American Journal of Biomedical and Life Sciences
2026, Vol. 14, No. 3, pp. 45–52
https://doi.org/10.11648/j.ajbls.20261403.13

Research Article

Prevalence of Plasmodium Species and Associated Risk Factors Among Patients in Daloa, Côte d'Ivoire

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Abstract

In Côte d'Ivoire, malaria remains a major public health concern due to its persistent and high transmission. This study aimed to determine the prevalence of *Plasmodium* species and associated risk factors among 179 patients in Daloa. A cross-sectional study was conducted at the Regional Center for Mutuality and Social Welfare in the School Environment (CREMOSS) of Daloa. Malaria diagnosis was performed using both microscopy and molecular biology techniques (PCR). The prevalence of malaria was 37.98% by microscopy compared with 54.18% by PCR, highlighting an underestimation of infections by conventional diagnostic methods. Plasmodium falciparum was the predominant species; however, other species such as *P. ovale*, *P. malariae*, and *P. vivax* were also detected, including mixed infections. Children aged 6 to 15 years showed the highest prevalence, with a significantly greater risk of infection compared to adults (OR = 2.19; p = 0.029). The non-use of insecticide-treated bed nets was associated with an increased risk of infection, while fever appeared to be a strong predictive factor for malaria (OR = 4.83; p < 0.001). These findings confirm the high circulation of malaria in the study area and emphasize the importance of molecular tools in improving the detection of submicroscopic infections and non-falciparum species. They also highlight the need to strengthen prevention and surveillance strategies, particularly targeting school-aged children in malaria-endemic areas.

Keywords

Malaria, *Plasmodium Falciparum*, Non-falciparum Species, Risk Factors, Côte d'Ivoire

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Received: 11 May 2026; **Accepted:** 29 May 2026; **Published:** 18 June 2026

1. Introduction

Malaria remains a major global public health problem, particularly in sub-Saharan Africa, where it constitutes one of the leading causes of morbidity and mortality [1]. This potentially life-threatening parasitic disease, caused by protozoa of the genus Plasmodium and transmitted to humans through the bites of infected Anopheles mosquitoes, represents a considerable health, economic, and social burden [2]. Despite international control efforts, malaria transmission remains intense and stable in many endemic regions, with the African region accounting for the majority of cases and deaths worldwide [2].

In Côte d'Ivoire, malaria remains endemic despite the implementation of several control strategies, including the distribution of insecticide-treated bed nets, intermittent preventive treatment, and improvements in case management. It continues to represent one of the leading causes of consultation in healthcare facilities, particularly among children and pregnant women [3]. Although *Plasmodium falciparum* is the predominant species and the main cause of severe malaria, the circulation of other species such as *Plasmodium malariae*, *Plasmodium ovale*, and, more rarely, *Plasmodium vivax*, has increasingly been reported due to advances in diagnostic tools. The distribution of these species varies according to geographical and ecological contexts, thereby influencing diagnostic, therapeutic, and preventive strategies.

Furthermore, the occurrence of malaria is closely associated with several risk factors, including environmental conditions, sociodemographic characteristics, individual behaviors, and access to preventive measures [4]. In this context, accurate and early diagnosis represents a key component of malaria control. Traditionally, malaria diagnosis relies on microscopy (thick and thin blood smears) and rapid diagnostic tests (RDTs). However, these methods have important limitations, including reduced sensitivity in cases of low parasitemia, dependence on the expertise of the microscopist, and difficulty in accurately identifying Plasmodium species or mixed infections. These shortcomings justify the increasing use of molecular biology techniques such as polymerase chain reaction (PCR), which are recognized for their higher sensitivity and specificity [5].

The city of Daloa, located in a high-rainfall area and characterized by intense agricultural activity, provides ecological conditions favorable for the proliferation of Anopheles mosquito vectors. However, updated data on the distribution of *Plasmodium* species and associated risk factors among patients attending healthcare facilities in this area remain limited. A better understanding of the prevalence of different Plasmodium species and the associated risk factors is essential for guiding prevention strategies, improving therapeutic management, and strengthening epidemiological surveillance. In this context, the present study aimed to determine the prevalence of *Plasmodium* species among patients attending the Regional Center for Mutuality and Social Welfare in the School Environment (CREMOSS) of Daloa and to identify the main risk factors associated with malaria infection in this locality. The expected findings will contribute to a better understanding of the epidemiological profile of malaria in Daloa and may support local malaria control interventions.

2. Methodology

2.1. Study Area

This cross-sectional study was conducted at the Regional Center for Mutuality and Social Welfare in the School Environment (CREMOSS), located in the city of Daloa, in west-central Côte d'Ivoire, from April to June 2025. This center was selected as the sampling site because it is a referral healthcare facility attended by a large number of students as well as a diverse population composed of both adults and children living in different neighborhoods. Its status as a community healthcare center makes it a primary healthcare facility frequently visited by local residents.

2.2. Sampling and Data Collection

The participants included in this study were patients of all age groups who were permanent residents of one of the departments of the Haut-Sassandra region and who had received a medical prescription for malaria diagnosis. Epidemiological data were collected using a structured questionnaire designed to obtain information on sociodemographic characteristics (age and sex), household socioeconomic status, and the use of preventive measures, particularly insecticide-treated bed nets. In addition, fever, the main clinical sign associated with malaria, was systematically documented.

Following the survey, a 5 mL venous blood sample was collected from each participant into ethylenediaminetetraacetic acid (EDTA) tubes by a qualified laboratory technician. Parasitological examination was performed by microscopy after staining thick blood smears with 10% Giemsa, according to the standard method described by [6], in order to determine parasitemia. Furthermore, 2 mL blood aliquots were collected from each sample, regardless of the thick smear result, and stored at −20°C in cryotubes for subsequent molecular analyses.

2.3. Ethical and Regulatory Considerations

Participants were informed about the objectives and methodology of the study in French or in a local language. Informed consent was obtained from the parents or legal guardians prior to inclusion in the study. Upon agreement, a consent form specifically designed for this purpose was provided to the participant or their legal representative and signed by both the latter and the investigator. No sample collection was per-

formed without prior informed consent. To ensure the confidentiality of participants' data, a unique identification code, known only to the principal investigator, was assigned to each enrolled participant.

2.4. Molecular Analysis

DNA was extracted from 200 µL of venous blood using the commercial QIAGEN kit (QIAamp DNA Blood Mini Kit), according to the manufacturer's instructions.

The 18S ribosomal DNA of *Plasmodium* species, also referred to as the small subunit ribosomal RNA (SSU rRNA) gene, was amplified using the polymerase chain reaction (PCR) technique. Amplification was performed using a two-step approach known as nested PCR. During the first amplification, a pair of primers targeting the conserved regions of the 18S ribosomal DNA of the genus *Plasmodium* was used (Table 1). A second amplification was subsequently carried out using the products of the first PCR as template DNA. This step relied on species-specific primers for the detection of different Plasmodium species, including *Plasmodium falciparum*, *Plasmodium malariae*, *Plasmodium ovale*, and *Plasmodium vivax* (Table 1).

Reaction mixtures were prepared for each sample in a final volume of 25 µL, containing 4 µL of template DNA (1 ng/µL), 1 µL (10 pmol/µL) of each primer (Eurogentec, France), and 12.5 µL of GoTaq Green Master Mix (Promega, USA). The final volume was adjusted with sterile nuclease-free water (Eurogentec, France).

The thermal cycling program for the first PCR consisted of an initial denaturation at 95°C for 5 min, followed by 45 cycles including denaturation at 94°C for 1 min, annealing at 57°C for 2 min, and extension at 72°C for 2 min, with a final extension at 72°C for 5 min. The second PCR was performed under the same reaction conditions, replacing the template DNA with 4 µL of the first-round PCR product. Cycling conditions included an initial denaturation at 95°C for 5 min, followed by 45 cycles of denaturation at 94°C for 1 min, annealing at 55°C for 2 min for P. falciparum and P. malariae, and at 54°C for P. vivax and P. ovale, followed by extension at 72°C for 2 min. A final extension step was performed at 72°C for 5 min. All amplifications were carried out using a conventional Bio-Rad thermal cycler.

Amplification products obtained from the second PCR were visualized under ultraviolet (UV) light following electrophoresis on a 2% agarose gel stained with GelRed. The sizes of amplified fragments were estimated by comparison with a 100 bp molecular weight marker. The presence of species-specific bands corresponding to the expected fragment sizes was interpreted as confirmation of infection.

Table 1. *List of primers used for the diagnosis of Plasmodium species [7].*

PCR	Primer sequence (5'-3')	Amplicon size (bp)
First PCR (genus Plasmodium)	F- TTTTTATAAGGATAACTACGGAAA R- CCTGTTGTTGCCTTAAACTTC	1200
Second PCR		
P. falciparum	F- TTAAACTGGTTTGGGAAAACCAAATA R- ACACAATGAACTCAATCATGACTACCCGTC	206
P. vivax	F- CGCTTCTAGCTTAATCCACATAACT R- ACTTCCAAGCCGAAGCAAAGAAAGTCCTTA	120
P. ovale	F- ATCTCTTTTGCTATTTTTTAGTATTGGAGA R- ATCTAAGAATTTCACCTCTGACATCTG	800
P. malariae	F- ATAACATAGTTGTACGTTAAGAATAACCG R- AAAATTCCCATGCATAAAAAATTATACAAA	144

2.5. Statistical Data Analysis

The collected data were entered into Microsoft Excel spreadsheet software (Version 2016) and analyzed using appropriate statistical tests with R software (version 3.2.2, 2015) and its associated interfaces. Ninety-five percent confidence intervals (95% CI) were estimated according to the binomial distribution using the Wald approximation. Pearson's Chi-square test of independence or Fisher's exact test (for $n < 5$) was used to determine the association between malaria infection and qualitative variables. Statistical significance was set at a p-value of 0.05 with a 95% confidence interval.

3. Results

3.1. Characteristics of the Study Population

A total of 179 patients were included in this study, comprising 111 (62%) females and 68 (38%) males, resulting in an overall male-to-female sex ratio of 0.61. The age of the participants ranged from 5 months to 64 years, with a mean age of 21 years.

3.2. Malaria Prevalence According to Parasitological Tests

The overall prevalence of malaria determined by microscopy was 37.98% (68/179). A predominance was observed among children aged 6 to 15 years, who accounted for 38% of this age group and 19.55% of all malaria cases (35/179).

Molecular analyses revealed a higher prevalence, estimated at 54.18% (97/179). These analyses also enabled a more precise characterization of the distribution of *Plasmodium* species, which was dominated by *Plasmodium falciparum* with a frequency of 50.27% (90/179) (Table 2). The other identified species included *Plasmodium ovale* (5.29%; 9/179), *Plasmodium vivax* (2.28%; 4/179), and *Plasmodium malariae* (1.12%; 2/179) (Table 2).

A total of seven (7) mixed infections were identified, including five co-infections involving *P. falciparum* and *P. ovale*, and two involving *P. falciparum* and *P. malariae* (Figure 1). Furthermore, monospecific infections were predominantly caused by *P. falciparum* (n = 83), followed by *P. ovale* (n = 3) and *P. vivax* (n = 4). However, no co-infection involving *Plasmodium vivax* was observed (Figure 1).

Table 2. *Distribution of Plasmodium species identified by PCR with 95% CI (N = 179).*

Species	Number (N)	Percentage (%)	95% CI
Plasmodium falciparum	90	50,27	[42,95 – 57,61]
Plasmodium ovale	9	5,29	[1,83 – 8,23]
Plasmodium vivax	4	2,28	[0,07 – 4,39]
Plasmodium malariae	2	1,12	[0,00 – 2,67]

95% CI: 95% Confidence interval

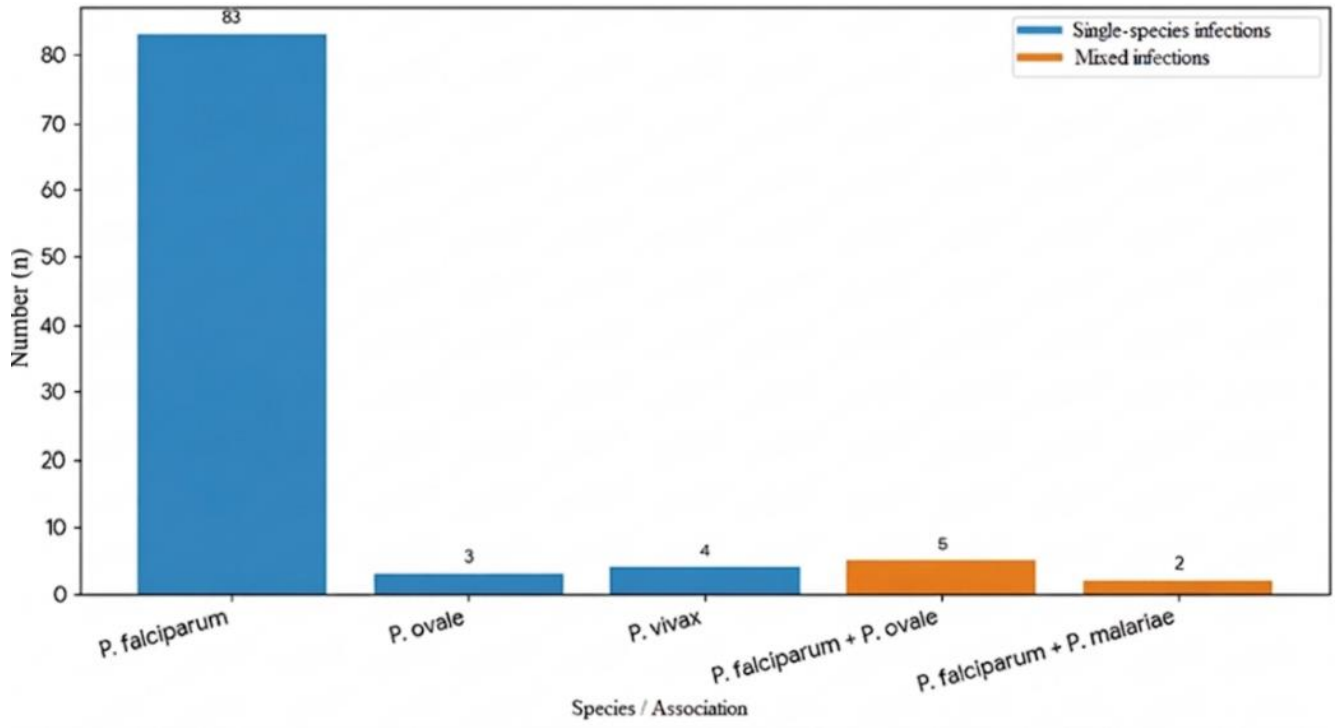

Figure 1. *Distribution of single-species and mixed infections (N = 179).*

3.3. Risk Factors Associated with Malaria

The crude prevalence of malaria infection was slightly higher among females (59.46%) than males. However, statistical analysis did not reveal any significant association between gender and the risk of malaria infection in the study population.

In contrast, age appeared to be a determining factor. The 6–15 years age group showed the highest prevalence (67.65%) (Table 3). A statistically significant difference was observed between children in this age group and individuals older than 15 years ($p = 0.029$). Moreover, children aged 6–15 years had a 2.19-fold higher risk of malaria infection compared to adults (Table 3).

The prevalence of infection was markedly lower among individuals using insecticide-treated bed nets (47.37%) compared to those who did not use them (63.11%). A significant association (p = 0.009) was observed between malaria infection exposure and the use of insecticide-treated bed nets. Individuals not using insecticide-treated nets were 2.35-fold more likely to be exposed to malaria infection compared to those who reported using them (Table 3).

The results indicate that fever is strongly associated with malaria infection. Nearly 74% of febrile individuals were infected, compared with only 37% of those without fever. Fever is a major clinical indicator and a highly reliable predictor of infection ($p < 0.001$). Furthermore, individuals presenting with fever were nearly five times more likely (OR = 4.83) to be infected with malaria than those without fever in this study (Table 3).

Table 3. *Factors Associated with Malaria Infection Detected by PCR in the Study Population.*

Variable		Total	PCR positive (%)	OR	95% CI	p-value
Sex						
	F	111	66 (59.46)	1.16	[0.63-2.13]	0.753
	M	68	38 (55.88)			
Age						
	0-5	25	16 (64)	1.86	[0.74-4.67]	0.268
	6-15	68	46 (67.65)	2.19	[1.13-4.24]	0.029
	> 15	86	42 (48.84)	-		-
ITN						
	yes	57	27 (47.37)	2.35	[1.24-4.48]	0.009
	no	122	77 (63.11)			
Ins						
	yes	118	70 (59.32)	1.16	[0.62-2.16]	0.764
	no	61	34 (55.74)			
Fever						
	yes	103	76 (73.79)	4.83	[2.54-9.15]	< 0.001
	no	76	28 (36.84)			

ITN: Insecticide-treated net; Ins: Insecticide; OR: Odds ratio; 95% CI: 95% Confidence interval

4. Discussion

This study aimed to assess the prevalence of malaria and associated risk factors in a symptomatic population of 179 patients. The malaria prevalence obtained by microscopy (37.98%) reflects a substantial circulation of malaria parasites in the Daloa area [8]. This level is comparable to those reported in several sub-Saharan African regions where malaria remains highly endemic, particularly among school-aged children [9]. The high proportion of malaria observed in children aged 6–15 years confirms their particular vulnerability to malaria infection, as reported in several studies conducted in sub-Saharan Africa where school-aged children constitute an im-

portant reservoir for parasite transmission [9, 10]. This situation may be explained by increased exposure to mosquito bites due to frequent outdoor activities, as well as incomplete acquisition of anti-malarial immunity [11]. Several studies have indeed shown that school-aged children represent a major asymptomatic reservoir of parasites in areas of stable transmission [10, 11].

The overall malaria prevalence varied considerably depending on the diagnostic method used, ranging from 37.98% by microscopy to 54.18% by molecular biology (PCR). This significant discrepancy highlights the limited sensitivity of light microscopy, often considered the "gold standard" but less effective in detecting low parasitemia levels [12]. The use of PCR revealed a substantial proportion of infections that would have gone undetected in routine clinical practice, confirming the need for molecular tools for accurate estimation of endemicity, particularly in settings with complex transmission patterns [13]. These submicroscopic infections represent a major challenge for malaria control and elimination programs, as they may silently contribute to sustained transmission.

The distribution of *Plasmodium* species observed in this study was largely dominated by *Plasmodium falciparum*, accounting for more than 50% of detected infections. This predominance is consistent with epidemiological data reported in sub-Saharan Africa, where *P. falciparum* remains the most widespread and pathogenic species [14]. However, the detection of other species such as *Plasmodium ovale*, *Plasmodium vivax*, and *Plasmodium malariae* highlights a non-negligible parasite diversity in the study area.

The detection of *Plasmodium vivax* deserves particular attention, as this species has long been considered rare in West Africa due to the high prevalence of the Duffy-negative phenotype in African populations. However, recent studies have reported its circulation in this region, particularly in Senegal, suggesting parasite adaptation or alternative erythrocyte invasion mechanisms [15]. Its presence in our study may therefore reflect an evolving epidemiological pattern of malaria in the region.

Furthermore, the identified mixed infections, mainly involving *P. falciparum* with *P. ovale* or *P. malariae*, indicate co-circulation of multiple *Plasmodium* species in the study population. These co-infections are generally underestimated by conventional diagnostic methods, particularly microscopy, due to the dominance of *P. falciparum*, which can mask minor species [16]. The absence of co-infection involving *P. vivax* may be related to its low prevalence in the study population or insufficient parasitemia levels preventing simultaneous detection with other species. Indeed, several recent studies have shown that *P. vivax* infections are often characterized by low parasite densities, which may escape conventional diagnostic methods, especially in the presence of *P. falciparum* [17].

The strong predominance of monospecific *Plasmodium falciparum* infections observed in this study confirms the major role of this species in local malaria dynamics. However, as reported by [18] in Côte d'Ivoire, the detection of non-falciparum species as well as mixed infections highlights the importance of integrating molecular diagnostic tools into epidemiological surveillance systems. Such approaches would improve the sensitivity of detection of circulating species, including mixed and submicroscopic infections, thereby strengthening malaria control and prevention strategies.

Risk factor analysis shows that age is a major determinant of infection. Children aged 6–15 years presented the highest prevalence (67.65%) with a 2.19-fold increased risk compared to adults ($p = 0.029$). This finding is consistent with several recent studies conducted in sub-Saharan Africa identifying school-aged children as a particularly vulnerable group and a major reservoir of malaria transmission [19]. Indeed, unlike adults who gradually acquire partial immunity after repeated exposure to the parasite, younger children still have incomplete immunity, making them more susceptible to malaria infection. Several recent studies have also shown that school-aged children constitute a major asymptomatic reservoir of *Plasmodium*, actively contributing to sustained community transmission [10, 19].

The use of insecticide-treated bed nets appears to be associated with a reduced malaria prevalence in this study. Individuals who did not sleep under bed nets were nearly twice as likely to be infected compared to users. Although the difference was at the threshold of statistical significance, this trend is consistent with extensive evidence demonstrating the effectiveness of insecticide-treated nets in preventing *Anopheles* bites and reducing malaria transmission [20]. The lack of statistical significance may be due to the relatively small sample size or reporting bias regarding actual and consistent bed net use. Nevertheless, these findings reinforce the importance of strengthening distribution and awareness strategies regarding proper and sustained use of insecticide-treated bed nets in at-risk populations.

Moreover, fever emerged as a strong clinical predictor of malaria infection in this population (OR = 4.83; $p < 0.001$). The strong association between fever and malaria observed in this study is consistent with the literature, which identifies fever as one of the primary clinical signs of malaria in endemic areas [21]. The high proportion of infected individuals among febrile subjects confirms that fever remains a key clinical indicator of malaria in endemic settings. The nearly fivefold increased risk of infection among febrile individuals underscores the diagnostic relevance of this symptom. However, the presence of a substantial proportion of infected but afebrile individuals also highlights the likely existence of asymptomatic infections in the study population [19]. These asymptomatic infections represent a major challenge for malaria control programs, as they constitute silent reservoirs capable of sustaining parasite transmission despite ongoing control efforts.

5. Conclusion

This study highlights a high prevalence of malaria in Daloa,

with a predominance of *Plasmodium falciparum*, while also revealing the presence of non-falciparum species and mixed infections. The findings further show that children aged 6–15 years constitute the most exposed group to malaria infection. The discrepancy observed between microscopy and PCR underscores the value of molecular approaches in improving the detection of submicroscopic infections and enhancing malaria epidemiological surveillance. In addition, the use of insecticide-treated bed nets appears to contribute to a reduction in infection risk, while fever remains an important clinical indicator of malaria. These results emphasize the need to strengthen prevention strategies, molecular diagnostics, and surveillance efforts, particularly targeting school-aged children in endemic areas.

Abbreviations

PCR	Polymerase Chain Reaction
RDT	Rapid Diagnostic Tests
CREMOSS	Regional Center for Mutuality and Social Welfare in the School Environment
EDTA	Ethylenediaminetetraacetic Acid
DNA	Deoxyribonucleic Acid

Acknowledgments

The authors would like to thank the staff of the Regional Center for Mutuality and Social Welfare in the School Environment (CREMOSS) of Daloa for allowing this study to be conducted in their facility. The authors also express their gratitude to the healthcare workers who assisted with sample collection. Special thanks are extended to all patients attending the center who agreed to participate in this study.

Author Contributions

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Data Availability Statement

The datasets used and/or analyzed during this study are available from the corresponding author upon reasonable request.

Conflicts of Interest

The author declares no conflict of interest.

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