Texila International Journal of Public Health
ISSN: 2520-3134
DOI: 10.21522/TIJPH.2013.10.04.Art031

The Prevalence of Asymptomatic Malaria Amongst Nursery and Primary School Pupils in Bui, Northwest Cameroon: A Cross-Sectional Study

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Abstract

Malaria is a public health concern worldwide, accounting for 31% of hospital consultations, 44% of hospitalisations, and 18% of deaths, and a cause of school absenteeism amongst pupils in Cameroon. The negative cognitive impact of malaria on pupils is a call for concern. This study aimed at determining the prevalence of asymptomatic malaria among pupils. A cross-sectional study was conducted from December 2015 to April 2016 in eight Nursery and Primary Schools in Bui. A total of 1,152 pupils were systematically screened for malaria using light microscopy. All anaemic and malaria positive cases were administered ferum folate/ferrous sulphate and artemether-lumefantrine for treatment. The prevalence of malaria and anaemia were 12% and 18.4%, respectively; anaemia accounted for 26.1% of all positive diagnostic tests. Malaria infection was significantly higher in adolescents aged 5–12 years (73.9%), as well as amongst males (51.4%). The proportion of pupils with asymptomatic malaria and anaemia was high. Age, sex, school, and community were associated with asymptomatic malaria as well as anaemia. Malaria intervention programmes should target asymptomatic malaria carriers for treatment to minimise transmission.

Keywords: *Asymptomatic malaria, Bui, Cameroon, Prevalence, School Children.*

Received: 28.09.2022 ***Accepted: 13.10.2022*** ***Published on: 29.12.2022***

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Introduction

Malaria, a preventable mosquito-borne, and curable disease that remains a major public health problem in the tropical countries of the world, especially sub-Saharan Africa [1-4], even after the scaling up of prevention with long-lasting insecticide nets [1, 5-7]. It is caused by unicellular protozoan parasites of the genus *Plasmodium*, which are transmitted to humans through the bites of infected female *Anopheles* mosquitoes [1, 7, 8]. Four species of *Plasmodium* are found across sub-tropical Africa including Cameroon: *Plasmodium falciparum*, *P. malariae*, *P. ovale* and *P. vivax* [2, 4, 9], of which *P. falciparum* is the most prevalent [10-13]. *Plasmodium knowlesi* has not been known to cause malaria among humans in Cameroon [10]. The major *Anopheles species* incriminated for malaria transmission in Cameroon include *Anopheles gambiae*, *Anopheles arabiensis*, *Anopheles funestus*, *Anopheles moucheti*, and *Anopheles Nili* [8, 14].

The period between 2001 and 2013 experienced a substantial expansion of malaria interventions, contributing to a 47% decline in malaria mortality rates globally [13]. Between 2019 and 2020, malaria was responsible for an estimated 227 – 241 million clinical cases of illness (in 85 endemic countries) and 558,000 – 627,000 deaths worldwide, 213 - 228 million cases, and deaths from 534,000 – 602,000 in the Sub-Saharan African Region [1], with under-five children (U5C) being the most affected [15]. In 2010, malaria accounted for 50% of all deaths with 5 – 9% occurring among the 5 – 14 years age group in Africa [16]. Malaria infection also affects the economy as it is a risk factor for stunting and wasting [17-19], as well as academic and cognitive performance among children [16, 20, 21], school absenteeism [16], and deaths in general [8].

In Cameroon, the epidemiology of malaria varies geographically depending on the local malaria transmission intensity, class, as well as the *Plasmodium* species [8, 22]. Cameroon has experienced a gradual increase in malaria yearly, from 369,178 in 2012 to 652,661 in 2018 [11, 23]. The prevalence of malaria in Cameroon is 29% [24]; being among countries accounting for more than 80% of malaria cases in the Central African sub-region [1]. Records from the Cameroon's Ministry of Health (MOH) reveal that 31% of consultations, 44% of hospitalisations, and 18% of deaths in health facilities are attributed to malaria [8]. Of the hospitalisations and deaths reported by the MOH in 2018, 31.5% and 28.4%, respectively, were among children who were less than five years old [11].

Studies in Cameroon and beyond have shown consistently that malaria is, and remains a public health problem [1, 2, 7, 25], with under-five years children suffering between one-to-five or more malaria episodes per year [23]. Thus, in this study, we seek to determine the incidence of malaria as well as anaemia amongst asymptomatic children in the study area.

Materials and Methods

Study Design and Setting

This was a community-based cross-sectional survey conducted from December 2015 to April 2016 amongst Nursery and Primary school children in the Djottin and Shisong communities of Bui Division in the Northwest Region of Cameroon (Table 1, Figure 1). Before the study, Head Teachers of each school were approached, the aims of the study were explained, and dates were proposed for health education and sample collection. After obtaining written approval from the Basic Educational Inspectors, Head Teachers in each school informed pupils about the study. Pupils who met the eligibility criteria after clinical examination were included in the study.

Table 1. Schools Distributed by School Type, Community, and Health Area

School	Type of school	Community	Health Area	Health district	2018 Population
CNS Shisong	Nursery	Shisong	Shisong	Kumbo East	18,841
GS Shisong	Primary	Shisong			
CS Djottin	Primary	Djottin	Djottin	Oku	10,147
GS Bamti	Primary	Djottin			
GS Chamkfung	Primary	Djottin			
GS Febwe	Primary	Djottin			
GS Gaggi	Primary	Djottin			
GS Meyessi	Primary	Djottin			

CNS; Catholic Nursery School, CS; Catholic School, GS; Government School

Bui Division is located between latitudes 6'000–6'20 N and longitudes 10°30–10'60, spanning an area of 2795 km^2 with six administrative sub-divisions (Figure 1) [26]. These "grass field" localities have two main seasons: the rainy season, which lasts from mid-March to October, sometimes lasting up to eight months, and the dry season, which lasts from November to early March [2, 27, 28]. Bui Division is in the High Western Plateau epidemiological strata of Cameroon [10], which is considered to be hypo-endemic with seasonal malaria parasite transmission occurring at a very low level [2]. Subsistence farming constitutes the mainstay of the inhabitants of these communities. Rivers and streams serve as the primary sources of drinking water, while pipe-borne water is the secondary source. The planning of these communities is natural, with scattered settlements around farmlands, linear settlements along main roads, and nucleated settlements. However, farms are clustered around settlements. Hospital records show that malaria occurs all year round, with peaks at the beginning of the rainy season [29-30]. The population of these communities relies on the Saint Elizabeth General Hospital Complex – Shisong and the Holy Family Health Centre – Djottin for medical care.

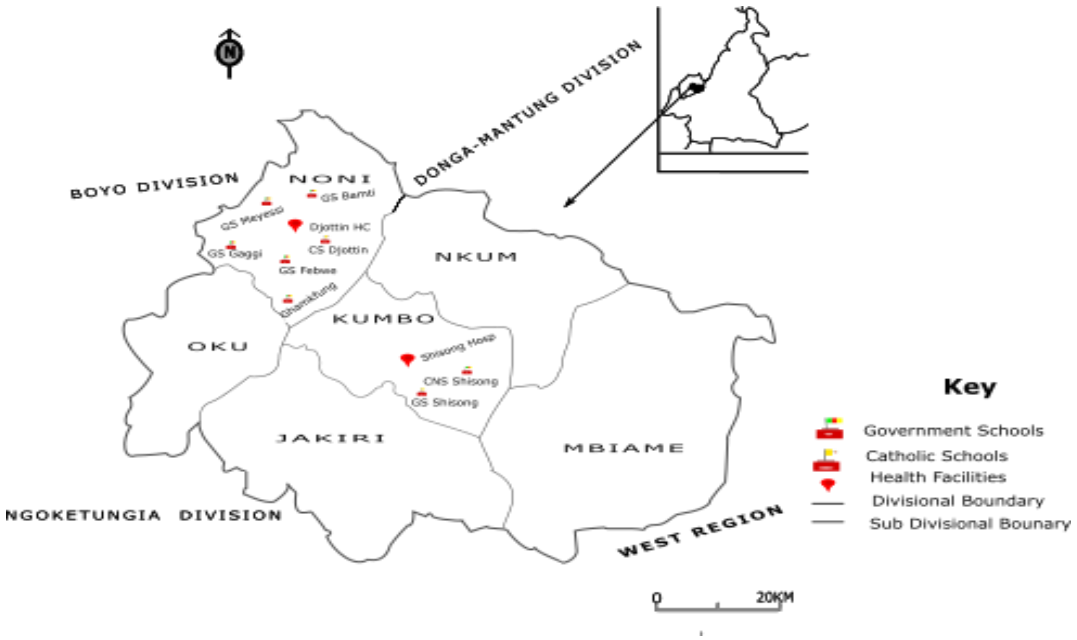

Figure 1. The Map of Bui in the Northwest Region of Cameroon

Study Population and Sample Size

The study population constituted Nursery and Primary School Pupils of selected schools in Djottin and Shisong. The following criteria were used to include pupils in this study: i) Nursery and Primary School Pupils in the selected communities, ii) of both sexes, iii) aged 2 - 12 years old, iv) having given their permission following parents'/guardian's signed informed consent. Pupils who did not fulfil these criteria were not enrolled in the study. Pupils with signs and symptoms of malaria; headache, malaise, weakness/lassitude, nausea/vomiting/diarrhoea, anorexia, and myalgia [4, 11, 31, 32], for three or more days [20, 33], were excluded from the study.

Pupils were enrolled consecutively to reduce selection bias. The minimum sample size was calculated based on a malaria prevalence of 33.9% [12] with simple microscopy [12, 34], with the CDC Epi Info version 7.2.2.6 StatCalc. The following characteristics: an average population of Shisong and Djottin Health areas of 28,988 in 2018 [35, 36], an accepted error margin of 5%, a design effect of 1.0, and two communities, were considered for the sample size determination. The minimum sample size per cluster/community was estimated to be 170. Considering a possible loss of samples due to breakage of slides and nonconsenting to participants, the sample size was adjusted by 10% to a minimum of 187 per cluster/community. A total of 1,319 Pupils (1,117 from Djottin and 202 from Shisong) were enumerated for the study, and 1,152 (973 from Djottin and 179 from Shisong) were enrolled.

Collection of Data and Specimens

A Microsoft Excel sheet was designed to document the ages and sex of pupils from the class registers and to enter data obtained from malaria microscopy and haemoglobin determination using the haemoglobinometer. The ages of the pupils were double-checked by asking some of them verbally for confirmation. After health education and clinical evaluation for signs and symptoms of malaria, blood for microscopy and haemoglobin were collected by needle prick.

Laboratory Analysis for Malaria Diagnosis and Determination of Haemoglobin Levels

Finger prick blood was collected from each pupil with signed consent from the parents. The first drop of blood was immediately wiped off. The next drop was placed on the haemoglobin (Hb) test strip already inserted into the URIT-12 haemoglobin meter (URIT Medical Electronic Co., Ltd, London, United Kingdom) for Hb determination. The Hb value displayed was recorded. A participant was considered anaemic when Hb concentration fell below the reference values [(> 11.0 g/dL for children 2 – 5 years, and > 11.5 g/dL for children 6 – 12 years) [37-39] as well as sex. The severity of anaemia was further classified and graded as mild (10 – 11 g/dL), moderate (8 – 9 g/dL), and marked (6 – 7 g/dL) [27, 37, 40].

Two drops of whole blood samples were placed on a pre-labelled, grease-free glass slide 1 mm apart for the preparation of thin and thick films for detection and speciation of malaria parasites, as described in other studies [34, 38, 39], by light microscopy (Olympus Optical Co., Ltd, Japan). Both thin and thick blood films prepared in the field were air-dried [12, 38, 39], and transported to the Catholic School of Health Sciences Parasitology Laboratory in Shisong, for staining and microscopy. The thin blood films were fixed in absolute alcohol, and both thick and thin blood films were stained using 10% Giemsa (1 in 20 dilution) solution for 15 minutes [4, 34, 41]. Slides were then examined microscopically for the presence of malaria parasites by two independent Medical Laboratory Scientists, and in the case of any disparity, they were read by a third Medical Laboratory Scientist, whose results were considered final. Slides were considered

positive when asexual forms and/or gametocytes of any *Plasmodium* species were observed on the thick blood film. A thick film was declared negative after observing more than 150 fields at x100 high power magnification, and no malaria parasite was seen. All anaemic and malaria-positive cases were administered ferum folate/ferrous sulphate (Haemaforte®) and malaria treatment; artemether/artesunate lumefantrine (Coartem®) as recommended by WHO for the African region and the Cameroon's Ministry of Health [2, 11, 20, 31].

Ethical Consideration

The study was approved by the Divisional Inspectorate of Basic Education of Bui (Ref Nº: B881-15 MINEDUB/DRNO/DDB/SAF/GA), the Director of the Saint Elizabeth General Hospital Complex, the Educational Authority for Catholic Primary schools, and was conducted following the Helsinki declaration [42]. Administrative clearances were obtained from the various Head Teachers. Moreover, parents or guardians signed consent forms on behalf of their children and permission was obtained from the pupils.

Outcome Variables

The main outcome variables were:

Prevalence of Malaria: defined as the proportion of pupils who tested positive for malaria, where the numerator comprises the number of pupils who tested positive for malaria and the denominator is the total number of pupils tested.

Asymptomatic Malaria: defined as a child/person with no recent history of symptoms or signs of malaria but who shows laboratory confirmation of parasitaemia [43, 44].

Prevalence of Anaemia: defined as the proportion of pupils with anaemia (using a haemoglobinometer) where the numerator comprises the number of pupils with anaemia and the denominator is the total number of pupils tested.

Statistical Analysis

Data were entered into a Microsoft Excel spreadsheet, double-checked for consistency, exported, and analysed with CDC Epi Info version 7.2.2.6 (Centre for Disease Control, Georgia, USA). Qualitative variables were expressed as percentages. The Pearson's Chi-square (χ^2) test was used to compare the population's general characteristics in association with malaria and anaemia. The level of statistical significance was set at $p < 0.05$.

Strengths and Limitations of the Study

Strengths

The data used in this study was collected by indigenous Medical Laboratory Technicians/Scientists and State Registered Nurses, who had mastery of all health areas in the study area. Basic education stakeholders were consulted for the list of nursery and primary schools in the selected communities. The quality of the data collected was assured through double-checking during data entry, as well as the reading of each slide by two independent Medical Laboratory Technicians/Scientists.

Limitations

This was a community-based study, carried out only in two communities. Samples were collected from asymptomatic pupils on the base of a simple clinical examination, without the reading of body temperature.

Results

Characteristics of Study Participants

In all, 1,319 pupils were surveyed from class registers; 33 (2.5%) of their parents/guardians refused consent, 76 (5,8%) whose parents/guardians signed consent refused assent, and 58 (4.4%) were absent from school on the day of specimen collection. A total of 1,152 (87.3%) of the pupils (609 females and

543 males) were surveyed in the two communities as shown in Table 2. The mean (±SD) age of the study participants was 6.0 ± 2.0 years old (range: 2 – 12). The mean (±SD) haemoglobin concentration was 13.0 ± 2.0 g/dL (range: 7.0 – 18.0), which was significantly ($F = 37.09$, $p < 0.0001$) higher in Shisong (14.0 ±2.0 g/dL, range: 7.0 – 18.0) with just two schools than in Djottin (12.0 ± 2.0 g/dL, range: 7.0 – 17.0) with six schools. The majority (74%) were adolescents, while most (52.9%) of the respondents were females.

Prevalence of Malaria and Anaemia with Respect to General Characteristics

A total of 138 slides were positive for malaria parasites giving an overall prevalence of 12%. The overall prevalence of anaemia amongst the study participants was 18.4% (212/1,152). In terms of severity, 5.5% of all pupils had mild anaemia, 12.2% had moderate anaemia, while 0.7% had marked anaemia. In bivariate analysis, the prevalence of malaria and anaemia was significantly associated with sex, age, and community (Table 3).

Table 2. Characteristics of the Study Population

Characteristic	Subclass	Frequency	Percentage
Age groups (in years)	Toddlers (2 – 4 years)	299	26.0
	Adolescents (5 –12 years)	853	74.0
Sex	Female	609	52.9
	Male	543	47.1
Locality	Djottin	973	84.5
	Shisong	179	15.5

Prevalence of Malaria and Anaemia with Respect to Communities and Schools

The prevalence of malaria varied significantly with regard to the communities ($\chi^2 = 3.00$; $p < 0.0001$), as well as schools ($\chi^2 = 3.00$; $p < 0.0001$). The prevalence of anaemia also varied significantly with regard to communities ($\chi^2 = 27.00$; $p < 0.0001$) and schools ($\chi^2 = 67.00$; $p < 0.0001$).

Globally, asymptomatic malaria infection rates were high, with the highest value reported in CS Djottin (28.3%) and the lowest value found in GS Meyessi (5.8%) (Figure 2). In a like manner, the lowest rates of anaemia were in GS Meyessi (5.2%) and the highest was in CS Djottin (33.1%). The level of severity of anaemia was also investigated across schools and communities (Table 2).

Table 3. Prevalence of Malaria/Anaemia by Age, Sex, and Community

Characteristic (Subclass)	Malaria		*p* value
	Negative (*n* = 1,014)	Positive (*n* = 138)	
Age groups (in years)			
Toddlers (2–4 years)	263 (25.9)	36 (26.1)	< **0.0001**
Adolescents (5–12 years)	751 (74.1)	102 (73.9)	
Sex			
Female	542 (53.5)	67 (48.6)	< **0.0001**
Male	472 (46.5)	71 (51.4)	
Community			
Djottin	863 (85.1)	110 (73.2)	< **0.0001**
Shisong	151 (14.9)	28 (20.3)	
	Anaemia		
	Normal (*n* = 940)	**Anaemic** (*n* =212)	

Age groups (in years)			
Toddlers (2–4 years)	291 (31.0)	8 (3.8)	< **0.0001**
Adolescents (5–12 years)	649 (69.0)	204 (96.2)	
Sex			
Female	492 (52.3)	117 (55.2)	< **0.0001**
Male	448 (47.7)	95 (44.8)	
Community			
Shisong	171 (18.2)	8 (3.8)	< **0.0001**
Djottin	769 (81.8)	204 (96.2)	

Boldface numbers indicate significant p – values

Association of Anaemia with Malaria

A total of 138 (12%) and 212 (18.4%) pupils were enumerated with positive malaria microscopy and anaemia, respectively. Thirty-six (26.1%) of children with malaria were anaemic, while 832 (82.6%) with negative malaria results had normal haemoglobin levels; showing a significant (χ^2 =6.00; p = 0.013) association between malaria and anaemia. The severity of anaemia also showed a significant association with asymptomatic malaria (Table 4).

Table 4. Association of Low Haemoglobin with Asymptomatic Malaria

Haemoglobin	**Malaria**			χ^2	***p* – value**
	Negative (*n* = 1,014)	**Positive (*n* = 138)**	**Total**		
Normal	838 (82.6)	102 (73.9)	940	6.00	**0.013**
Mild anaemia	53 (5.2)	10 (7.2)	63	14.00	**0.002**
Moderate anaemia	119 (11.7)	22 (15.9)	141		
Marked anaemia	4 (0.4)	4 (2.9)	8		
Overall anaemia	176 (17.4)	36 (26.1)	212		

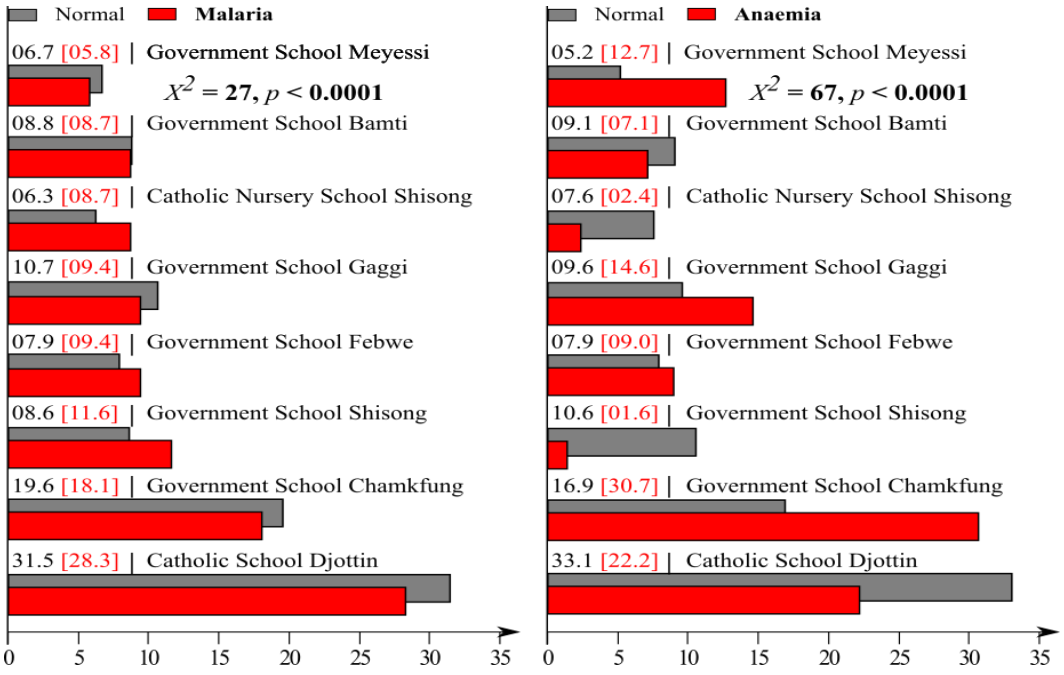

Figure 2. Prevalence of Asymptomatic Malaria and Anaemia with Respect to Schools

Discussion

This study explored the incidence of asymptomatic malaria and anaemia amongst nursery and primary school children in two communities in Bui. The incidence of asymptomatic malaria was 12% while that of anaemia was 18.4%. Thirty-six (3.1%) of the pupils were analysed to suffer from both malaria and anaemia, and 12.2% had moderate anaemia.

Prevalence of Malaria/Anaemia in Association with General Characteristics

The rate of 12% prevalence of malaria in this study is less than 26.1%, 17.2 – 53.2%, 28.1%, 30.1%, and 33.9% reported among U5C [45], febrile patients at the Mbakong Health Centre of the Bafut Health District [3], children at the Douala gynaeco-obstetric and paediatric hospital [46], employees at the workplace [19] and school-aged children [12] respectively in Cameroon. It is also less than the 25%, 30%, 38.3%, 41.52 – 42.8% (females vs males), 42%, 50%, and 69.9% reported among U5C and school children in the Democratic Republic of Congo [47], Thai-Myanmar boarder [21], the hyper-endemic Kombewa area of Western Kenya [48], Ghana [49], Niger [50], Senegal [51], and asymptomatic malaria among households members in Abuja – Nigeria [52].

Our findings were similar to those of other studies in Cameroon; 10.8% of asymptomatic malaria among school children in the Nkongho-mbeng rural area of the Southwest Region [53], 14.1% among HIV patients in Limbe [54], 17.6% amongst children from five epidemiological strata of malaria in Cameroon [10], and 18.9% in Douala [18]. Out of Cameroon, it was similar to the 16.4% among U5C in the meso-endemic Iguhu of Western Kenya [48], 18.4% among asymptomatic migrant labourers in Armachiho District – Ethiopia [9], and the 1 – 19% among people in the Amazonian indigenous Yanomami community area [55].

Our results were higher than those reported elsewhere in Africa and Asia; 2.8 – 9.5% for mother-to-child transmissions in Burkina Faso [44], 2.6% asymptomatic malaria amongst Dak Nong children in Vietnam [56], 3.9 – 8.7%% among Sherkole refugee camp and Wogera district U5C in Ethiopia [15, 57], 5.2% reported in Thailand-Myanmar [22], 6.4% among U5C in endemic prone Marani – Western Kenya [48], and 6.8% reported among school children in Northwest Ethiopia [43].

The differences between our findings and other studies are evidence of the variability of malaria endemicity in Cameroon [2, 3] and maybe also due to the differences in study designs.

The 18.4% anaemia in this study was far less than 62 – 68.7% amongst children around Mount Cameroon [33, 58] and the 64.1 vs 78.4% reported among U5C in sub-Saharan Africa [59] vs Ghana [60]. It was, however, almost similar to the 23.1% reported amongst school age children in Belo and Bui Divisions [27]. The significant differences are a result of differences in the study populations; wherein the two studies around Mount Cameroon involved febrile patients, while ours involved asymptomatic school pupils.

The overall moderate anaemia of 2% is less than 29.6% reported under-fives in Buea [33], 34% vs 48% reported amongst U5C in sub-Sahara Africa [59] vs Ghana [60].

In this study, sex, age, malaria infection, community, and school were significantly associated with anaemia, which was similar to that of a study reported by Belo and Bui amongst school-aged children [27], and different from the findings of [59] among U5C in sub-Sahara Africa.

Determinants of Malaria/Anaemia amongst Study Participants

The association of age and sex with malaria in our study was similar to findings reported in the Nkongho-mbeng rural area of the Southwest Region [53], the Mbakong Health Centre [3],

among children at the Douala gynaeco-obstetric paediatric hospital [46], and among school-aged children in Belo and Bui [27] of the Northwest Region of Cameroon. In our study, malaria was also associated with communities which is also similar to another study in the Democratic Republic of Congo and Ethiopia [47].

Our study also showed that anaemia was dependent on age which is similar to the findings reported elsewhere in Cameroon; Belo and Bui, Douala, Limbe, and Buea [18, 27, 54, 58] and among under-fives in Ghana [60].

Conclusion

This is the first study assessing the prevalence of malaria and anaemia amongst asymptomatic nursery and Primary School Pupils in Bui. The proportion of asymptomatic pupils with malaria and anaemia was significantly high. Of the few risk factors examined, age, sex, and community were significantly associated with asymptomatic malaria as well as anaemia. To reduce the malaria burden, intervention programmes such as the National Malaria Control Program and other stakeholders have to target asymptomatic malaria carriers to prevent the transmission of the parasite. There is also a need to identify mechanisms for ensuring that everybody has uninterrupted easy access to mosquito nets as well as regular utilisation. The Ministry of Health, in collaboration with stakeholders should intensify education on the effective use of mosquito nets by all members of the household, especially the vulnerable populations.

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