35.6%). Participants live in different types of houses with vegetation almost all around. A summary of the characteristics of the study population is provided in Table 1.

3.2. Prevalence of malaria infection

The prevalence of malaria varied with the diagnostic tool used. Out of the 406 participants sampled, 170 (41.9%) were found infected with malaria parasites using mRDT, while 156 (38.4%) were found positive after microscopic examination (Table 2). The sensitivity and specificity of the mRDT obtained by considering microscopy as the reference method were 88.5% and 87.2%, respectively. Out of the 237 households surveyed, 110 (46.4%) had at least one case of malaria with an average of 1.41 ± 0.91 infected individuals per household (with 0–6 infected people per household).

3.3. Overall mRDT performance

Table 3 presents the sensitivity values obtained by comparing the performance of mRDT used (SD Bioline) according to microscopy. It showed that, the highest sensitivities (close to the reference) were obtained for values greater than 500 parasites/μl of blood. Beyond 5000 parasites/μl of blood, the sensitivity level reached 100%.

3.4. Plasmodium species circulating in Makenene

Only one species of *Plasmodium* (*P. falciparum*) was detected during the study, despite the double P.f/Pan band displayed on the mRDT. To understand if the presence of double bands was linked to parasite density (parasitaemia), the proportion of samples with double bands was calculated for several parasitemia ranges. It was found that the proportion (frequency) of samples with double band (Pf/Pf-Pan) increased with the level of parasitemia (Fig. 1). The mean density of *Plasmodium* (parasitemia) was higher in case of the presence of a double band (P.f/Pan bands) compared to one band (Pf band) (Kruskal-Wallis-Wilcoxon test; $W = 1123.5$, $P = 0.0003$). This means that double bands (Pf/Pan) are more likely to appear when the parasite density (parasitaemia) exceeds a certain threshold.

3.5. Gender, age and malaria infection

Malaria prevalence was correlated with some demographic characteristics of the population as presented in Table 4. Malaria prevalence did not vary significantly according to gender (Chi square test: $\chi^2 = 0.14$, $P = 0.71$) while significant variation was observed between age groups

Table 1
Sociodemographic characteristics of the participants.

Category	Variables	*N*	%
Gender	Male	158	38.9
	Female	248	61.1
Age group (in years)	< 5	65	16.1
	5–14	100	24.8
	15–24	82	20.3
	25–64	144	35.6
	≥ 65	13	3.2
House construction material	Mud and cement	113	27.8
	Cement blocks	149	36.7
	Earth briks	144	35.5
Grass around house	Present	307	75.6
	Absent	99	24.4
Swamp/stagnant water around house	Present	104	25.6
	Absent	302	74.4
Opening between roof and walls (eaves)	Present	84	20.9
	Absent	318	79.1

Abbreviation: *N*, number of participants per group.

Table 2
Malaria prevalence according to the diagnostic tests.

Diagnostic test	Participants tested	*N*	Prevalence (%)
Microscopic examination[a]	Positive	156	38.4
	Negative	250	61.6
	Total	406	100
SD Malaria P.f/Pan Ag RDT[b]	Positive	170	41.9
	Negative	236	58.1
	Total	406	100

Abbreviation: *N*, number of participants sampled.
[a] Reference method for malaria diagnostic test.
[b] SD BIOLINE Malaria Ag P.f/Pan rapid diagnostic test (RDT).

Table 3
Sensitivity obtained by SD BIOLINE Malaria Ag P.f/Pan according to parasitaemia.

No. of parasites/µl of blood	No. of positive cases		Sensitivity (%)	Reference sensitivity[a] (%)
	Microscopy	SD Bioline Malaria Ag P.f/Pan		
1–50	2	1	50.00	50.0
51–100	10	4	40.00	90.0
101–500	43	35	81.40	96.0
501–1000	23	22	95.65	100
1001–5000	43	41	95.35	100
> 5000	35	35	100	100
Total	156	138	88.50	95.5

[a] Sensitivity indicated by the manufacturer.

(Fisher's exact test: $F = 10.6$, $P = 0.03$). Participants of the age group 5–14 years-old were more frequently infected than others. Concerning parasite density, no significant differences were observed between gender and age groups (Table 4).

3.6. Sleeping behaviours, bednet usage and malaria prevalence

Of all the participants who tested positive, 93 (38.3%) declared to use mosquito nets as means of protection against malaria. The majority of participants declared going to bed before 22:00 h and wake up after 5:00 h. Table 5 shows that the chances of being infected were almost the same for the different variables related to sleeping behaviour. Yet, no significant differences were observed even after variables adjustment.

3.7. House type, environmental characteristics and malaria prevalence

Some environmental characteristics were recorded in order to determine whether they are related to malaria prevalence or not. Logistic regression analyses were carried out between the type of habitat and the probability of occurrence of malaria. Even though the prevalence was higher in houses built with earth bricks, the likelihood of being infected with malaria parasites was almost the same for all types of habitats as well as for all environmental factors considered with no significant difference observed even after adjusting variables (Table 6).

4. Discussion

Accurate assessment of malaria infection, and therefore prevalence, may help expand malaria control and surveillance interventions (Iqbal et al., 2008). This study aimed to assess malaria prevalence and risk factors in Makenene, Centre Region of Cameroon, to provide information that could be used by the public health sector to improve its control strategy in this locality.

A prevalence of 38.4% was recorded from the asymptomatic sampled participants with more than 46% of households infected and with an average of 1.41 ± 0.91 infected individuals per household. This result is close to the prevalence observed in previous studies in Cameroon (Kimbi et al., 2012; Ndamukong-Nyanga et al., 2014; Lehman et al., 2018). This can be explained by the fact that, in areas with local malaria

Table 4
Malaria parasite prevalence and geometric mean parasite density (GMPD) according to gender and age.

Variable	No. tested	No. infected	Prevalence (%)	P_1	GMPD ± SD	P_2
Gender				0.71		1.00
Male	158	63	39.87		1391 ± 38,114	
Female	248	93	37.50		1490 ± 72,376	
Age group (in years)				0.03		0.46
0–4	65	25	38.41		1564 ± 56,678	
5–14	100	51	51.00		1746 ± 78,790	
15–24	82	30	36.58		1601 ± 18,190	
25–64	144	46	31.94		920 ± 34,890	
≥ 65	13	3	23.08		1664 ± 2880	

Note: P_1 is the *P*-level obtained using the Chi-square and Fisher's exact test; P_2 is the *P*-level obtained using the Mann-Whitney-Wilcoxon and Kruskal-Wallis test.

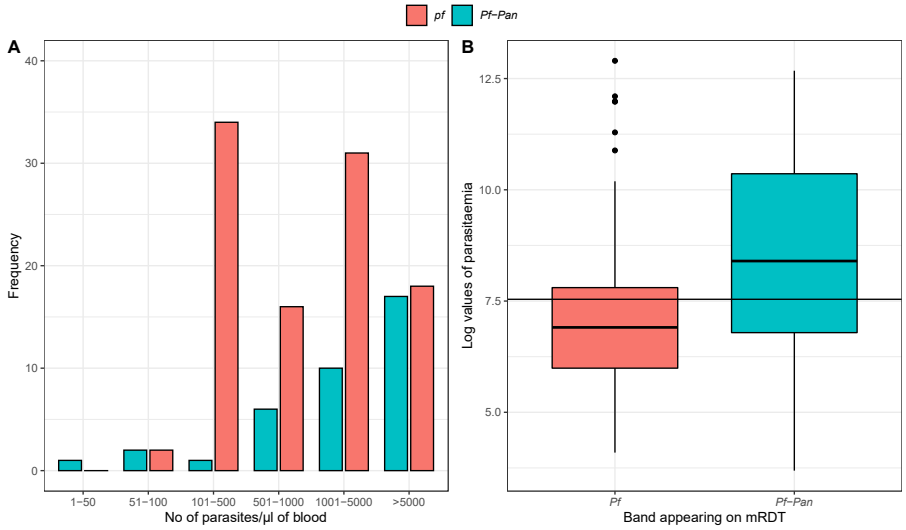

Fig. 1. Band displayed on SD Bioline mRDT and parasitaemia. **A** Variation of the proportion of samples with single and double bands displayed with respect to parasitaemia ranges. **B** Comparison of the parasitaemia distribution between samples with single and double bands.

Table 5
A logistic regression model of the impact of human sleeping behaviours on malaria parasite prevalence.

Variable	*n* (*N*)	Prevalence (%)	naOR (95% CI)	*P*-value	aOR (95% CI)	*P*-value
Sleeping times						
Before 22:00 h	115 (285)	40.35	Reference	0.08	Reference	0.98
After 22:00 h	26 (88)	29.54	1.61 (0.96–2.70)		1.01 (0.43–2.47)	
Waking up times						
After 5:00 h	122 (315)	38.73	Reference	0.46	Reference	0.35
Before 5.00 h	19 (58)	32.76	0.77 (0.42–1.39)		0.81 (0.53–1.26)	
Bednet use						
Yes	93 (243)	38.27	Reference	1.00	Reference	0.59
No	63 (163)	38.65	1.009 (0.79–1.30)		0.88 (0.57–1.37)	

Abbreviations: *n*, no. of positive cases; *N*, total no. of participants; naOR, non-adjusted OR; aOR, adjusted OR; OR, odds ratio; CI, confidence interval.

Table 6
A logistic regression model of the effect of habitat type and environmental characteristics on malaria parasite prevalence.

Variable	*n* (*N*)	Prevalence (%)	naOR (95% CI)	*P*-value	aOR (95% CI)	*P*-value
House type						
Earth brick	62 (144)	43.05	Reference		Reference	
Semi-hard	40 (113)	35.40	0.72 (0.43–1.20)	0.24	1.42 (0.84–2.44)	0.19
Hard	54 (149)	36.24	0.75 (0.47–1.20)	0.28	1.48 (0.88–2.49)	0.13
Vegetation						
Present	114 (307)	37.13	Reference		Reference	
Absent	42 (99)	42.42	1.14 (0.87–1.50)	0.34	0.68 (0.41–1.13)	0.13
Swamps/Stagnant water						
Absent	114 (302)	37.75	Reference		Reference	
Present	42 (104)	40.38	1.07 (0.81–1.40)	0.64	0.68 (0.41–1.14)	0.14
Opening between roof and walls						
Absent	128 (318)	40.25	Reference		Reference	
Present	25 (84)	29.77	0.62 (0.37–1.05)	1.00	1.60 (0.93–2.82)	0.09

Abbreviations: *n*, no. of positive cases; *N*, total no. of participants; naOR, non-adjusted OR; aOR, adjusted OR; OR, odds ratio; CI, confidence interval.

transmission, repeated exposures to bites of infective mosquitoes carrying malaria parasites make the host immune system more able to control parasite density thus preventing the onset of clinical symptoms (Carrasco-Escobar et al., 2017). In other words, the study population of Makenene might have acquired an immunity which prevents them from developing signs/symptoms of the disease but does not protect them from the infection with the parasite (Hassanpour et al., 2017).

Results of this study underline the potential limits of the use of multispecies Pf/Pan mRDTs for the detection of malaria infection by *P. falciparum versus* co-infections. The false positives and negatives observed in this study, and which had an impact on the prevalence obtained by mRDTs, could be assigned to recent antimalarial treatments taken by some participants and to a low parasite density. It is well established that antimalarial treatment leads to false positives (FP-RDT) because the PfHRP2 antigen persists in the blood for days to weeks after the disappearance of the parasite (Iqbal et al., 2004; Houzé et al., 2009; Dalrymple et al., 2018). The presence of FP-RDTs and FN-RDTs are reported in several studies (Wu et al., 2015; Bamou et al., 2021). Malaria surveillance based on RDTs only as diagnosis should benefit from well-integrated, quality control procedures assessing the potential impact of reduced sensitivity and specificity as done previously (Hosch et al., 2022). On the other hand, blood smears examination revealed the presence of only *P. falciparum* in the samples even in those that displayed a Pf/Pan band. Three hypotheses may account for this: (i) the mutation of the PfHRP2 antigen in the P.f/Pan samples; (ii) the increase in parasitaemia that leads to the appearance of the Pan band on the multispecies mRDTs; or (iii) the presence of several undetected *Plasmodium* species in the thin smear which could only be confirmed by molecular methods. Hosch et al. (2022) speculated that, if PfHRP mutations carrying *P. falciparum* exist, they would have most likely been masked by co-infecting *P. falciparum* isolates gene deletions leading to the overall high frequency of polyclonal *P. falciparum* infections. Previous studies have also shown that malaria diagnosis by polymerase chain reaction (PCR) revealed differential distribution of mono- and mixed species infections with *Plasmodium* spp. in Cameroon (Akindeh et al., 2021; Kojom-Foko et al., 2021). One of the limitations of this study remains the lack of PCR confirmation for *Plasmodium* spp.

Malaria prevalence was assessed in relation to other parameters to determine whether certain factors could contribute to malaria occurrence and persistence. A significant difference was observed in malaria prevalence between the different age groups. Participants aged between 5 and 14 years-old had a higher prevalence and the same was true for parasitaemia except that no significant difference was observed in the latter case (parasitaemia). This association between age and malaria was also observed by Lehman et al. (2018) and by Siddiki et al. (2020) in other cities in Cameroon. The different infection rates observed among these age groups could be attributed to the level of acquired immunity that increases with age, which may also be associated with protection against malaria infection. In general, in endemic areas, acquired immunity against malaria is lower in children than in the elderly. However, children of lower ages most often benefit from better protection since their immune system is weak (Doolan et al., 2009; Fowkes et al., 2016). In these regions, the immune system becomes increasingly strong as a result of repeated infections by vector *Anopheles* mosquito bites. This immunity protects individuals against non-severe and severe malaria (Stevenson & Riley, 2004; Doolan et al., 2009).

Owning an LLIN is not always synonymous with its good use. Although people consider LLINs as efficient means to avoid mosquito bites, their bad usage and their longevity could lead to the loss of their effectiveness and therefore LLINs no longer provide protection against mosquito vectors of malaria (Ngongang-Yipmo et al., 2022). In addition, previous studies have shown that there is sometimes a gap between the possession of mosquito nets and their daily use (Doutoum et al., 2019). This could explain why no significant difference was observed between participants with LLINs and those who did not have regardless of sleeping and waking up times. The majority of mosquito nets present in Makenene are those from the 2016 LLINs mass distribution campaign (i.e. 6 years of existence/usage). However, mosquito nets are generally effective for three years. It will be important to check the status of LLINs (integrity) and their efficacy against the malaria vectors present in the locality. The

decline in malaria prevalence according of the use of mosquito nets is reported in Foumban in West Cameroon (Siddiki et al., 2020).

Even though our results did not show significant differences of malaria prevalence according to house type and environmental characteristics, they can be considered as factors that predispose populations to malaria infection. A greater prevalence of malaria was obtained among participants living in earth brick houses and found near watercourses. Doutoum et al. (2019) explained that malaria is a disease caused mainly by poverty, especially in rural areas where poor quality housing is observed (holes in the walls, openings in the ceilings, surrounding vegetation and mosquito breeding sites) creating an environment conducive to the spread of malaria (Githeko et al., 2012). Previous studies in Africa have shown that better-built houses with very good drainage systems are associated with a lower risk of malaria infection (Ayele et al., 2012; Osterbauer et al., 2012). The content of communication in the fight against malaria and the means of prevention should consider the sociodemographic realities, the environmental factors and the living habits of the populations in terms of housing.

5. Conclusions

This study shows high prevalence of malaria among asymptomatic people in Makenene. People of the locality have almost the same chances of being infected with *P. falciparum* parasites despite differences in their sleeping behavior, bednet usage and characteristics of their habitats. Free diagnostic campaigns for malaria screening using standard techniques and control measures should be implemented considering the environmental context of this locality. Furthermore, additional studies are needed to identify the vectors responsible for malaria transmission in the locality and their insecticide resistance status.

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Ethical approval

Prior to the study, an authorization was granted from the Director of the District Medical Center (DMC) of Makenene and the territorial administrative authorization was obtained from the Divisional Officer of the district of Makenene (No. 04/AR/JOR-04/SP). This study has been also approved by the Regional Committee for Ethics and Human Health Research of the Center Region (CE No. 1289/CRERSHC/2021). After obtaining verbal authorizations from the heads of households of different neighborhoods, written consent forms were collected from each participant before their inclusion in the study. Participants were informed about the confidentiality and willingness aspects of the study and were free to withdraw at any time.

CRediT author statement

Conceived and designed the experiments: DJ, TT, MMPA, RB and ANC. Performed survey: DJ, LDM and FJV. Analysed the data: DJ, FJV and RB. Contributed materials: ANC, TT, RT and AD. Drafted the paper: DJ, MMPA and BR. Revised critically the paper: all authors. All authors read and approved the final manuscript.

Declaration of competing interests

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

The data supporting the conclusions of this article are included within the article and its supplementary files.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.crpvbd.2022.100104.

Supplementary file S1. Questionnaire used during malaria survey in Makenene to collect demographic information of the population.

Supplementary file S2. Malaria study database with all information provided in this manuscript.

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