American Journal of
Biomedical Science & Research

www.biomedgrid.com
ISSN: 2642-1747

Research Article

Risk Factors Influencing Malaria Infected Children in Côte D'Ivoire

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To Cite This article: *Mocket A Ehouman*, Rufin Kouassi Assare, Wossan Currie Pamela Ehouman RN, Ahossan André Ehouman SHI, Pamela Nassinata Dosso and Kouakou Eliezer Ngoran, Risk Factors Influencing Malaria Infected Children in Côte D'Ivoire. Am J Biomed Sci & Res. 2026 31(6) AJBSR.MS.ID.004107,* **DOI:** 10.34297/AJBSR.2026.31.004107

Received: August 08, 2026 **Published:** August 18, 2026

Abstract

Background: Malaria is the most widespread parasitological disease that currently affects pregnant women and young children in low-income countries and particularly in West Africa Region where only few countries carry the high burden of malaria crisis due to its high transmission tropism during the increased humidity and raining seasons. During the year 2022, malaria killed almost four people every day including three children under the age of five in Côte d'Ivoire. This research study aims to update the malaria prevalence and the risk factors influencing malaria infected children less than 7 years in the Western Côte d'Ivoire, in order to raise public awareness and recommend a sound public health strategy of care to alleviate this scourging problem.

Methods: A cohort of 1028 male and female children aged 3 months to 6 years were recruited from the period of March 2020 to June 2021 in 46 villages. Each of the 907 enrolled children provided venous blood samples for the Diagnosis of Anaemia (FBC) and malaria (Giemsa staining thick and thin smears) and Rapid Diagnostic Tests (RDTs). Pearson's Chi-2 test ($\chi 2$) was used for comparison of malaria prevalence between groups. Logistic regression models using data of the participants diagnosed positive by microscopy were employed to identify independent risk factors and morbidity patterns associated with *Plasmodium falciparum* mono-infection and co-infections. Significant test was considered at a threshold of 0.05.

Results: Out of the 907 enrolled children, 471(51.9%) were male and 436(48.1%) females. Malaria prevalence by RDT was 82.8% (751/907) with *P. falciparum* infections accounting for 57.9% (435) and mixed infections species 34.8% (316), while the prevalence by microscopy was 60.0% (180/300). Furthermore, the microscopy findings showed 75.0% of P. falciparum, 7.2% of *P. malariae* and 16.7% of *P. falciparum /P. malariae*. On the other hand, 65.5% of children were anaemic (25.8% mild, 37.9% moderate and 1.76% severe). The logistic regression analysis indicated that variables such as Anaemia, Age category, Body temperature, Platelet, and WBC impact positively on malaria while Sex has no influence. The highest MPD (>10,000 parasites/µl of blood) was observed in younger children (70.1%) compare to older ones (43.9%).

Conclusion: Female and male (60.3% versus 59.7%) were almost equally infected and *P. falciparum* was the most prevalent parasites (75.0%) beside the recrudescence of mixed *P.f/P.m* species (16.7%) in Western Côte d'Ivoire.

Keywords: Malaria, Anaemia, Prevalence, Risk factors of malaria, Children less than 7 years, Côte d'Ivoire

Introduction

The mosquito-borne infectious disease named Malaria caused by protozoan parasites of the genus Plasmodium is responsible for severe public health and development challenge in West Africa where the disease is endemic across the region. Five (5) countries (Burkina Faso, Ghana, Mali, Niger, and Nigeria) amongst the eleven identified as "High Burden to High Impact" (HBHI) malaria infected countries that accounts for 66% of the global Malaria cases (173 million and 408 000(68%) of deaths in 2023) are West African countries. Furthermore, the African Region carried the highest malaria burden in 2023, with 94% representing 263 million malaria cases and 95% of deaths (597 000) of globally [40]. In Côte d'Ivoire, a country with tropical climate that is influenced by the impact of rainfall patterns and now coupled with the increasing climate changes is directly linked to mosquito breeding's. These breeding's increase malarial risk outbreaks meaningfully during the high humidity and rainy seasons. Indeed, this study was conducted in a humid tropical or mountainous climate in the Western region. There are two seasons, characterized by a long rainy season from April to October and a short dry season from November to March. The 2018 WHO report highlighted the prevalence of malaria in 2017 Côte d'Ivoire to be estimated at 49% and 52% based on Giemsa Staining (GS) of thick blood smear and Rapid Diagnostic Testing (RDT) respectively [38]. In addition, there was no difference in malaria case incidence in 2023 in comparison to 2015 in Côte d'Ivoire [41]. Reported death cases were 3,000 deaths with 8,750 000 malaria cases observed in Côte d'Ivoire in 2023 [41]. The main objective of this research was, to determine the prevalence of malaria and the associated risk factors influencing malarial infection in children aged 3 months to 6 years old in the Western Côte d`Ivoire in order to stimulate public and governmental awareness to the scourge of the mosquito-borne infectious disease for a better care strategy.

Methods

Study Design and Study Sites

This is an observational clinical research study carried out from March 2020 to May 2021 in 4 Health District Departments (Biankouma, Man, Facobly, and Duékoué), 13 Sous-Prefectures (Blapleu, Fagnampleu, Sangouiné, Guezon, Totodrou, Ouyably-Gnondrou, Guéhiébly, Podiagouine, Man, Gbangbégouiné-Yati, Kpata, Gbangbegouine, Biankouma) and forty-six villages of "TONKPI" Region (Man) (Figure 1).

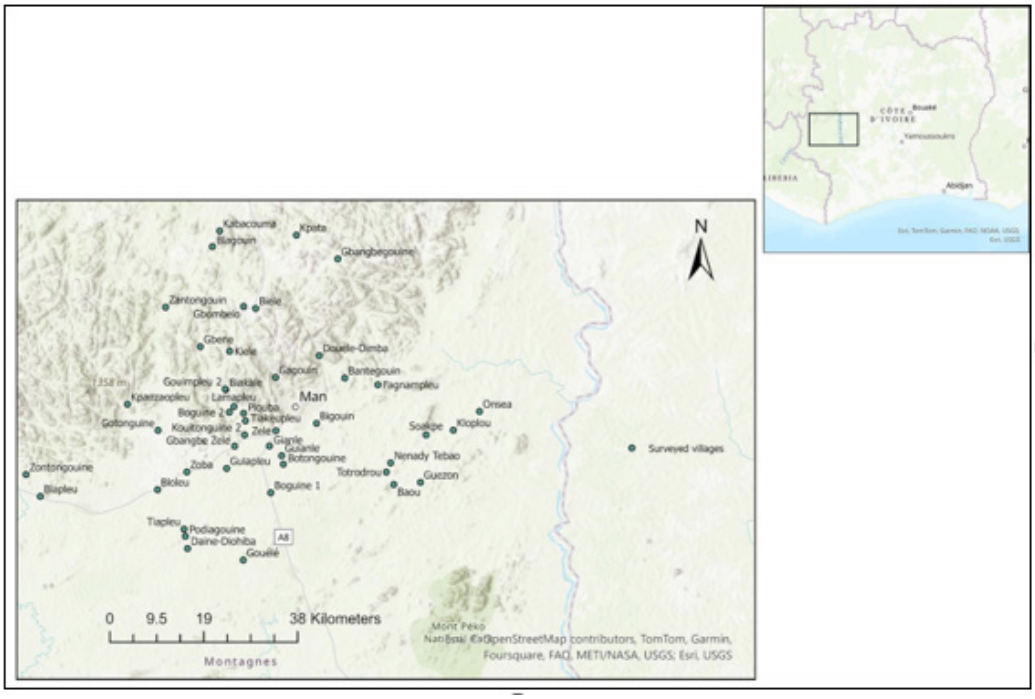

Figure 1: Map of the Villages and Towns enrolled in the study (Study area).

Data Collection

Recruitment of Study Participants: We screened 1028 participants (children) aged 3 months to 6 years old among which, 907 fulfilled the protocol requirements by providing both the clinical samples i.e. venous blood for the diagnosis of anaemia and malaria and the body temperature (auxiliary) which lead to their enrolment in the study. All enrolled study participants reside in rural area with similar life style patterns where both parents or guardians are mostly farmers, house wives or commercial traders.

Socio-Demographic Data: Socio-demographic data including age, sex, height (to the nearest centimetre), weight (to the nearest

0.5 kg), parent's profession and body temperature were collected using a structured questionnaire administered to each enrolled participant who signed a written informed consent or gave a fingerprint (illiterate participants).

Blood collection: A total of 2 mL of venous blood were drawn into ethylenediaminetetraacetic acid (EDTA) treated vacutainer tubes from each of the 907 enrolled study participants. Drawn blood samples were kept on ice until transported to the central laboratory located at the Centre Hospitalier Regional of Man (CHR Man) where the Full Blood Count was conducted (haematology testing). In addition, about 20 µl of blood was collected from each pricked child's finger or the remaining of the collected blood to perform directly the malaria RDTs.

Malaria Microscopy and Malaria Rapid Diagnostic Test

Malaria Microscopy (Giemsa Stained- Thick and Thin Blood Smear): Giemsa thick and thin blood smear on a single slide (malaria microscopy) was prepared using approximately 10 µl from the 2 ml of venous blood collected in the EDTA-treated tube as per Trape JF procedure [35] for the count of malaria parasitemia [39]. The slides were analysed and quality controlled by well-trained and experienced laboratory technicians. Slides were considered positive when asexual forms and/or gametocytes of any Plasmodium species were observed on the blood film. Parasite density per microliter of blood was determined by the number of malarial parasites per 200 leukocytes on a thick blood film, considering a white blood cell (WBC) count of 8000 leucocytes/µl of blood and then transformed into malaria parasite density by multiplying by a factor of 40. Malaria parasite density was classified as mild (<1,000 parasites/µl of blood), moderate [1,000-10,000] or severe (≥10,000 parasites/µl of blood) [35,39]. The thick and thin blood smear (GS) slides were scored either negative or positive. Positive scores were further classified into any of the plasmodium species e.g. Plasmodium falciparum, P. malariae, P. vivax, or P. ovale, P. knowlesi or as undetermined if the technician experienced difficulty clearly identifying the species.

Malaria Rapid Diagnostic Test (RDT): About 10-20 µl of blood collected from the pricked finger of each child was tested for malaria, making use of the plastic capillary tube provided in the RDT kit, as per manufacturer instructions (Humasis Malaria Pf/Pan Antigen Test; Humasis Co. Ltd, Humasis, South Korea). The result was scored as negative if no Plasmodium species were detected, or positive if *P. falciparum* or mixed if *P. falciparum* and other species of Plasmodium were detected [21].

Haemoglobin Determination and Classification of Anaemia: The Full Blood Count (FBC) was performed using URIT 3000 PLUS analyser, Urit Medical Electronics, China and the result printed and further analysed. Anaemia was assessed and classified according to the age, sex of the participants and the concentration value of the Haemoglobin (Hb) as per WHO guidelines [26]. Thus, children from 3 months to 6 years with an Hb level of less than 11 g/dL were considered as anaemic while those with an Hb level ≥11 g/dL were considered as normal (non-anaemic). Anaemia was further categorized as mild if the Hb level was less than 10 g/dL, moderate between 7 and 10 g/dL and severe below 7 g/dL [26].

Data Analysis

Study data were collected and entered into a database using the double-entry system in Epi-data version 3.1 (EpiData, Odense Denmark, 2004). Inconsistencies were cleaned and validate. All statistical analyses were completed after validation by importing the data into SPSS version 20 (IBM Corp; 2011) and STATA 18 software (STATA Corporation, College Station, Texas, USA). Univariate analysis, Chi-2 test (χ^2) and P: (Probability) were used for comparison of proportions between groups. Logistic regression models using data of the participants diagnosed positive by microscopy were employed to identify independent risk factors and morbidity patterns associated with Plasmodium falciparum mono-infection and co-infections. Significant test was considered at a threshold of 0.05.

Ethics Consideration

Ethical approval was sought and obtained from the "Comité National d'Ethique des Sciences de la Vie et de la Santé (CNESVS) de la Côte d'Ivoire" (N/Ref: 024- 21/MSHP/CNESVS-km) prior to the commencement of the study. Additional permission was obtained from the 46 visited village chiefs to conduct the study. Overall, the aims, the procedures, the potential risks and the benefits of the study were explained to the physicians, nurses, and assistant nurses of each local Health Centre involved as well as the villagers before the start of the study during community meetings. A signed consent from either one the biological parent or legal representative was sought and obtained before any study procedure was completed due to the fact that all study participants were minors. Only participants who voluntary consented for the study were included. Participants diagnosed with malaria related sickness were treated free of charge. Those who required additional assistance were referred to the local government health centre for assistance. Moreover, participants were informed that their information will be anonymized by using a coding system instead of their real names.

Results

Characteristics of the Study Population

Overall, the 907 children enrolled in the study were less than 7 years old. Amongst this total, 471 (51.9%) were Male and 436 (48.1%) were Female. Children aged [3M-2Y] or younger accounted for 105 (11.6%) of the sample, follow by the middle age [3-5Y] representing 466 (51.3%), while the older children [6-7 Y[accounted for 336 (37.1%). And so, children between [3-5Y] of age constituted the largest proportion (51.3% percent) of the study population. They all fulfilled the study requirement by providing the venous blood and the body temperature. Furthermore, the venous blood volume was considered enough to prepare Giemsa stained-thick and thin blood smear slides for only 300 (33.1%) participants out of the 907 while all participants were tested for RDT (100%).

Prevalence of Malaria (RDT testing) by Sex and Age

The results of the RDT testing's summarized in Table 1 indicated no difference in terms of gender. *P. falciparum* infected male children represented 83.2%; (95% CI:79.5-86.5) *versus* the female counterparts 82.3%; (95% CI: 78.4-85.8). In terms of the age group, the older children [6-7 Y [were significantly infected than the younger ones [3M-2Y] with a prevalence of 89.0%; (95% CI: 85.1-92.1) and 61.0%; (95% CI: 50.9-70.3) respectively (p<0.001). In terms of mixed Infections, a total of 34.8%; (95% CI:31.7-38.0) children (n =316) were positive amongst which 171 (36.3%) were male and 145 (33.3%) were female. Still, the oldest children [6-7 Y [were more infected than youngest ones [3M-2Y] at a prevalence of 33.6%; (95% CI:28.6-39.0) and 22.9%; (95% CI:15.2-32.1) respectively (p = 0.009) (Table 1).

Table 1: Prevalence of malaria (RDT testing) by sex and age in Western Côte d'Ivoire.

Tested*		Non-Infected	*P.f* Infected	% (95% CI)	χ^2	P-Value	Mixed Inf.	% (95% CI)	χ^2	P-Value
Sex										
Male	471 (51.9)	79 (16.8)	392	83.2 (79.5-86.5)			171	36.3 (32.0-40.8)		
Female	436 (48.1)	77 (17.7)	359	82.3 (78.4-85.8)	0.13	0.723	145	33.3 (28.8-37.9)	0.93	0.336
Age Group										
[3M-2Y]	105 (11.6)	41 (39.1)	64	61.0 (50.9-70.3)			24	22.9 (15.2-32.1)		
[3 – 5Y]	466 (51.3)	78 (17.0)	388	83.3 (79.6-86.5)			179	38.4 (34.0-43.0)		
[6 – 7 Y]	336 (37.1)	37 (11.0)	299	89.0 (85.1-92.1)	44.30	<0.001**	113	33.6 (28.6-39.0)	9.48	0.009**
Total	**907**	**156 (17.2)**	**751**	**82.8 (80.2-85.2)**			**316**	**34.8 (31.7-38.0)**		

***Note:** M: Month, Y: Year, *RDT: Rapid diagnostic test and P.f = Plasmodium falciparum, X^2: Chi-2 test, M: Month, Y: Year, X^2: Chi-2 test, P: Probability, %: Prevalence, Mixed Inf.: mixed infection, CI: Confidence interval, and **Significant test at the threshold of 0.05.

Prevalence of Malaria (Microscopy Testing) Testing by Sex and Age

Out of the 300 smear slides read by the Laboratory technicians, 180 (60.0%) participants were positive for malaria and 120 (40.0%) were negative (Table 2). Data of the positive malaria slides are summarized in Table 2 where 135 participants were diagnosed with *P. falciparum* 75.0%; (95% CI: 68.0-81.1), 13 with *P. malariae* 7.2%; (95% CI: 3.9-12.0), 30 with mixed species (*P. falciparum* /*P. malariae*) accounting for 16.7%; (95% CI: 11.5-22.9), and 2 undetermined species (1.1%). In terms of the age category, there was no difference in the infection rates regarding the two identified Plasmodium species. All three age categories were almost equally infected with no statistical significance noted whether in mono or co-infection status. Indeed, *P. falciparum* prevalence in children aged between [3M-2Y] was 74.5%; (95% CI: 60.4-85.7) while amongst the [3-5Y] old it was 76.2%; (95% CI: 65.7-84.8) and 73.3%; (95% CI: (58.1-85.4) in the [6-7Y [old children. Similar trends were observed for *P. malariae* as well as in the mixed infection (further details are available in (Table 2).

Table 2: Prevalence of malaria (Microscopy testing) by sex and age in Western Côte d'Ivoire.

Test.*		*P.f*	% (95% CI)	χ^2	p-value	*P.m*	% (95% CI)	χ^2	p-value	*P.f*/ *P.m*	% (95% CI)	χ^2	p-Value		Undet.
Sex															
Male	89	70	78.7 (68.7-86.7)			5	5.6 (1.8-12.6)			13	18.7 (11.3-28.2)				1
Female	91	65	71.4 (61.0-80.4)	1.25	0.263	8	8.8 (3.8-16.6)	0.68	0.411	17	14.6 (8.0-23.7)	0.54	0.463		1
Age Group															

[3M-2Y]	51	38	74.5 (60.4-85.7)			2	3.9 (0.5-13.5)			11	21.6 (11.3-35.3)			0
[3-5Y]	84	64	76.2 (65.7-84.8)			6	7.1 (2.7-14.9)			12	14.3 (7.6-23.6)			2
[6-7 Y]	45	33	73.3 (58.1-85.4)	0.14	0.934	5	11.1 (3.7-24.1)	1.85	0.397	7	15.6 (6.5-29.5)	1.27	0.531	0
Total	**180**	**135**	**75.0 (68.0-81.1)**			**13**	**7.2 (3.9-12.0)**			**30**	**16.7 (11.5-22.9)**			**2 (1.1)**

***Note:** *Test..: Tested Positive by microscopy, P.f: Plasmodium falciparum, P.m: Plasmodium malaraie, Undet.: Undetermined, CI: Confidence interval, χ^2: Chi-2 test, M: Month, Y: Year, P: Probability, %: Prevalence.

Relationship between Age and Prevalence of Plasmodium Infection

Figure 2 depicts the relation between the Age and the prevalence of Plasmodium infection where the infection rate increased in younger children less than 1 year (<50 weeks) to 3 years (<149 weeks) before dropping in the 3 to 4 years' children (149 to 199 weeks) till a new recrudescence in the older children from 5 years onwards (>249 weeks) (Figure 2).

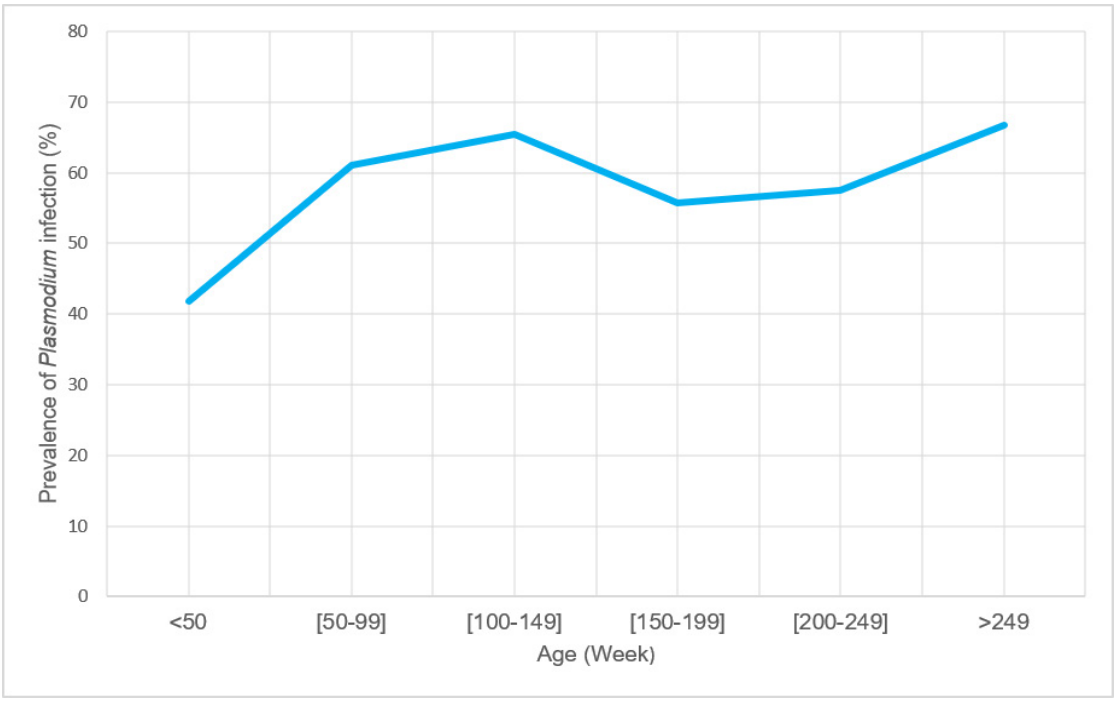

Figure 2: Relationship between Age and the prevalence of Plasmodium species infection.

Relationship between Plasmodium density and Sex/Age

In terms of gender regarding Malaria Parasite Density (MPD) or the intensity of MPD our findings showed the absence of correlation. Plasmodium parasites infected with almost equally the male 11413.5; (95% CI: 7603.0-15223.9) and female sex participants 9520.4; (95% CI: 5423.9-13616.8). Table 3 indicates that although male participants had slightly higher malaria parasite density compared to female participants, that was not statistically significant (p = 0.122). With regard to the age category, in contrary, the malaria parasite density (p=0.002) or the intensity of MPD (p=0.008) were statistically significant. Indeed, younger children, [3M-2Y] had prominently higher malaria parasite density 18,936; 95% CI: 11729.9-26142.1) compared to the older children 5,751.4; 95% CI: 2940.7-8562.1). Idem for the intensity of malaria parasite density where 70.1% of the younger children had MPD >10,000 parasites/µl of blood compare to 43.9% in the older children (Table 3).

Table 3: Relationship between Age category, Malaria parasite density, and Intensity of parasite density.

Tested*		Infected	MPD (95% CI)	χ^2	P-Value	Intensity of MPD			χ^2	P-Value
					Mild <1,000	Moderate [1,000-10,000]	Severe >10,000			
Sex										
Male	149	89 (59.7)	11413.5 (7603.0-15223.9)	0.121	0.122	24 (16.1)	36 (24.2)	89 (59.7)	2.32	0.313
Female	151	91 (60.3)	9520.4 (5423.9-13616.8)			30 (19.9)	44 (29.1)	77 (51.0)		
Age Group										
[3M-2Y]	97	51	18936 (11729.9-26142.1)	12.8	0.002*	12 (12.4)	17 (17.5)	68 (70.1)	13.71	0.008*
[3-5Y]	137	84	7828.6 (4306.8-11350.4)			26 (19.0)	42 (30.7)	69 (50.4)		
[6-7 Y]	66	45	5751.4 (2940.7-8562.1)			16 (24.2)	21 (31.8)	29 (43.9)		
Total	**300 (33.1)**	**180**	**10456.4 (7680.1-3232.7)**			**54 (18.0)**	**80 (26.7)**	**166 (55.3)**		

***Note:** *: Tested by microscopy, MPD: Malaria parasite density, CI: Confidence interval, χ^2: Chi-2 test, M: Month, Y: Year, P: Probability, %: Prevalence.

Prevalence and Severity of Anaemia

The analysis of the FBC results revealed that 594 (65.5%) participants out of the 907 enrolled were anaemic while 313 (34.5%) were non-anaemic. The Grading of Anemia as per WHO criteria indicated the magnitude of mild, moderate, and severe anemia to be 25.8% (n = 234), 37.9% (n = 344) and 1.8% (n = 16) respectively [37].

Prevalence and Severity of the Body Temperature

The recorded body temperature (axillary data) of the 907 enrolled study participants after analysis indicated the following: mean temperature 37.01 °C, minimum 35.4 °C, maximum 40.4 °C, and the Standard deviation was 0.5733. The categorization as per clinical guidelines from the American Academy of Pediatrics (AAP), NHS, WHO, CDC guidelines as Low grade (35.0 - 36.0°C), Normal: 36.1 - 37.5 °C, Mild grade: 37.6 - 37.9°C, Moderate- grade (38.0 - 39.0°C), High grade (39,1- 41 °C), and Hyperpyrexia/Hyperthermia (> 41 °C) (AAP, 2026), revealed the following prevalence: 32 participants had Low grade temperature (3.5%), 786 participants had Normal temperature (86.6%), 46 participants had Mild grade temperature (5.1%) follow by 34 Moderate grade temperature (3.8%), 9 High grade temperature (1.0%) and 0 Hyperpyrexia.

Prevalence and Severity of the White Blood Cell (WBC) Counts

The WBC counts obtained from the FBC results, were analysed and then categorized as per WHO guidelines [1]. The mean WBC counts was 10094.65, the minimum 14.1, the maximum 170000, and the Standard deviation was 7821.181. The categorization of WBC counts as Normal (4,500-13,000 cells/mm^3) or low (Leukopenia: < 4,500 cells/mm^3), or high (Leukocytosis: > 13,000 cells/mm^3) showed that amongst the 907 participants enrolled in the study 778 (85.8%) had a normal WBC value, 17 (1.9%) had Leukopenia and 112 (12.4%) had Leukocytosis.

Prevalence and Severity of the Platelet (PLT) Counts

The analysis of the total enrolled study participants Platelet counts indicated the mean at 282256.1, the minimum at 5000, the maximum at 969000, with the Standard deviation at 121367.7. Following categorization as per WHO guidelines, as Normal (150,000 - 400, 000/mm^3), Low (Thrombocytopenia: < 150,000 cells/mm^3), High (Thrombocytosis: > 400, 000 cells/mm^3), the prevalence of PLT counts was determined (AAP, 2026). And so, 717 (79.0%) participants had a Normal PLT count, follow by 23 (7.10%) Thrombocytopenia, then 126 13.9% Thrombocytosis.

Associated between Plasmodium species infections (GeFs_Pos) and Anaemia, Platelet, WBC, Sex, Body Temperature, and Age category

The multivariate logistic regression analysis summarized in Table 4 showed that being infected with plasmodium species (malaria) strongly increased the risks for variables such as the Anaemia, Platelet (PLT), Body Temperature and Age Category and a lesser degree for WBC, while no influence was observed in relation to the participant gender (Sex). Indeed, anaemic children have 3.18-time risks of being infected by plasmodia (p = <0.001; 95% CI: 1.85-5.49) than the non-anaemic ones. On the other hand, older children

aged between [6-7Y [years have 2.90-time risks to get infected by malaria (p = 0.005; 95% CI: 1.38-6.09) compared to the younger ones [3M-2Y]. Similarly, children with moderate and mild grade body temperature have 2.15 times (p = 0.419; 95% CI: 0.34-13.67), and 1.10 times (p = 0.868; 95% CI: 0.37-3.24) risk of having malaria infection respectively compared to a child with a normal body temperature. Furthermore, the abnormal WBC counts such as Leukopenia had no impact, while children with Leucocytosis had 1.18 times risk (p = 0.658; 95% CI: 0.57-2.45) of having plasmodium species infection compared to children with a normal WBC count. Lastly, gender (Sex) in particular has no risk influence (p = 0.800; 95% CI (0.57-1.55) on malaria infection (Table 4).

Table 4: Association between Plasmodium species infections (GeFs_Pos) and Anaemia, Platelet, WBC, Sex, Body Temperature, and Age category.

	Association	OR[a]	P-Value	95% CI
	Anaemia			
GeFs_Pos	Non anaemic	1		
	Anaemic	3.18	<0.001*	(1.85-5.49)
	Platelet			
	Normal	1		
	Thrombocytopenia	2.23	0.094	(0.87-5.72)
	Thrombocytosis	0.39	0.007*	(0.19-0.77)
	WBC			
	Normal	1		
	Leukopenia	0.95	0.951	(0.17-5.32)
	Leucocytosis	1.18	0.658	(0.57-2.45)
	Body Temperature			
	Normal	1		
	Low	0.47	0.394	(0.85-2.64)
	Mild	1.1	0.868	(0.37-3.24)
	Moderate	2.15	0.419	(0.34-13.67)
	High	ND	ND	ND
	Gender (Sex)			
	Female	1		
	Male	0.94	0.8	(0.57-1.55)
	Age Category			
	[3 M-2Y]	1		
	[3-5Y]	1.71	0.07	(0.96-3.07)
	[6-7Y]	2.9	0.005*	(1.38-6.09)

***Note**: GeFs_Pos = Positive Plasmodium species, WBC: White Blood Cell, Y = Year, M = Month, OR[a] = adjusted Odd ratio, CI = Confidence Interval, and *Significant test at the threshold of 0.05.

Discussion

This study, aimed to determine the risk factors influencing malaria infected children aged 3 months to 6-year-old in Western Côte d'Ivoire. Using this observational study design to collect data on participant gender and age, haemoglobin concentrations, WBC counts, PLT counts and the body temperature, we collected venous blood and body temperature from 907 children living in rural area and analysed their Full Blood Count (FBC) results. Overall, the haemoglobin concentrations analysis from our findings revealed 65.5% prevalence of anaemia that is predominantly moderate (37.9%), followed by 25.8% mild, then 1.8% of severe cases. This 65.5% prevalence even though significantly higher than the global WHO average of 40% [42] is in line with the reported rates in West and Central African Region by UNICEF of 65.2% using data from 17 countries including Côte d'Ivoire. In UNICEF report, the

anaemia prevalence in children aged 6 to 59 months was 35.0% moderate, 26.5% mild, and 3.7% severe [36]. Elsewhere, various studies have also reported a much higher prevalence than our study results namely a study by Mghanga and collaborators in Southern Tanzania which found 83.2% of anaemia in the same age group. In *Mghanga et al.,* study, the moderate anemia accounted for 44.8% of all anaemic children, follow by 9.2% mild, then 46.0% severe cases [23]. Similarly, *Wirth et al.,* study in Sierra Leone reported 76.3% anaemia in children < 5 years old with moderate, mild, and severe anemia accounting for 45.8%, 25.2%, and 5.4% respectively [43]. Our RDT study results showed no difference in relation to gender infection rates. Male and Female participants were almost equally infected (83.2% versus 82.3% respectively). Same as the microscopy results where 78.7% male were infected compared to 71.4 % female even though the microscopy samples were relatively small in size in comparison to the RDT samples. This observation, may be explained by the use of Insecticide-Treated Nets (ITNs), Long-Lasting Insecticidal Nets (LLINs), and Indoor Residual Spraying (IRS) by parents following the yearly country-wide National Malaria LLINs distribution program year, and also reported elsewhere by other authors [32,13,11]. A contrario, Ikpeama, in Nigeria reported an increased malaria prevalence in favour of the male participants (91.1%) compared to the females (70%) in same age groups. In addition, younger children (15%) were less infected than older ones (89.5%) [15]. The latter was in line with our RDT testing findings although that contrasts with our microscopy results. Our microscopic results also differ from *Bousema et al.,* in Kenya where blood-smear positive malaria infections were greater in young children < 5 years (74%) compared to older children (30-50%) [6].

The Malaria Parasite Density (MPD) and its intensity was both statistically significant in our study (p = 0.002 and p = 0.008 respectively). In fact, younger children with 18,936 parasites/µl of blood had an intensity of 70.1% compared to the older children with 5751.4 parasites/µl of blood and an intensity of 43.9%. However, there was no significant differences in terms of gender between male participants and female counterparts (11,413.5 parasites/µl versus 9,520.4 parasites /µl respectively). A study by *Adu-Gyasi et al.,* in Ghana reported similar gender prevalence with no major difference (males: 11058/ul versus females: 10217/ul) [3]. This significant variation in terms of MPD may be due to the maturity of the older children aged between 6 to 7 years' immune system in comparison to the younger children [3M-2Y], with an immature immune system relying mainly on the mother`s transmitted immune protection (maternal antibodies) or the presence of Fetal Hb (HbF), which is protective and causes poor parasite growth [28,7,44]. The prevalence of malaria by age category results showed that younger children aged [3M-2Y] (61.0%) were less infected than children aged [6-7 Y] (89.0%). These results are in line with *Rugiranka et al.,* study in Malawi or that of Alemneh and collaborators in Ethiopia. In *Malawi, Rugiranka et al.,* found that 25.6% children of 6-23 months and 43.4% of children aged 32-59 months were infected with malaria [30]. On the other hands, *Alemneh et al.,* reported that 6.9% of children aged 6 month-1 year, 14.6% of those aged 1-2 years and others 77.9% aged >2-5 years were infected with malaria i.e. children aged 6 months to 2 years are less malaria infected [5]. These observations can be explained by the fact that infants under 6 months may benefit from maternal antibodies, while older children gradually acquire immunity through repeated exposure [22].

Associated Between Plasmodium Species Infections (Gefs_Pos) And Anaemia

The findings from this study revealed that plasmodia infected children were over 3 times more likely to have anaemia compared to children who were not infected. In other words, the risk of having anaemia increases by 3 times as the child is infected with plasmodium species (has malaria). Our findings are aligned with the studies by Rugiranka and Ramroop (2020) in Malawi where their 2020's study showed that children without anaemia have a lower prevalence of testing positive to malaria compared to anaemic children (aOR = 0.233; p =<0.001) [30]. Furthermore, *Sultana and et al.,* reported in Kenya similar finding where children with malaria had 3.52 times to be anaemic compared to non-anaemic children [33].

Associated Between Plasmodium Species Infections (Gefs_Pos) and Age Category

Furthermore, children belonging to the age category [6-7Y [and [3-5Y] year are almost 3 and 2 times respectively at risks of being infected by malaria plasmodia than children of [3M-2Y] year old [2]. These findings are corroborated by several studies conducted by *Kihwele et al.,* in Tanzania, Carlucci in *Mozambique,* and *Akello et al.,* in Uganda whose results supported the understanding that children under 6 months had a significant reduced risk of malaria infection compared to children older than 2 years. This highlights the protective impact of early infancy from maternal antibodies during pregnancy and breastfeeding [8,4]. Furthermore, *Mhelembe et al.,* in Nigeria reported (Children aged 12-24 Months: aOR = 0.73, p<0.0001 vs. 37-48 Months: aOR = 0.33, p<0.0001 vs. 49-59 Months: aOR = 0.31, p<0.0001; Reference was 6-12 Months); Zgambo et al., in Malawi reported (Children aged 12-23 Months: aOR = 1.1 vs. 24-35 Months: aOR= 2.0 vs. Children > 40 Months aOR= 2.1, p = 0.045; Reference was 6-11 Months) and *Sultana et al.,* in Kenya reported (Children aged 5-9years: aOR = 2.92 vs. under 5 years as a Reference category) [24,45,33]. These researchers highlighted the fact that the risk of a positive malaria increased as the child's age increased which is in line with our study findings. Indeed, younger children less than one-year old particularly, may sleep under Insecticide- Treated Net (ITN) mostly with their parents compare to their older counterparts, thereby reducing their exposure to mosquitoes [8,22]. Furthermore, older children tend to spend more time outdoors and may engage in activities that increase their exposure to mosquitoes [18].

Associated Between Plasmodium Species Infections (Gefs_Pos) and Temperature

In our study, children with abnormal body temperature like mild and moderate temperature grade increase the likelihood of

having malaria (aOR = 1.10; p = 0.868; aOR = 2.15; p = 0.419 respectively). This association is consistent with Kiemde and collaborators study in Burkina Faso that reported a strong correlation between infections with Plasmodium species and temperature. Indeed, as per Kiemde and collaborators study, a Temperature > 39.5 °C had a higher risk of having malaria (OR = 2.06, p = 0.002). This significant association observed in our study may be due to either the rupture of erythrocytic cells (rupture of erythrocytic-stage schizonts) or the fact that parasitized red cells have obstructed the capillaries and post-capillary venules leading to local hypoxia and the release of toxic cellular products (e.g. TNF-a, IL-6) [9,10].

Associated Between Plasmodium Species Infections (Gefs_Pos) and Platelet

In terms of the association between Plasmodium species infection (malaria) and Platelets, our study results showed that abnormal platelets count (Thrombocytopenia and Thrombocytosis) had a positive impact in children, even though the association was not strong in our cohort regarding the Thrombocytosis (aOR = 0.39; p = 007) but stronger in terms of Thrombocytopenia (aOR = 2.23, p = 0.094) [14]. In Thailand, *Kotepui et al.,* study revealed a more significant association in the sense that patients with thrombocytopenia were 32 times more at risk of having malaria infection (OR = 31.8; p = 5.6) than those with normal platelets count. [20]. We further reported 7.1% Thrombocytopenia, and 13.9% Thrombocytosis prevalence's in our study. Elsewhere, three Pakistani researchers *Taha et al., Hassan et al., Mumtaz et al.,* also reported higher degrees of thrombocytopenia at 69.2%, 87.3%, and 85.5% in patients infected with malaria respectively [34,12,25]. The observed thrombocytopenia can be explained by different mechanisms such as immune mediated mechanisms including immune destruction of circulating platelets, splenic pooling, and reduced platelet lifespan. Indeed, platelets can kill circulating parasites of all major Plasmodium species during human malaria [31,16,27] or else the plasmodia parasites can induce either a splenic sequestration of platelets or an immune-mediated platelet destruction when interacting with infected erythrocytes during an acute malaria infection [17,27].

Associated Between Plasmodium Species Infections (Gefs_Pos) and Sex

There was no association between Plasmodium species infections and Sex in our study (aOR=0.94; p = 0.800) same as *Houngbedji et al.,* study in Côte d'Ivoire (aOR = 0.78; p<0.001) [14]. Elsewhere, a study by *Roosihermiatien et al.,* in Indonosia also shared the same result revealing an absence of association between the gender and the risk of plasmodium species infection (uOR= 0.960; p = 0.74) [29]. *Furthermore, Zgambo et al.,* study in Malawi (Male: uOR= 0.9, p<0.001 versus Female: uOR=1.00) and *Sultana et al.,* in Kenya study's results (Male: aOR = 1.09 versus Female: aOR = 1.00) are consistent with our findings [33,45]. In contrast, *Kiemde et al.,* study in Burkina Faso reported a positive association (OR = 1.19; p= 0.255) in children aged less than 5 years [19]. This lack of association observed in our study which is consistent with various published studies existing in the literature i.e. no significant association between sex and the prevalence of Plasmodium infection, may be explained by the fact that both males and females have almost equal risks of infection rates and exposure mostly because they share the same environment (e.g. rural) like it is the case in our study.

Associated between Plasmodium Species Infections (GeFs_Pos) and WBC

In our study there was no association between Plasmodium species infection and abnormal White Blood Cells (WBC) counts in regards to Leukopenia (aOR = 0.95; p = 0.951) in contrast to Leucocytosis that shows a slight association (aOR = 1.18; p = 0.658). The earlier contrast with *Kotepui et al., and Kiemde et al.,* results. Indeed, our results in relation to Leukopenia is in line with Kotepui and collaborators study in Thailand, where infected patients with Leucopenia were 3 times more at risks of having malaria infection (OR = 3; p = 2.7) in comparison to those with normal leucocytes count [20]. Similarly, Kiemde et al., reported in Burkina Faso a less strong positive correlation in terms of Leucopenia (OR = 1.18; p = 0.803) [19]. Lastly, several studies have reported Leucocytosis among the malaria patients and its attachment to a poor prognosis in relation to the malaria disease (21,7,19].

Strengths and Limitations

This prospective study leveraged a substantial large number of participants to enable a comprehensive analysis. The limitations of this study included the small size of the Giemsa stained-thick and thin blood smear samples in comparison to the overall total participants. Nevertheless, a combination of microscopy and RDT was used to enhance diagnostic sensitivity even though the use of additional diagnostic tools such as molecular *viz*. the Polymerase Chain Reaction (PCR) technic that will add a highly sensitive advantage and thus would allow to clarify the high number of false-negative or false-positive diagnoses obtained with RDT compared to microscopy.

Conclusion

This study aimed to presents the risk factors influencing malaria infected children under 7 years old in Western Côte d'Ivoire. Based on the important insights brought by the malaria microscopy tests analysis, our findings presented a much stronger association in relation to variables like anaemia, moderate body temperature, thrombocytopenia and older children [6-7Y], with a total absence of association in particular to the participant sex and leukopenic children.

Acknowledgments

The authors are grateful to all the 46 communities and their chiefs for their willing participation in field study as well as the committed study participants for their willingness to collaborate and to Dr. Tra Bi Joel, Head of Laboratory at CHR of Man,

Conflict of Interest Statement

The authors have declared that no competing interests exist.

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