

Epidemiology of malaria using LED fluorescence microscopy among schoolchildren in Douala, Cameroon

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.

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].

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

29 In endemic areas, malaria is a first cause of school absenteeism and many aspects of
30 cognition disorders [7]. Over time, repeated malaria episodes reduce the performance of
31 schoolchildren [8]. Few studies addressed the deleterious effects of malaria among
32 schoolchildren in Cameroon [9-11]. Kimbi and colleagues previously estimated up to 14
33 absenteeism days per month among schoolchildren in the Southwest region of the country
34 [9].

35 Understanding the epidemiology of malaria especially among schoolchildren can be
36 enhanced by the use of new diagnostic tools, given the many limitations inherent to the
37 classical Giemsa-stained thick blood film which is time-consuming, labour-intensive and
38 requires good quality reagents and equipment as well as skilled microscopists [12, 13].

39

40 The attempts for more efficient hospital and/or community-based management of malaria
41 could be also improved with new methods. Developing, testing and implementing simple,
42 cost effective, rapid and accurate alternative diagnostic tools requiring little or no training
43 could greatly improve and scale up all these aforementioned composite items of malaria
44 control. In this sense, fluorescence-based methods could be interesting alternatives
45 diagnostic tools. They were proven to have almost equal malaria diagnosis-related
46 performances with the Giemsa-stained blood film when real-time PCR was used as gold
47 standard [12]. The authors of this study reported sensitivity, specificity, positive and negative
48 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. MATERIAL 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.

76 **2.3 Study sample size**

77 Children were recruited on a convenient approach in each school through random sampling
78 method. Based on a malaria prevalence of 58.4% with fluorescence microscopy as reported
79 by Kimbi et al. [14], the sample size was determined using the Lorentz's formula $n = Z^2pq/d^2$
80 where n = the required sample size, Z =statistic for the desired confidence level (1.96 for
81 95% confidence level), p = assumed malaria prevalence in schoolchildren, $q = 1-p$:
82 proportion of malaria negative participants and d = accepted margin of error (5%). The
83 minimum sample size was estimated as $n=373$. A total of 785 schoolchildren were included
84 in the study.

85 .

86 **2.4 Study design**

87 This cross-sectional study took place in three districts of the city of Douala from November
88 2013 to January 2014. Prior to field data collection, school officials and teachers were
89 informed on the aim and objectives of the survey. Only five schools were willing to
90 participate in the study upon the sensitization phase. Forms were distributed to
91 schoolchildren in order to seek for parental/guardian consent for their participation in the
92 study. Only children whose parents/guardians provided signed parental consent forms were
93 finally enrolled in the study. Work space was provided to the study team by officials of the
94 schools. A questionnaire was administered to parents/guardians; clinical examination of the
95 child was made and blood collected for malaria diagnosis.

96

97 **2.5 Questionnaire**

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

101 **2.6 Clinical examination**

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

105 **2.7 Malaria diagnosis**

106 A drop of blood was collected from finger prick and used for the diagnosis of malaria
107 infection. CyScope® fluorescence microscopy (Partec-Sysmex® GmbH, Germany) was used
108 for diagnosis. The CyScope® microscope has two light sources emitting normal and
109 fluorescent lights. Fluorescent light allows the detection of malaria parasites through an
110 incident ultraviolet (UV) emission. Readily-prepared and ready to-use test slides labeled with
111 4',6-Diamidino-2-Phenylindole (DAPI), a DNA-specific fluorescent dye allowing for detection
112 of intraerythrocytic *Plasmodium* DNA at 443 nm wavelength [18, 19] were used. For the test,
113 10µL of capillary blood was placed on the dye-containing area of a labeled slide, covered
114 with a cover slip, incubated for one minute at room temperature in the dark and then
115 observed under x 40 objective. The presence of bright shiny intracellular tiny dots observed
116 under UV light indicates the presence of malaria parasites in red blood cells (Figure 1).
117 Results were interpreted as valid (negative or positive) and invalid [20].

118

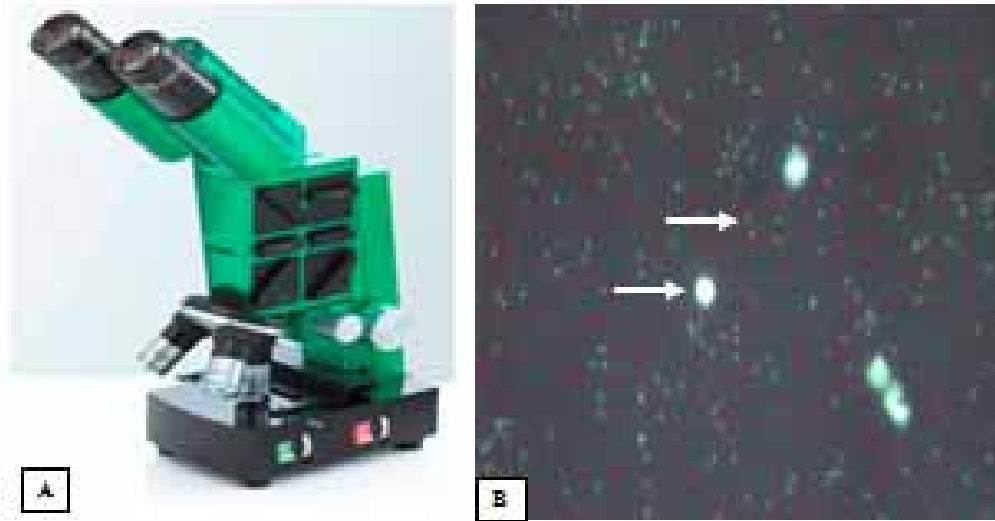


Figure 1. A. CysCope® (Partec-Sysmex, Japan); **B.** Positive malaria slide under observation with CyScope®. Bigger spots correspond to White Blood Cells (WBC) and the smaller spots to malaria parasites (Photography provided by the authors).

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].

138 2.9 Ethical statements

139 The study was carried in accordance with guidelines for human experimental models in
140 clinical research as stated by the Cameroon Ministry of Public Health. Signed informed
141 consent form was obtained from each parent/guardian for their child to participate in the
142 study and assent was obtained from all participating children aged nine and above. Negative
143 malaria cases with fever were referred to the nearest health facility for further examination.
144 Participation in the study was strictly voluntary and parents were free to decline answering
145 any question or withdraw their children if they so wished at any time. In addition, the study
146 was approved by the Regional Delegation of Public Health for the Littoral under
147 N°2853/11/L/MINESEC/DLP/SDAG/IMS. All positive cases were treated on the spot
148 following the protocol recommended by the Ministry of Public Health of Cameroon with
149 Artemisinin-based combination therapy (Artesunate-Amodiaquine).

150

151 2.10 Statistical analysis

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

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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).

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)
Gender				
Male	156 (51.83)	128 (45.88)	116 (57.14)	385 (49.05)
Female	145 (48.17)	151 (54.12)	87 (42.86)	400 (50.95)
M/F Sex ratio	1.06	0.85	1.33	0.96
Age groups (years)*				
< 5	0 (0)	63 (22.58)	0 (0)	63 (8.05)
5-10	217 (72.09)	216 (77.42)	0 (0)	433 (55.30)
> 10	84 (27.91)	0 (0)	203 (100)	287 (36.65)
Mean age $\pm$ SD (years)	8 ± 2	7 ± 2	12 ± 1	9 ± 3
Clinical status*				
Non febrile ($< 37.5^{\circ}\text{C}$)	215 (71.43)	258 (92.47)	189 (93.10)	662 (84.55)
Febrile ($\geq 37.5^{\circ}\text{C}$)	86 (28.57)	21 (7.53)	14 (6.90)	121 (15.45)
Temperature $\pm$ SD ($^{\circ}\text{C}$)	36.63 ± 0.25	36.43 ± 0.33	37.17 ± 0.31	36.70 ± 0.41
Use ITNs*				
Yes	138 (51.11)	107 (40.53)	93 (45.81)	338 (45.86)

No 132 (48.89) 157 (59.47) 110 (54.19) 399 (54.14)

176 Data are presented as frequency (percentage) and mean $\pm$ standard deviation (SD); F =

177 Female; M = Male; ITNs = Insecticide-treated nets; * = data were missing in a few children

178

179 3.2 Prevalence and factors associated with malaria

180 Two (2) slides were unreadable; parasitological results were thus available for 783

181 participants. The prevalence of malaria parasite infection was 45.47% (356; 95%CI = 42.01-

182 48.97).

183 The risk of malaria infection significantly varied with respect to age, district and body

184 temperature. Indeed, the highest prevalence of malaria 80.95% was recorded in the

185 youngest (< 5 years) age group while the lowest was recorded in more than 10 years

186 (22.30%) as presented in Table 2. In addition, the multivariate logistic analysis showed that

187 the risk of malaria were significantly lower in aged > 10 years old (OR = 0.19; $P = .0009$).

188 Besides, the risk of malaria infection was more than two fold (OR = 2.44; $P = .0274$) higher

189 in children from the Ndogpassi II district compared with their counterpart from the Cite-SIC

190 district.

191 The risk of malaria parasites infection was about five folds higher in febrile compared with

192 their afebrile counterparts (OR = 4.86; $P = .0033$). The prevalence of malaria was 77.78%

193 and 44.71% respectively in both groups (Table 2).

194

195 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
Age (years old)						
< 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*
Gender						
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
Districts						
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
Schools^{&}						
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*	-	-
Temperature (°C)						
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*
Use ITNs						
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

196 *: significant; N°: Number; ITNs: Insecticide-treated Nets; OR: Odds Ratio; 95%CI: Confidence Interval
197 at 95%; &: The variable was excluded from the multivariate logistic model because of redundancy of
198 information.
199

200 3.3 Prevalence of asymptomatic malaria and its distribution with regard to 201 sociodemographic variables

202 The overall prevalence of asymptomatic malaria was 40.74% (319/783; 95%CI = 37.35 –
203 44.22). Thus, 89.61 % (319/356; 95%CI: 86.01-92.37) of all malaria parasite infection was
204 asymptomatic. It should be noted that no severe malaria case was recorded in this study.

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

210

211 **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		

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

214

215 **3.4 Prevalence of fever of non-malarial origin**

216 A total of 84 malaria negative children presented with fever during this study; the overall
 217 prevalence of fever of non-malarial origin was 10.73% (84/783; 95%CI = 8.75 – 13.09). The
 218 majority of the cases were found in 5-10 years old (65 cases), the Nylon district (68 cases)
 219 and La Bonte school (68 cases). In addition, the differences were significant ($P < .0001$)
 220 (Table 4).

221

222

223

224

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

Variables	Categories	N° of fever of non-		Statistics	P-value
		Total	malarial origin (%)		
Gender	Male	383	44 (11.49)	/	.56 ^s
	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)		

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

228

229

230 **3.5 Factors associated with the use of Insecticides-treated Nets (ITNs)**

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

233

234

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

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

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

237

238 4. DISCUSSION

239

240 4.1 Prevalence of malaria

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

248

249

250 4.2 Prevalence of malaria with respect to district, age, body temperature and ITNs

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

255 Malaria infection significantly decreased with age of the children, the highest prevalence
256 (80.95 %) was recorded in the youngest (< 5 years age group) while the lowest (22.30 %) was
257 recorded in the more than 10 years age group. This finding is consistent with previous
258 studies [31, 32] and may be explained by the fact that immunity against malaria becomes
259 stronger over time following recurrent infecting mosquito bites [33] especially in highly
260 endemic areas. Our finding outlines the fact that underfives are more vulnerable to the
261 infection [1] even though its link with malaria infection risk is far from merely linear.

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

268 The prevalence of malaria was lower in children sleeping under ITN (41.42 %) compare to
269 those in those not sleeping under the ITN (47.87 %) although the difference was not
270 significant. This is not in line with some studies which outlined the effectiveness of ITNs for
271 malaria control [36-38]. The absence protective effect found in our study might be explained
272 by possible resistance of mosquitoes to insecticides (pyrethroids) used for treating mosquito
273 nets since an important proportion of insecticide-resistant mosquitoes was previously
274 reported in Ndogpassi [16], a district included in our study. Besides, loss of ITNs integrity
275 and failure to follow guidelines for their effective use might result in a poor protective effect
276 [38].

277

278 **4.3 Prevalence of fever of unknown origin**

279 An important proportion of fever cases of non malarial origin were recorded in this study
280 (10.73 %). At least two explanations may be given for this. First, the method of diagnosis
281 used may have missed some cases of malaria infection. The method has previously been
282 associated with some false negative results, especially at low parasite density (i.e., below
283 400 parasites per μL of blood) [39]. One major limitation of this study is that parasite density
284 was not estimated. Second, fever cases could be attributable to other diseases especially
285 bacterial or viral infections [40, 41]. Infections of bacterial origin are reported to be
286 responsible for 45.26% of all fever cases of non malarial origin [40]. Thus, it would be helpful
287 to depict the etiology of fever cases in our setting in order to properly manage febrile
288 individuals in health facilities.

289

290 **4.4 Prevalence of asymptomatic malaria**

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

301 Asymptomatic infections represent an obvious concern since associated with onward
302 transmission of parasites, recurrent episodes of symptomatic malaria, maternal and neonatal
303 mortality, chronic anemia, co-infection with invasive bacterial disease and cognitive
304 impairment [43]. The high proportion of asymptomatic parasite infection as well as low levels

305 of uncomplicated malaria found in this study reinforces the idea that systematic screening
306 and treatment of asymptomatic carriers is an important strategy to be implemented for
307 malaria control, elimination and eradication [44]. In a context of malaria control and later
308 elimination over the country, it would be critical and worthwhile to track and treat all cases of
309 malaria infection in community. CyScope® fluorescence microscope could be a valuable tool
310 to achieve this objective. A recent study in Nigeria showed that the CyScope® fluorescence
311 microscope was cost-effective, has high sensitivity and specificity as well as a short
312 turnaround time compared with other rapid diagnosis tools evaluated [45]. A benefit-risk
313 analysis of this diagnosis tool in our setting is worth thinking over in further studies.

314

315 **4.5 Rate and factors associated with use of ITNs**

316 The overall ITNs use rate found in this study (45.74%) is much lower than the 80% targeted
317 by the Ministry of Public Health in its 2011 guidelines [5]. Importantly, Cameroon government
318 has carried out a second nationwide distribution campaign of LLINs to all households from
319 2015 to 2016 [46]. LLINs coverage rate is likely to have greatly improved over the country
320 with this second campaign. Besides, the utilization of ITNs was significantly lower in males
321 compared to females (20.70 % versus 25.03 %; $P = .04$). The same trend has previously
322 been reported by Tchinda et al. [47] in the MFOU health facility in the Centre Region of
323 Cameroon. This finding might outline the fact that girls would have access more easily to this
324 prevention tool even though sampling statistical fluctuations may also explain this result.

325

326 **5. CONCLUSION**

327

328 This study shows high malaria prevalence in schoolchildren in Douala. Malaria epidemiology
329 is influenced by some variables such as area and age. This also highlights the fact that, in
330 spite of implementation and scaling up of malaria control interventions over the country,
331 there is a large proportion of asymptomatic malaria parasites carriers who constitute parasite
332 reservoirs. Thus the urgent need for interventions based on active tracking and treating of all

333 cases of malaria infection in community especially in children who pay the heaviest tribute to
334 the disease. CyScope® fluorescence microscopy could be a valuable diagnosis tool to
335 achieve this objective.

336

337

338 **CONSENT**

339

340 All authors declare that 'written informed consent was obtained from the patient (or other
341 approved parties) for publication of this original research article. A copy of the written
342 consent is available for review by the Editorial office/Chief Editor/Editorial Board members of
343 this journal.

344

345 **DISCLAIMER**

346 The findings and conclusions in this paper are those of the authors and do not necessarily
347 represent the views of any of their affiliated Research Institutions. Furthermore, mention of
348 any company name or product does not constitute endorsement by the authors. None
349 financial support has been allocated for this study.

350 **ETHICAL APPROVAL**

351

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

356

357 **Disclaimer: - This study was presented at the conference**

358 **"22nd Annual conference of the Cameroon Bioscience Society (CBS), At Nkolbisson,**
359 **Yaoundé, Cameroon".**

360 **Available link is**

361 ["https://www.researchgate.net/publication/309428883 Epidemiology of malaria using L](https://www.researchgate.net/publication/309428883_Epidemiology_of_malaria_using_Light-emitting_diode_LED_fluorescence_microscopy_in_schoolchildren_in_Douala_Cameroon)
362 [ight-](https://www.researchgate.net/publication/309428883_Epidemiology_of_malaria_using_Light-emitting_diode_LED_fluorescence_microscopy_in_schoolchildren_in_Douala_Cameroon)
363 [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)

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