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Original Research Article

Asymptomatic malaria parasitaemia in school children of Ekondo Titi sub-division, Cameroon

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Bonaventure Tientche, Damian Nota Anong* Smith Asaah , Jerome Fru-Cho and Theresa K. Nkuo-Akenji

Department of Microbiology and Parasitology, Faculty of Science, University of Buea, P.O.Box 63 Buea Cameroon

*Corresponding Author
Email: damian.anong@ubuea.cm

Asymptomatic malaria is a new challenge for National Malaria Control Programs. As transmission declines, control interventions often become increasingly focal and programs need to adapt and target remaining parasite reservoirs. This study was to assess the prevalence and proportion of asymptomatic malaria infections among school children in Ekondo Titi Sub-Division. A cross-sectional study was conducted among 529 school pupils aged between 4 and 14 years in six primary schools of Ekondo Titi Sub-Division, South West Cameroon. Blood samples were collected from children and used for the preparation of blood films for the detection of malaria parasites, determination of malaria parasite density and hemoglobin concentration. Parasites were present in the peripheral blood of 74.2 % (360) of school children. The prevalence of anaemia was 44. 7 %(217). The Mean Parasite density of asexual parasites was higher in children with moderate and severe anaemia when compared with non-anaemic children but the difference was not significant. There was no significant difference in the levels of parasitaemia between sexes and age groups. This study indicates that, despite scaling up of malaria elimination programs in Cameroon, there is still a substantial proportion of asymptomatic, individuals with parasitaemia in the country that may act as a silent reservoir for malaria transmission.

Keywords: Asymptomatic, transmission, anaemia, reservoirs, malaria

INTRODUCTION

More than 500 million school-age children worldwide are at risk of malaria infection (Gething et al., 2011) As a result of the scaling up of effective interventions such as insecticide-treated bed nets (ITNs), indoor residual spraying (IRS),intermittent preventive treatment (TPT), treatment with artemisinin- based combinations (ACT) and important improvements in access to effective treatment for clinical malaria, a remarkable 17% decline in the number of malaria cases between 2000 and 2010 and a 26% decrease in the malaria-specific child mortality rate globally between 2000 and 2011 have been reported in Africa (Lindblade et al., 2013) .However, countries with the highest incidence rate reported the least decrease in number of clinical cases. Malaria is strongly associated with poverty. Within countries, parasite prevalence rates in children are highest among poorer populations and in rural areas (USAID, 2009).

In 2011, the Cameroonian government scaled up interventions by making available insecticides treated bed net across the ten regions of the country. Other malaria control activities included a pilot program to use rapid diagnostic tests (RDTs) in health facilities through community health workers that was brought to national scale in the second half of 2012 and subsidized first-line treatment of artemisinin-based combination therapy(ACT) (Bowen, 2013).

Recent records from the Cameroon Ministry of Public Health have indicated the fact that of the 20 million total population, over 90% are at risk and about 41% have at least one episode of malaria each year (Antonio-Nkondjio et al., 2008; Minsanté, 2008). This represents 8.2 million people suffering from malaria attacks each year (Ndo et al., 2011). In Cameroon malaria morbidity rate stood at 40 % of hospital recorded deaths, 50 % of morbidity in children less than five years old, 40 % of deaths in children between 0-5 years and 40-45 % of medical consultations (Bigoga et al., 2007). Thus, Cameroon is one of the major contributors to the sub-Saharan African malaria incidences and therefore, takes an enormous toll on lives in terms of medical costs, days of labour lost and negative effects on learning, especially in school aged children (Kimbi et al., 2005).

The burden of malaria in school-age children is poorly defined because this age group is not included routinely in household-based cluster surveys. Most of the primary school age children in endemic areas carry malaria parasites without symptoms due to the developing immunity following constant exposure (Barger et al., 2009). Asymptomatic carriers (AC) are individuals who harbour the Plasmodium asexual forms, with or without gametocytes, but do not present clinical symptoms of the disease (Kern et al., 2011). Asymptomatic cases provide a fundamental reservoir of parasites and they might become gametocyte carriers, contributing to the persistence of malaria transmission (Alves et al., 2005; Ouédraogo et al., 2009). There are also reports that parasites from asymptomatic carriers are more infectious than from symptomatic individuals (Laishram et al., 2012). The existence of asymptomatic malaria infections is crucial to the spread of infections in the community which then results in absenteeism at work as well as school, hindering development and worsening poverty as a result of developing symptomatic disease (Barger et al., 2009).

The progress recorded in global malaria control has prompted a renewed emphasis on malaria elimination, leading to a shift in focus from targeting only clinical malaria to also identifying and treating asymptomatic malaria parasitaemia (Yeka et al., 2012) although the ability of the parasite to develop resistance to antimalarial drugs and increasing insecticide resistance of the vector threatens progress towards elimination. Surveys conducted in school-age children in the slope of mount of Cameroon (Nkuo-Akenji et al., 2002; Achidi et al., 2008; Nkuo- Akenji et al., 2008; Kimbi et al., 2013) found parasite rates in school age children of about 50%, with a lower rate among those living higher up Mount Cameroon.

Asymptomatic malaria is a new challenge for strategic plans for National Malaria Control Programs, a situation in which a human Plasmodium reservoir is maintained, with individuals who are not treated because they are not diagnosed, since they are asymptomatic. On the other hand, the diagnosing of such cases becomes difficult because of the low level of parasitaemia (Worku et al., 2014). Furthermore, with the move towards elimination in low-moderate transmission settings, there is a need to tackle untreated reservoirs of infection, to which school children are important contributors (Githeko et al., 1992; Bousema et al., 2004). Malaria is still a major public health problem and an obstacle to socioeconomic development in Cameron (Swagata et al., 2013). It is even more important to assess the situation because since 2003, the Cameroon government has subscribed to some known malaria control and preventive measures, such as the free distribution of insecticide treated nets (ITNs) to households (Bowen, 2013).

The method of malaria control adopted by the National Malaria Control Programme (NMCP) is the early detection of parasites in symptomatic people followed by treatment with artemisinin combination therapies (ACTs) to curb the disease burden. Therefore, the malaria control program in Cameroon usually leaves out individuals chronically infected with malaria parasites, but showing no symptoms (MPH, 2010). Asymptomatic infection, mainly by *P. falciparum*, is an important obstacle to reaching the Millennium Development Goal 4 (Swagata et al., 2013). Asymptomatic careers do not seek treatment for infection and as such the life cycle of the infection continues. As transmission declines, control interventions often become increasingly focal (Macauley, 2005) and programs need to adapt and target remaining parasite reservoirs.

No study on the prevalence of malaria has been reported in Ekondo Titi Subdivision, a rural community till date. Due to a shortage of staff in health care facilities and shortcomings in surveillance and information systems there is still a significant lack of data on malaria from this region. The objective of this study was to assess the prevalence and proportion of asymptomatic malaria infections among school children in the Ekondo Titi Subdivision.

MATERIALS AND METHODS

Study Area

The study was carried out in Ekondo Titi sub-division in Ndian division, South West Region, Cameroon which is located at the coastal plain with an estimated population of about 77,648 inhabitants and a total land surface area of 1839.25 km^2 (BUCREP, 2005). Land use is predominantly agricultural but urbanization and industrialization are proceeding rapidly (Itie, 2006). Ekondo Titi is 120 km from Buea the regional capital, 1.0 meter above sea level and located between latitudes $4^05'$ North and 5^o North of the Equator and between longitudes 8^0 E and 9^0 E of the Greenwich Meridian. The annual average rainfall is about 2743.5mm and the average annual temperature is approximately 27.3°C (Kometa and Kimengsi, 2013). The climate is equatorial with a long rainy season from March to October. The relative humidity is 90 % (Pamol, 2008).

Ekondo Titi has neither a water distribution nor a sewage disposal system. Houses are typically made up of planks, blocks, straw and mud, with crevices serving as hiding places for mosquitoes. Many houses do not have electricity. People living in these areas are subsistence farmers of cassava, plantain, maize, industrial cocoa and palm trees in addition to Pamol Plantations Plc. workers who are engaged in the maintenance of extensive palm tree plantations.

Study design

This was a cross-sectional study designed to determine the prevalence of malaria in children residing in rural communities of Ekondo Titi. The study was carried out from March to May and from September to October 2014 which corresponds to the beginning and the end of the rainy season respectively when malaria is generally high.

Primary school children of both sexes aged 4 -14 years attending 6 schools namely: Kita government primary school at Kita Balue, Lipenja Barombi government primary school at Lipenja Barombi, Ngolo Metoko government Primary school at Ngolo Metoko, Bongongo Barombi II government Primary school at Bongongo Barombi II, Lobe Estate government primary school group III at Lobe Estate and Sacred Heart Nursing and Primary school at Ekondo Titi were enrolled for the study. Pupils were enrolled only if they had presented a consent form signed by parent/legal guardian, and from whom blood was collected.

Eligibility Criteria

Inclusion Criteria

All primary school children in grade 1 to grade 6 who agreed to participate with no clinical signs or symptoms of ill health as of the time of the investigation, with a signed informed consent form from their parents or guardians and attended school on a day when data collection was done were included.

Exclusion Criteria

School children who were severely ill, had a body temperature ≥37.5 °C or who had not attended school during the period of research and those who had no signed informed consent form from their parents or guardians were excluded from the study.

Ethical consideration

Before commencement of the study, an ethical clearance was obtained from the South West Regional Delegation of Public Health while administrative clearance was obtained from the Inspectorate of Basic Education. The consenting process involved the explanation of the content of the information sheets to parents or guardians by a responsible research team member in the language (English, French or Pidgin) the caretaker best understood and opportunities were given for questions/clarification. Emphasis was laid on the voluntary nature of participation and that they could withdraw at any time without any explanation.

Measurement of clinical parameters

Body temperature was measured using a digital thermometer (Citizen Systems, Japan). A child was considered febrile when he/she had a body temperature ≥37.5 °C. Weight and height were measured using a Terraillon weighing scale (Terraillon, Paris) and a height board, respectively (Nkuo- Akenji et al., 2008). The ages of the children were obtained from the school register.

Laboratory Investigations

Collection of blood samples

Prior to the collection of blood samples, data such as age, sex and axillary temperature were recorded. Capillary blood from a finger prick was used for the preparation of thin and thick blood films.

Malaria parasite density

Giemsa-stained thin and thick blood films were observed microscopically under the 100x objective. Malaria parasites were counted against 200 leucocytes in thick films to obtain the parasite density. This was expressed as the number of parasites per microlitre (μL) of blood assuming an average leucocyte count of 8000 cells per μL of blood. Slides were considered positive when asexual forms and/or gametocytes of any Plasmodium species were observed on the blood film. A slide was declared negative only after having examined at least 100 high power fields (Cheesbrough, 2006). Parasitaemia was classified as low (< 500 parasite /μl of blood), moderate (501-5000 parasites/μl of blood) and high (>5000 parasites/μl of blood) (Nkuo- Akenji et al., 2008). Each sample was read by two independent microscopists, blinded to each others interpretations, first at Lobe Estate Hospital using the MS 300 (Micros Handelegesellshaft, Austria) microscope and later at the Biotechnology Unit of the University of Buea.

Determination of haemoglobin concentration (Hb in g/dl)

Measurement of Hb was done on blood from a finger prick using a URIT-1systems (URIT Medical Electronic Co. Ltd, China). Blood was collected by finger prick, blotted on the test strip and immediately placed into a portable spectrophotometeric instrument. The hemoglobin value was displayed within the 15-60 seconds. Anaemia was classified as mild to moderate and severe when haemoglobin concentrations were respectively 10.9-7.0 g/dl and <7 g/dl (Mogensen et al., 2006).

Statistical Analysis

Data were analysed using EXCEL(Microsoft) and SPSS for Windows,version 11.0 (SPSS Inc.,Chicago, IL, USA). The analysis of variance (ANOVA), and Fisher exact tests were used to compare group means. Proportions were compared using the chi-square test. Parasite densities were $\log_{10}$ transformed, before analysis. The statistical significance level was set at $p<0.05$.

Table 1. Characteristics of study participants from various schools of Ekondo-Titi

School	Bongongo	Kita	Lipenja	Lobe E.	Ngolo	Sacred H	Total	P value
variable s%(n)								
Sex								
Male	50.0(23)	62.3(43)	65.7(44)	44.8(43)	54.2(45)	43.5(54)	52.0(252)	
Female	50.0(23)	37.7(26)	34.3(23)	55.2(53)	45.8(38)	56.5(70)	48.0(233)	0.017
Mean(±SD) age(years)	7.91±2.393	8.99±2.731	8.22±2.49	6.85±1.95	8.39±2.289	8.13±1.40	8.04±2.243	0.000
Age group								
≤5	21.7(10)	14.5(10)	14.9(10)	31.2(30)	10.8(9)	2.4(3)	14.8(72)	
6-9	41.3(19)	43.5(30)	56.7(38)	59.4(57)	56.6(47)	79.8(99)	59.8(290)	
10-14	37.0(17)	42.0(29)	28.4(19)	9.4(9)	32.5(27)	17.7(22)	25.4(123)	0.000
Mean(±SD) weight(kg)	23.73±6.197	25.32±7.84	25.2±7.24	21.05±4.8	24.34±5.53	23.81±7.18	23.76±6.56	0.000
Mean(±SD) height(m)	1.186±0.177	1.24±0.173	1.20±0.13	1.15±0.12	1.19±0.133	1.21±0.16	1.199±0.15	0.006
Mean(±SD) temp(°C)	36.439±0.6	36.36±0.61	36.24±1.1	36.13±0.5	36.52±0.46	36.62±0.67	36.39±0.74	0.000

Table 2. Levels of parasitaemia by sex and age group among the school children

Parameter	**Parasite density distribution per micro litre of blood**				
	1-499%(n)	**500-999**%(n)	**1000-4999**%(n)	**≥5000**%(n)	**p-value**
Sex					
Female	48.8(81)	19.3(32)	31.3(52)	0.6(1)	
Male	46.4(90)	21.6(42)	30.9(60)	1.0(2)	
Total	**47.5(171)**	**20.6(74)**	**31.1(112)**	**0.8(3)**	**0.907**
Age group(years)					
≤5	32.8(19)	31.0(18)	34.5(20)	1.7(1)	
6-9	51.4(107)	16.8(35)	31.2(65)	0.5(1)	
10-14	47.5(45)	22.3(21)	28.7(27)	1.1(1)	
Total	**47.5(171)**	**20.6(74)**	**31.1(112)**	**0.8(3)**	**0.158**

RESULTS

Demographic characteristics of the study population

A total of 529 pupils from six primary schools were enrolled in the study but blood films from 14 children were unreadable and 30 pupils had a temperature ≥37.5 °C and were excluded from the analyses. Only data from 485 school children were analyzed (Table 1).

The presence of parasites and body temperature

Parasites were present in the peripheral blood of 74.2% (360) of school children without malaria. Body temperature of the pupils ranged from 31.5.^{0}C to 37.4 °C with a mean of 36.39 °C (SD ± 0.74). The majority of children with parasitaemia had a low parasite density 47.5 % (171) (Table 2). Prevalence of parasites in pupils with asymptomatic malaria was higher in males 77.0% (194) compared with females 71.2b% (166) but the difference was not statistically significant (X^2 =2.74, P=0.098) (Table 3).

Parasitaemia with respect to age and sex

The Prevalence of asymptomatic malaria was higher in males 77.0% (194) compared with females 71.2% (166) but the difference was not statistically significant (X^2 =2.085, P=0.149) The distribution of parasite densities among the asymptomatic malaria participants with respect to age and sex is shown in Table 3. The mean parasite density was (830.41±1546.747, P=0.590). There was no significant difference in the levels of parasitaemia between the sexes (X^2 =2.085, P=0.149), and the age groups (X^2=2.767, P=0.251) (Table 3). The distribution of Plasmodium parasitaemia with relation to age (Figure 1) showed that low parasitaemia of < 500 parasites/ul of blood was highest in the age group 6-9 years (51.4%) followed by the age group of 10-14 years with 47.5 %. Only 32.8 % of children < 5 years old had parasitaemia of < 500

Table 3. The relationship of sex, age group and anaemia status with prevalence and density of asexual stages of Plasmodium

characteristic	category	Number examined	% infected (number)	Mean Parasite density (±SD)
Sex	Female	233	71.2(166)	767.51±1131.278
	Male	252	77.0(194)	888.57±1850.217
Total		**485**	74.2(360)	830.41±1546.747
Level of significance			**X^2 =2.085, P=0.149**	**F=0.741, P=0.390**
Age group (years)	≤5	72	80.6(58)	1003.61±1286.836
	6-9	290	71.7(208)	800.31±1739.877
	10-14	123	76.4(94)	800.00±1155.789
Total		**485**	74.2(360)	830.41±1546.747
Level of significance			**X^2=2.767, P=0.251**	**F=0.529, P=0.590**
anaemic status	Non anaemic	268	73.5(197)	866.38±1828.212
	Moderate	213	75.6(161)	779.72±1101.960
	Severe	4	50.0(2)	1120.00±1512.261
Total		**485**	74.2(360)	830.41±1546.747
Level of significance			**X^2=1.506, P=0.471**	**F=0.256, P=0.774**

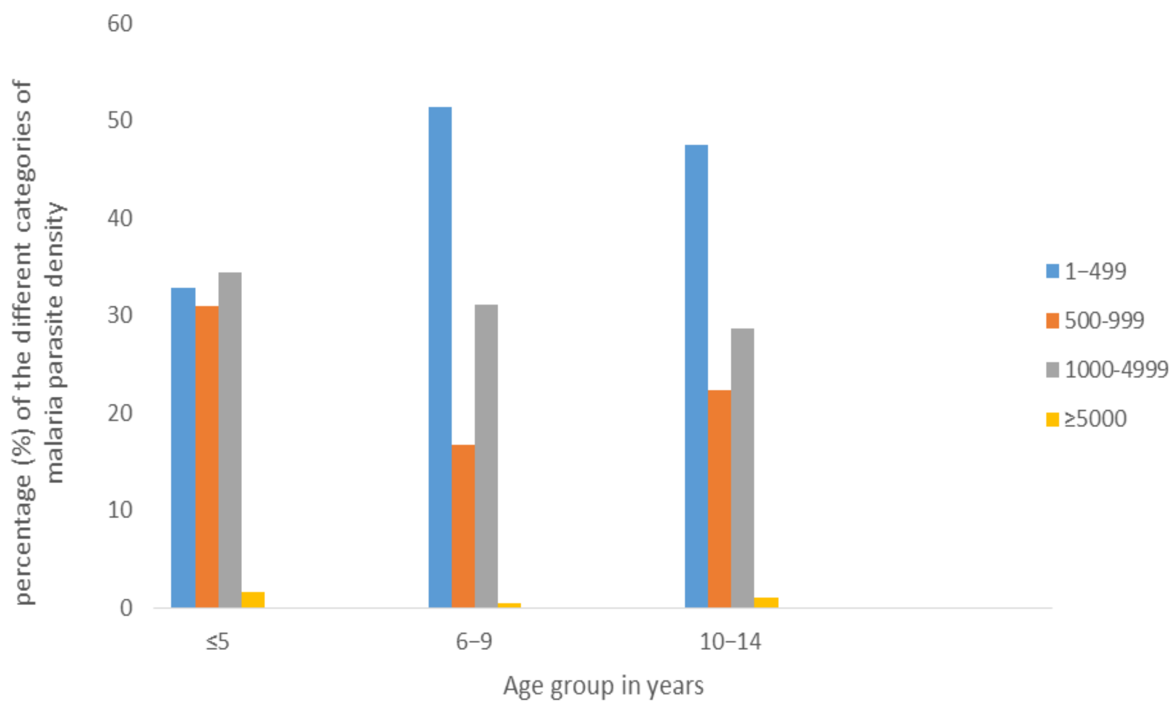

Figure 1: Distribution of Plasmodium parasitaemia in children with age group

parasites/ul of blood.

Haemoglobin concentration

The overall prevalence of anaemia (Table 4) in the study population was 44.7% (217). The prevalence of asexual parasites of Plasmodium was higher in children with anaemia 75.1% (163) when compared with those without anaemia 73.5% (197), but the difference was not significant (X^2=0.837, P=0.658). The mean haemoglobin concentration in the study population was 10.09±1.25 (Table 4). Overall 55.3% (268) of children were non-anaemic (Hb ≥ 11.0 g/dl), 43.9% (213**)** had mild to moderate anaemia (Hb 10.9–7 g/dl) and 0.8 %(4) had severe anaemia. The prevalence of anaemia differed considerably between schools (p<0.05). The Mean Parasite density of asexual parasites was higher in children with moderate anaemia and severe anaemia when compared with children with no

Table 4. Prevalence of parasitaemia and anaemia in pupils of various schools

Location	Number Examined	Parasitaemia		Haemoglobin(g/dl)			Mean Hb±SD
		Negative%(n)	Positive%(n)	≥11%(n)	10.9-7	<7	
Bongongo	46	23.9(11)	76.1(35)	15.2(7)	80.4(37)	4.3(2)	9.58±1.42
Kita	69	26.1(18)	73.9(51)	68.1(47)	31.9(22)	0.0(0)	11.20±0.86
Lipenja	67	10.4(7)	89.6(60)	56.7(38)	41.8(28)	1.5(1)	10.95±1.32
Lobe E.	96	27.1(26)	72.9(70)	58.3(56)	40.6(39)	1.0(1)	11.08±1.56
Ngolo M.	83	16.9(14)	83.1(69)	53.0(44)	47.0(39)	0.0(0)	10.92±1.14
Sacred H.	124	39.5(49)	60.5(75)	61.3(76)	38.7(48)	0.0(0)	11.05±0.72
Total	**485**	**25.8(125)**	**74.2(360)**	**55.3(268)**	**43.9(213)**	**0.8(4)**	**10.09±1.25**
p-value		**0.000**			**0.000**		**0.000**

anaemia although the difference was not significant (F=0.256, P=0.774) as shown in Table 3. There was a significant positive correlation between age and haemoglobin concentration(r=0.128; p=0.01).

DISCUSSION

Mapping of malaria high-risk areas is essential for planning health interventions (Dev et al., 2004). The primary objective of this investigation was to establish baseline data on the prevalence of asymptomatic carriers of Plasmodium parasites in Ekondo-Titi an enclave of the South West Region of Cameroon.

The results of the study show that the overall prevalence of asymptomatic malaria cases in this population of school children at the time of survey was 74.2 %. These findings are far higher than data reported in Somalia (Noor et al., 2008), Gabon (Nkoghe et al., 2011) and Nigeria (Singh et al., 2014) but lower than that of Cote d'Ivoire (Nzeyimana et al., 2002). This study shows that the most exposed households to asymptomatic malaria are those in the more remote parts of a region as shown in other studies (Kreuels et al., 2008; Sumbele et al., 2015). The prevalence reached 89.6 % in Lipenja Barombi community, one of the highest rates ever reported in the country. High prevalence of asymptomatic Plasmodium infection observed may be due to the fact that schools were located in an enclave, deep in the African rain forest where intervention and management programs were yet to be implemented. Parasite density was determined by microscopy, which has been known to be less sensitive than PCR (Golassa et al., 2013). This is an indication that the actual prevalence may be higher than observed. This large reservoir of asymptomatic school children with parasitaemia is likely to act as a source of infection to other children in school and also at home.

The prevalence and density of asexual stages of Plasmodium parasites which was higher in males than in females, as shown in other studies (Nzeyimana et al., 2002; Kimbi et al., 2012 & Worku et al., 2014) could be due to the difference in behaviour of male and female children. Male are generally more active than females and spend more time playing outdoors in the evenings after school than females. Therefore, they are more exposed to mosquito bites. In addition, males preferably sleep without shirts while female cover their bodies, thereby protecting themselves from mosquito bites to some extent.

Age is one of the most important factors that correlate with protective immunity in areas in which malaria is endemic (Swagata et al., 2013). Adults and older children have a lower prevalence of infection and a lower incidence of clinical symptoms (Djimdé et al., 2003; Sharma et al., 2006). This might be due to repeated exposure to malaria infection during the early part of their lives hence acquired immunity (Nankabirwa et al., 2013). Clinical immunity starts to appear with increasing age. Apart from the presence of asymptomatic infection in these children, reduced parasite density was observed, thus suggesting the beginning of anti-parasite immunity (Owusu-Agyei et al., 2001). In our present study, we observed that malaria prevalence and parasite density were higher in children under 5 years and decreased with age. This is in line with other reports by Nkuo- Akenji (2002) and Ugwu (2015). The lack of sterile immunity in malaria also helps to maintain the parasites in individuals and enhance the spread through the bites of mosquitoes as is evident in these apparently healthy children carrying parasites which the immune system can control without any clinical manifestation for some time.

Continuous exposure to Plasmodium parasites may lead to the development of partial immunity for protection against further complication and consequently, the creation of asymptomatic carriers (Githeko et al., 1992). In general, high parasitaemia has been associated with increased disease severity. However, this is not always the case as peripheral parasitaemia does not always accurately reflect the number of parasites due to sequestration (Laishram et al., 2012). The presence of such low parasitaemia would most likely have a ‘booster effect’ on the immune system and during clinical attacks in children with normal haemoglobin levels, preventing the occurrence of severe malaria (Nkuo-Akenji et al., 2002).Although asymptomatic carrier state may help in maintaining the spread of parasites in the population which is a draw back in control strategies, evidence from other studies (Le Port et al., 2008; Males et al., 2008), suggests that long-term asymptomatic carriage of malaria parasites protects against subsequent disease possibly by reducing the risk of super infection with more virulent strains We found that asymptomatic malaria in school children in the Ekondo titi Sub-division was accompanied by low grade parasitaemia, which did not seem to have a significant effect on haemoglobin levels. This definitely reflects the role of the immune system in limiting the effects of the parasite that usually become more visible with clinical malaria.

An important reason underlying a recent interest in malaria in school-age children is the concern that malaria may interfere with a child's educational development (Nankabirwa et al., 2014). A study undertaken in Nigeria showed that malaria caused an average loss of three school days per episode (Erinoso and Bamgboye, 1988). Also, in another study of asymptomatic Plasmodium infection and cognition among Primary School children in a high malaria transmission setting in Uganda, Nankabirwa (2013) reported that infected children had lower test scores for abstract reasoning and sustained attention compared with uninfected children.

In conclusion, three major observations can be deduced from this cross-sectional survey. First, this study demonstrates that there is high level of malaria in rural communities. Environmental factors, such as proximity to forest, household density, type of houses and socio-economic status have been shown previously to be significantly and positively correlated with risk of malaria (Nkuo-Akenji et al*.,* 2006). Secondly, school children represent a large reservoir of the parasites that are likely to act as a source of infection. Lastly, asymptomatic malaria parasitaemia, in this rural and remote area did not seem to have a significant effect on haemoglobin levels.

This study indicates that, despite scaling up of malaria elimination programs in Cameroon, there is still a substantial proportion of asymptomatic, individuals with parasitaemia in the country that may act as a silent reservoir for malaria transmission. Considering ongoing elimination efforts, these findings highlight the need for new intervention strategies targeting rural and remote communities. Therefore, there is a need for further evaluation of the burden of asymptomatic malaria.

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Conflict of Interests

The authors declare that there is no conflict of interests regarding the publication of the paper

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