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Plasmodium species and their prevalence in the city of Mokolo, Far North Cameroon

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Abstract
Objective: In order to identify the types of *Plasmodium* species and to determine the parasites prevalence, a parasitological study based on thick drops and thin smears was carried out in March and September 2022 (Two passages), in four sites (Ouro Tada, Tasha Haman Gawar, Tasha Koutourou and Mbikem) in the town of Mokolo, Far North Cameroon.
Methodology: Blood samples were taken in accordance with laboratory techniques and procedures for the microscopic diagnosis of malaria as recommended by WHO (2010). A total of 3840 individuals were samples according to age (0 to 5 years, 6 to 9 years, 10 to 15 years and 16 to over). So 4 age groups x 4 sites x 2 passages + 60 girls + 60 boys) = 3840 individuals.
Results: Of all the *Plasmodium* species identified (*P. falciparum* and *P. malaria*e**),** *P. falciparum* showed a higher prevalence in the wet and dry seasons. The Ouro Tada site was the most contaminated in both seasons. *Plasmodium falciparum* showed a prevalence of 70.61% in Ouro Tada, 68.62% in Tasha Haman Gawar, 68.12% in Tasha Koutourou and 69.27% in Mbikem. As for *Lasmodium malariae* showed a prevalence of 31.04% in Ouro Tada, 31.37% in Tasha Haman Gawar, 31.18% in Tasha Koutourou and 30.71% in Mbikem.
Conclusion: Among all the *Plasmodium* species identified (*P. falciparum*, *P. malariae***)**, *P. falciparum* showed a higher prevalence, followed by *P. malariae*.

Keywords: Typology, *Plasmodium* species, parasite prevalence, Mokolo, Cameroon

Introduction
Mosquitoes are insects responsible for major public health problems by their role as excellent vectors of infectious and harmful diseases that affect humans [1]. These diseases contribute enormously to worldwide underdevelopment and particularly in tropical countries [2]. During their blood meal they can transmit agents responsible for several diseases such as Malaria, dengue, yellow fever, encephalitis, filariasis [3]. Malaria is an infectious disease caused by the Protozoa *Plasmodium* spp. and transmitted by the infecting bite of a female mosquito to the genus *Anopheles*. It is a major public health problem in tropical countries [4]. In 2021, 84 countries worldwide reported a total of 247 million cases of malaria, with 95% (234 million) occurring in Africa WHO region. The global tally of malaria deaths reached 619 000 deaths with 93% occurring in African region [5]. Children aged less than 5 years are the most vulnerable group affected by malaria. In 2021, they accounted for 95.79% (593 000) of all malaria deaths worldwide. *Plasmodium falciparum* is the most prevalent malaria parasite in Sub-Saharan Africa, with *Anopheles gambiae* Giles as its major vector [5]. In Cameroon, 3.327.381 million malaria cases were reported with 2.481 deaths, with children under five accounting for 79%. Far North region recorded 17.6% mortality [6]. Despite the efforts made by the government through seasonal chemoprevention campaigns for children under five, and the distribution of long action impregnated mosquito nets, malaria remains a major endemic and the leading cause of mortality and morbidity. Today, there are no data on the parasite prevalence of malaria in the four sites chosen for our study. Yet people in this part of the country regularly go to hospital because of malaria. In view of this observation, we urgently need to carry out a parasitological study to establish baseline data in the Mokolo locality. Through this research, we want to identify the types of *Plasmodium* and determine their prevalence in each of the study sites.

2. Materials and Methods

2.1. Presentation of study sites

The study took place at four sites in Mokolo (10.74° ; 13.8°), capital of the Mayo-Tsanaga department in the Far North Region Cameroon (figure 1): Ouro Tada (13°38'13.92''N; 13°77'94.10'' E), Tasha Haman Gawar (10°73'85.90''N; 13°79'41.03'' E), Tasha Koutourou (10°73'66.31''N; 13°78'72.85''E) and Mbikem (10°44'32.78''N; 13°48'8.17''E) are the rural villages around ten kilometers west of Mokolo town (figure 1). The relief is made up of plains surrounded by the Mandara and Kapsiki mountains, which reach heights of 800 and 1500 m respectively [7]. The sahelian climate is characterized by two seasons of inequal length: a long dry season lasting seven to nine months (October to June) and a short rainy season lasting two to three months (July to September). The average annual temperature is 32.5 °C. Annual rainfall is generally low at between 700 and 800 mm. The particularity of this Mokolo locality is linked to the existence of two hydraulic dams, one 3km (large dam) and the other 7km (small dam) from the town center. The presence of these dams encourages the local populations to grow market-garden crops in both the dry and rainy seasons [7]. Our work was carried out around the small dam, framed by the four study sites.

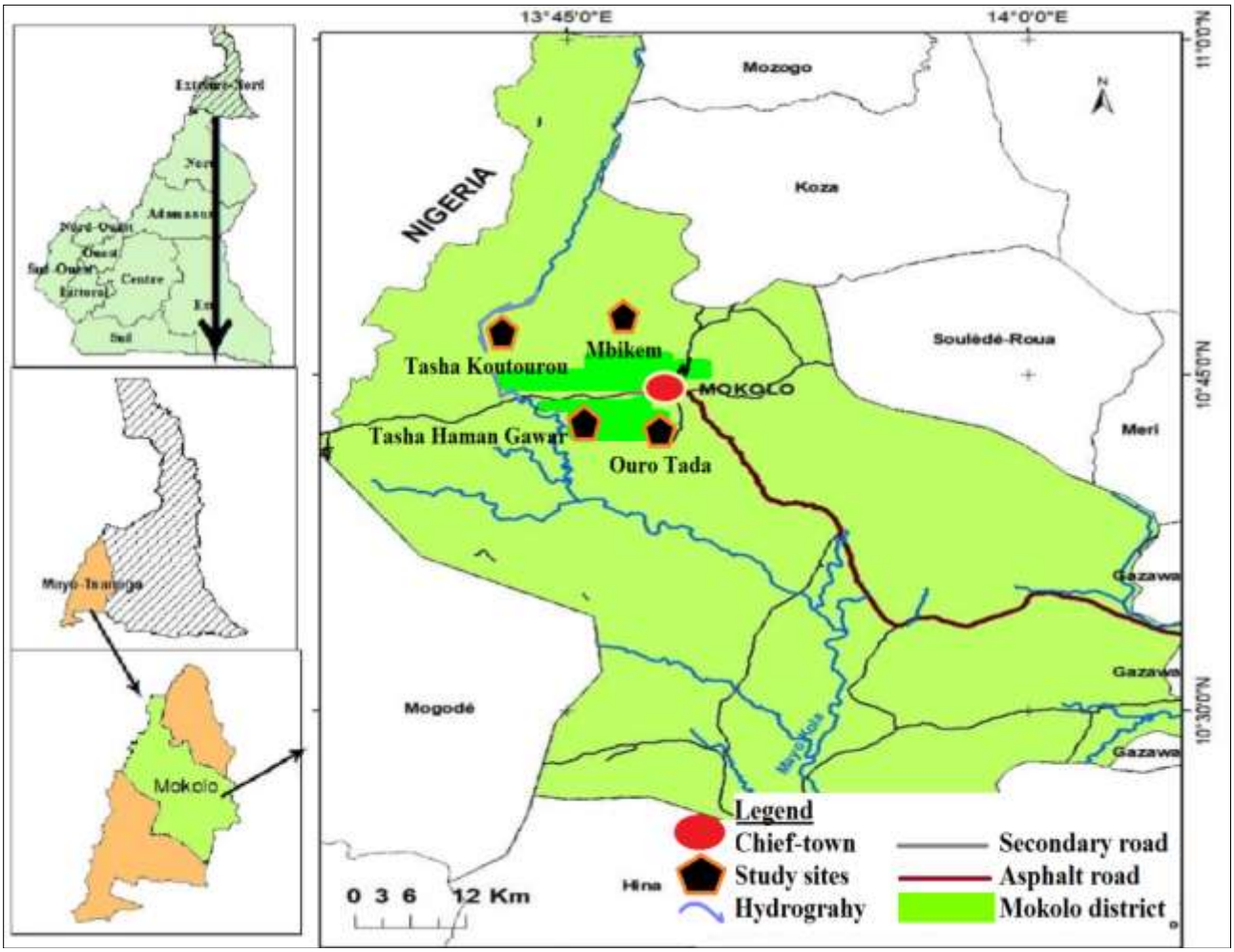

Fig 1: Location of study sites

2.2. Sampling method

The blood sample was taken from 3840 individuals at four different sites (Ouro Tada, Tasha Haman Gawar, Tasha Koutourou and Mbikem), according to age, season and gender.

2.2.1. Passage sampling at the four sites

During the two passage (March and September), the simple size did not vary at any of the four sites (table I).

Table 1: Distribution of sampled by passage and site

Sites	Ouro Tada	Tasha Haman Gawar	Tasha Koutourou	Mbikem	Total
March	480	480	480	480	1920
September	480	480	480	480	1920
Total	960	960	960	960	3840

2.2.2. Sampling by age group and gender

During the two passage (March and September), the simple size did not also vary at any of the four sites. Table II shows the distribution of sampled by age group and gender for the March period.

Table 2: Distribution of sampled by age group and gender

Period	Sites	0 to 5 years			6 to 9 years			10 to 15 years			16 to over			Total
		Gender			Gender			Gender			Gender			
		G	B	T	G	B	T	G	B	T	G	B	T	
March 2022	OT	60	60	120	60	60	120	60	60	120	60	60	120	480
	THG	60	60	120	60	60	120	60	60	120	60	60	120	480
	TK	60	60	120	60	60	120	60	60	120	60	60	120	480
	Mb	60	60	120	60	60	120	60	60	120	60	60	120	480
	Total	240	240	480	240	240	480	240	240	480	240	240	480	1920

OT: Ouro Tada; THG: Tasha Haman Gawar; TK: Tasha Koutourou; Mb: Mbikem; G: Girls; B: Boys; T: Total

2.3. Identification of the *Plasmodium* species

2.3.1. Technical steps for blood smear preparation and staining

The blood smear was prepared using the Cheesbrough technique [8]. A small drop of blood was placed on the end of an object slide (figure 2). Without waiting, the short side of another slide was moved to touch the drop of blood. The two slides should form an angle of between 30° and 45°. After a few seconds, the blood spread by capillary action between the two blades. The slide is then tilted towards the other end of the object slide, carrying the blood behind it, which spreads out in a thin layer. Immediately after making the smear, the slide was shaken to ensure rapid drying. The slide was then fixed by dripping 2 or 3 drops of absolute methanol onto the vertically positioned smear. After evaporation of the methanol, the slide was stained with Giemsa diluted to 3.5% in water buffered to a pH appropriate for the parasites of interest. The slide preparation was left to stain for 20-30 minutes, then drained and carefully rinsed under running water. It was then left to dry and examined under an immersion microscope with a 100x objective.

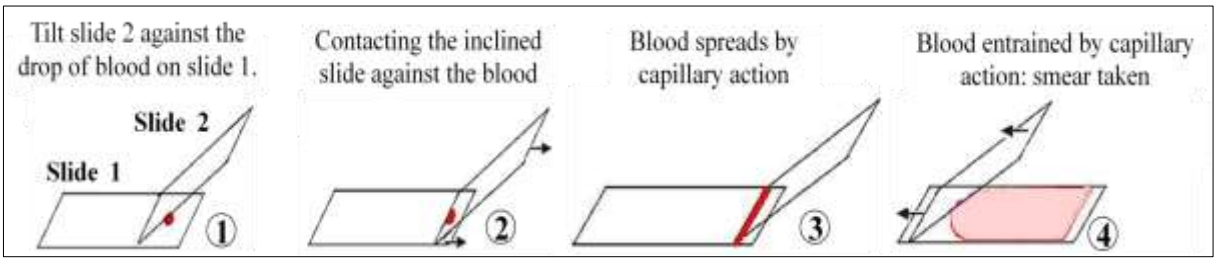

Fig 2: Taking a blood smear

2.3.2. Technical steps for reading blood smear

The blood smear was also read using the Cheesbrough technique [8]. Look for gametocytes, schizonts and possibly trophozoites (very small and invisible to *P. falciparum*). *P. malariae* parasite is often found in the vicinity of the smear [8].

2.3.3. Distinctive characteristics of human malaria parasites on thin blood smears

The advantage of blood smears is that they can be used to determine the *Plasmodium* species. Laboratory diagnostics is based on parasite characteristics related to the host cell, such as size, shape, granulation, rate of infestation of host cells [9]. Table III shows the distinctive characteristics of human malaria parasites on thin blood smears according to Kokoskin [9].

2.4. Determination of parasite prevalence

2.4.1. Sampling and Thick drop preparation

The tip of the patient's finger is cleaned with cotton soaked in 70° alcohol, then pricked in a single stroke with a sterile vaccinostyle. The first drop is cleaned. The second drop is collected on a slide, spread out and crushed with the corner the corner of another slide for defibration (figure 3). The slide is dried at room temperature. It was then rinsed with neutral water for 15 minutes, then immersed in 6% Giemsa solution for approximately 30 minutes. The slide is rinsed and dried. After drying, the slide is ready for reading. To avoid confusion between slides, the patient's code and name, as well as the date on which the thick drop was made, are noted 2 cm from the tip of the slide.

Table 3: Distinctive characteristics of human malaria parasites on thin blood smears

Characters	*P. falciparum*	*P. malariae*
Size	Normal	Normal
Shape	Round, crenellated	Round
Granulations	Few purplish seeds	Rare and delicate
Infected cells rate	All cells	Only aged cells,

2.4.2. Thick drop reading

The slide is read under a photonic microscope with an immersion objective (×100). Parasites can be seen in three forms: trophozoites, schizonts and gametocytes. The reading is made by counting parasites per 100 leukocytes [10]. Sexed forms are counted separately over 200 fields and are not considered in the parasite density. Parasite density is therefore expressed as the number of parasites per 100 leukocytes. Parasite density can also be expressed as the number of parasites per microliter of blood by multiplying by 80 (one microliter of blood contains an average of 8,000 leukocytes according to the WHO. Microscopic examination of 200 fields covers around 0.5µl of blood [11].

Fig 3: Sampling and thick drop preparation

2.5. Data Analysis

Data were analyzed using R software version Rx64 4.1.0. Standard descriptive statistics such as proportion, means and standard deviation were used in the tables presenting the results. The exact binomial test and the chi-square test of conformity were used to compare the observed percentage with the theoretical percentage. The chi-square test of homogeneity or independence was used to compare several observed distribution.

The p-value was considered significant when $p< 0.05$.

3. Results and Discussion

3.1. *Plasmodium* species identified

3.1.1. Global distribution of *Plasmodium* species

Blood examination and microscopic observation of the slides identified two plasmodial species (figure 4) at the four sites in the Mokolo locality. Overall, these were *P. falciparum* (69.15%) and *P. malariae* (31.27%). A non-significant difference was noted between these observed representativeness rates (χ^2= 0.031339, df = 1, p-value = 0.9845). The predominance of *P. falciparum* in this study can be explained by the fact that this species is the main vector of malaria transmission in Sub-Saharan Africa in general and in Cameroon in particular. Our results are in line with the World Health Organization's report, which states that of the five species of malaria parasites responsible for human malaria, two are particularly dangerous: *P. falciparum* and *P. vivax*. The first parasite causes more deaths and is also the most widespread on the African continent. The second is the dominant species in most countries outside Sub-Saharan Africa [5].

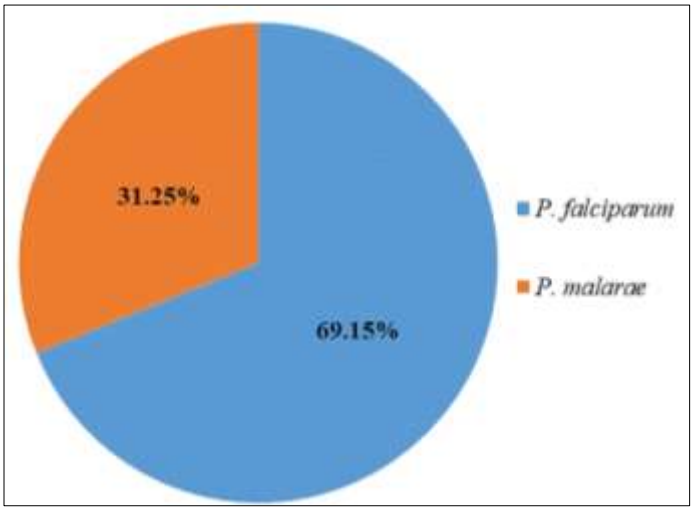

Fig 4: Global distribution of plasmodial species

3.1.2. Distribution of Plasmodial species by sites

The distribution of *Plasmodium* species in the four sites is heterogeneous (figure 5). Ouro Tada site: *P. falciparum* (70.61%) and *P. malariae* (31.04%); Tasha Haman Gawar site: 68.62%, and 31.37% respectively for *P. falciparum* and *P. malariae*; Tasha Koutourou site: *P. falciparum* (68.12%) and *P. malariae* (31, 86%); Mbikem site: 69.27% and 30.71% for *P. falciparum* and *P. malariae* respectively. A non-significant difference was noted between these observed representativeness rates (χ^2= 2.4444, df = 3, p-value = 0.8746). The dynamics of plasmodial species abundance explored in our samples at Ouro Tada, Tasha Haman Gawar, Tasha Koutourou and Mbikem revealed a large difference in *Plasmodium* infection between the four sites. This makes it possible to confirm and understand the disparity in the epidemiological characteristics of *Plasmodium* infection in Mokolo. This disparity was also highlighted by a study carried out in Libreville (Gabon), which showed that urbanization, housing type and socio-economic level could have an impact on parasite transmission [12].

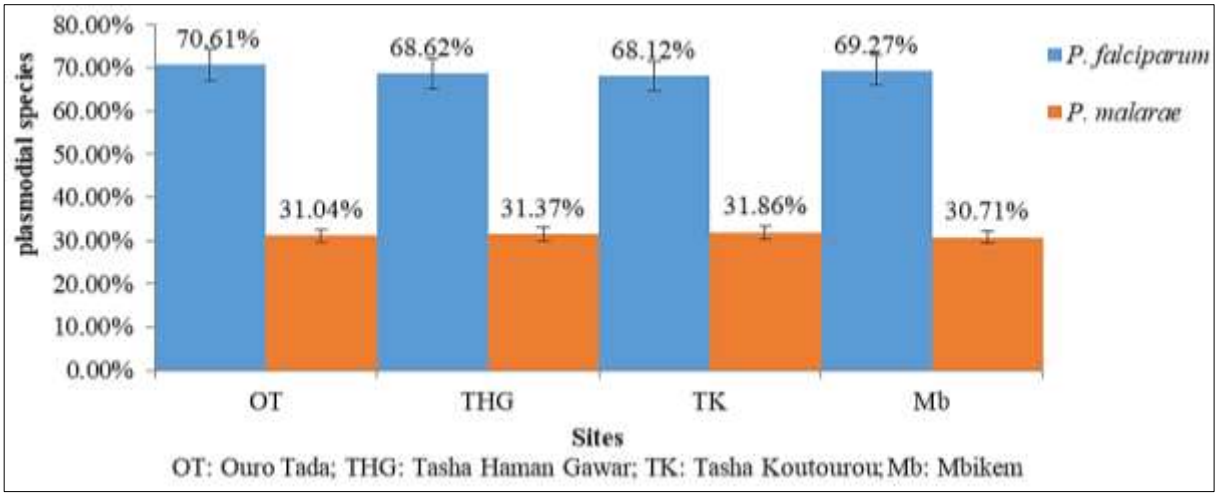

Fig 5: Distribution of Plasmodial species by sites

3.1.3. Distribution of *Plasmodium* species by passage

The distribution of *Plasmodium* species by passage in the four sites is also heterogeneous (figure 6). So, on the first pass in March 2022, the Mbikem site recorded more plasmodial species with 87.49% *P. falciparum* and 12.50% *P. malariae*; then comes the Ouro Tada site with 82.60% *P. falciparum* and 17. 39% *P. malariae*; and then the Tasha Koutourou site with 77.26% *P. falciparum* and 23.63% *P. malariae*; lastly, the Tasha Haman Gawar site with 76.35% *P. falciparum* and 23.63% *P. malariae*. A non-significant difference was noted between these observed representativeness rates (χ^2= 0.59823, df = 3, p-value = 0.9964). On the second pass in September 2022, there was a drop in the number of *Plasmodium* species at all study sites. In Ouro Tada *P. falciparum* led the way with 64.83%, followed by *P. malariae* 34.15%. In Tasha Haman Gawar, *P. falciparum* led also the way with 67.00%, followed by *P. malariae* 33.50%. The same applies to Tasha Koutourou (*P. falciparum* 66.87%, *P. malariae* 33.12%) and Mbikem (*P. falciparum* 65.51%, *P. malariae* 34.48%). A non-significant difference was noted between these observed representativeness rates (χ^2= 3.8681, df = 3, p-value = 0.6945). In view of the data obtained, the proportions of plasmodial species in March 2022 are high compared with those obtained in September. This can be explained by the presence of water retention dams which create permanent breeding sites for mosquitoes, and by the fact that, in March, the populations do not use impregnated mosquito bites, due to the heat. On the other hand, in September, the phenomenon of washing out mosquito breeding sites leads to mosquito instability. Our results corroborate those of Bouba *et al.* [13] in their work on Diversity of Culicidae, determination of entomological parameters of the transmission of *Plasmodium* spp. in Maga, Far North Cameroon. These authors showed that there was a non-significant difference in the proportion of mosquitoes in the dry and rainy seasons, and that, this could be explained by the presence of water in all seasons in Maga due to the existence of the lake [13]. For Saotoing [14], in the dry season, there is less leaching in the breeding grounds and water in rather stagnant, favoring the laying and development of mosquito larvae [14]. The same observation was made by Konan *et al.* [15] in Tiassalekro, an irrigated rice growing village in the southern forest zone of Côte d'Ivoire. The high numbers of *Anopheles gambiae* individuals can be justified by the proximity of the dwelling houses selected for captures [15].

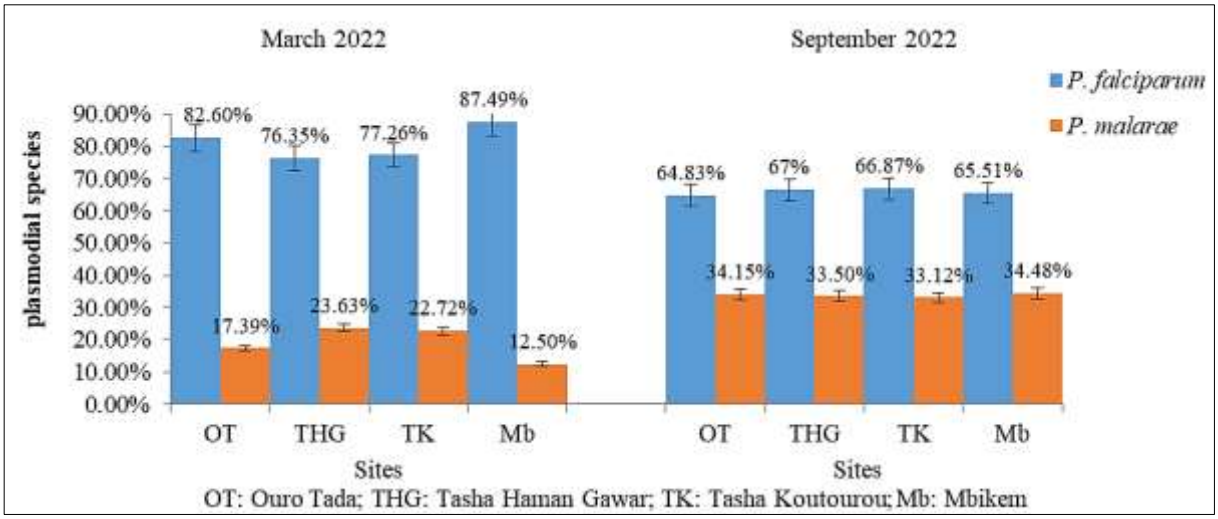

Fig 6: Distribution of Plasmodial species by passage

3.2. Malaria prevalence according Thick Drop Test

The results of Thick Drop Test are shown in table VI. The data in the table show that, irrespective of age group, gender or site, of the 3840 individuals sampled, 1333 were positive to the thick drop test, representing a prevalence of 34.71%. Results by age show that the prevalence of malaria is relatively high in children aged 0 to 5 and 6 to 10. In fact, it is 38.85% and 38.12% respectively. In the other age groups, the prevalence is lower and it reaches 33.33% in individuals aged between 10 and 15, and 28.54% in those over 16. There was no significant difference between the two tests (P>0.05, table III). With regard to results by sex, 36.09% of boys and 33.33% of girls were positive. On the other hand, whatever the study site, the prevalence of malaria is disproportionate high. The Ouro Tada site showed the highest prevalence (43.33%), followed by Tasha Haman Gawar (37.39%), then Tasha Koutourou (32.39%) and Mbikem (25.72%). Our results are contrary to those obtained by the National Malaria Control Program (PNLP) in Côte d'Ivoire. Indeed, a study of the 2016 malaria and anemia parasite prevalence survey by the PNLP revealed that parasite prevalence was higher in children under five. In addition, this prevalence decreased as the age of the individuals sampled increased [6]. The difference between our results and those of the Côte d'Ivoire PNLP can be explained by the sampling period, as seasonal variations have a strong influence on the transmission of this parasitic infection [6].

Table 4: Malaria prevalence Thick Drop Test

Socio-demographic characteristics	Prevalence by Thick Drop Test (TDT)					
	TDT+	TDT-	n	Prevalence (%)	P-value	CI95%
Age group						
0 to 5 years	373	587	960	38.85[d]	0.59*	[0.35 - 0.42]
6 to 9 years	366	594	960	38.12[d]	0.94*	[0.35 - 0.41]
10 to 15 years	320	640	960	33.33[d]	0.83*	[0.30 - 0.36]
16 to over	274	686	960	28.54[d]	0.71*	[0.25 - 0.31]
Gender						
Male	693	1227	1920	36.09[f]	0.94*	[0.33 - 0.38]
Female	640	1320	1920	33.33[f]	0.75*	[0.31 - 0.35]
Sites						
OT	416	544	960	43.33[h]	0.84*	[0.40 - 0.46]
THG	359	601	960	37.39[h]	0.81*	[0.34 - 0.40]
TK	311	649	960	32.39[h]	0.80*	[0.29 - 0.35]
Mb	247	713	960	25.72[h]	0.60*	[0.22 - 0.28]
Set results	1333	2507	3840	34.71		[0.21 - 0.26]

P (%): Prevalence; p-val: p-value; CI95%: 95 percent confidence interval OT: Ouro Tada; THG: Tasha Haman Gawar; TK: Tasha Koutourou; Mb: Mbikem; +: positive test; -: negative test; n= number of sampled persons. Values followed by same letters in the same column are not significantly different at α=5%. *: *p*>0.05 (not significant).

3.3. *Plasmodium*'s prevalence

3.3.1. Prevalence of *Plasmodium falciparum*

During the study period, the prevalence of *P. falciparum* (figure 7 varied according to age group and period of passage. For the first passage and according to age, individuals aged 0 to 5 and 6 to 9 are the most infested and the parasite prevalence in these age intervals are: from 0 to 5 years, 12.5%, 12.5%, 6.66% and 4.16% respectively for the Ouro Tada, Tasha Haman Gawar, Tasha Koutourou and Mbikem sites; from 6 to 9 years, 10%, 10%, 3.33% and 5.83% for the same sites. In the second passage, parasitic prevalence for people aged between 0 and 5 years are: 20%, 19.16%, 18.33%, and 13.33% respectively for the Ouro Tada, Tasha Haman Gawar, Tasha Koutourou and Mbikem sites; 20.83%, 20.83%, 15.83% and 15% for people aged between 6 and 9 years for the same sites. A non-significant difference was noted between these observed prevalence rates (χ^2= 4.5953, df = 9, p-value = 0.8681). The high prevalence rate in these age group (0 to 5 and 6 to 9 years) is explained by the vulnerability of children under five, and even under ten, to parasites as their weak immune systems are unable to defend themselves effectively against any parasitic aggression. Our results corroborate those of Snow *et al.* [16] in their work on the global distribution of clinical episodes of *Plasmodium falciparum* malaria. Nature, which showed that children under five and pregnant women are the most vulnerable. The Prevalence of *P. falciparum* in the second pass (September) are high compared with those obtained in the first pass (March). In fact, the Prevalence of *P. falciparum* evolved biphasilically during the course of our work, whatever the age group: a first phase with relatively low prevalence in March 2022 and second with high prevalence in September 2022. Thus, we obtained an average of 20% in September versus 8.96% in March for children under five, 18.12% versus 7% for under 10, 12.29% versus 5.42% for under 15 and 11% versus 1.04% for over 16. There was a highly significant variation between the passages (χ^2= 17.33, df = 3, p-value = 0.0006046). This could be explained by the abundance of water in September due to the rainy season, which is responsible for the diversification of breeding sites and, consequently mosquito outbreaks. A study carried out by Ibrahima [17] showed a highly significant difference (χ^2= 136,107 and *P*< 0,001) in the variation of the plasmodic index to the passage and age group in their study population [17].

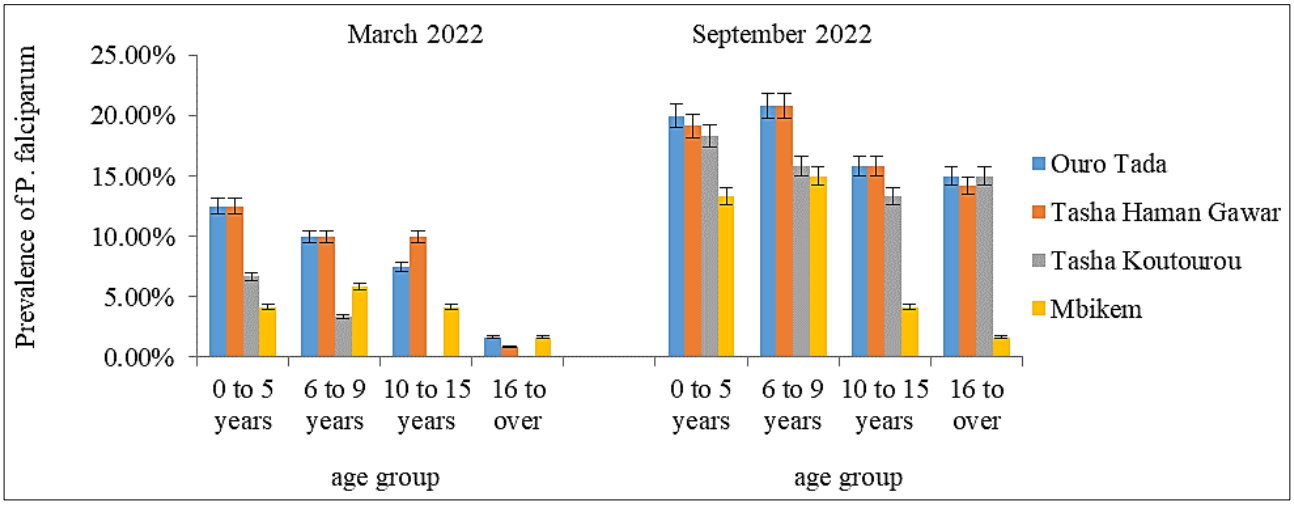

Fig 7: Prevalence of *P. falciparum* by age group

3.3.2. Prevalence of *Lasmodium malariae*

The prevalences of *P. malariae* (figure 8) varied also according to age group and period of passage. For the first passage (March), persons aged 0 to 5 and 6 to 9 are the most infested. These prevalences are: 4.16%, 1.66%, 0.83% and 0% respectively for the Ouro Tada, Tasha Haman Gawar, Tasha Koutourou and Mbikem sites. From 6 to 9 years, the prevalences are: 5.83%, 4.16%, 0% and 0.83% for the same sites. From 10 to 16 years, the prevalences are: 3.33%, 0%, 0.83% and 0%. From over 16 age group, the prevalences are: 0.83%, 1.66%, 0% and 0%. A non-significant difference was noted between these observed prevalence rates (χ^2= 4.9968, df = 9, p-value = 0.8346). In the second passage (September), parasitic prevalence for people aged between 0 and 5 years are: 10%, 19.16%, 16.16% and 11.16% respectively for the Ouro Tada, Tasha Haman Gawar, Tasha Koutourou and Mbikem sites; 18.33%, 15.83%, 15.83% and 5.83% for people aged between 6 and 9 years; 9.16%, 15%, 10% and 10% for people aged between 10 and 15 years; 8.33%, 14.16%, 8.33%, and 2.50% for people over 16 years old in the same sites. A non-significant difference was noted between these observed prevalence rates (χ^2= 9.017, df = 9, p-value = 0.4357). In view of this result, we observe that the prevalence of *P. malariae* decreases with age and period of passage. Older people did not suffer from malaria, as the incidence density was low compared to younger people. This difference in the risk of malaria attacks may be due to the level of acquired immunity to *P. malariae* in the four sites, resulting in a considerable age difference in the occurrence of attacks. Indeed, it has been shown that by the age of 60, an adult will have had 43 malaria attacks since birth, with 23% occurring in adulthood [19]. Rogier's work shows that the risk of malaria attacks is 10 to 20 times higher after the age of 10 [21]. In our results, clinical immunity clearly played a role, as adults exposed to high levels of perennial transmission are consequently more immune than children exposed only to seasonal transmission. Rasamoel *et al.* [22] pointed out that low parasitic inoculation in a population conditions a considerable delay in premonition, whereas perennial transmission favor rapid acquisition of premonition [22].

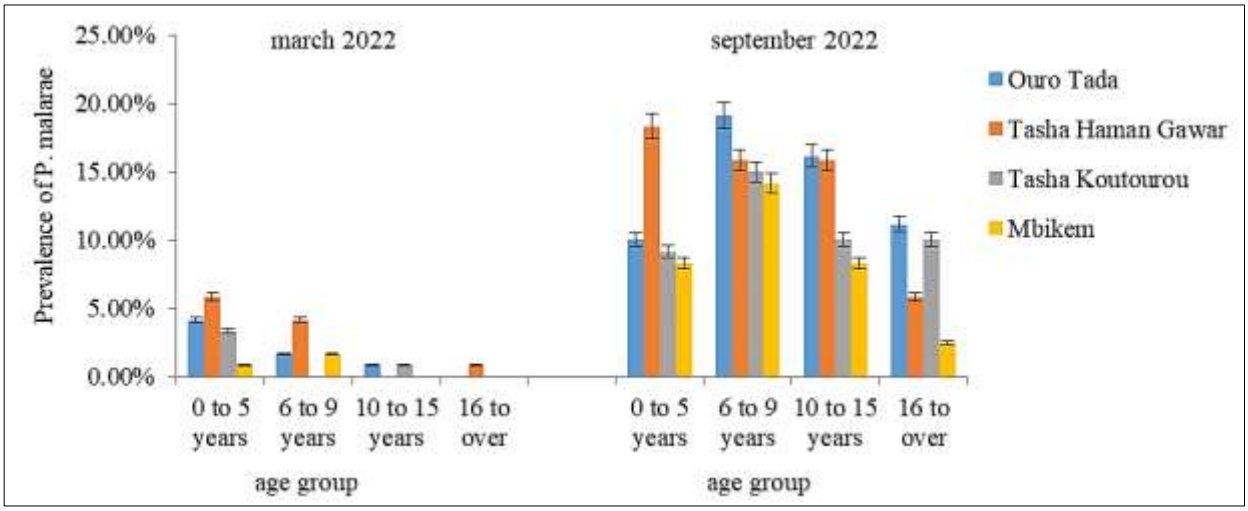

Fig 8: Prevalence of *P. malariae* by age group

4. Conclusion

Thick drop and thin smear blood tests at the four study sites confirmed the existence of three *Plasmodium* species with unequal proposals, varying from site to site. These were *P. falciparum* and *P. malariae*. The Ouro tada site showed the highest prevalence, followed by the Tasha Haman Gawar site, then the Tasha Koutourou site and finally the Mbikem site.

5. Acknowledgement
The authors of this article would like to thank the administrative and traditional authorities of the four sites for their contribution in facilitating the blood sampling of individuals. The authors would also like to express their gratitude to Mr Boubakari for his help in carrying out thick drop and smear blood tests.

6. Conflicts of Interest
The authors confirm that the content of this article does not present any conflict of interest.

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