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# A Cross-sectional Study on the Influence of Altitude and Urbanisation on Co-infection of Malaria and Soil-transmitted Helminths in Fako Division, South West Cameroon

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### *Authors' contributions*

*This work was carried out in collaboration between all authors. Author JLNN designed the study, wrote the protocol, carried out field and laboratory work and wrote the first draft of the manuscript. Author HKK designed the study, wrote the protocol, carried out field work, read and corrected the manuscript. Author IUNS performed the statistical analysis, read and corrected the manuscript. Authors YN and SCB participated in the data collection and literature searches. Authors KJNN and LGL read and corrected the manuscript. All authors read and approved the final manuscript.*

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## ABSTRACT

**Aims:** Malaria and soil-transmitted helminth (STH) infections are parasitic diseases afflicting populations that are impoverished and malnourished. The aim of this study was to assess the influence of altitude and urbanisation on Co-infection of malaria and soil-transmitted helminths in Fako Division, South West Cameroon.

**Study Design/Place and Duration of Study:** It was a cross-sectional survey carried out from 2012 to 2014 involving 1138 children aged 4 – 15 years in Fako Division.

**Methodology:** Structured questionnaire was administered to obtain demographic and socio-economic data. Blood samples were collected by pricking the finger. Malaria parasite prevalence, density and species were determined from Giemsa-stained thick and thin blood smears respectively. Quantitative estimation of helminth eggs was done by Kato-Katz thick smear technique. Based on height above sea level (a.s.l.), the study sites were classified as Lowland (<200 m.a.s.l), Lower middle belt (>200 but ≤ 400 m.a.s.l), Upper middle belt (>400 but ≤600 m.a.s.l.) and Highland (>600 m.a.s.l). The study communities were also classified into rural, semi-urban and urban areas.

**Results:** The overall prevalence was 38.1% (433) for malaria, 2.5% (29) for STHs and 0.9% (10) for malaria-STH co-infection. The prevalence of malaria was significantly highest ($\chi^2$ = 84.6, P <0.001) in urban areas (52.2%, 263) than in the semi-urban (29.4%, 152) and rural areas (15.4%, 18). Malaria prevalence was significantly highest ($\chi^2$ = 123.4, P <0.001) at the lowest altitude (60.5%, 182) and decreased as altitude increased to a minimum of 15.4% (18) at highland (>600 m.a.s.l). Only two species of soil-transmitted STH [*Ascaris lumbricoides* (1.9%) and *Trichuris trichiura* (0.6%)] were found. The prevalence of STH was significantly higher ($\chi^2$= 33.8, P <0.0001) in rural (13.6%, 16) than urban (0.39%, 2) areas. The prevalence of STH was significantly highest ($\chi^2$ = 33.8, P <0.0001) at high altitude (13.6%, 16) than the upper middle-belt (2.0%, 8), lower middle-belt (1.2%, 4) and lowlands (0.7%, 2). The prevalence of co-infection varied significantly ($\chi^2$ = 72.2, P <0.0001) with level of urbanisation with the highest level of co-infection occurring in the rural areas (2.6%, 3) and the lowest level in the urban areas (0.2%, 1).

**Conclusion:** Malaria control measures need to be intensified especially in the lowland and urban areas. Deworming campaigns are yielding positive results in Fako Division.

*Keywords: Malaria; soil-transmitted helminths; co-infection, prevalence; altitude; urbanisation; socio-economic class; anaemia.*

## 1. INTRODUCTION

Malaria and soil-transmitted helminth infections are parasitic diseases that cause high rates of morbidity and have similar geographical distributions in the tropics and sub-tropics [1]. These diseases afflict populations that are both impoverished and malnourished [2,3]. Malaria is endemic in sub-Saharan Africa including Cameroon where the disease is prevalent throughout the year [4,5]. Health statistics for Cameroon indicate that the prevalence of malaria in the country in 2013 was approximately 41% [6,7]. Malaria is generally considered to be a disease of rural areas, but many factors linked to a rapid and uncontrolled urbanization lead to an increase in malaria transmission in cities across Africa [8]. The prevalence of the disease has been shown to coincide with the distribution of the *Anopheles* vector [9], and is more prevalent in lowland than highland areas [10]. The disease usually results in severe health consequences such as anaemia, fever, splenomegaly, liver inflammation, abdominal disorders, muscle and joint pains [11] and its burden (clinical symptoms and pathology) becomes more severe when it co-exists with other infections such as STHs [12].

WHO [4], estimated that more than 880 million children suffer from STHs, and the total annual death toll due to STHs was about 135,000. The main species that infect man are the roundworm (*Ascaris lumbricoides*), the whipworm (*Trichuris trichiura*) and the hookworms (*Necator americanus* and *Ancylostoma duodenale*) as reported by WHO [13]. Apart from anaemia, helminth infections can also have severe consequences such as stomach upset, fever, stomach ache, “potted-belly”, stomach occlusion and dysentery [14]. STHs can also contribute to nutritional deficiency diseases due to competition for digested food between them and their hosts

[15] and this contributes to malnutrition and iron-deficiency anaemia [16].

In many areas where human malaria and STHs co-exist, school age children often harbour the heaviest infections and suffer much of the associated morbidity [3,17] especially when they are co-infected [12]. Several factors predispose children to both malaria and intestinal helminths and some of these include climatic conditions, poverty and sanitary practices [4,13,18,19].

According to de Silva et al. [20], each country needs to analyse its own disease infection pattern in order to plan effective control measures. This can be achieved by collecting data in all regions and localities in the country. Fako Division is located along the slope of Mount Cameroon and different localities there have different levels of urbanization and are located at different altitudes. These factors may affect malaria and STH prevalence [19] and subsequently anaemia prevalence, and may also be taking their toll on the health status of the individual, weakening the individual's ability to fight off other infections [21]. As such an evaluation of the burden of malaria co-infection with STHs among school children in Fako Division is necessary to better plan control measures and improve on the health of children. Few studies have been done on malaria and STH co-infection in South West Cameroon in the past fifteen years [12,16,22,23,24]. However, the findings of these studies may have changed over time as a result of rapid urbanisation, population movements as well as development and modifications of control policies. The findings of the reported studies were however limited to a few localities and could not be generalised for the whole of Fako Division. Also, none of these studies combined the prevalence of malaria and STH co-infection in relation to anaemia status of children, altitude and urbanization. Recently, there have been general national deworming campaigns for school age children every three to six months and the distribution of free insecticide treated bed nets for malaria control [6,25]. It is therefore necessary to assess the prevalence of STHs as an evaluation of the control programme against intestinal helminths. Against this background, the aim of this study was to assess the prevalence of malaria and STH co-infections among school age children in relation to levels of urbanization and altitudes in Fako Division, southwest Cameroon.

## 2. MATERIALS AND METHODS

### 2.1 Study Areas and Period

This study was carried out in the Mount Cameroon area from January 2012 to April 2014 beginning from the localities along the banks of the Atlantic ocean (Limbe) to the last settlements along the slope of Mount Cameroon (Bova and Bonkanda) with coordinates ranging from longitude 09º18' 47"E to longitude 09º18' 82"E and latitudes 04º05' 09''N to 04º05'57'' N. The localities included Limbe (0m a.s.l., longitude 09º18' 75"E and latitudes 04º05'52'' N), Mutengene (250m.a.s.l., longitude 09º18' 82"E and latitudes 04º05'57'' N), Dibanda (386m.a.s.l., longitude 09º18' 47"E and latitudes 04º06' 94''N), Bolifamba (487 m.a.s.l., longitude 09º18' 51"E and latitudes 04º05' 09''N), Bomaka (507 m.a.s.l., longitude 09º18' 52"E and latitudes 04º05' 11''N), Muea (562 m.a.s.l., longitude 09º18' 53"E and latitudes 04º05' 14''N), Bova (980m.a.s.l., longitude 09º19' 47''E and latitudes 04º07' 13N) and Bonakanda (1000 m.a.s.l., longitude 09º19' 50''E and latitudes 04º07' 13N). The study sites were classified according to altitude as Lowland (<200 m.a.s.l: Limbe); Lower middle belt (>200 but ≤400 m.a.s.l: Mutengene and Dibanda); Upper middle belt (>400 but ≤600 m.a.s.l: Bolifamba, Bomaka and Muea) and Highland (>600 m.a.s.l, Bonakanda and Bova). Many houses in the lowland areas usually have standing water around them especially during the rainy season. Given the sloppy nature of the highland areas, there is hardly any standing water found around houses. These communities were also classified into rural (Bonakanda, Bova); semi-urban (Dibanda, Bolifamba, Muea and Bomaka) and urban (Limbe and Mutengene) areas. In the rural communities in the Mount Cameroon region, the inhabitants are mainly subsistence farmers. Most of them are poor and live in houses made up of planks with holes and crevices in the walls. Most houses in the rural areas have farmlands and bushes around them. With the lack of latrines in some homes, defecation on the soil may not be uncommon. In the semi-urban areas, there is a mixture of plank and block houses, the rich, middle and poor classes as well as people of all works of life including subsistence farmers who periodically go to farm in the rural areas. On the other hand the inhabitants of the urbanised areas generally have higher standards of living and hygiene with most of them having steady jobs while some are traders. Over 70% of houses in the urban areas are made up of cement bricks while the rest are made out of plank. The environment is generally cleaner and there are proper latrines in all homes. Weather records for the Mount Cameroon area from the Cameroon Development Corporation indicate a mean relative humidity of 80%, an average rainfall of 4000 mm and a temperature range of 18 - 32ºC. There are two distinct seasons - a cold rainy season which spans from mid-March to October and a warm dry season with frequent light showers which runs from November to mid-March.

## 2.2 Study Population

The study included 1138 pupils of both sexes aged 4 - 15 years. The schools to be included in the study were chosen by random sampling. Pupils were enrolled into the study only if they fulfilled the following inclusion criteria: were pupils in one of the chosen schools had received parental/guardian consent (came with signed consent forms), gave their assent by succumbing to the blood as well as stool collection procedures.

## 2.3 Study Design and Participants

The study design was cross-sectional involving school children aged 4 to 15 years. An ethical clearance was obtained from the Ethics Committee of the Delegation of Public Health, while administrative clearances were obtained from the Delegation of Basic Education and Catholic Education Secretariat, of the South West Region, Cameroon. Prior to sample collection, visits were made to each school to explain the objectives and potential benefits of the study to the head teachers / teachers of the schools. The purpose and methodology of the survey was explained and pre-arrangements were made with the teachers of each school on when to collect blood and stool samples. Informed consent forms were distributed in the various schools to the children to take to their parents / legal guardians asking for their consent before sample collection. The sample size was calculated using the previous prevalence of malaria (44.26%) observed in primary school children in the Mount Cameroon area by Kimbi et al. [26]. The sample size was determined using the formula $n = Z^2pq/d^2$ [27] where n = the sample size required, z = 1.96: confidence level test statistic at the desired level of significance, p = 44.26%: proportion of malaria prevalence, q = 1-p: proportion of malaria negative children and d = acceptable error willing to be committed. The optimum sample size was estimated as n = 379. To achieve the minimum sample size of 1138 children in urban, semi urban and rural areas, a total of 1800 informed consent/assent forms (600 in each area) were given out to pupils. The 1800 children were randomly selected by drawing from a list of names of children per class. Out of the 1800 consent forms given out, 1150 children returned a signed form. Those included in the study were those whose parents / legal guardians consented by signing the consent / assent forms and who succumbed to the sample collection procedure. Investigative methods included a questionnaire, clinical assessment and laboratory analyses.

## 2.4 Administration of the Questionnaire

A simple structured questionnaire was administered in English and exceptionally in Pidgin English where necessary to children to obtain data on each child’s name, sex, age and the socio-economic status. The socio-economic status was classified as poor (those living in plank houses, had no television / radio, no car, used firewood kitchen and pit toilets), middle class (those living in block houses, had flush toilets, television / radio, gas kitchen, but no car) and rich (those having all what the middle class children had and a car) [28].

## 2.5 Fever Assessment

The axiliary temperature of each child was recorded before blood collection. Fever was defined as a temperature ≥37.5ºC.

## 2.6 Collection, Preparation and Examination of Blood Samples

After cleaning the lobe of a finger with cotton wool soaked in methylated spirit, the finger was pricked using a sterile lancet. The first drop of blood was wiped off with clean gauze and a drop of blood was applied to a haemoglobin meter strip for the measurement of haemoglobin using a haemoglobinometer (URIT 12 digital haemoglobinometer). Briefly, the strip was inserted into the haemoglobinometer which gave the reading in g/dL. The results were recorded and haemoglobin levels were used to classify anaemia as follows: children 6 months – 4 years <11.0 g/dL, children 5 – 11 years <11.5 g/dL, children 12 – 14 years <12.0 g/dL [15]. Blood drop from the finger was used to prepare thick and thin blood films on labelled slides for the assessment of parasite presence and density. The slides were air dried and Giemsa-stained as described by Cheesbrough [15]. The blood films were examined under the oil immersion (x100) objective of an Olympus® BX 40F light microscope (Olympus optical Co. Ltd., Japan). Thick films were examined and considered positive when asexual forms and or gametocytes were present in the blood film. Parasites were counted against 200 leucocytes and expressed as parasites per microlitre (μL) of blood, assuming a white blood cell count of 8000/μL of blood. Slides were declared negative after observing at least 100 high power fields without detecting any parasites.

Parasitaemia was classified as low (<500 parasite/μL of blood), moderate (501 - 5000 parasites/μL of blood) and high (> 5000 parasites/μL of blood [29]. The four human *Plasmodium* species were differentiated using thin blood films with the aid of identification charts of Cheesbrough [15]. All positive malaria patients were treated with Artesunate + amodiaquin in consultation with staff of the Tole Health centre.

### 2.7 Collection and Examination of Stool Samples

As part of the screening exercise, all children were given labelled universal bottles and instructed on how to do proper faeces collection. The younger children were assisted in the stool collection process by a laboratory technician. The stool samples were put in a mobile cooler containing ice packs to preserve the eggs. Stool samples were then transported to the laboratory and processed within 12 hours after collection and microscopically examined within 1 hour of preparation to avoid missing hookworm ova. The Kato-Katz thick smear technique was used for the quantitative estimation of helminth eggs in stool [30]. Duplicate smears were prepared from each specimen. As a control measure, 10% of randomly selected smears were re-examined by a third experienced parasitologist who was blinded to the previous results. The number of eggs per gram of faeces, was calculated based on the amount of faeces (41.7 mg) filled in the hole of the plastic template. The number of eggs in the 41.7 g of faeces were counted and multiplied by the factor of 24 to give the number of eggs per gram (g) of faeces [30]. The intensity of infection of the species of worms was expressed as the number of eggs per gram (epg) of faeces [15,30]. After collection of stool samples all pupils were treated with mebendazole (irrespective of helminth status) as part of the national deworming programmes.

### 2.8 Statistical Analysis

Data was entered into spread sheets using Microsoft excel, validated and analyzed with the statistical package for social sciences (SPSS) version 17 (SPSS, Inc., Chicago, IL, USA). Data was summarized into means and standard deviations, and percentages were used in the evaluation of the descriptive statistics. Proportions were compared using the chi-square test ($\chi^2$). The analysis of variance (ANOVA, F) or student t-test was used where appropriate to compare geometric mean parasite densities (GMPD) and geometric mean egg density (GMED after $\log_{10}$ transformation of the data. Pearson's correlation coefficient was used to evaluate relationship between variables. Significant levels were measured at 95% confidence level with significant differences recorded at $P < 0.05$.

## 3. RESULTS

### 3.1 Characteristics of the Study Population

A total of 1138 children (609 females and 529 males) were involved in the study. Out of these, 433 were positive for malaria giving an overall prevalence of 38.0%. The majority of the study participants were in the age group 6 to 10 years (68.3%, 777) and the least were in the age group ≤ 5 years (12.4%. 141). The overall prevalence of fever in the study population was (26.6%, 303) and that of anaemia was 44.2% (Table 1). The majority of the participants were found in the semi-urban areas (45.4%, 517) while the least were found in the rural areas (10.3%, 117). With respect to altitude, majority of the participants lived in the upper middle belt (34.7%, 395), while the least lived in the highland areas (10.3%, 117). The overall prevalence of STHs was 2.5% (n = 29) and the overall prevalence of malaria and STH co-infection was 0.9% (n = 10).

### 3.2 Sex, Age, Fever Status and Malaria Parasite

Malaria parasite prevalence was significantly higher ($\chi^2 = 3.2$, $P = 0.041$) in males (40.8%) than females (35.6%). The overall GMPD was 701 with a range of 25 – 80000 parasites/μL of blood. The GMPD/μL of blood was comparable in males (710) and females (692). The prevalence of malaria parasite was significantly highest ($\chi^2$ = 10.4, P = 0.005) in children of the ≤5 years age group (1.8%) and lowest in the age group 10 – 15 years (28.6%). Although not significantly different, the GMPD/μL was highest in pupils in the age group ≤5 years than in the other age groups and higher in those with fever (2214) than those without (2101). Pupils with fever, had a significantly higher ($\chi^2$ = 3.02, P = 0.048) prevalence of malaria parasites (42.2%) when compared with those without fever (36.6%) as shown in Table 2.

## 3.3 Altitude, Levels of Urbanisation and Malaria Parasite

Malaria parasite prevalence was significantly highest ($\chi^2$= 123.4, P <0.001) at the lowest altitude (60.5%) and decreased as altitude increased to a minimum of 15.4% at highland. GMPD/μL of blood was significantly highest (F= 41.147, P<0.0001) in the upper middle belt (2131) and lowest at low altitude (397). With respect to the level of urbanisation, the prevalence of malaria parasite was significantly highest ($\chi^2$ = 84.6, P <0.001) in pupils from urban areas (52.2%) than the semi-urban (29.4%) and rural areas (15.4%). Also, the GMPD/μL of blood was significantly highest (P<0.001) in pupils from the semi-urban areas (1299) than rural (526) and urban (397) areas as shown in Table 3.

## 3.4 Prevalence and Intensity of STHs

Only two species of STHs (*A. lumbricoides* and *T. trichiura*) were found among the study participants. Although not significant *A. lumbricoides* was more prevalent (1.9%) than *T. trichiura (*0.6%). In relation to sex, the prevalence of STHs was similar in males (2.5%) and females (2.6%). Pupils of the ≤5 years age group had the highest prevalence of STH (2.8%) while the lowest was observed in the 10 - 15 years age group (1.8%), but the difference was not statistically significant ($\chi^2$ = 59, P = 0.74).

Those who did not have toilets had the highest prevalence of STHs (33.3%, 1) followed by those who used pit toilets (2.7%, 27) and lastly by those who used flushed toilets (0.7%, 1) and the difference was significant ($\chi^2$ = 13.3, P = 0.001) as shown in Table 4. Pupils in the middle class had the highest prevalence of STH (3.4%, 14) when compared with their poor (2.2%, 15) and rich (0%, 0) counterparts although the difference was not statistically significant ($\chi^2$ = 2.7, P = 0.255).

In relation to species, the prevalence of *A. lumbricoides* and *T. trichiura* were significantly highest ($\chi^2$ = 33.8, P <0.001) in the rural areas (13.7%) than in the semi-urban (2.1%) and urban areas (0.39%). The prevalence of STHs was significantly highest ($\chi^2$ = 67.8, P <0.0001) at the highland (13.7%) and decreased with altitude to the lowest value at the lowland (0.7%).

The geometric mean egg density (GMED) of *A. lumbricoides (*720, epg) was higher than that of *T. trichiura* (259 epg) but the difference was not statistically significant (t = 2 and P = 0.512). GMED was significantly highest (t = 1.8, P <0.0001) in the age group 6 – 10 years (4320 epg) and lowest in the age group 10 – 15 (720 epg) and comparable in males (1625 epg) and females (1226 epg) as shown in Fig. 1.

The GMED was significantly highest (t = 0.41, P <0.0001) in those who had no toilets (2880 epg) and lowest in those who used flushed toilets (720 epg), as shown in Fig. 2. In relation to social class, the GMED was significantly highest (t = 0.33, P = 0.031) in pupils in the middle class (1242 epg), and lowest in pupils of the rich class (96 epg). Regarding urbanisation the GMED was significantly highest (F = 32.9, P <0.0001) in pupils from the urban areas (1488 epg) and lowest in those from rural areas (1210 epg) while with respect to altitude, the GMED was significantly highest (F = 32.9, P <0.0001) in pupils from lowland (1488 epg) and lowest in those from highland (1210 epg) as shown in Fig. 2.

## 3.5 Malaria and STH Helminth Co-infection

The highest prevalence of co-infection was recorded among pupils of the highland (2.6%) and the value decreased with a decrease in altitude to the least in the lowland (0.3%) and the difference was statistically significant ($\chi^2$ = 86.7, P <0.0001). The highest prevalence of single infections was recorded in pupils of the lowland areas (60.5%) when compared with those at other altitudes and the difference was significant ($\chi^2$ = 83.7, P = <0.0001). The level of single infection, co-infection and no infection varied significantly with altitude ($\chi^2$ = 71.9, P = <0.0001) as shown in Table 5.

**Table 1. Baseline characteristics of the study population, Fako Division, Southwest Cameroon, 2014**

| Factor | Category | Number examined | Proportion % (n) |
|---|---|---|---|
| Sex | Females | 609 | 53.5 |
| | Males | 529 | 46.5 |
| Age (years) | ≤5 | 141 | 12.4 |
| | 6 - 10 | 777 | 68.3 |
| | >10 | 220 | 19.3 |
| Level of urbanisation | Rural | 117 | 10.3 |
| | Semi-urban | 517 | 45.4 |
| | Urban | 504 | 44.3 |
| Altitude | Highland | 117 | 10.3 |
| | Upper middle belt | 395 | 34.7 |
| | Lower middle belt | 325 | 28.6 |
| | Lowland | 301 | 26.5 |
| Prevalence of malaria | | 1138 | 38.0 (433) |
| Prevalence of fever | | 1138 | 26.6 (303) |
| Prevalence of anaemia | | 1138 | 44.2 (503) |
| Prevalence of STH | | 1138 | 2.5 (29) |
| Prevalence of malaria-STH co-infection | | 1138 | 0.9 (10) |

**Table 2. Malaria parasite prevalence and GMPD with respect to sex, age and fever status**

| Factor | Category | Number examined | Prevalence of malaria % (N) | Test statistics | GMPD/ µL of blood | Range/ µL of blood | Test statistics |
|---|---|---|---|---|---|---|---|
| Sex | Females | 609 | 35.6 (217) | $\chi^2$ = 3.2<br>P = 0.041 | 692 | 25 - 80000 | t = 2.058<br>P = 0.152 |
| | Males | 529 | 40.8 (216) | | 711 | 80 - 5333 | |
| Age in years | ≤ 5 | 141 | 41.8 (59) | $\chi^2$ = 10.4<br>P = 0.005 | 862 | 80 - 20000 | F = 0.933<br>P = 0.39 |
| | 6 - 10 | 777 | 40.0 (311) | | 672 | 40 - 80000 | |
| | > 10 | 220 | 28.6 (63) | | 716 | 25 - 16000 | |
| Fever status | Fever | 303 | 42.2 (128) | $\chi^2$ = 3.02<br>P = 0.048 | 2214 | 666 - 80000 | t = 0.39<br>P = 0.70 |
| | No fever | 835 | 36.4(304) | | 2101 | 25 - 5600 | |
| Total | | 1138 | 38 (433) | | 701 | 25 - 80000 | |

**Table 3. Malaria parasite prevalence and GMPDs with respect to altitude and level of urbanisation**

| Factor | Category | Number examined | Malaria parasite prevalence % (N) | Test statistics | GMPD /µL of blood | Range/µL of blood | Test statistics |
|---|---|---|---|---|---|---|---|
| Altitude | Lowland | 301 | 60.5 (182) | $\chi^2$ = 123.4<br>P < 0.001 | 397 | 40 - 80000 | F = 41.1<br>P <0.001 |
| | Lower middle belt | 325 | 42.2 (137) | | 2131 | 218 -16000 | |
| | Upper middle belt | 395 | 24.3 (96) | | 447 | 25 - 5000 | |
| | Highland | 117 | 15.4 (18) | | 526 | 140 - 1500 | |
| Level of urbanisation | Rural | 117 | 15.4 (18) | $\chi^2$ = 84.6<br>P <0.001 | 526 | 140 - 1500 | F = 40.9<br>P <0.0001 |
| | Semi-urban | 517 | 29.4 (152) | | 1299 | 25 - 16000 | |
| | Urban | 504 | 52.2 (263) | | 397 | 40 - 80000 | |
| Total | | 1138 | 38 (433) | | 701 | 25 - 80000 | |

**Table 4. Prevalence of STH in the various categories of children**

| Variable | Category | Number examined | Prevalence of STH % (N) | Test statistics |
|---|---|---|---|---|
| STH Species | *A. lumbricoides* | 1138 | 1.9 (22) | $\chi^2 = 1.67$ |
| | *T. trichiura* | 1138 | 0.6% (7) | P = 0.51 |
| Sex | Females | 609 | 2.6 (16) | $\chi^2 = 0.033$ |
| | Males | 529 | 2.5 (13) | P = 0.86 |
| Age group (Years) | ≤ 5 | 141 | 2.8 (4) | $\chi^2 = 0.59$ |
| | 6 – 10 | 777 | 2.7 (21) | P = 0.74 |
| | > 10 | 220 | 1.8 (4) | |
| Anaemia | Yes | 503 | 3.4 (17) | $\chi^2 = 2.21$ |
| | No | 615 | 2.0 (12) | P = 0.14 |
| Malaria parasite | Positive | 433 | 2.1 (9) | $\chi^2 = 0.40$ |
| | Negative | 705 | 2.8 (20) | P = 0.66 |
| Socio-economic status | Poor | 683 | 2.2 (15) | $\chi^2 = 0.2.7$ |
| | Middle class | 408 | 3.4 (14) | P = 0.26 |
| | Rich | 47 | 0 (0) | |
| Toilet type | Flush | 134 | 0.7 (1) | $\chi^2 = 13.3$ |
| | Pit | 1001 | 2.7 (27) | P = 0.001 |
| | No toilet | 3 | 33.3 (1) | |
| Altitude | Lowland | 301 | 0.7 (2) | $\chi^2 = 67.8$ |
| | Lower middle belt | 325 | 1.2 (4) | P <0.001 |
| | Upper middle belt | 395 | 2.0 (8) | |
| | Highland | 117 | 13.7 (16) | |
| Urbanization | Rural | 117 | 13.7 (16) | $\chi^2 = 33.8$ |
| | Semi-urban | 517 | 2.1 (11) | P <0.001 |
| | Urban | 504 | 0.39 (2) | |
| Total | | 1138 | 2.5 (29) | |

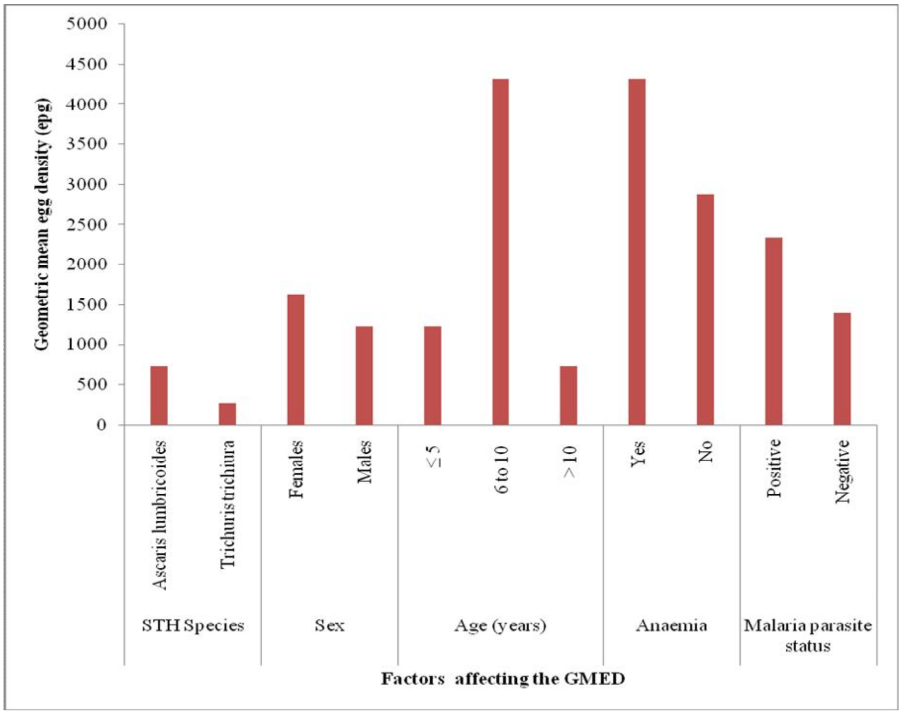

**Fig. 1. GMED of STHs in relation to sex, age, anaemia and malaria parasite status**

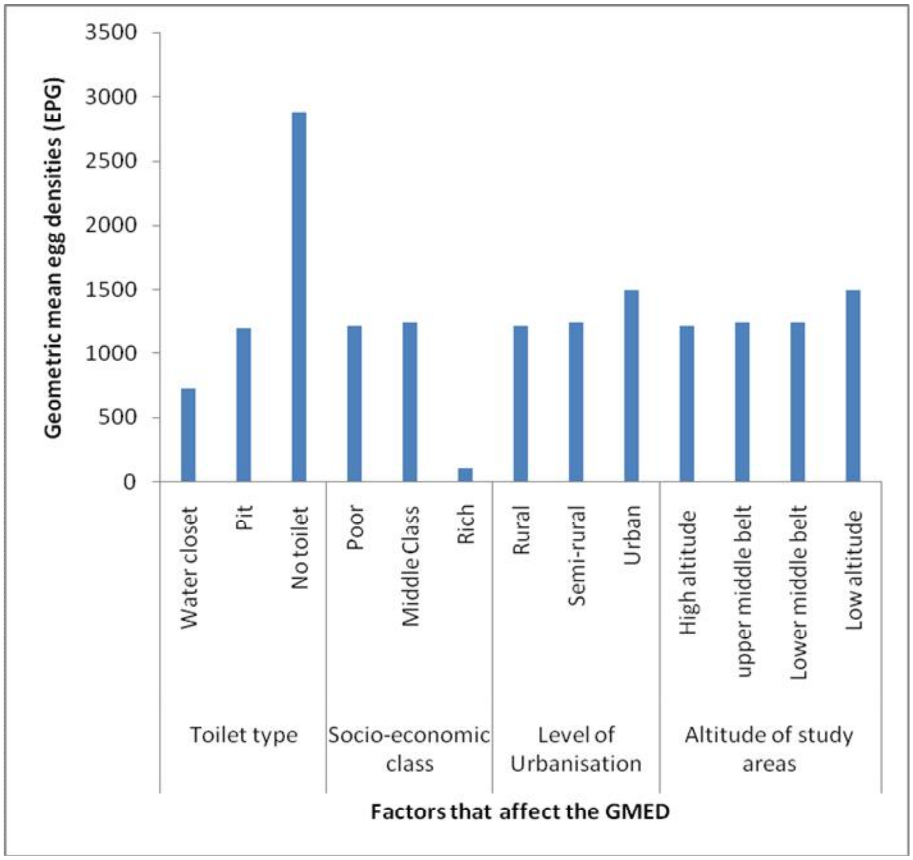

**Fig. 2. GMED of STHs in relation to toilet type, social class, level of urbanisation and altitude**

**Table 5. STH co-infection and single infection in relation to altitude and urbanization**

| **Factor** | **Category** | **Number examined** | **Co-infection (malaria parasitaemia + STHs)** | **Single infection (malaria parasitaemia / STHs) % (N)** | **No infection** | **Test statistics** |
|---|---|---|---|---|---|---|
| Altitude | Lowland | 301 | 0.3 (01) | 60.5 (182) | 39.2 (118) | $\chi^2$ = 71.9<br>P <0.0001 |
| | Lower middle belt | 324 | 0.9 (3) | 25.0 (81) | 74.1 (240) | |
| | Upper middle belt | 395 | 1.15 (3) | 38.5 (152) | 60.8 (240) | |
| | Highland | 117 | 2.6 (3) | 23.9 (28) | 73.5 (86) | |
| Level of significance | | | $\chi^2$ = 86.7<br>P <0.0001 | $\chi^2$ = 83.7<br>P <0.0001 | | |
| Level of urbanisation | Rural | 117 | 2.6 (3) | 23.9 (28) | 73.5 (86) | $\chi^2$ = 72.1<br>P <0.0001 |
| | Semi-urban | 516 | 1.2 (6) | 29.5 (152) | 69.4 (358) | |
| | Urban | 504 | 0.2 (1) | 52.2 (263) | 47.6 (240) | |
| Level of significance | | | $\chi^2$ = 72.2<br>P <0.0001 | $\chi^2$ = 76.4<br>P <0.0001 | | |
| Total | | 1137 | 0.9 (10) | 39.0 (443) | 60.2 (684) | |

The prevalence of co-infection varied significantly ($\chi^2$ = 72.2, P <0.0001) with level of urbanisation with the highest level of co-infection in the rural areas (2.6%) and the lowest level in the urban areas (0.2%). Contrary to co-infection, the highest level of single infection was recorded in the urban areas (52.2%) and the lowest level in the rural areas (23.5%) and the difference was statistically significant ($\chi^2$ = 76.4, P <0.0001) as shown in Table 5. There was also a statistically significant difference between co-infection, single infection and no infection with respect to level of urbanisation ($\chi^2$ = 72.1, P <0.0001).

## 4. DISCUSSION

Where human malaria and STHs are co-endemic, children often harbour the heaviest infections and suffer much of the associated morbidity [3,17]. Several factors predispose children to both malaria and STH infections and some of these include climatic conditions (humidity), poverty and sanitary practices [4,18].

The prevalence of malaria in this study was 38.0%, a value lower than that reported by other researchers in previous years in Fako Division [13,23,24]. However, this prevalence is higher than expected considering the control measures that have been put in place by MINSANTE Cameroon which include free malaria screening and treatment among the 0 - 5 years age group available in public and privately owned hospitals, clinics and health centres, the subsidization of the prices of anti-malarial drugs for other age groups as found in the pro-pharmacies in Cameroon, the free distribution of insecticide-treated bed nets (ITNs) as well as LLINs for households and massive sensitization on radio and television [31,32].

The prevalence of malaria decreased with an increase in the age of the pupils with the least in the age group 11-15 years. This could be due to the acquisition of protective immunity after repeated exposure to malaria infection [16,33]. Children in this age group are also likely to be more knowledgeable about malaria preventive measures such as protective clothing. Such children can also undertake physical killing of malaria vectors [34].

The prevalence of malaria was significantly highest in urban areas when compared with the semi-urban and rural areas. The urban areas (Limbe and Mutengene) are found at the lowest altitude where climatic conditions such as high temperatures, humidity and rainfall which all favour the rapid growth of the *Anopheles* mosquitoes (and consequently malaria transmission) might have played a major role in the parasite prevalence. These urban areas also have relatively crowded neighbourhoods where houses are constructed in close proximity to one another. Thus, infected mosquitoes do not need to fly for long distances from their breeding sites to get attracted to stimuli emanating from the human body where they can bite and inoculate the parasites [35]. The close proximity of houses in the urban areas is an indication that more humans are found within a small geographical area. As such during intermittent feeding by mosquitoes [36], they can feed on several persons in one night and if infected the parasite can be transmitted to several people in one night. The reverse is the case in the rural areas (Bova and Bonakanda) where houses are more dispersed. A reduction in the number of mosquito bites logically implies a reduction in transmission leading to a reduction in malaria prevalence.

Additionally, the fact that humans out-number animals in the urban localities and most *Anopheles* vectors prefer human blood to animal blood [37,38] could also explain the higher prevalence of malaria parasites in these areas. Although it was not investigated in this study, domestic animals such as goats were seen grazing in farms and around houses in the rural areas. At night some *Anopheles* mosquitoes might have been attracted to feed on these domestic animals, thus reducing the mosquitoes' chances of feeding on humans. This might have contributed to the lower prevalence of malaria parasites in the rural areas. This finding however contradicts the postulations of Keating et al. [39] who stated that increased urbanisation reduces the number of breeding places for *Anopheles* mosquitoes and malaria transmission. They however linked this to more polluted water in urban areas which is improper for the *Anopheles* mosquito development.

Malaria prevalence was highest at the lowest altitude and decreased as altitude increased. At low altitudes, the malaria parasite usually completes its sporogonic cycle within a shorter time in the mosquito vector due to higher temperatures that favour the rapid growth of both parasites and mosquitoes and thus increase malaria transmission [40]. There are also more streams, rivers, springs and stagnant water at low altitudinal areas in this study area and these serve as potential breeding sites for mosquitoes.

Environmental conditions vary with change in altitude. At high altitudes, the life expectancy for *Anopheles* is very short [41]. They therefore face more adverse climatic conditions which reduce their longevity compared with that recorded at lower altitudes [41] and thus reduce their ability to transmit malaria. It has been reported that low temperatures reduce vector dynamics [42], their reproductive fitness [10] and thus malaria transmission potentials. Localities found at a high altitude in this study hardly had stagnant water around homes due to swift run-offs after rainfall. The low prevalence of malaria at high altitudes agrees with the stipulations of Tchuinkam et al. [10] who stated that highland areas of Africa are mostly hypoendemic for malaria.

The overall prevalence of fever in the study population was 26.6% and the prevalence of malaria parasites was significantly higher in febrile pupils than their afebrile counterparts. The GMPD had the same pattern as that of malaria parasite prevalence though without any significant difference. These results indicate the presence of both asymptomatic and symptomatic malaria among apparently healthy school children. This observation agrees with the findings of Ojurongbe et al. [1] in Nigeria who explained that the presence of fever among participants in a malaria study is characteristic of both symptomatic and asymptomatic infection in malaria endemic regions.

The overall prevalence of intestinal helminths (2.5%) was lower than that reported by Ndamukong et al. [22]; Achidi et al. [12], Kimbi et al. [24] and Makoge et al. [16] who all recorded higher prevalence values of STHs in the South West Region of Cameroon. The low prevalence recorded in this study when compared with previous studies is probably an indication of improved hygienic conditions. In these study sites most of the children had toilets thus reducing possible faecal soil contamination. In other parts of the world other authors have also recorded higher prevalence' of STH than that of this study [1,42,43,44,45,46]. The low prevalence recorded in this study when compared with other countries could be due to the deworming campaigns implemented by the Ministry of Public Health in Cameroon organised every three to six months [5,6,7]. This policy calls for the free deworming of all school age children by community agents and sensitization of people of all other ages to deworm themselves. This is further enhanced by the subsidization of drugs in pro-pharmacies and health centres all over the country and education on television and Radio [5,6]. This control strategy is in line with WHO's control intervention which is based on the periodic administration of anthelminthics to groups of people at risk, supported by the need for improvement in sanitation and health education [13]. WHO also recommends annual treatment in areas where prevalence rate of soil-transmitted helminthiasis is between 20% and 50%, and a bi-annual treatment in areas with prevalence rates of over 50% [13].

Only two species of STHs (*A. lumbricoides and T. trichiura*) were found among the study participants and *A. lumbricoides* was more prevalent (1.9%) than *T. trichiura (*0.6%) *A. lumbricoides* and *T. trichiura* are known to have a worldwide distribution especially in the tropics and subtropics [13,47]. Again, the fact that both *A. lumbricoides* and *T. trichiura* have basically the same ecological requirements and transmission routes may also explain their co-existence in the study area. This finding agrees with the findings of Makoge et al. [16] in Mbonge, Southwest Cameroon. Hookworms were absent among the pupils in this study. A low prevalence of hookworms had earlier been recorded by Nkuo-Akenji et al. [23] in the Mount Cameroon area and this was linked to the soil type.

There was a significantly higher prevalence of *A. lumbricoides* and *T trichiura* in the rural areas (13.6%) than in the urban areas (0.39%, 2) and the prevalence of co-infection had a similar pattern. The high altitude areas in this study were rural areas and had significantly highest prevalence of STH compared with the middle belt and lowland. The prevalence of STHs has been linked to poverty and improper hygiene. This could be due to the fact that the rural areas in Fako Division are occupied mostly by people of the middle and the poor socio-economic classes (who had a higher prevalence of STHs than the pupils in the rich class in this study). This could be due to the fact that the main activity in the rural areas is subsistence farming and the generally low level of sanitation in such areas exposes the inhabitants to STH infections. It is worth noting that sometimes unwashed fruits are eaten on farms without washing hands thus pre-disposing such persons to STHs [13,48].

In relation to social class, the lowest prevalence of STH was recorded among the pupils in the rich class (0%), than the poor (2.2%) and lastly the middle class (3.4%). This could be explained by the fact that rich people often use water closet toilets and as such have available water and hand sanitizers for hand washing after using the toilet than poor people. The rich also live in cleaner environments than the middle and poor class pupils. A significant difference (P = 0.001) in prevalence of STH based on toilet type was observed and those who did not have toilets had the highest prevalence of STH while those who used flushed toilets had the lowest. The concept of personal hygiene may explain why the prevalence of STHs was higher among those who did not have toilets or used pit toilets than those who used water closet toilets [18]. The use of water-closet toilets indicates the availability of water. As such hand-washing is made easier and faeco-oral route transmission of helminth eggs is reduced.

The prevalence of malaria and STH co-infection observed in this study was lower (0.9%) than that recorded by Achiidi et al. [12], Kimbi et al. [24] and Makoge et al. [16]) in Cameroon; Ojurongbe et al. [1], Dada-Agdebola et al. [49] and Abanyie et al. [50] in Nigeria; and Fleming et al. [44] in Brazil who all recorded malaria-STHs co-infections ranging from 4.3% to 60.6%. This low prevalence could be due to increased awareness of preventive measures and control efforts against both malaria and STH infections over the years.

The findings of this study indicated that the younger pupils harboured a higher level of both single and mixed infection(s) than the older pupils and this is likely because of their playing habits on faecally contaminated ground [51]. The highest level of co-infection was recorded among pupils in the high altitude areas and reduced as altitude decreased. The distribution coincided with the distribution of the STHs. The highest level of single infections was recorded in the lowland areas and decreased as altitude increased. This corresponded to the distribution of malaria and most probably for the same reasons.

## 5. CONCLUSION

From this study it was concluded that the prevalence of malaria in the study population was high and reduced with altitude indicating that control measures have to be intensified against malaria especially in the lowland areas. The prevalence of STHs was generally low in Fako Division indicating that the deworming campaigns by The Ministry of Public Health in Cameroon are yielding positive results. The prevalence of STHs and co-infections of malaria and STHs were higher in rural areas than in urban areas indicating that the level of urbanisation has an influence on disease prevalence.

## CONSENT

All pupils were issued consent/assent forms to seek for their parents' approval. Pupils were accepted for screening when they brought back signed informed consent / assent forms following the approval of their parents / guardians.

## ETHICAL APPROVAL

An ethical clearance was obtained from the South West Regional Delegation of Public Health. Administrative clearances were obtained from the Regional Delegation of Basic Education as well as from the Catholic Education Board. All pupils were issued informed consent forms to seek for their parents' approval. Pupils were only included in the study when they brought back signed informed consent forms following the approval of their parents and succumbed to the sample collection procedure.

## COMPETING INTERESTS

Authors have declared that no competing interests exist.

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