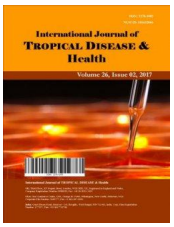

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Malaria and Geohelminthiasis: Their Prevalence and Impact on Iron Stores Parameters of School Aged Children 5 to 10 Years in the Buea Municipality, Cameroon

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Authors' contributions

This work was carried out in collaboration between all authors. All authors read and approved the final manuscript.

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ABSTRACT

Aims: To determine the prevalence of malaria and geohelminthiasis and to assess their impact on iron stores of school children around Buea Municipality, Cameroon.
Study Design: It was a cross-sectional comparative study.
Place and Duration of Study: The study was carried out amongst primary school children in Buea Municipality, Cameroon, between the 1st of May to 27th of July 2015.
Methodology: We included 388 primary school children (188 males, 200 females; age range 5-10 years). Structured questionnaires were used to obtain sociodemographic data and venepuncture technique was used to collect blood samples. Stool samples were collected in stool containers. Malaria prevalence, red cell indices and iron studies were determined using Giemsa-stained thin and thick films, automated haematology analyser and ELISA methods respectively. Quantitative estimation geohelminthe ova was done using the Kato-katz method. The participants were divided into different infectious groups.
Results: The overall prevalence of malaria and geohelminthiasis were 38.4%, 149 (95% CI: 33.2, 43.6) and 19.8%, 77 (95% CI: 15.8, 23.8) respectively. There was a significant increase in ferritin levels (P=.03) and soluble transferrin receptors (P<.001) in the infection groups when compared to the control group. Conversely, haemoglobin levels in the various infection groups were significantly reduced (P<.001) when compared to the control group.
Conclusion: Malaria and geohelminthiasis continues to infect school children with some of the iron store parameters being significantly raised across the various infection groups including malaria only and geohelminthiasis only groups, with haemoglobin levels significantly reduced when compared to the control group. Anaemia caused by malaria is as result of iron sequestration in the storage compartment.

Keywords: Malaria; geohelminthiasis; iron store; ferritin; soluble transferrin receptor.

1. INTRODUCTION

Malaria still stands out as one of the greatest well-known parasitosis of the entire human race, posing a threat to approximately more than 3.3 billion individuals in the whole world in 97 different countries [1]. It is a problem to the general public with children and pregnant women who live in the areas of Africa with widespread infections especially Sub-Saharan Africa being those mostly affected as well as non-immune travellers into these areas. Almost all deaths due to malaria occur in this continent and children below 5years of age are responsible for more than 78% of all deaths [1]. Africa accounts for more than 90% of world's death tolls as a result of malaria.

Geohelminthes are soil-transmitted parasites whose immature stages (eggs) need a growth period or incubation on the earth prior to the time that they can infect humans [2]. The commonest and well known of such parasites are Hookworm, *Strongyloides stercoralis*, *Trichuris trichiura* and *Ascaris lumbricoides* [3]. The prevalence of geohelminthe infection world-wide is about 2 billion with school going children being the ones that suffer most with the greatest number featuring in children in Sub-Saharan Africa (SSA) [4].

Iron is an essential nutrient for nearly every living organism including humans and the malaria parasite. Iron impacts a broad range of biological processes; including host and parasite cellular function, erythropoiesis and immune function [5]. Access to iron is particularly important in the context of host-pathogen interactions. When confronted with infection and inflammation the human host reallocates its iron reservoirs in an effort to deprive invading pathogens of iron. The human protein hepcidin—a rheostat of systemic iron homeostasis—signals the body to decrease absorption of iron in the proximal duodenum and orchestrates the movement of iron from serum into storage within the liver and macrophages [6]. As a result of reduced serum iron, erythropoiesis—a process exquisitely sensitive to iron levels—slows in the face of infection as well as inflammation. The human host's active reduction in bioavailable iron protects against a wide range of pathogens [7].

Geohelminthes and *Plasmodium* species can both infect the same individual as they share the same environmental conditions that predispose them to co-infection, whose prevalence is high in Sub-Saharan Africa [8]. Twenty five percent of school-aged children in SSA, were estimated to be at risk of *P. falciparum* and hookworm co-infection [9]. It has been reported that

geohelminthiasis robs the body of some important nutrients thus leading to the sub-normal levels of such nutrients, stunted growth, mental retardation and poor academic capability [10]. Geohelminthic infections are prevalent in the tropical regions and contribute to anaemia by inhibiting iron absorption and metabolism as well as other nutrients such as vitamins which are present in very small amount in the body or can even cause them to be lost from the body [11]. *P. falciparum* also leads to anaemia as a result of increased erythrocyte destruction [12]. In Cameroon, malaria and geohelminthiasis are common and co-exist. Young children, expectant mothers and children of school-going age are most affected by the combined burden of these infections and their consequences on their health because of their vulnerability [13-16]. Even with some studies carried out in this area on asymptomatic malarial and geohelminthiasis on children that stay in Buea Municipality of the Mount Cameroon area, in Cameroon, it is not quite clear if such infections or their co-infection have any impact on the iron stores parameters of such children [15,17-18].

2. MATERIALS AND METHODS

2.1 Study Design and Ethical Issues

The study was a cross-sectional comparative analytical study. The study participants were divided into four groups viz those infected with malaria only, geohelminthiasis only, malaria co-infection with geohelminthiasis and the control group (negative for both infections). Ethical approval was obtained from the faculty of health sciences institutional review board (IRB) of the University of Buea (n^o: 2015-02-0368) while administrative clearances to collect samples were collected from south west regional delegations of public health and basic education, inspector for basic education and catholic education secretary for buea diocese. These were presented to the different head teachers for endorsement. Informed consent was gotten from the pupils' parents and/or guardians through a consent form which was sent to them one week before sample collection; to read, understand-ask questions and approve or disapprove their child/ren's participation in the study. The pupils' assents were sought and a structured questionnaire was filled through an interview prior to sample collection.

2.2 Study Area and Population

This study was conducted in the Buea Municipality with the participants drawn from primary schools in Tole, Molyko, Muea and Bwitingi with geographical coordinates and height above sea levels of these study sites being 9°24'E/4°11N; 636m, 9°30'E /4°17'N; 533m, 9°30'E/ 4°13'N; 545m and 9°28'E/ 4°17'N; 647m respectively (Fig. 1). Both males and females school children aged 5 to 10years were selected using a volunteer sampling technique. This municipality is found on the eastern slope and at the bottom of Mount Cameroon-the highest mountain in West and Central Africa. The relief is hilly with only 10% of the total land being fairly undulating [19]. The climate is that of the equatorial rainforest since it is located around the equator. During the entire year there is the spread of malaria with the intensity being very high during the rainy season and at lower altitudes. Majority of the malaria parasites species observed within this area is *P. falciparum* [20]. The plank houses are the major housing type in the rural area while cement block houses predominate in the urban setting. The major occupation around this vicinity is farming and the populace gets its major livelihood from agriculture [21].

2.3 Sample Size

The sample size was calculated using the formula postulated by Bryan [23]:

$$n = \frac{t^2 p(1-p)}{e^2}$$

Where n =sample size, t= confidence level of 95% (standard value of 1.96); p= estimated prevalence in the study area (59%) as published by Achidi *et al*. [24]; e = margin of error at 5 per cent (standard value of 0.05). The optimum sample size was estimated at 384. To achieve this sample size a total of 900 participants' information with consent form attached were distributed to pupils in the four different study sites. Out of the 900 consents forms given out, 391 returned signed consent forms. Those included in the study were those whose parents/guardians consented by signing the consent form and who succumbed to the sample collection procedure. From the number that returned the signed consent form, samples were collected from 388 pupils.

2.4 Selection Criteria

The following were excluded from the study: participants below 5years and above 10 years, those whose parents/guardians refused them from participating and those from other primary schools that were not selected for the study. Those who had phobia for venipuncture even if the parents had consented were excluded.

2.5 Data Collection

Data for the study was collected using a structured questionnaire to obtain the socio-demographic data and risks factors of malaria and geohelminthiasis of the study participants. For each pupil, blood and stool samples were collected.

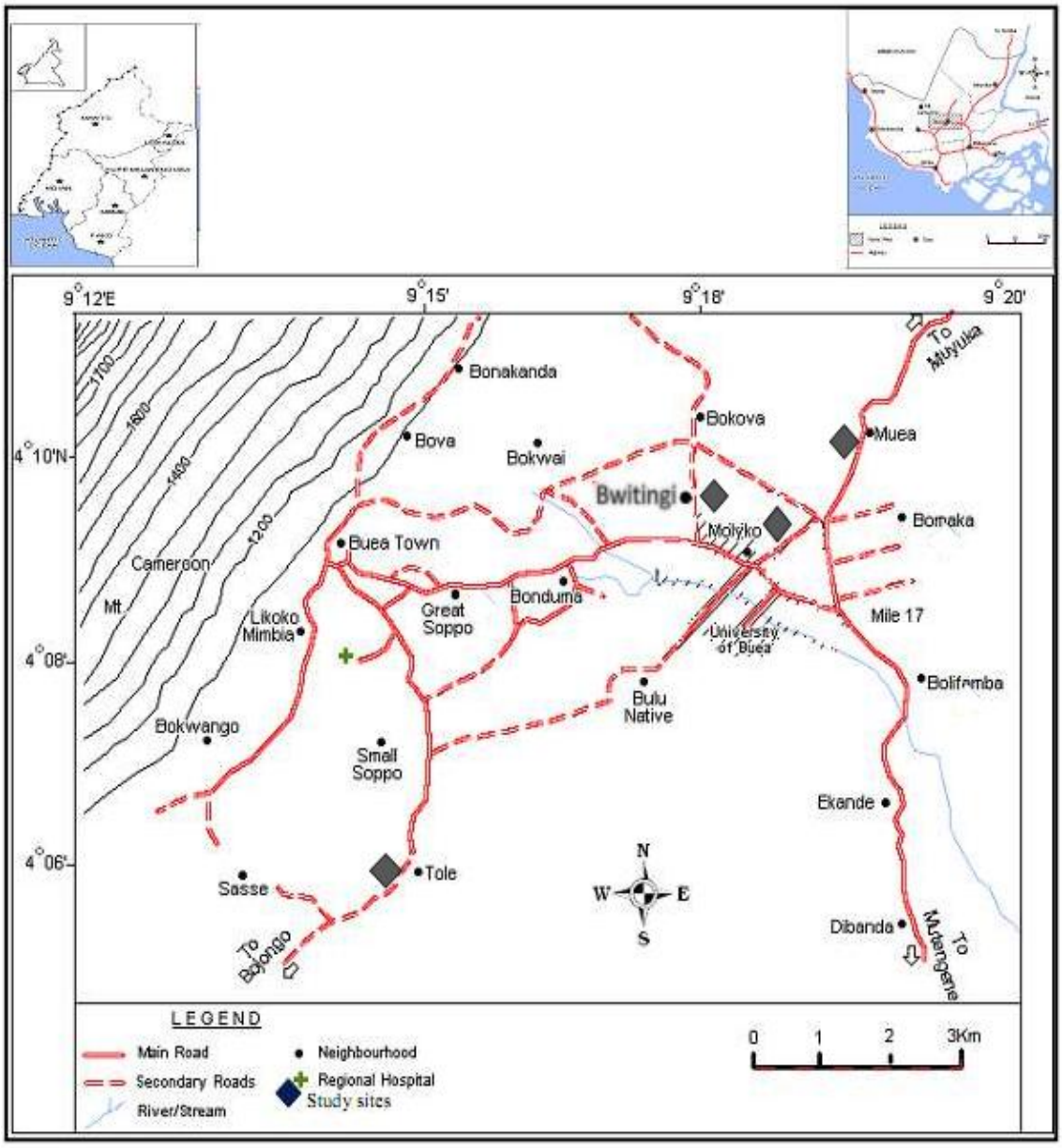

Fig. 1. Spatial Layout of Buea Sub-Division. Insert Location of the South West Region in Cameroon and Location of Fako Division in the South West Region of Cameroon (Adapted from the Map of Buea [22])

2.5.1 Blood sample collection

Five millilitres of whole blood was collected from each of the study participants by venipuncture, a drop of the blood was used to prepare a thick and thin film on a pre-labelled microscope slide, two milliliters was dispensed into potassium EDTA anticoagulated vacutainer tube (BD Vacutainer Systems, Plymouth, UK) of three milliters capacity for full blood count and three milliliters was transferred into a dry glass vacutainer tube (BD Vacutainer Systems, Plymouth, UK) after which it was centrifuged and the serum used for iron studies. These samples were placed in a cooler with ice packs and transported to the Malaria Research Laboratory of the University of Buea for full blood count analysis on that same day and serum was extracted from the dry vacutainer tube aseptically after centrifugation at 3000rpm for 5minutes and stored at -70ºC for iron studies.

2.5.2 Stool sample collection

Stool containers enclosed in a transparent polyethene bag were given to the study participants whose parents had consented and it was explained to them during a health talk on how to collect the stool samples. The stool samples were collected in the stool containers and enclosed in the transparent polyethene bag. Each sample was collected from the pupil and labeled accordingly during blood collection and transported to the laboratory for analysis within 12 hours of collection.

2.6 Laboratory Methods

A drop of blood collected by venipuncture was used to prepare thick and thin films on a pre-labelled microscope slide for the assessment of malaria parasite and density using light microscopy as described by Cheesbrough [3]. The blood films were examined under the oil immersion (X100) objective of the Olympus Microscope (Olympus Optical Co., Ltd, Japan). Thick films were considered positive when there was the presence of asexual forms and/or gametocytes. Parasites were counted against 200 leucocytes and expressed as parasites per microlitre of blood, using the total leucocytes counts generated from haematological analysis. Slides were declared negative after observing at least 100 high power fields without detecting any parasites. The slides were cross-checked and confirmed by a WHO certified malaria microscopist. Full blood count was determined using the Automated Haematology Analyser (URIT3300, Guangxi, China), with reagents bought from the same company and following the manufacturer's instructions. Only some red cell associated parameters (Haemoglobin [Hb] and red cell indices such as mean cell volume (MCV), mean cell haemoglobin (MCH) and mean cell haemoglobin concentration (MCHC)]) and total leucocytes counts were obtained. Anaemia was defined as Hb < 110g/l, microcytosis as MCV < 80fl and hypochromia as MCHC <31.5g/dl or MCH <27pg [3]. The quantitative determination of serum ferritin and soluble transferrin receptors (sTfR) was carried out using reagents purchased from Diagnostic Automation/Cortez Diagnostics, Inc., California, USA. The ELISA method of measurement was employed and the analyses were done in the Biotechnology Laboratory of the University of Buea. The instructions provided by the manufacturer of the test kits (Diagnostic Automation/Cortez Diagnostics, Inc., California, USA) were strictly followed. Reference range for sTfR was 1.8 – 4.6 mg/L (25.13 – 64.22 nmol/L) while that for ferritin was 32.0-501.0µg/l for males and 3.5-223.5µg/l for females. The stool samples were prepared and processed using the Kato Katz technique employing a 41.7mg template. In the technique faeces is pressed through a mesh screen to remove large particles. A portion of the sieved sample is then transferred to the hole of a template on a slide. After filling the hole, the template is removed and the remaining sample (approx. 41.7mg depending on size of template) is covered with a piece of cellophane soaked in glycerol (glycerine). The glycerol 'clears' the faecal material from around the eggs. The eggs are then counted and the number calculated per gram (g) of faeces [3]. The prepared slide was mounted and screened using the X10 objective and the X40 objective of the Olympus Microscope was used to confirm the type of egg seen using the identification charts of Cheesbrough [3] The prepared smears were examined within 1 hour of preparation to avoid missing hookworm ova. Duplicate smears were prepared from each specimen. Standard operating procedure was used for stool collection and processing so as to maintain a good quality study as described by the World Health Organization [25,3]. An experienced parasitologist; who was previously blinded to the previous results confirmed 10% of randomly selected slides each day after laboratory diagnosis.

2.7 Statistical Analysis

Data was entered into spread sheets using Microsoft Excel 2007, validated and analysed

using Statistical Package for Social Science (SPSS) version 20 (SPSS, Inc., Chicago, IL, USA) with use of frequency tables and figures to present the results. The data was summarised as mean, 95% confidence interval and standard deviation while percentages were used in the evaluation of the descriptive statistics. The analysis of variance (ANOVA) was used to compare the red cell and iron parameters in the various groups. The margin of error used was $P<0.05$.

3. RESULTS

Table 1 shows the socio-demographic characteristics of the study participants. A total of 388 school children aged 5 to 10years voluntarily participated in the study. Out of these 48.5% (188/388) were males and 51.5% (200/388) were females. Two hundred and eleven (54.3%) came from the urban setting while 45.7% (177/388) were drawn from the rural setting of the municipality. The mean age for the pupils was 8.09±1.57years. Most of the pupils 62.4% (242/388) were of the age range 8 to 10years compared to those of the age group 5 to 7years 37.6% (146/388). Farming was the predominant paternal occupation of the pupils (45.1%, 175/388). This was closely followed by those whose paternal occupation was business (39.3%, 153/388). The least here were those whose paternal occupation was industrial workers. The two principal housing types in this municipality were block and plank houses. Majority (60.6%, 235/388) of the pupils lived in the traditional plank houses while the remaining proportion (39.4%, 153/388) lived in block houses. Again majority 86.5%, (336/388) of the participants were afebrile as defined by temperatures less than 37.5ºC, while the remaining proportion were febrile (temperature greater or equal to 37.5ºC).

Table 2 shows the overall iron, red cell indices and parasitological parameters of the study population. The overall mean haemoglobin and prevalence of anaemia (Hb <110g/l) in the study population was 103.2±13.1g/l and 71.2%, 274(95% CI: 66.8, 75.6) respectively. Also the overall mean of ferritin and sTfR were 68.64±46.31 µg/l and 2.08±0.62 µg/ml respectively. Again the prevalence of iron deficiency by ferritin using cutoff <30 µg/l and sTfR values ≥1.76µg/ml were 20.5%, 80 (95% CI: 15.2, 25.3) and 66.3%, 257 (95% CI: 59.7, 70.8) respectively. The prevalence of malaria and geohelminthiasis amongst the studied subjects was 38.4%, 149 (95% CI: 33.2, 43.6) and 19.8%, 77 (95% CI: 15.8, 23.8).

Table 1. Socio-demographic characteristics of study population

Parameters	Mean±SD	% Proportion
Age in years (n=388)	8.09±1.57	
5-7 (n=146)		37.6
8-10 (n=242)		62.4
Gender (n=388)		
Male (n=188)		48.5
Female (n=200)		51.5
Urbanisation (n=388)		
Urban (n=211)		54.3
Rural (n=177)		45.7
House Type (n=388)		
Plank (n=235)		60.6
Block (n=153)		39.4
Febrile Status (n=388)	36.93±0.49	
Febrile (n=52)	37.62±0.30	13.5
Afebrile (n=336)	36.82±0.49	86.5
Paternal Occupation (n=388)		
Farming (n=175)		45.1
Government Worker (n=33)		8.6
Industrial Workers (n=27)		7.0
Business (n=153)		39.3

Table 2. Iron, red cell indices and parasitological parameters of study population

Parameters	Mean±SD	% Prevalence*
Ferritin in µg/l (n=388)	68.64±46.31	
Abnormal (Ferritin<30µg/l, n=80)	23.40±6.39	20.5 (15.2, 25.3)
sTfR in µg/ml (n=388)	2.08±0.62	
Abnormal (sTfR>1.76µg/ml, n=257)	2.41±0.25	66.3 (59.7, 70.8)
Haemoglobin in g/dl (n=388)	103.2±13.1	
Anaemia (Haemoglobin<110g/l, n=276)	97.7±9.4	71.2 (66.8, 75.6)
Mean Cell Volume in fl (n=388)	77.70±7.01	
Microcytosis (MCV<80fl, n=150)	70.83±5.60	38.7 (33.0, 44.4)
Mean Cell Haemoglobin in pg(n=388)	24.98±2.10	
Hypochromia (MCH<27pg, n=338)	24.51±1.70	87.0 (83.0, 91.0)
Mean Cell Haemoglobin Concentration	31.05±3.59	
Hypochromia (MCHC<31.5g/dl, n=175)	29.96±3.23	46.6 (42.4, 50.8)
Malaria (n=388)		
Positive (n=149)	1134±12442	38.4 (33.2, 43.6)
Negative (n=239)	-	61.6 (56.6, 66,6)
Geohelminthiasis (n=388)		
Positive (n=77)	3126±4471	19.8 (15.8, 23.8)
Negative (n=311)	-	80.1 (76.3, 84.2)
Malaria-Geohelminthiasis Co-infection (n=37)		9.5 (5.2, 13.8)

** % Prevalence (95% confidence interval), sTfR-soluble transferrin receptor, µg/l-microgram per litre, µg/ml-microgram per milliliter, g/l-gram per litre, fl-femtolitres, pg-picogram,*

Table 3. Mean levels and standard deviations of ferritin, sTfR, haemoglobin and red cell indices of children based on infection states

Parameters	Malaria (n=112)	Geohelminthiasis (n=40)	Co-infection** (n=37)	Control (n=197)	P-value
Ferritin (µg/l)	**81.44±51.37**	67.31±57.05	70.22±38.36	61.11±41.64	.03
sTfR (µg/ml)	**2.34±0.63**	**2.27±0.65**	2.16±0.56	1.90±0.59	<.001
Hb (g/l)	97.7±12.5	99.7±13.5	**95.0±10.2**	106.7±10.9	<.001
MCV (fl)	77.46±5.93	77.35±7.19	79.42±5.00	77.60±7.81	.48
MCH (pg)	24.85±2.05	24.77±1.93	24.84±1.75	25.11±2.21	.58
MCHC (g/dl)	29.58±4.40	31.14±3.04	**28.42±3.96**	31.81±2.47	<.001

***Malaria co-infection with geohelminthiasis, s-significant, ns-not significant, Hb-haemoglobin, MCV-mean cell volume, MCH-mean cell haemoglobin, MCHC-mean cell haemoglobin concentration, µg/l-microgram per litre, µg/ml-microgram per milliliter, g/l-gram per litre, fl-femtolitres, pg-picogram, g/dl-gram per deciliter*

Table 3 compares the mean levels of ferritin, sTfR and red cell indices between the groups of infections and the control group. The mean levels of ferritin and sTfR were compared to the various infection groups of the pupils, with those who were not infected acting as the control group. There was a significant increase of ferritin when compared across the infectious groups (P=0.032). Further posthoc analysis revealed that the pupils infected with malaria only had significantly increased (P=0.016) ferritin when compared to the control group. Soluble transferrin receptors levels were also significantly elevated across the various infection groups (P<0.0001). On the other hand, a posthoc analysis of soluble transferrin receptors levels were also found to significantly increase in pupils who were infected with only malaria and geohelminthiasis (P=0.000 versus P=0.026 respectively). Haemoglobin and MCHC values were significantly reduced (P<0.0001) in the various infection groups and raised in the control group.

4. DISCUSSION

Malaria parasites attack and destroy red blood cells; which results to anaemia. Anaemia increases the risk of death and a drop in school performance and attendance [26]. *Plasmodium* parasites required iron for its essential cellular and metabolic activities [27]. Geohelminthiasis also causes anaemia by disturbing iron absorption in the body and also causes increase loss in essential end products macromolecule breakdown needed by the body [11]. Children

that are exposed to both infections and are asymptomatic or clinically well have a greater risk, since both infectious need iron for their metabolic activities.

Malaria is transmitted throughout the entire year with high rates during the rainy season. This and other infections have been shown to modify ferritin and other parameters that have been used to evaluate the iron parameters of the community. At the same time, malaria and other haemoglobin variants cause increase haemolysis which would have led to the higher values of sTfR. This finding differed significantly with a work almost similar to this where the authors noted lower mean ferritin levels and a higher proportion of iron deficiency in children aged 0 to 5 years in a national research in Cameroon [28]. This same work reported a high prevalence of iron deficiency defined by elevated sTfR values and this could be attributed to the high frequency and episodes of malaria seen in this area of Cameroon. However, the present prevalence seen in this work did not corroborate with that of a study conducted in Abia State, Nigeria amongst school-age kids in a setting situated on the outskirt of the town [29].

The prevalences of malaria parasitaemia and geohelminthiasis were 38.4% (149/388) and 19.8% (77/388) respectively in Buea; the place where this study was carried out. This study was done in the rainy season that favours the growth of bushes around homes and standing water in pot holes that predispose to the transmission of these infections. The frequency of malaria parasitaemia was similar to another study carried out in this area that reported a prevalence of 38.0% [30]. Malaria parasitaemia proportion was lower compared to other works cited by two previous studies and a recent study of 59.3%, 44.26% and 41.7% [12,20,31] and could have been as a result of the measures put in place by the Ministry of Public Health to control the spread of the disease [30]. The prevalence of geohelminthiasis dropped considerably when mirrored to previous works [15,32]. The reason for this low prevalence could have been the regular deworming (twice yearly) organised by the Ministry of Public health [30]. Recent studies show a rise in the prevalence of soil-transmitted helminthiasis to 22% amongst children aged 4 to 12years in Bolifamba; a community in the Buea municipality six months after mass deworming [33].

Findings from this present study revealed that ferritin and sTfR values in the various infectious groups were significantly raised when compared to the control group, while haemoglobin and MCHC results significantly dropped when compared to the control group. The level of Ferritin was also significantly raised in children in the malaria only group when matched up to those that were negative for both malaria and geohelminthiasis that acted as the reference or control group. During infection there is re-distribution or sequestration of iron in the stores and thus making it unavailable for the synthesis of haemoglobin and the malaria parasite. The sequestration of iron in the stores serves as a protective mechanism put forth by the body to eliminate the malaria parasite. Also ferritin is an acute phase reactants. Malaria presents with an acute inflammation, with release of cytokines which have an impact on these acute phase proteins. These have to be considered when drawing cut-off for iron deficiency in malaria endemic areas using ferritin. This finding corroborates with other studies which concluded that malaria parasitaemia was significantly related with some iron status indicators like ferritin (raised levels), Zinc protoporphyrin and transferrin receptors amongst preschool children in Rural Western Kenya [34-37]. Also studies in Zambia amongst children aged 4 to 8 years reveal that ferritin levels were significantly raised in seasons of high malaria transmission when compared to seasons of low malaria transmission [38]. These findings uphold this fact that prolong confinement of iron inside the stores as a result of inflammatory conditions and haemolysis of the red blood cells brought about by malarial infection, are possibly the main causes of decrease in haemoglobin levels and MCHC observed in this study and consequently anaemia in the developing world [39].

Levels of sTfR were significantly increased in malaria only and geohelminthiasis only groups when compared to the control group as shown in table 3. sTfR are surface receptors seen in almost all the cells in humans, with about 80% of it in blood coming from immature red blood cells and is a marker of erythropoiesis. Immature red blood cells allegedly; are considered to be the main spring of sTfR in the body and during haemolytic destruction of red blood cells in malaria episodes; more of them are released into the circulation causing the levels to increase. Malaria parasite density increases with sTfR levels [37,40]. This finding agrees with some previous studies [12,35,37] which all reported increased levels in school pupils with clinical and asymptomatic malaria infection and the levels

were higher than those seen in cases of iron deficiency anaemia. sTfR is used to measure erythropoietic activity, with increased levels seen during haemolytic conditions, iron deficiency, ineffective erythropoiesis, and megaloblastic anaemia. Many ancient authors have argued that sTfR remains as a better and responsive marker that can be used to appraised body iron stores as it increases during the early stages of iron deficiency and that it is not influenced by inflammation [41]. However, the results does not agree with other research works carried out on individuals in high pressure malaria infection area in which the serum level of sTfR were significantly reduced or did not show any significant increase in the presence of clinical malaria. They attributed this discrepancy to the fact the bone marrow did not respond erythropoietin and that was why the sTfR in those with clinical malarial infection in Venuatu was low [42-43].

Geohelminthiasis also affects sTfR in that the geohelminths compete for the available nutrients including iron necessary for red blood cell production, thus causing anaemia and this could have been one of the reasons that accounted for raised sTfR levels. Anaemia and geohelminthiasis always go together and they have a relationship [44]. Immature red cells that are seen during most anaemic cases are the major source of sTfR, more of them are released into the circulation causing their levels to increase [40].

5. CONCLUSION

In conclusion, this study has demonstrated that malaria and geohelminthiasis continues to infect school children in Buea municipality. From this study one of the mechanisms that causes anaemia during a malaria episode in this municipality is the prolong sequestration of iron in the storage compartment as shown by elevated of ferritin. Consequently iron is not readily available to the developing red blood cells in the bone marrow. Geohelminthes cause anaemia by competing for available nutrients with iron inclusive as indicated by raised levels of sTfR. It is also worth noting that malaria has an impact on iron stores parameters such as ferritin thus making it difficult for it to be used in assessing the iron status of the population.

ETHICAL APPROVAL AND CONSENT

Ethical approval was obtained from the faculty of health sciences institutional review board (IRB) of the University of Buea (n^o: 2015-02-0368) while administrative clearances to collect samples were collected from south west regional delegations of public health and basic education, inspector for basic education and catholic education secretary for buea diocese. These were presented to the different head teachers for endorsement. Informed written consent was gotten from the pupils' parents and/or guardians through a consent form which was sent to them one week before sample collection; to read, understand-ask questions and approve or disapprove their child/ren's participation in the study. The pupils' assents were sought and a structured questionnaire was filled through an interview prior to sample collection.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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