ISSN: 2155-9597

Journal of Bacteriology & Parasitology

The International Open Access Journal of Bacteriology & Parasitology

Digital Object Identifier: http://dx.doi.org/10.4172/2155-9597.1000154

Bacteriology & Parasitology

Research Article Open Access

An Update of Asymptomatic *Falciparum* Malaria in School Children in Muea, Southwest Cameroon

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Abstract

Despite efforts put in by the international community to reduce malaria burden in Africa, the disease especially that is caused by *Plasmodium falciparum*, still remains a major health problem in sub-Saharan Africa. A cross-sectional study involving 366 pupils was conducted in Muea, Southwest Cameroon to assess an update of the burden of falciparum malaria in school children. Capillary blood samples were collected and Giemsa-stained blood films were observed microscopically for the identification and quantification of malaria parasites. Capillary tubes were filled with blood and spun at 10,000 rpm for 5 minutes, for the determination of packed cell volume (PCV). The overall prevalence of asexual parasites and anaemia were compared with the values, previously reported in a similar study at the same site in 2005. The overall prevalence of *P. falciparum* asexual parasites was 44.26%, as opposed to a value of 98% reported in 2005. The prevalence of asexual parasites significantly decreased with age (X^2 =20.86, p<0.0001). The values were similar in the sexes. The overall geometric mean parasite density (GMPD) of asexual *P. falciparum* was 1490.00 ± 1674.92 and the value was similar in the sexes and age groups. *P. falciparum* gametocyte prevalence was 17.49% and the value decreased significantly with age (X^2=22.88, p< 0.0001). The overall GMPD of gametocytes was 23.48 ± 6.96 parasites/µl. Gametocytaemia decreased with age and the difference was significant (F=62.61, *p*<0001). The overall prevalence of anaemia was 3.83%, as opposed to 10.6% in 2005. Generally, there was a significant drop in prevalence of asexual malarial parasites and anaemia in school children, compared to the previously reported values in 2005, and this is ascribed to the use of intervention strategies in recent years in the area.

Keywords: Update; Malaria; Prevalence; Density; Anaemia; Sexual; Asexual; School children; Cameroon

Introduction

Despite efforts put in by the international community to reduce the burden of malaria in Africa, the disease especially that is caused by *Plasmodium falciparum*, is still a major health problem in sub-Saharan Africa and is responsible for over one million deaths annually [1]. Regular updates on the burden of the disease (including asymptomatic cases) in different epidemiological settings are therefore, necessary to provide valuable information for policy makers to plan appropriate management and control strategies.

In malaria-endemic countries, a large proportion of *P. falciparum* infections are asymptomatic, and asymptomatic carriers do not usually seek treatment for their infection and therefore, constitute a reservoir of parasites available for transmission by *Anopheles* mosquitoes. It is thought that long term asymptomatic carriage may represent a form of tolerance to the parasite in children, building up their immune response, thereby protecting the children from developing either a mild malarial attack or a more severe one, by keeping their immunity effective [2]. Conversely, asymptomatic carriage may represent a mode of entry to symptomatic malaria, as well as transmission, especially in young children [3].

In Cameroon, malaria accounts for over 40% hospital attendance [4] and therefore, takes an enormous toll on the lives in terms of medical costs, days of labour lost and negative effects on learning, especially in school aged children [5]. Kimbi et al. [5] reported a 98% prevalence of asymptomatic malaria parasites in Muea, a semi-urban area in Southwest Cameroon, with *P. falciparum* (the most fatal species) responsible for 93% of all malaria parasite species present. The study however, did not assess the transmission index of gametocytaemia, which is very vital for the transmission of the disease from one person to another. Considering the high levels of morbidity and mortality of malaria especially that due to *P. falciparum*, it is important to monitor the situation in this area, in order to find out whether it is improving or worsening. For malaria control measures to be effective, both symptomatic as well as asymptomatic individuals must be included in the management strategies. 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 [6]. There have been reports on the general decline in malaria burden in some African countries [7-9], and this has been attributed to the introduction of several national malaria scale-up programmes. It is not known whether these measures have registered any impact on malaria burden in any part of Cameroon, including Muea. This study therefore, sought to update the level of malaria parasite prevalence and density of both asexual and sexual stages of *P. falciparum,* as well as anaemia in asymptomatic school children who are naturally exposed to malaria in the Muea area of Southwest Cameroon.

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Received October 01, 2012; **Accepted** October 17, 2012; **Published** October 22, 2012

Citation: Kimbi HK, Keka FC, Nyabeyeu HN, Ajeagah HU, Tonga CF, et al. (2012) An Update of Asymptomatic *Falciparum* Malaria in School Children in Muea, Southwest Cameroon. J Bacteriol Parasitol 3:154. doi:10.4172/2155-9597.1000154

Materials and Methods

Study area

The study was conducted in Catholic School, Muea (the same area in which a similar study was reported in 2005, between March and June, 2011 [5]. Muea is a semi-urban area in Southwest Cameroon, situated at an altitude of 548m above sea level. It has two distinct seasons: a cold rainy season from mid-March to October, and a warm dry season that lasts for the rest of the year. The mean annual temperature is 25°C, with a relative humidity of over 80% and an annual rainfall of approximately 4000 mm. The high rainfall in this area, together with warm temperatures and the rich volcanic soil, favour the growth of a dense tropical forest. Part of this forest has been transformed into banana plantations as well as maize, cocoyam, cassava and sugar-cane farms. Falciparum malaria is endemic in the area, accounting for up to 97% of malaria infections.

Study population

The study population consisted of 366 asymptomatic pupils aged 4 to 15 years, and of both sexes. Visits were made to the schools to explain the objectives and potential benefits of the study (free malaria treatment for positive cases) to the teachers. The purpose and methodology of the survey was explained and planned with the teachers. Informed consent forms were distributed to the children for parents'/legal guardians' consents before sample collection. The forms were filled and returned through the children. Those included in the study were (i) those whose parents consented by signing the informed consent forms, (ii) were willing to participate in the study, and (iii) were infected only with *P. falciparum* infections.

An ethical clearance for the study was obtained from the Ethical Review Board of the South West Regional Delegation of Public Health, Cameroon, while an administrative clearance was obtained from the South West Regional Delegation of Basic Education, Cameroon.

Collection of blood samples

Prior to the collection of blood samples, data such as age, sex and axillary temperature were recorded in a structured questionnaire. Capillary blood from a finger prick was used for the preparation of thin and thick blood films. A heparinized capillary tube was filled with blood for the determination of packed cell volume (PCV).

Processing of blood films and microscopy

Thick and thin blood films were stained with 10% Giemsa for 20 minutes, after fixing thin films with absolute methanol for one minute [10]. The thick and thin films were observed using the 100X (oil immersion) objective of the microscope (Optical Co. Ltd, Japan), for the detection of parasites and species, respectively. A blood smear was declared negative only after examining 100 high power fields, without seeing any parasites. Only specimens that had *P. falciparum* alone were considered in this study. Asexual parasite density and gametocytaemia were determined by counting parasites against 200 WBC and 500 WBC, respectively assuming a white cell count of 8000 leucocytes/µl of blood, and expressed as parasites per µl [10]. Slides were read separately and independently by two experienced microscopists and in the case of any discrepancies, slides were read again. Children positive for malaria were treated with a combination of malartin (artesunate) and amodiaquine, in collaboration with the authorities of a nearby public health centre.

Assessment of anaemia

Capillary tubes containing blood were centrifuged at 10,000 rpm for 5 minutes and PCV values were read using a microhaematocrit reader. Pupils were considered to be non-anemic, if PCV was ≥31% [5]. Those diagnosed of anaemia were given iron sulphate.

Statistical analysis

Data was entered into Microsoft Excel and analyzed using the Statistical Package for Social Sciences (SPSS), version 11.0 for Windows (Chicago, IL, USA). The analysis of variance (ANOVA), (F-test) was used to compare group means. The Pearson Chi square test was used to compare proportions. Parasite densities were $\log_{10}$ transformed, before analysis. The statistical significance level was set at $p<0.05$.

Results

Characteristics of the study population

Out of a total of 590 forms given out, 372 pupils returned signed forms. Six children diagnosed with mixed infections were excluded from the study. A total of 366 children aged 4-15 years and of both sexes (158 males, 208 females), satisfied the inclusion criteria and were included in the study. The mean age of the study subjects was 8.48 ± 2.78 years. The highest number of study subjects (166) was recorded in the 6-9 years age group, while the least (54) was in the age group ≤5 years. The mean PCV and asexual geometric mean parasite density (GMPD) were 36.55 ± 3.89 and 1490.00 ± 1674.92 parasites/µl, respectively. The overall sexual GMPD was 23.48 ± 6.96 parasites/µl. The mean temperature of the study population was 36.74 ± 0.45.

Prevalence and density of *P. falciparum* asexual parasites with respect to sex, age and anaemic status

The overall prevalence of asexual stage parasites was 44.26% (166/366), as opposed to 98% (241/244) reported in 2005. Prevalence in males was similar (48.10%) to that in females (41.35%), as shown in table 1 (X^2=1.66, P=0.19). The highest prevalence of asexual parasites was recorded in the age group ≤5 years (70.37%), while the lowest occurred in the age group 10-15 years (34.25%) and the difference was significant (X^2=20.86, P<0.0001).

The GMPD of asexual parasites was similar in the sexes (F=0.67, P=0.57). The highest GMPD of asexual parasites (1557.25 ± 2435.51 parasites/µl) was recorded in the youngest age group, while the lowest

Characteristic	Category	Number examined	Number infected with asexual parasites (%)	GMPD ± SD of asexual parasites
Sex	Male	158	76 (48.10)	1539.92 ± 1624.69
	Female	208	86 (41.35)	1447.23 ± 1721.16
Total		366	162 (44.26)	1490.00 ± 1674.92
Level of significance			X^2 = 1.66, P = 0.19	F = 0.67, P = 0.41
Age group (years)	≤ 5	54	38 (70.37)	1557.25 ± 2435.51
	6 - 9	166	74 (44.58)	1607.05 ± 1307.39
	10 - 15	146	50 (34.25)	1288.27 ± 1458.46
Total		366	162 (44.26)	1490.00 ± 1674.92
Level of significance			X^2 = 20.86, P < 0.0001	F = 0.56, P = 0.57
Anaemic status	Anaemic	14	8 (57.14)	3356.38 ± 1190.19
	Non-anaemic	352	154 (43.75)	1428.45 ± 1663.38
Total		366	162 (44.26)	1490.00 ± 1674.92
Level of significance			X^2 = 0.98, P = 0.32	F = 6.80, P = 0.01

GMPD: Geometric Mean Parasite Density; SD: Standard Deviation

Table 1: The influence of sex, age and anaemic status on prevalence and density of asexual stages of *P. falciparum*.

(1288.27 ± 1458.46 parasites/µl) was recorded in the 10-15 years age group, but the difference was not significant (F=0.56, P=0.57). A similar trend in age groups was observed in 2005.

The overall prevalence of anaemia in the study population was 3.83% (14/366), while that reported in 2005 was 10.6% (26/246). No case of severe anaemia was reported during both study periods. The prevalence of asexual parasites of *P. falciparum* was higher in anaemic (57.14%, 8/14) than in non anaemic children (43.75%, 154/352), but the difference was not significant (X^2=0.98, P=0.32). The GMPD of asexual parasites was higher in anaemic (3356.38 ± 1190.19 parasites/µl) than in non-anaemic children (1428.45 ± 1663.38 parasites/µl), and the difference was significant (F=6.8, P=0.01), as shown in table 1.

Prevalence and density of *P. falciparum* gametocytes with respect to sex, age and anaemic status

The overall prevalence of *P. falciparum* gametocytes was 17.49% (64/366). Generally, more males (21.52%, 34/158) harbored gametocytes than females (14.42%, 30/208), but the difference was not significant (X^2=3.13, P=0.08). The prevalence of *P. falciparum* sexual parasites was significantly higher (X^2=22.88, P<0.0001) in the ≤5 years age group, when compared with the other age groups and decreased with age (Table 2).

GMPD of *P. falciparum* gametocytes was higher in males (24.24 ± 7.47) than females (22.63 ± 6.28), but the difference was not significant (F=1.17, P=0.28). The highest GMPD of gametocytes/µl (32.89 ± 5.83) also occurred in the ≤ 5years age group, while the lowest (19.88 ± 3.15) was recorded in the 10-15years age group, and the difference between age groups was significant (F=62.61, P<0.0001), as shown in table 2.

The prevalence of *P. falciparum* gametocytes was higher (57.14%, 8/14) in anaemic than non-anaemic children (15.91%, 56/352), and the difference was significant (X^2=15.87, P<0.0001). The density of gametocytes was also higher (26.23 ± 8.40) in anaemic than in non-anaemic children (23.12 ± 6.71), but the difference was not significant (F=1.76, P=0.19).

Characteristic	Category	Number examined	Number infected with gametocytes (%)	GMPD ± SD
Sex	Male	158	34 (21.52)	24.24 ± 7.47
	Female	208	30 (14.42)	22.63 ± 6.28
	Total	366	64 (17.49)	23.48 ± 6.96
	Level of significance		X^2 = 3.13, P = 0.08	F = 1.17, P = 0.28
Age group (years)				
	≤ 5	54	18 (33.33)	32.89 ± 5.83
	6 - 9	166	36 (21.69)	20.87 ± 3.16
	10 - 15	146	10 (6.85)	19.88 ± 3.15
	Total	366	64 (17.49)	23.48 ± 6.96
	Level of significance		X^2 = 22.88 P < 0.0001	F = 62.61 P < 0.0001
Anaemic status	Anaemic	14	8 (57.14)	26.23 ± 8.40
	Non-anaemic	352	56 (15.91)	23.12 ± 6.71
	Total	366	64 (17.49)	23.48 ± 6.96
	Level of significance		X^2 = 15.87, P < 0.0001	F = 1.76, P = 0.19

GMPD = Geometric Mean Parasite Density; SD = Standard Deviation

Table 2: Influence of sex, age and anaemic status on prevalence and density of gametocytes of *P. falciparum*.

Discussion

This study was aimed at assessing an update of the burden of asymptomatic malaria and anaemia due to *P. falciparum* in asymptomatic school children in Muea, Southwest Cameroon. The results show a decline in the prevalence of both asexual malaria parasites and anaemia in the study area, when compared to the values reported in 2005 in the same area and during the same season. Similar studies carried out earlier in other parts of Southwest Cameroon also showed higher values for malaria parasite prevalence and anaemia, than that reported in the present study [4,11,12]. This report also contrasts with the observations of Font et al. [13] who reported that in endemic areas, the prevalence of asymptomatic parasitaemia at any given time in children, ranges from 60 to 100%. This shows a remarkable improvement in the health of pupils, and also confirms that malaria is a major contributor to anaemia [9].

The decline in prevalence of malaria parasites and anaemia could be due to the recent introduction of several educative health campaigns on malaria prevention in public and private media (radio and television channels), in Cameroon. The anti-malarial campaign messages and slogans have been broadcasted in English, French and the local languages, giving the literate as well as illiterate members of the public, the opportunity to understand. Such messages have included the recognition of common signs and symptoms of malaria, protective and prophylactic measures and the advice to seek prompt and appropriate diagnosis, and treatment of malaria once signs and symptoms of the disease are suspected. These educative talks have probably led to a change in behavior and contributed to the decline in malaria parasite prevalence. Some inhabitants do no longer farm around their homes, in order to avoid mosquito bites. Klinkenberg et al. [14] reported that one of the ways to eliminate resting sites for adult *Anopheles* mosquitoes was to avoid farming around homes.

The dramatic decline in the prevalence of asymptomatic malaria parasites and anaemia might also be due to an expansion in the coverage of Insecticide-treated Nets (ITNs) in recent years. The National Malaria Control Program (NMCP) in Cameroon distributed ITNs to pregnant women and children under five in 2003 and 2005. Since 2007, ITNs have been systematically distributed to all households in different health districts, across the national territory. This exercise was initiated as a follow-up of resolutions and commitments taken by African Heads of State at the African Summit on Roll Back Malaria in Abuja [15]. Although, concrete statistics on the distribution and effective utilization of ITNs in Muea could not be analyzed, the most important change in recent years has been the increased use of freely distributed ITNs in the study area. Some authors also reported a decline in malaria burden in Kenya [7] and Tanzania [9], and this was attributed to the use of interventions such as ITNs. Steketee and Campbell [8] reported that prior to 2005, most countries in malaria endemic areas recorded less than 5% of households having at least one ITN, but by 2009, a number of countries had exceeded 25% use of ITNs and Intermittent Preventive Treatment (IPTp) in rural pregnant women. This scale up coincided with documented reductions in child morbidity and mortality, out-patient malaria diagnosis and hospital admission, and deaths attributed to malaria.

The Cameroon Government has also made available, basic malaria drugs at relatively low prices in pro-pharmacies across the national territory, in order to reduce the cost of malaria treatment, since poverty is one of the factors that contribute to the high prevalence of malaria in endemic areas. In 2010, the government instituted as a policy that, all children below five would be treated free of charge in all hospitals.

Artemisinin-based combination therapies (ACTs) were also adopted as first line treatment for uncomplicated malaria in Cameroon in 2004, to replace sulphadoxine-pyrimethamine to which widespread resistance had already developed [16]. The National Malaria Control Programme organized workshops across all regions in Cameroon, to educate and inform health workers at public, mission and private health facilities of the change in policy and the need to adhere to it. The free treatment of malaria in children below five, availability of anti-malarial drugs at relatively low cost, and educative talks are important factors that are likely to have contributed to the decline in malaria and anaemia prevalence in Muea, although, these have not been investigated in a controlled study. Okiro et al. [7] attributed the decline in malaria burden in paediatric malaria admissions on the coast of Kenya, to the availability of anti-malarial medicines.

Muea has also witnessed some degree of urbanization in recent years, which has led to an increase in the number and quality of social facilities in the area. The fact that some of the farm lands in this area have been replaced by buildings and many plank houses (owned by the Cameroon Development Corporation, CDC), which often had crevices through which mosquitoes could enter the houses *ad libitum* (free-feeding) have been replaced by block houses. This has likely reduced the mosquito biting rate and consequently, transmission of the disease. This agrees with reports from other studies, which have reported that urbanization is likely to reduce the frequency and transmission dynamics of malaria [17,18].

The prevalence and density of asexual stages of *P. falciparum* were higher in males than in females, even though the difference was not significant. This is in line with an earlier study by Adio et al. [11]. This could be due to the fact that, male children play outdoors in the evenings more often and are therefore, more exposed to mosquito bites. The prevalence of asexual stages of *P. falciparum* decreased significantly with age. This could be attributed to the fact that Muea is an endemic area and the children could have gradually developed some degree of immunity against the malaria parasite, as a result of repeated infections. This is in line with the findings of Paul et al. [19] and Baliraine et al. [20] who also reported that asymptomatic parasitaemia may be beneficial in inducing and sustaining partial immunity against malaria, although a significant drawback is the suppression of haematocrit levels [21].

Children who were anemic had a higher density of asexual stages of *P. falciparum* than those who were non-anemic, and the difference was significant. This was probably due to the destruction of red blood cells by the parasites, as they fed on the haemoglobin of red blood cells rendering the subjects anaemic. Therefore, the higher the parasite loads, the lower the PCV, which is directly related to the concentration of haemoglobin [22].

Gametocytes are the sexual, non-proliferating blood-stage forms of *Plasmodium* species that are transmission agents to the mosquito vector. They are products of the asexual, replicating, non-transmissible forms, with which the pathology of malaria is associated. The prevalence of *P. falciparum* gametocytes in the study population was probably a reflection of the level of asexual parasitaemia. Bousema et al. [23] and Ouedraogo et al. [24] reported high asexual parasitaemia, as an important risk factor for gametocyte carriage. Both prevalence and density of gametocytes decreased significantly with increasing age. This is probably a reflection of the degree of immunity to the disease, within the age groups. Similar observations were reported by Quakyi et al. [25]. In a study in The Gambia, Seidlein et al. [26] identified anaemia, absence of fever and low asexual parasitaemia as independent risk factors for gametocyte carriage in a similar age group, as in this study. This implies that control measures need to be intensified in the youngest age group, whose level of immunity is low, and have the highest level of gametocytes as well as anaemia and consequently, constitute reservoirs for malaria transmission. Since the values for gametocytes were not assessed in the 2005 study, we did not have any basis for comparison, and so it is difficult to say whether the situation is improving or worsening, as far as gametocyte carriage is concerned. However, the present results form the basis for comparison in future.

There's been no documentation of changes in disease burden associated with increase in access and use of interventions funded by the government, or international agencies in Southwest Cameroon. It is important to analyze hospital-based data, in order to compare with the data in the present study. It is also recommended that entomological studies be carried out and compared with that of previous studies, in order to know whether the situation has improved or not. It is understood that there is generally an inherent interval between programme scale-up and documenting programme outcomes [8]. This report shows that a profile of programme impact is likely emerging in the study area.

To conclude, it is not possible to discuss with certainty, the factors that have led to the decline in malaria parasite and anaemia prevalences in the study area, but the drastic change in intervention strategies like ITNs, use of ACTs, rapid urbanization in the area, health education through public media and National Malaria Control Programme (NMCP) seem to have played major roles. It is hoped that the authorities in charge of these management programmes will not relent efforts as malaria is still a major public health problem in Cameroon.

Acknowledgements

We are grateful to all the parents/guardians for their consent and the children who participated in this study.

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