



## **Epidemiology of Malaria Using LED Fluorescence Microscopy among Schoolchildren in Douala, Cameroon**

**Léopold Gustave Lehman<sup>1\*</sup>, Loick Pradel Kojom Foko<sup>1</sup>, Calvin Tonga<sup>1</sup>,  
Hervé Nyabeyeu Nyabeyeu<sup>1</sup>, Else Carole Eboumbou<sup>2</sup>, Larissa Kouodjip Nono<sup>3</sup>,  
Lafortune Kangam<sup>3</sup>, Arlette Linda Ngapmen<sup>3</sup>, Peguy Brice Assomo Ndemba<sup>1</sup>,  
Isabelle Matip<sup>3</sup> and Nicolas Policarpe Nolla<sup>4</sup>**

<sup>1</sup>*Parasitology and Entomology Research Unit, Department of Animal Organisms, Faculty of Science,  
The University of Douala, P.O Box 24157, Douala, Cameroon.*

<sup>2</sup>*Faculty of Medicine and Pharmaceutical sciences, The University of Douala, P.O Box 24157, Douala,  
Cameroon.*

<sup>3</sup>*Department of Animal Organisms, Faculty of Science, University of Yaoundé I, P.O Box 812, Douala,  
Cameroon.*

<sup>4</sup>*Department of Biochemistry, Faculty of Science, The University of Douala, P.O Box 24157, Douala,  
Cameroon.*

### **Authors' contributions**

*This work was carried out in collaboration between all authors. Author LGL designed the study, wrote the protocol and wrote the first draft of the manuscript. Author LPKF designed the study, performed the statistical analysis, wrote the protocol and wrote the first draft of the manuscript. Authors CT and ECE managed the analyses of the study. Authors LPKF, CT, HNN, ECE, LKN, LK, ALN, PBAN, IM and NPN managed the literature searches. All authors read and approved the final manuscript.*

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### **ABSTRACT**

**Aims:** Determine factors associated with malaria infection, the prevalence of asymptomatic carriage of malaria parasites and fevers of non malarial origin.

**Study Design:** This was a cross-sectional study.

\*Corresponding author: Email: [leopoldlehman@gmail.com](mailto:leopoldlehman@gmail.com);  
Email: [kojomloick@gmail.com](mailto:kojomloick@gmail.com);

**Place and Duration of Study:** The study was carried out in three districts of the town of Douala, Cameroon in 2013.

**Methodology:** Seven hundred and eighty five (785) schoolchildren aged 3 to 17 years were enrolled upon parental consent and tested for the presence of malaria parasites in capillary blood. In addition, sociodemographic and clinical data were also documented.

**Results:** The overall prevalence of malaria parasite infection was 45.47% and significantly varied with respect to age, health district and body temperature. Asymptomatic infections accounted for 89.61% of all malaria infection cases. Fevers of non malarial origin were found in 10.73% of the pupils. The overall ITNs use rate found was 45.74% with value significantly lower in males compared to females (20.70% versus 25.03%;  $P = .04$ ).

**Conclusion:** There is an urgent need for the implementation of interventions based on active detection and treatment of all cases of malaria infection in community especially in children who pay the heaviest tribute to the disease. CyScope fluorescence microscopy could be a valuable diagnostic tool to achieve this objective.

*Keywords: Malaria; epidemiology; fever; schoolchildren; fluorescence microscopy; Douala.*

## 1. INTRODUCTION

Malaria remains the first endemic parasitic disease worldwide [1]. In 2015, malaria was responsible for nearly 212 million clinical cases with subsequent 429,000 deaths worldwide. sub-Saharan African (SSA) countries account for about 90% and 92% of cases and deaths respectively, children under five and pregnant women being the most vulnerable groups [1]. Malaria-related burden has declined over the last decades, probably due to the implementation of control strategies in many countries [1-4].

In Cameroon, malaria is a major public health problem with 40 to 45% medical consultations and 30 to 47% hospitalizations [5]. The main control strategies adopted by the National Malaria Control Program (NMCP) include the intermittent presumptive treatment (IPT) of pregnant women, free management of simple malaria in children under five with Artemisinin-based combination therapies (ACTs), indoor residual spraying (IRS), and more recently free distribution of long-lasting insecticidal nets [1,6].

In endemic areas, malaria is a first cause of school absenteeism and many aspects of cognition disorders [7]. Over time, repeated malaria episodes reduce the performance of schoolchildren [8]. Few studies addressed the deleterious effects of malaria among schoolchildren in Cameroon [9-11]. Kimbi and colleagues previously estimated up to 14 absenteeism days per month among schoolchildren in the Southwest region of the country [9].

Understanding the epidemiology of malaria especially among schoolchildren can be

enhanced by the use of new diagnostic tools, given the many limitations inherent to the classical Giemsa-stained thick blood film which is time-consuming, labour-intensive and requires good quality reagents and equipment as well as skilled microscopists [12,13].

The attempts for more efficient hospital and/or community-based management of malaria could be also improved with new methods. Developing, testing and implementing simple, cost effective, rapid and accurate alternative diagnostic tools requiring little or no training could greatly improve and scale up all these aforementioned composite items of malaria control. In this sense, fluorescence-based methods could be interesting alternatives diagnostic tools. They were proven to have almost equal malaria diagnosis-related performances with the Giemsa-stained blood film when real-time PCR was used as gold standard [12]. The authors of this study reported sensitivity, specificity, positive and negative predictive values of 61.8%, 98%, 95.7% and 63.9% respectively against 62.2%, 96%, 95.7% and 63.9% for Giemsa-based method [12]. Besides, fluorescence microscopy is increasingly accepted and used in Cameroon by some researchers [14,15]. This study is aimed at accessing the prevalence and factors associated with malaria, and determining the prevalence of asymptomatic carriage of malaria parasites and fevers of non malarial origin using Light Emitting Diodes (LED) Cyscope fluorescence microscopy.

## 2. MATERIALS AND METHODS

### 2.1 Study Sites

This cross-sectional study was conducted in three districts of the city of Douala (Nylon,

Logbaba and Deido), Littoral Region of Cameroon. Douala is located at 3°48'N, 10°08'E, near the Atlantic coast, within the Congo-Guinean phytogeographical zone characterized by a typical equatorial climate with two rainy seasons extending from March to June and from September to November [16]. The city is 1 m above sea level and receives over 3,500 mm rainfall annually. It is the main business city in Cameroon characterized by a poor urbanization. These environmental conditions are favorable for the creation of breeding sites for malaria vectors.

Four primary schools and one secondary school were selected for the study; La Carrière Evangelic School and Le Messie School Group in the Ndogpassi II District, La Clarté School Group and La Bonté Maternal and Primary School in the Nylon district and finally the Government Secondary School Cite-SIC in the Cite-SIC District. These are subsequently referred to as La Carriere, Le Messie, La Clarte, La Bonte and Cite-SIC respectively within the further sections of the manuscript.

## 2.2 Study Population

The study population consisted of school children aged 3-16 years of both sexes whom written and signed parental/guardian consent was obtained for their participation in the study. They were divided into three age groups: < 5 years, 5-10 years and >10 years.

## 2.3 Study Sample Size

Children were recruited on a convenient approach in each school through random sampling method. Based on a malaria prevalence of 58.4% with fluorescence microscopy as reported by Kimbi et al. [14], the sample size was determined using the Lorentz's formula  $n = Z^2pq/d^2$  where  $n$  = the required sample size,  $Z$ =statistic for the desired confidence level (1.96 for 95% confidence level),  $p$  = assumed malaria prevalence in schoolchildren,  $q$  = 1- $p$ : proportion of malaria negative participants and  $d$  = accepted margin of error (5%). The minimum sample size was estimated as  $n=373$ . A total of 785 schoolchildren were included in the study.

## 2.4 Study Design

This cross-sectional study took place in three districts of the city of Douala from November

2013 to January 2014. Prior to field data collection, school officials and teachers were informed on the aim and objectives of the survey. Only five schools were willing to participate in the study upon the sensitization phase. Forms were distributed to schoolchildren in order to seek for parental/guardian consent for their participation in the study. Only children whose parents/guardians provided signed parental consent forms were finally enrolled in the study. Work space was provided to the study team by officials of the schools. A questionnaire was administered to parents/guardians; clinical examination of the child was made and blood collected for malaria diagnosis.

## 2.5 Questionnaire

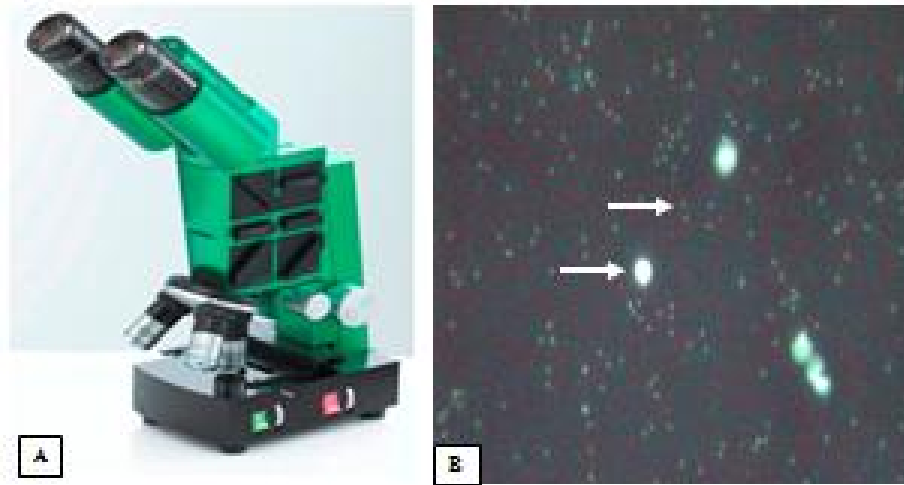
A trained interviewer administered the structured questionnaire to parents/guardians in order to collect sociodemographic and behavioral information concerning their children. Clinical information was also collected.

## 2.6 Clinical Examination

Armpit temperature was measured for each child using a clinical thermometer. Signs and symptoms of simple or severe malaria were checked for, in accordance with the WHO guidelines on malaria diagnosis [17].

## 2.7 Malaria Diagnosis

A drop of blood was collected from finger prick and used for the diagnosis of malaria infection. CyScope<sup>®</sup> fluorescence microscopy (Partec-Sysmex<sup>®</sup> GmbH, Germany) was used for diagnosis. The CyScope<sup>®</sup> microscope has two light sources emitting normal and fluorescent lights. Fluorescent light allows the detection of malaria parasites through an incident ultraviolet (UV) emission. Readily-prepared and ready to-use test slides labeled with 4',6-Diamidino-2-Phenylindole (DAPI), a DNA-specific fluorescent dye allowing for detection of intraerythrocytic *Plasmodium* DNA at 443 nm wavelength [18,19] were used. For the test, 10  $\mu$ L of capillary blood was placed on the dye-containing area of a labeled slide, covered with a cover slip, incubated for one minute at room temperature in the dark and then observed under x 40 objective. The presence of bright shiny intracellular tiny dots observed under UV light indicates the presence of malaria parasites in red blood cells (Fig. 1). Results were interpreted as valid (negative or positive) and invalid [20].



**Fig. 1. A. CysCope® (Partec-Sysmex, Japan); B. Positive malaria slide under observation with CysCope®. Bigger spots correspond to White Blood Cells (WBC) and the smaller spots to malaria parasites (Photography provided by the authors)**

## 2.8 Operational Definitions

- Fever was considered as armpit temperature  $\geq 37.5^{\circ}\text{C}$  [21].
- Asymptomatic malaria was defined as the presence of malaria parasite with an axillary temperature  $< 37.5^{\circ}\text{C}$  [22].
- Simple malaria was defined as the presence of malaria parasite with an axillary temperature  $\geq 37.5^{\circ}\text{C}$  in the absence of signs of severity [22].
- Severe malaria was defined as the presence of malaria parasite with an axillary temperature  $\geq 37.5^{\circ}\text{C}$  and the presence of at least one sign of severity [17].
- Fever of non-malarial origin was defined as armpit temperature  $\geq 37.5^{\circ}\text{C}$  in the absence of malaria parasite in children as diagnosed by fluorescent microscopy.
- Children who had slept under a bed net the day before data collection were considered bed nets users [23].

## 2.9 Ethical Statements

The study was carried in accordance with guidelines for human experimental models in clinical research as stated by the Cameroon Ministry of Public Health. Signed informed consent form was obtained from each parent/guardian for their child to participate in the study and assent was obtained from all participating children aged nine and above. Negative malaria cases with fever were referred

to the nearest health facility for further examination. Participation in the study was strictly voluntary and parents were free to decline answering any question or withdraw their children if they so wished at any time. In addition, the study was approved by the Littoral under N°2853/11/L/MINESEC/DLP/SDAG/IMS. All positive cases were treated on the spot following the protocol recommended by the Ministry of Public Health of Cameroon with Artemisinin-based combination therapy (Artesunate-Amodiaquine).

## 2.10 Statistical Analysis

All data were keyed in an Excel spreadsheet and statistical analyses were performed with SPSS 22 for Windows (SPSS, Inc., Chicago, IL, USA). Descriptive statistics were used where appropriate. Independence Chi-square, Fisher's exact tests and Goodness-of-fit Chi-square were used to compare proportions. Logistic regression analysis was used to identify factors associated with malaria infection and the use of insecticide-treated bed nets (ITNs). Crude and adjusted Odds Ratios (OR) as well as their 95% Confidence Intervals (CI) was computed. All variables of interest were used in univariate logistic model and thereafter a selection between "Schools" and "district" variables was made for multivariate logistic in order to avoid the redundancy of information [24]. Statistical significance was set at  $P < .05$ .

### 3. RESULTS

#### 3.1 Characteristics of Schoolchildren

A total of 785 children were enrolled in the study including 400 females (50.95%) and 385 males (49.05%) for an overall male-to-female sex ratio of 0.96. Schoolchildren from the Cite-SIC district were older on average than their counterparts from Nylon and Ndogpassi II ( $P < .0001$ ). Pupils aged > 10 years were predominant in the study population (44.21%). No fever was found in 84.55% of the participants (Table 1). Furthermore, the rate of mosquito nets usage was 45.74% (338/739; 95%CI: 42.18-49.34).

#### 3.2 Prevalence and Factors Associated with Malaria

Two (2) slides were unreadable; parasitological results were thus available for 783 participants. The prevalence of malaria parasite infection was 45.47% (356; 95%CI = 42.01-48.97).

The risk of malaria infection significantly varied with respect to age, district and body temperature. Indeed, the highest prevalence of malaria 80.95% was recorded in the youngest

(< 5 years) age group while the lowest was recorded in more than 10 years (22.30%) as presented in Table 2. In addition, the multivariate logistic analysis showed that the risk of malaria were significantly lower in aged > 10 years old (OR = 0.19;  $P = .0009$ ).

Besides, the risk of malaria infection was more than two fold (OR = 2.44;  $P = .0274$ ) higher in children from the Ndogpassi II district compared with their counterpart from the Cite-SIC district.

The risk of malaria parasites infection was about five folds higher in febrile compared with their afebrile counterparts (OR = 4.86;  $P = .0033$ ). The prevalence of malaria was 77.78% and 44.71% respectively in both groups (Table 2).

#### 3.3 Prevalence of Asymptomatic Malaria and Its Distribution with Regard to Sociodemographic Variables

The overall prevalence of asymptomatic malaria was 40.74% (319/783; 95%CI = 37.35 – 44.22). Thus, 89.61% (319/356; 95%CI: 86.01-92.37) of all malaria parasite infection was asymptomatic. It should be noted that no severe malaria case was recorded in this study.

**Table 1. Distribution of participants in districts with respect to socio-demographic and clinical variables**

Variables	Nylon (N = 301)	Ndogpassi II (N = 279)	Cite-SIC (N = 203)	Total (N = 785)
<b>Gender</b>				
Male	156 (51.83)	128 (45.88)	116 (57.14)	<b>385 (49.05)</b>
Female	145 (48.17)	151 (54.12)	87 (42.86)	<b>400 (50.95)</b>
<b>M/F Sex ratio</b>	1.06	0.85	1.33	<b>0.96</b>
<b>Age groups (years)*</b>				
< 5	0 (0)	63 (22.58)	0 (0)	<b>63 (8.05)</b>
5-10	217 (72.09)	216 (77.42)	0 (0)	<b>433 (55.30)</b>
> 10	84 (27.91)	0 (0)	203 (100)	<b>287 (36.65)</b>
<b>Mean age ± SD (years)</b>	8 ± 2	7 ± 2	12 ± 1	<b>9 ± 3</b>
<b>Clinical status*</b>				
Non febrile (< 37.5°C)	215 (71.43)	258 (92.47)	189 (93.10)	<b>662 (84.55)</b>
Febrile (≥ 37.5°C)	86 (28.57)	21 (7.53)	14 (6.90)	<b>121 (15.45)</b>
<b>Temperature ± SD (°C)</b>	36.63 ± 0.25	36.43 ± 0.33	37.17 ± 0.31	<b>36.70 ± 0.41</b>
<b>Use ITNs*</b>				
Yes	138 (51.11)	107 (40.53)	93 (45.81)	<b>338 (45.86)</b>
No	132 (48.89)	157 (59.47)	110 (54.19)	<b>399 (54.14)</b>

Data are presented as frequency (percentage) and mean ± standard deviation (SD); F = Female; M = Male; ITNs = Insecticide-treated nets; \* = data were missing in a few children

Table 2. Factors associated with malaria prevalence

Variables	N° of children examined	N° of children Infected	Crude OR (95%CI)	P-value	Adjusted OR (95%CI)	P-value
<b>Age (years old)</b>						
< 5	63	51 (80.95)	1		1	
5-10	433	241 (55.66)	0.29 (0.15 - 0.57)	.0003*	0.69 (0.34 - 1.39)	.30
> 10	287	64 (22.30)	0.07 (0.03 - 0.13)	< .0001*	0.19 (0.08 - 0.52)	.0009*
<b>Gender</b>						
Male	383	182 (47.52)	1		1	
Female	400	174 (43.50)	1.18 (0.89 - 1.56)	.26	0.96 (0.69 - 1.34)	.81
<b>Districts</b>						
Cite-SIC	203	50 (24.63)	1		1	
Ndogpassi II	279	205 (73.48)	8.48 (5.59 - 12.84)	< .0001*	2.44 (1.10 - 5.40)	.0274*
Nylon	301	101 (33.55)	1.55 (1.04 - 2.30)	.0325*	0.66 (0.34 - 1.27)	.21
<b>Schools<sup>*</sup></b>						
Cite-SIC	203	50 (24.63)	1		-	-
La Bonte	157	70 (44.59)	0.72 (0.43 - 1.20)	.0124*	-	-
La Clarte	144	71 (49.31)	2.98 (1.89 - 4.70)	< .0001*	-	-
La Carriere	91	30 (32.97)	10.20 (5.69 - 18.27)	< .0001*	-	-
Le Messie	188	135 (71.80)	7.79 (4.97 - 12.23)	< .0001*	-	-
<b>Temperature (°C)</b>						
Afebrile	765	342 (44.71)	1		1	
Febrile	18	14 (77.78)	4.47 (1.31 - 27.72)	.0004*	4.86 (1.13 - 35.13)	.0033*
<b>Use ITNs</b>						
No	399	191 (47.87)	1		1	
Yes	338	140 (41.42)	0.77 (0.58 - 1.03)	.07	0.86 (0.62 - 1.20)	.38

\*: significant; N°: Number; ITNs: Insecticide-treated Nets; OR: Odds Ratio; 95%CI: Confidence Interval at 95%; &: The variable was excluded from the multivariate logistic model because of redundancy of information

As presented in Table 3, distribution of asymptomatic malaria case was similar between both sexes as no statistically significant difference was found ( $P = .40$ ). Conversely, the highest prevalence of asymptomatic malaria were recorded in 5-10 years old (66.46%), the Ndogpassi II district (60.19%) and Le Messie school (38.24%), and the difference was statistically significant ( $P = <.0001$ ).

### 3.4 Prevalence of Fever of Non-malarial Origin

A total of 84 malaria negative children presented with fever during this study; the overall

prevalence of fever of non-malarial origin was 10.73% (84/783; 95%CI = 8.75 – 13.09). The majority of the cases were found in 5-10 years old (65 cases), the Nylon district (68 cases) and La Bonte school (68 cases). In addition, the differences were significant ( $P < .0001$ ) (Table 4).

### 3.5 Factors Associated with the Use of Insecticides-treated Nets (ITNs)

Multivariate regression model showed that utilization of ITNs was significantly lower in males compared to females (20.70% versus 25.03% respectively; OR = 0.74;  $P = .04$ ).

**Table 3. Distribution of asymptomatic malaria with respect to variables**

Variables	Categories	Total	Percentage (%)	Statistics	P-value
Gender	Male	152	47.65	0.705	.40
	Female	167	52.35		
Age (years old)	< 5	51	15.99	255.6	<.0001*
	5-10	212	66.46		
	> 10	56	17.55		
Districts	Nylon	83	26.02	110.7	<.0001*
	Ndogpassi II	192	60.19		
	Cite-SIC	44	13.79		
Schools	La Bonte	12	3.76	102.7	<.0001*
	La Carriere	70	21.94		
	La Clarte	71	22.26		
	Le Messie	122	38.24		
	Cite SIC	44	13.79		

Data are presented as frequency (percentage); Goodness-of-fit chi-square was used to compare proportions; \*: Significant

**Table 4. Prevalence of fever of non-malarial origin with respect to selected variables**

Variables	Categories	Total	N° of fever of non-malarial origin (%)	Statistics	P-value
Gender	Male	383	44 (11.49)	/	.56 <sup>§</sup>
	Female	400	40 (10.00)		
Age (years old)	< 5	63	0 (0.00)	20.923	<.0001*
	5-10	433	65 (15.01)		
	> 10	287	19 (6.62)		
Districts	Nylon	301	68 (22.59)	71.998	<.0001*
	Ndogpassi II	279	8 (2.87)		
	Cite-SIC	203	8 (3.94)		
Schools	La Bonte	157	68 (43.31)	220.281	<.0001*
	La Carriere	91	0 (0.00)		
	La Clarte	144	0 (0.00)		
	Le Messie	188	8 (4.26)		
	Cite SIC	203	8 (3.94)		

Data are presented as frequency (percentage); Independence chi-square and Fisher's exact test (&) were used to compare proportions; \*: Significant

**Table 5. Factors associated with the utilization of Insecticide-treated nets**

<b>Variables</b>	<b>Total</b>	<b>Use of ITNs (%)</b>	<b>Crude OR (95%CI)</b>	<b>P-value</b>	<b>Adjusted OR (95%CI)</b>	<b>P-value</b>
<b>Gender (n = 737)</b>						
Male	<b>371</b>	185 (49.87)	1		1	
Female	<b>366</b>	153 (41.80)	0.72 (0.54 - 0.97)	.02*	0.73 (0.55 - 0.98)	.03*
<b>Age (years old) (n = 737)</b>						
< 5	<b>63</b>	22 (34.92)	1		1	
5-10	<b>388</b>	181 (46.65)	1.63 (0.94 - 2.84)	.08	1.39 (0.77 - 2.50)	.28
> 10	<b>286</b>	135 (47.20)	1.67 (0.94 - 2.94)	.07	1.35 (0.62 - 2.96)	.47
<b>Districts (n = 737)</b>						
Cite-SIC	<b>203</b>	93 (45.81)	1		1	
Ndogpassi II	<b>264</b>	107 (40.53)	0.81 (0.56 - 1.17)	.25	0.88 (0.46 - 1.69)	.70
Nylon	<b>270</b>	138 (51.11)	1.24 (0.86 - 1.78)	.25	1.24 (0.74 - 2.07)	.41

\*: significant; ITNs: Insecticide-treated Nets; OR: Odds Ratio; 95%CI: Confidence Interval at 95%



## 4. DISCUSSION

### 4.1 Prevalence of Malaria

Using the CyScope<sup>®</sup>, the prevalence of malaria parasite infection in this study (45.47%) is similar to that reported by Kimbi et al. [14] in schoolchildren from southwest Cameroon. However, Ndamukong et al. [25] found higher prevalence (66.2%) in the same region in preschool and schoolchildren. Highest prevalences were found using light microscopy in Ekondo-Titi (74.2%) and Muea (98%) respectively [9,26]. Tole, Ekondo-Titi and Muea are less urbanized than our study sites. In fact, urbanization is known to be associated with the decline of malaria prevalence [27].

### 4.2 Prevalence of Malaria with Respect to District, Age, Body Temperature and ITNs

The risk of malaria infection was more than two fold (OR = 2.44;  $P = .0274$ ) higher in children from the Ndogpassi II district compared to their counterpart from the Cite-SIC district. This is in line with many studies which pointed out the significant influence of environment in the epidemiology of malaria [28-30].

Malaria infection significantly decreased with age of the children, the highest prevalence (80.95%) was recorded in the youngest (< 5 years age group) while the lowest (22.30%) was recorded in the more than 10 years age group. This finding is consistent with previous studies [31,32] and may be explained by the fact that immunity against malaria becomes stronger over time following recurrent infecting mosquito bites [33] especially in highly endemic areas. Our finding outlines the fact that underfives are more vulnerable to the infection [1] even though its link with malaria infection risk is far from merely linear.

Malaria parasites were nearly five folds more likely to be detected in febrile children than in their afebrile counterparts (OR = 4.86;  $P = .0033$ ). This is consistent with finding of Sayang et al. [34] in Yaounde (Cameroon). Fever is a clinical symptom commonly presented by malaria infected individuals especially children even though not pathognomonic to the disease. Fever arises during intraerythrocytic parasite development and results from the direct and/or indirect action of malaria pigment referred to as hemozoin [35].

The prevalence of malaria was lower in children sleeping under ITN (41.42%) compare to those in those not sleeping under the ITN (47.87%) although the difference was not significant. This is not in line with some studies which outlined the effectiveness of ITNs for malaria control [36-38]. The absence protective effect found in our study might be explained by possible resistance of mosquitoes to insecticides (pyrethroids) used for treating mosquito nets since an important proportion of insecticide-resistant mosquitoes was previously reported in Ndogpassi [16], a district included in our study. Besides, loss of ITNs integrity and failure to follow guidelines for their effective use might result in a poor protective effect [38].

### 4.3 Prevalence of Fever of Unknown Origin

An important proportion of fever cases of non malarial origin were recorded in this study (10.73 %). At least two explanations may be given for this. First, the method of diagnosis used may have missed some cases of malaria infection. The method has previously been associated with some false negative results, especially at low parasite density (i.e., below 400 parasites per  $\mu\text{L}$  of blood) [39]. One major limitation of this study is that parasite density was not estimated. Second, fever cases could be attributable to other diseases especially bacterial or viral infections [40,41]. Infections of bacterial origin are reported to be responsible for 45.26% of all fever cases of non malarial origin [40]. Thus, it would be helpful to depict the etiology of fever cases in our setting in order to properly manage febrile individuals in health facilities.

### 4.4 Prevalence of Asymptomatic Malaria

Most of infected participants in our study were asymptomatic (99.36%). From an evolutionary view, asymptomatic malaria is understood as the result of a partial anti-parasite immunity [42]. Asymptomatic carriers are common in most malaria endemic countries [9,33]. Douala is an area of perennial malarial transmission [16]. Inhabitants are thus highly exposed to *Plasmodium* infection and therefore develop an immune response that protects them from presenting clinical manifestations of the disease, although not strong enough to totally clear the infection. This might also explain why no severe malaria case was recorded among participants although on the other hand children with severe malaria could simply have been absent at the

time of the study as they were either probably at home or in health facility for treatment.

Asymptomatic infections represent an obvious concern since associated with onward transmission of parasites, recurrent episodes of symptomatic malaria, maternal and neonatal mortality, chronic anemia, co-infection with invasive bacterial disease and cognitive impairment [43]. The high proportion of asymptomatic parasite infection as well as low levels of uncomplicated malaria found in this study reinforces the idea that systematic screening and treatment of asymptomatic carriers is an important strategy to be implemented for malaria control, elimination and eradication [44]. In a context of malaria control and later elimination over the country, it would be critical and worthwhile to track and treat all cases of malaria infection in community. CyScope® fluorescence microscope could be a valuable tool to achieve this objective. A recent study in Nigeria showed that the CyScope® fluorescence microscope was cost-effective, has high sensitivity and specificity as well as a short turnaround time compared with other rapid diagnosis tools evaluated [45]. A benefit-risk analysis of this diagnosis tool in our setting is worth thinking over in further studies.

#### 4.5 Rate and Factors Associated with Use of ITNs

The overall ITNs use rate found in this study (45.74%) is much lower than the 80% targeted by the Ministry of Public Health in its 2011 guidelines [5]. Importantly, Cameroon government has carried out a second nationwide distribution campaign of LLINs to all households from 2015 to 2016 [46]. LLINs coverage rate is likely to have greatly improved over the country with this second campaign. Besides, the utilization of ITNs was significantly lower in males compared to females (20.70% versus 25.03%;  $P = .04$ ). The same trend has previously been reported by Tchinda et al. [47] in the MFOU health facility in the Centre Region of Cameroon. This finding might outline the fact that girls would have access more easily to this prevention tool even though sampling statistical fluctuations may also explain this result.

#### 5. CONCLUSION

This study shows high malaria prevalence in schoolchildren in Douala. Malaria epidemiology is influenced by some variables such as area and

age. This also highlights the fact that, in spite of implementation and scaling up of malaria control interventions over the country, there is a large proportion of asymptomatic malaria parasites carriers who constitute parasite reservoirs. Thus the urgent need for interventions based on active tracking and treating of all cases of malaria infection in community especially in children who pay the heaviest tribute to the disease. CyScope® fluorescence microscopy could be a valuable diagnosis tool to achieve this objective.

#### CONSENT

All authors declare that written informed consent was obtained from the patient (or other approved parties) for publication of this original research article.

#### ETHICAL APPROVAL

All authors hereby declare that all experiments have been examined and approved by the appropriate ethics committee and have therefore been performed in accordance with the ethical standards laid down in the 1964 Declaration of Helsinki. An ethical clearance was sought close to Institutional Review Board (IRB) of Health Delegation of the town of Douala.

#### DISCLAIMER

This study was presented at the conference “22nd Annual conference of the Cameroon Bioscience Society (CBS), At Nkolbisson, Yaoundé, Cameroon”.

Available link is

[https://www.researchgate.net/publication/309428883\\_Epidemiology\\_of\\_malaria\\_using\\_Light-emitting\\_diode\\_LED\\_fluorescence\\_microscopy\\_in\\_schoolchildren\\_in\\_Douala\\_Cameroon](https://www.researchgate.net/publication/309428883_Epidemiology_of_malaria_using_Light-emitting_diode_LED_fluorescence_microscopy_in_schoolchildren_in_Douala_Cameroon)

#### COMPETING INTERESTS

Authors have declared that no competing interests exist.

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