

Original Research Article

Schistosomiasis and Malaria Co-Infection in School Aged Children in the Tiko Health District, South West Region, Cameroon

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ABSTRACT

Aims: Parasitic infections are a major cause of diseases and morbidity in Africa, with malaria inflicting the largest burden followed by schistosomiasis. Co-infection with schistosomiasis and malaria may lead to severe health effects on children harbouring both infections. Studies have suggested that co-infection with helminths and malaria alters haematological indices. This study investigated co-infection with *Schistosoma* species and *Plasmodium falciparum* amongst school-aged children in the Tiko Health District, South West Region of Cameroon.

Methods: A community based study was conducted in three health areas (Holforth, Tiko Town and Likomba) of the Tiko Health District. Participants aged 5 – 15 years were enrolled into the study. Blood, urine and stool samples were collected from the participants after obtaining consent from their parents. Samples were examined using Microscopy and Polymerase Chain Reaction. Haemoglobin levels were measured using a haemoglobinometer. A total of 397 participants were enrolled into the study.

Results: The prevalence of schistosomiasis, *falciparum* malaria and co-infection amongst school aged children were 16.1%, 44.3% and 7.8% respectively. Prevalence of anaemia was 38.0%. Those co-infected had the highest prevalence of anemia (54.8%) than the mono-infected participants though this difference was not statistically significant ($\chi^2 = 3.96, P = 0.138$). Co-infection was associated with the health area ($\chi^2 = 13.878, P = 0.001$) and visit to the stream ($\chi^2 = 7.223, P = 0.007$). There was an association between co-infection and malaria parasite density ($\chi^2 = 7.525, P = 0.0232$).

Conclusion: There was a relatively low prevalence of co-infection of schistosomiasis and malaria in school-aged children in the Tiko Health District. With respect to anaemia severity, there was no observed association between the infection category and severity of anaemia in the participants ($\chi^2 = 5.64, P = 0.464$).

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Brief about problem disease
Objective
Material and methods
Brief result without discussion main clear results
Finally conclusion

Keywords: Schistosomiasis; Malaria; Anemia; Co-infection; Prevalence; Microscopy; Polymerase Chain Reaction.

1. INTRODUCTION

Parasitic infections remain a major cause of diseases and morbidity in Africa [1]. Amongst these diseases, *Plasmodium falciparum*, the causative agent of *falciparum* malaria inflicts the largest burden [2, 3]. About 3.2 billion people, almost half of the world's population, are at risk of malaria [4]. There are about 219 million cases and 660,000 deaths that occur every

year [5]. It is estimated that about 90% of the population infected with malaria lives in the Sub-Saharan Africa where it is the commonest cause of death and serious morbidity, especially for children and pregnant women. Approximately 86% of malaria deaths globally are children under 5 years of age [6]. Children between six months and five years of age are at highest risk for severe disease and death. During this period, children are most vulnerable as they have lost maternal immunity and haven't yet developed specific immunity to infection [7]. The epidemiology of *falciparum* malaria has been changing over the past 10 years, with declining numbers of clinical cases reported in different parts of the world [7]. In Africa, malaria deaths have been cut by one third in the last decade. Outside of Africa, 35 out of 53 countries affected by malaria have reduced malaria cases by 50% in the same time period. In countries where access to malaria control interventions has significantly improved, child mortality rates have fallen by about 20% [8], a percentage more than twice that of all childhood death attributable to malaria [7]. Schistosomiasis is the second most common parasitic disease on the globe [9]. It is prevalent in tropical and subtropical areas especially in poor communities without access to safe drinking water and adequate sanitation. Transmission takes place only where snail vectors are present and where there is contact between the population and an infected fresh water body. Blood flukes of the genus *Schistosoma* are responsible for the disease which occurs in two forms: intestinal schistosomiasis caused by *S. mansoni*, *S. japonicum* and *S. intercalatum* and urinary (urogenital) schistosomiasis (US) caused by *S. haematobium*. It infects about 240 million people in more than 70 countries of the developing world and up to 300,000 deaths per year [10]. It is estimated that 20,000-200,000 people die of schistosomiasis each year [11]. Globally, up to 261 million people required treatment for schistosomiasis in 2013, but only 40 million received it [11]. Schistosomiasis remains a major health problem in Cameroon and contributes to a third of the morbidity among school-aged children [12]. Polyparasitism appears to be the rule, rather than the exception, both at the population level and among individuals residing in developing countries [13]. The effects of polyparasitism are often clinically unapparent, however, in some situations, co-infections may exacerbate disease symptoms due to one of the pathogens. It has also been reported that co-existent infections may also, under some circumstances, suppress clinical symptoms due to one or both pathogens [14]. Malaria and schistosomiasis often overlap in tropical and subtropical countries imposing tremendous disease burden [15, 16, 17]. The substantial epidemiological overlap of these two parasitic infections invariably results in frequent co-infections [18, 19]. The implications of concomitant malaria and helminth infections have been mainly explored in animals under laboratory conditions [20, 21, 22]. In human populations, some studies showed a protective role of helminths. Nacheret al.[23] found that a helminth infection was associated with a protection from cerebral malaria, and Murray et al.[24] showed that treatment of severe ascariasis was accompanied by recrudescence of malarial attacks in children. Conversely, other studies suggested a deleterious effect of co-infection [25, 26]. The challenges facing the development of a highly effective malaria vaccine have generated interest in understanding the interactions between malaria and co-endemic helminth infections such as those caused by *Schistosoma* that could impair vaccine efficacy by modulating host immune response to *Plasmodium* infection and treatment [17, 27]. It has been suggested that the immune response evoked by helminths infection may modify immune responses to plasmodia and consequently alter infection and disease risks [17, 28, 29]. Presently, the extent to which schistosomiasis modifies the rate of febrile malaria remains unclear [30]. This is because studies conducted to date have produced conflicting results [31, 32, 33] on the immune responses to *Plasmodia* with *Schistosoma* co-infection. In the South West Region of Cameroon, a few studies have investigated co-infection of urogenital schistosomiasis and malaria in Muyenge [34] and also soil transmitted helminths and *Plasmodium falciparum* co-infection in Ekona and Tole [35]. The prevalence of co-infection in these studies was 15.2% and 5.6% respectively. However, such co-infection studies have not been conducted in the Tiko Health District whose climate and ecology

favour the thriving of these parasites and their vectors. Such co-infection studies might elucidate the persistence and morbidity of *falciparum* malaria in the study area. The primary objective of this study was to determine the level of co-endemicity of schistosomiasis and *falciparum* malaria in school-aged children in the Tiko Health District and secondly to assess the severity of malaria in children co-infected with schistosomiasis in the study area.

2. MATERIALS AND METHODS

2.1 Study Area

This study was carried out in the Tiko Health District, located in Fako Division of the South West Region of Cameroon. Tiko has a hot climate favourable for the breeding of the vectors of these parasites. The health district has a total surface area of 484km² and located between longitude 8.6°10'E and latitude 4°5.2'N. The population of Tiko is currently estimated at 151,109 inhabitants. The health district is inhabited by the indigenous Bakweri people but also by Cameroonians from other tribes and the Igbos and Ijaws from Nigeria. Tiko has a coastal equatorial climate with daily temperatures ranging from 28°C to 33°C. The main source of income is Agriculture and trade. The Cameroon Development Corporation, an agro-industrial corporation, employs majority of the inhabitants. The area is characterized by sandy alluvial and volcanic soils with high agricultural potentials. The main water courses in the Tiko municipality include Rivers (Mungo, Ombe, Ndongo and Benyo) which empty into the Atlantic Ocean. The Tiko Health District is made up of 8 health areas (Fig. 1) namely; Holforth, Kange, Likomba, Mutengene, Mondoni, Mudeka, Missellele and Tiko town. It is headed by the District Medical Officer. This health district has 21 health facilities with about 90 communities.

The present study was conducted in three health areas (Holforth, Tiko town and Likomba) in the Tiko Health District.

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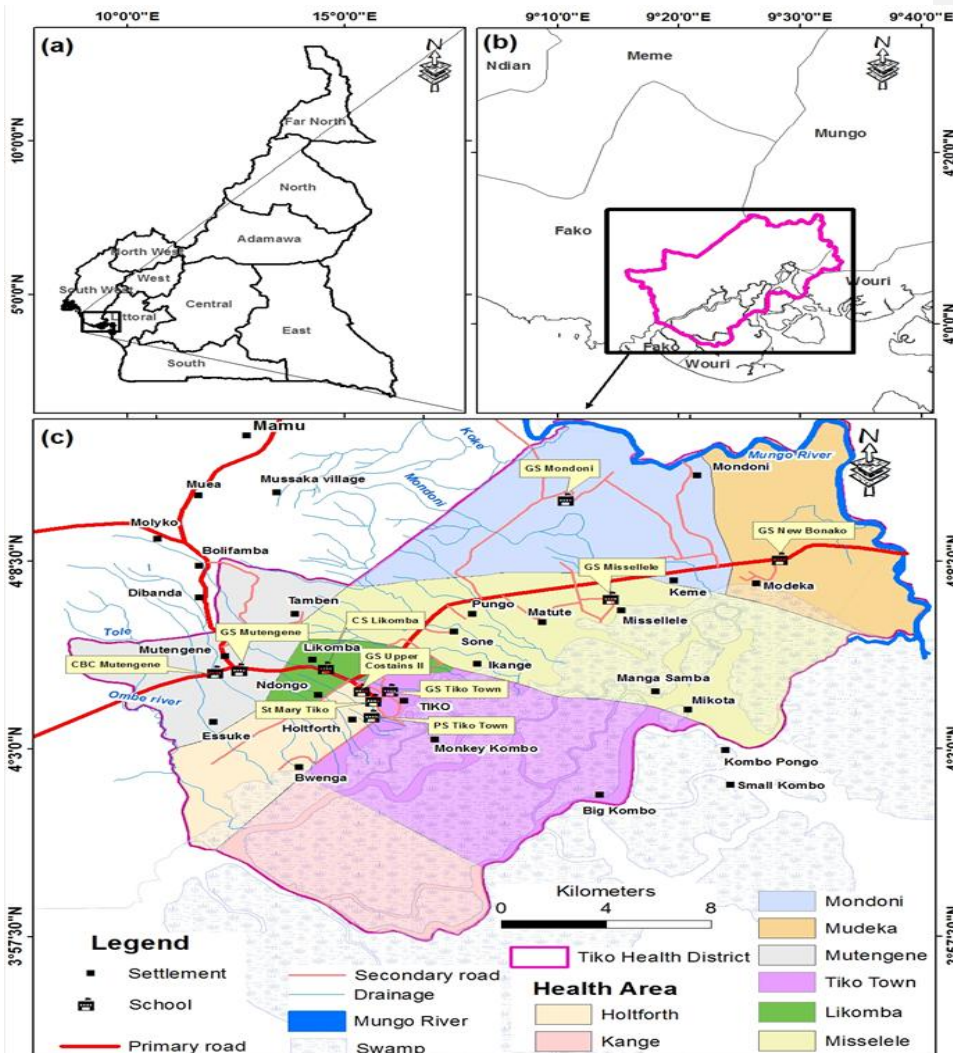


Fig. 1. A Map of the Tiko health district showing the study areas

2.2 Study Population

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The study included school-aged children ranging from 5 to 15 years. The Tiko Health District has 84 primary schools including public, private and confessional schools. Twelve percent (12.02%) of the population of Tiko are school-aged children. Children between 5 and 15 years of age, corresponding to the age group at risk for schistosomiasis were sampled from the communities. Only Children whose parents provided consent for their participation and who agreed to provide stool, urine and blood samples were enrolled for the study.

2.3 Study Design

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A community-based cross sectional study was carried out in three randomly selected health areas (Holforth, Tiko town and Likomba) in the Tiko Health District from April to July, 2021. The study was aimed at determining the level of co-endemicity of schistosomiasis and *falciparum* malaria and also assessing the severity of malaria in school-aged children co-infected with schistosomiasis in the study area. Children aged 5 – 15 years were randomly selected from households of randomly selected communities. Questionnaires were administered to eligible participants after which stool, urine and blood samples were collected (Fig. 2).

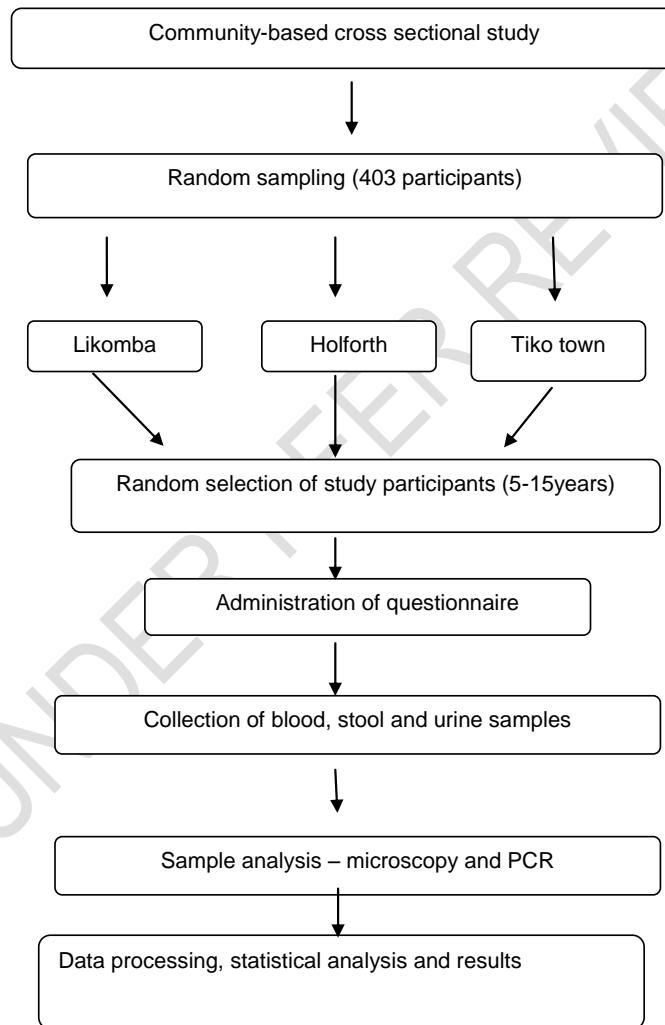


Fig. 2. A flow chart of the study design

2.4 Sample Size Calculation

The sample size was calculated using the Kish Leslie formula [36]. A prevalence of 15.2% of co-infection with *Schistosoma haematobium* and *Plasmodium falciparum* was considered from a similar study by Kimbi et al. [34]. Absolute Precision (implies margin of sampling error, d) tolerated was set at 5%, at 95% confidence interval (Z).

$$\text{Sample size } (n) = (Z^2 p (1-p)) \div d^2.$$

$$P=15.2\% (0.152)$$

$$n = \frac{1.96^2 * (0.152) * (0.848)}{0.05^2} = 197.98$$

n= 198 participants

The minimum sample size was 198 participants but finally a total of 397 participants were recruited into the study.

2.5. Samples Collection

A structured questionnaire was used to collect demographic data and behavioural factors linked to malaria and schistosomiasis from every participant. Blood samples were collected by finger pricking using a sterile lancet. Firstly, the finger was cleaned with an alcohol swab and then pricked with a lancet. About 100µL of blood was placed on a clean labelled slide for thick and thin blood films for malaria parasite identification and quantification. Another 100µL was used to measure Hb levels using the haemoglobin meter. For DNA extraction, another 100 µL of blood was placed on a Whatman filter paper, air dried and stored at room temperature. Each participant was given two labelled sterile stool and urine containers, toilet roll, applicator stick and an A4 sheet to provide about 1g of faeces and 20 mL of urine separately. Participants were instructed to pass out faeces on the A4 sheet and use the applicator stick to transfer a small portion of the stool into the container. The rest of the stool was discarded. Each child was instructed to pass out about 20ml of urine into the second sterile container. All the children were provided with detergents and clean water to wash their hands after stool and urine collection. The samples were placed in a cool ice box and transported immediately to the Tiko District Hospital Laboratory for processing and analysis.

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2.6 Detection and Quantitation of *P.falciparum*

2.6.1 Microscopy

The thin films were fixed with methanol for 30 seconds. The films were stained with 10% Giemsa at pH 7.0 for 20 minutes [37]. The films were examined under the microscope at X100 objective for identification of malaria parasite species. Considering the number of leucocytes/µL of blood as 8000, parasite density of blood using the thick film was expressed as: parasite count (x) 8,000 divided by number of WBCs counted [38]. More than 200 high power fields were examined before a smear was considered negative.

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2.6.2 DNA extraction and Nested Polymerase Chain Reaction (nPCR)

Parasite nucleic acid was identified using polymerase chain reaction. DNA was extracted from Whatman filter blots using 5% chelex resin. About 200µL of chelex resin was pipetted into a sterilized 0.5 mL eppendorf tube and heated at 100°C for 10 minutes. The blot spot on Whatman paper was then transferred into the eppendorf tube containing hot chelex and incubated for 12 minutes at 100°C in a water bath. The tube was removed and mixed for 1 minute and further incubated for 10 minutes at 100°C in a water bath. Eppendorf tubes from the incubator were centrifuged at 12000rpm for 2 minutes. The supernatant containing the DNA was pipetted and stored at -20°C for further use.

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2.6.3 DNA amplification by nPCR

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The nPCR amplification strategy was used for genotyping the 18S rRNA genes of *P. falciparum* in which rPLU6 and rPLU5 primers were used for the primary amplification while species specific primers rFAL1 and rFAL2 were used for secondary amplification for nested PCR (Table 1, 2 and 3). The PCR amplifications were performed in a 96-well thermocycler. The cycling conditions for primary and secondary PCR are shown on Table 4 and 5 respectively. The negative control contained all the components except the template DNA while the positive control had all the components and 1 μ L of the positive control DNA (3D7 strain). Amplicons from the second-round PCR were resolved on 1.2% agarose gels (1.2g of agarose diluted in 100 ml of tag buffer) following electrophoresis and visualized on a gel documentation system to check the band size of the PCR products.

Table 1. Sequences of 18S rRNA primers for Plasmodium PCR

Gene	Primers	Sequence
18SrRNA	Nest-1 forward rPLU5	5'CCT GTT GTT GCC TTA AAC TTC-3'
	Nest-1 reverse rPLU6	5'TTA AAA TTG TTG CAG TTA AAA CG-3'
	Nest-2 forward rFAL1	5'TTA AAC TGG TTT GGG AAA ACC AAA TAT ATT-3'
	Nest-2 reverse rFAL2	5'ACA CAA TGA ACT CAA TCA TGA CTA CCC GTC-3'

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Table 2. Pipetting instructions for Nest 1 PCR

Reagent	Volume (for 20 μ L reaction volume)
PCR water	14.8 μ L
Taq buffer	2 μ L
dNTP	0.8 μ L
Forward primer - rPLU5	0.2 μ L
Reverse primer - rPLU6	0.2 μ L
Taq polymerase	1 μ L
Template (DNA)	1 μ L

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Table 3. Pipetting instructions for Nest 2 PCR

Reagent	Volume (for 20 μ L reaction volume)
PCR water	14.8 μ L
Taq buffer	2 μ L
Dntp	0.8 μ L
Forward primer - rFAL1	0.2 μ L
Reverse primer - rFAL2	0.2 μ L
Taq polymerase	1 μ L
Template (DNA)	1 μ L

Table 4. Conditions for Primary nPCR – 25 cycles

PCR conditions	Temperature	Time
Initial denaturation	95°C	3minutes
Denaturation	94°C	30seconds
Annealing	55°C	1minute
Extension	68°C	2minutes
Final extension	68°C	3minutes

Table 5. Conditions for Secondary nPCR – 30 cycles

PCR conditions	Temperature	Time
Initial denaturation	94°C	3minutes
Denaturation	94°C	30seconds
Annealing	61°C	1minute
Extension	68°C	1minute
Final extension	68°C	3minutes

2.7 Detection and Quantitation of schistosome eggs

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2.7.1 Stool analysis by formol-ether sedimentation technique for *S. mansoni*

Stool samples were analysed by the formol-ether sedimentation technique for the presence of schistosome eggs. This technique uses a solution of lower specific gravity than the schistosome eggs, thus concentrating them in the sediment. The formalin fixed the eggs of the parasites such that they were no longer infectious and also preserved their morphology. Faecal debris was extracted into the ethyl acetate phase of the solution. After centrifugation, the sediments were observed under the x10 and x40 magnification objective of the microscope for identification and quantitation.

2.7.2 Urine sedimentation for the detection of *S. haematobium* eggs

A urine strip test was used to test for the presence of blood in urine by dipping a test strip into the urine for 1 minute. The strip was then removed and observed for any colour change. The samples were further examined for the ova of *Schistosoma haematobium* by urine sedimentation method, which allowed the intensity of the infection to be measured under the x10 magnification objective of the microscope. 10ml of urine samples were placed in sterile tubes and centrifuged for 2000rpm for 5 minutes to obtain sediment. The sediment was placed on a slide, covered with a cover slip and mounted on microscope for the quantitation of *S. haematobium* eggs.

2.8 Data management and Statistical analysis

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Data was entered into Excel version 2016 and analysed using IBM Statistical Package for Social Sciences (IBM SPSS version 25) (IBM Inc. 2012). Validated data was checked for normality by checking for skewedness before any statistical analysis was performed. Socio-demographic and clinical characteristics were presented as frequencies and percentages. The Pearson chi-square test was used to examine the association between prevalence and socio demographic factors. All significant levels were measured at 95% confidence interval (CI), with the level of significance set at $P < 0.05$. The Pearson chi-square test was used to compare the malaria parasite density and anaemia severity in co-infected and non-co-infected groups.

3. RESULTS

3.1 Socio-demographic characteristics of the study population

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A total of 397 children were enrolled into the study. The characteristics of the study participants are shown on Table 6. Over 63.7% of the study participants were within the age

range 9 – 12 years. The distribution of study participants in the three health areas was almost equal, with majority of the participants being females (51.1%). The stream was the main source of water (65.0% stream visit) for domestic use and bathing.

Table 6. Characteristics of the study population

Characteristics	Category	Number examined n (%)
Age	5 – 8years	107 (27.0)
	9 –12years	253 (63.7)
	13 – 15years	37 (9.3)
Gender	Male	194 (48.9)
	Female	203 (51.1)
Health area	Likomba	146 (36.8)
	Holforth	131 (33.0)
	Tiko town	120 (30.2)
Stream visit	Yes	258 (65.0)
	No	139 (35.0)

3.2 Prevalence of Schistosomiasis in Tiko Health District

Of the 397 participants enrolled into this study, 64(16.1%) were infected with *S. haematobium* parasite (Fig. 3).

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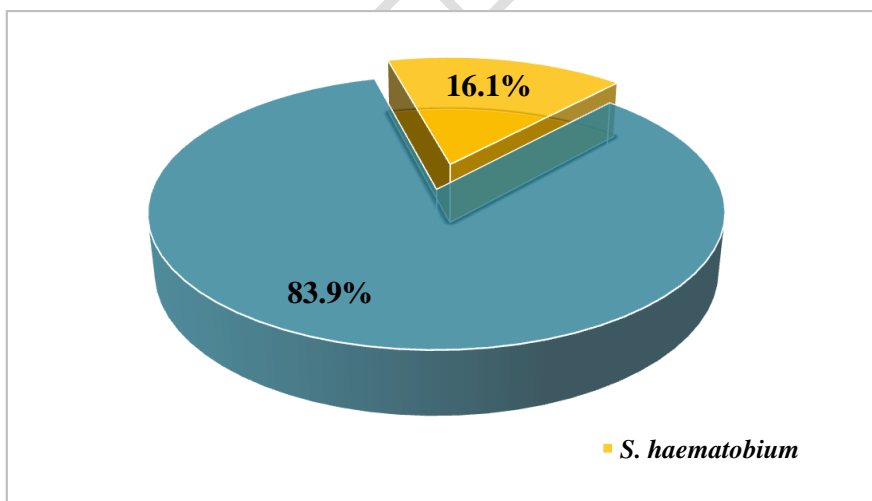


Fig. 3. Prevalence of Urogenital schistosomiasis in Tiko Health District

3.3 Prevalence of falciparum malaria in Tiko Health District

3.3.1 Prevalence of *falciparum* malaria by microscopy

Out of the 397 blood samples examined by microscopy, 138 were positive for the malaria parasite giving a prevalence of 34.8%. The only species identified was *Plasmodium falciparum* (Fig. 4).

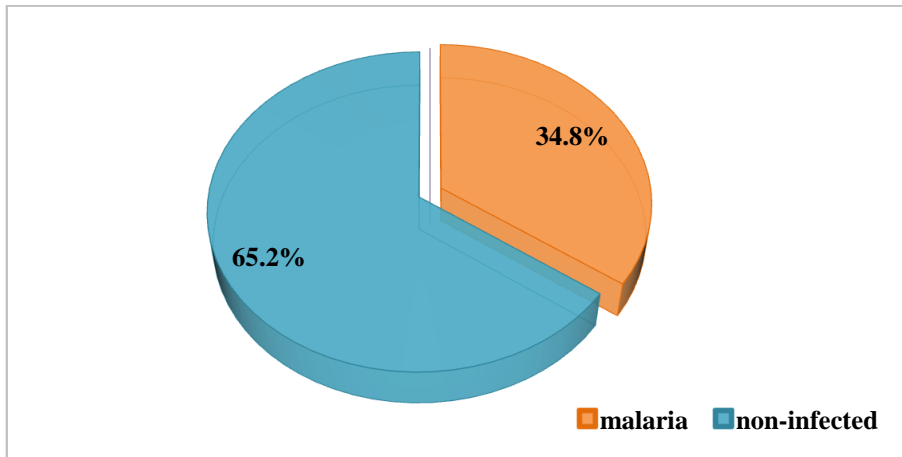


Fig. 4. Prevalence of *falciparum* malaria in Tiko Health District by microscopy

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3.3.2 Prevalence of *falciparum* malaria by nested PCR

Of the 397 blood samples tested by nPCR, 176 were positive for the malaria parasite giving a prevalence of 44.3% (Fig. 5).

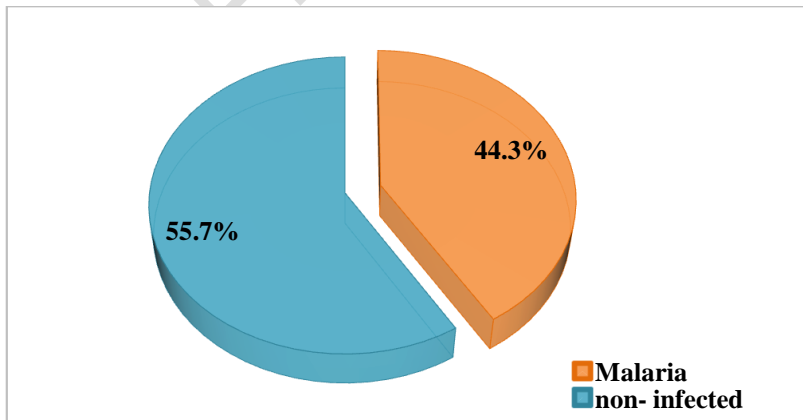


Fig. 5. Prevalence of *falciparum* malaria in Tiko Health District by nested PCR
3.3.3 Intensity of malaria infection in the study area

Out of the 138 children infected with *P. falciparum* by microscopy, the prevalence of low (<500 parasites/ μ L of blood), moderate (501–5000 parasite/ μ L of blood), and high (>5000 parasites/ μ L of blood) parasitaemia were 28.3% (39), 58.7% (81), and 13.0% (18), respectively. Moderate parasitaemia was more common among the study participants (Fig. 6).

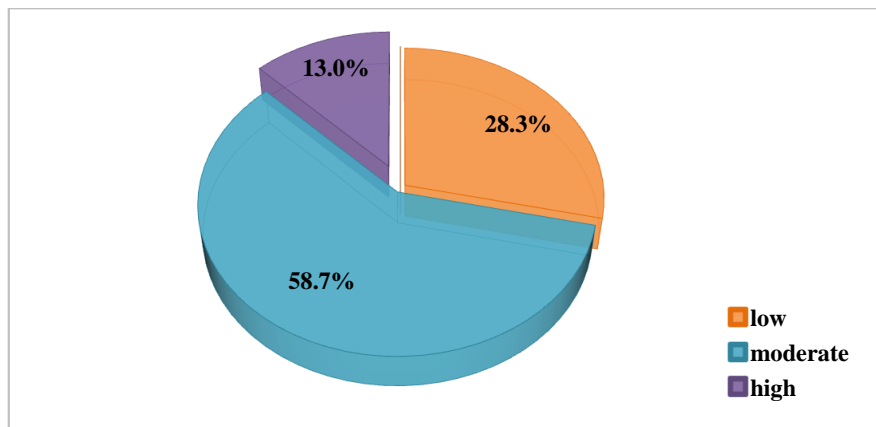


Fig. 6. Prevalence of malaria parasitaemia among study participants

3.4 Prevalence of Co-infection in Tiko Health District

From the 397 participants enrolled into this study, 176 (44.3%) were positive for *falciparum* malaria (nPCR) and 64 (16.1%) were positive for schistosomiasis. Thirty-one (31) children were co-infected with both parasites giving a prevalence of 7.8% (Fig. 7).

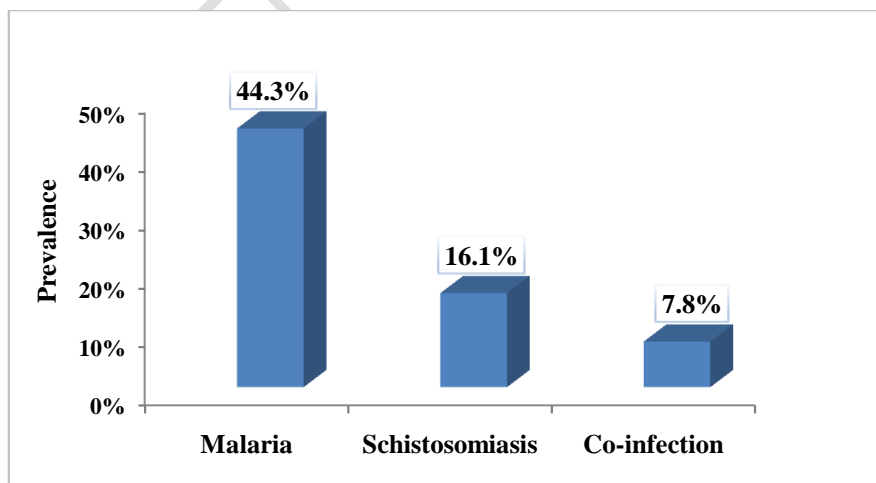


Fig. 7. Prevalence of *falciparum* malaria, urinary schistosomiasis and co-infection.

3.5 Prevalence of Anaemia in Tiko Health District

Of the 397 participants enrolled into this study, 151 were anaemic, giving a prevalence of 38.0% as shown in Fig 8.

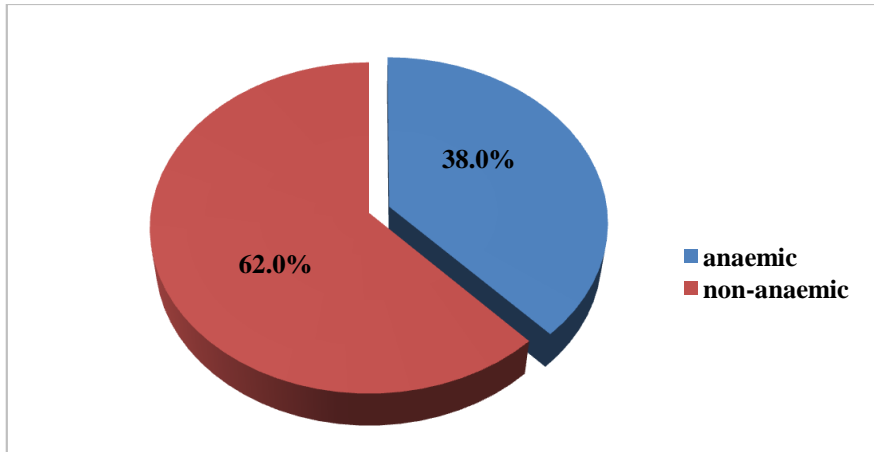


Fig. 8. Prevalence of anaemia in Tiko Health District.

3.5.1 Severity of anaemia in study participants

Out of the 151 participants that were anaemic, 1.3% (<7g/dl) had marked, 27.2% (7 – 9.9g/dl) moderate and 71.5% (10 – 11.4g/dl) mild anaemia respectively (Fig. 9).

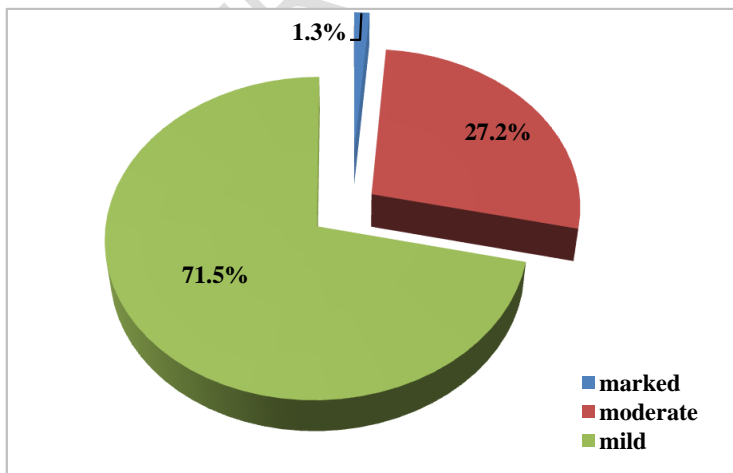


Fig. 9. Prevalence of anaemia severity among study participants

3.5.2 Association between anaemia and the different infection categories (co-infection and mono-infection with MP)

Out of the 397 participants, 36.8% (146) were infected with MP only, 8.3% (33) with Schistosomiasis only, 7.8% (31) were co-infected with MP and Schistosomiasis, and 47.1.4% (187) were uninfected. There was a significant difference ($F = 3.615$, $P = 0.0286$) when comparing mean haemoglobin values between the different infection categories; as those co-infected had the lowest mean hemoglobin value ($10.8 \pm 1.80\text{g/dL}$) when compared to mono-infected patients accordingly (Table 7).

The prevalence of anemia in the study population was 38.0% (153) and those co-infected had the highest prevalence of anaemia (54.8%) than the mono-infected participants though this difference was not statistically significant ($\chi^2 = 3.96$, $P = 0.138$).

With respect to anemia severity, there was no observed association between the infection category and severity of anemia in the participants ($\chi^2 = 5.64$, $p = 0.464$).

Table 7. Prevalence of anaemia, anaemia Severity and Mean (SD) Hb levels by infection category

Infection Category	No Examined	Prevalence of anaemia n (%)	Mean (SD) Hb (g/dL)	Prevalence of anaemia severity n(%)		
				Mild	Moderate	Marked
MP only	146	61(41.8)	11.3(1.65)	42(68.9)	17(27.8)	2(3.3)
Shisto only	33	10(30.3)	11.9(1.57)	8(80.0)	2(20.0)	0(0)
Coinfection	64	17(54.8)	10.8(1.80)	11(64.7)	6(35.3)	0(0)
		$P=0.138$ $\chi^2 = 3.96$	$P= 0.0286$ $F =3.615$		$P= 0.464$ $\chi^2 = 5.64$	

3.6 Association between parasitaemia and the different infection categories (co-infection and mono-infection with MP)

There was a significant difference in malaria parasite density between mono-infected and co-infected individuals, with malaria mono-infected participants possessing a higher mean parasite density of 2274.97 ± 4391.626 compared with 1597.6 ± 1863.15 parasites per microliter of co-infected participants ($F = 5.6053.372$, $P < 0.0001$) (Table 8). Mono-infected participants had a significantly ($P < 0.0232$) higher prevalence of low (28.3%) and high (15.1%) parasitaemia when compared with co-infected participants.

Table 8. Proportion of malaria parasitaemia level in malaria mono-infection and co-infection in the study population

Infection Category	Number Examined	Mean Parasitaemia	Plasmodium parasitaemia		
			Low	Moderate	High
MP mono infection	113	2274.97	32(28.3)	64(56.6)	17(15.1)
Co-infection	25	1597.6	7(28.0)	17(68.0)	1(4.0)
		$P < 0.0001$ $F = 5.6053$		$P = 0.0232$ $\chi^2 = 7.525$	

3.7 Relationship between some socio-demographic factors and co-infection in Tiko Health District.

The prevalence of co-infection in Tiko health District was 7.8%. With respect to the age prevalence, the age group 5 – 8 years had the highest prevalence of co-infection (9.3%), closely followed by children aged from 9 – 12 years (7.9%). However, there was no statistically significant difference in the prevalence of co-infection among the age groups, ($\chi^2 = 1.694$, $P = 0.429$).

With respect to gender prevalence, males were more infected (9.8 %) with both parasites than females (5.9%). The difference in the prevalence of co-infection between this two groups was not statistically significant ($\chi^2 = 2.077$, $P = 0.150$).

With regards to the health areas investigated, Likomba had the highest prevalence (14.4%) of co-infection with the least prevalence (3.8%) reported from Tiko town health area. The difference in the co-infection rates between the health areas was statistically significant, ($\chi^2 = 13.878$, $P = 0.001$).

Also, the prevalence of co-infection was higher (10.5%) in children who visited the stream than in those who did not go to the stream (2.9%). The difference in prevalence between these 2 groups was statistically significant ($\chi^2 = 7.223$, $P = 0.007$) as shown on Table 9.

Table 9. Relationship between socio-demographic factors and co-infection in THD.

Characteristics	Total		Co-infection Prevalence (%)	χ^2	P-value
	Examined	Positive			
Age group(Years)					
[5-8]	107	10	9.3	1.694	0.429
[9-12]	253	20	7.9		
[13-15]	37	1	2.7		
Gender					
Male	194	19	9.8	2.077	0.150
Female	203	12	5.9		
Health Area					
Likomba	146	21	14.4	13.878	0.001
holforth	120	5	4.2		
Tiko town	131	5	3.8		
Stream visit					
Yes	258	27	10.5	7.223	0.007
No	139	4	2.9		

χ^2 =chi square test

4. DISCUSSION

This study investigated the prevalence of co-infection with schistosomiasis and falciparum malaria in school-aged children in the Tiko Health District, South West Region of Cameroon. *Schistosoma haematobium* and falciparum malaria are common amongst school-aged children living in Tiko. The Prevalence of co-infection was 7.8% and it was associated to the health area and visits to the stream. The prevalence of *Schistosoma haematobium* was 16.1% which confirms the presence of *Schistosoma haematobium* focus in the Tiko Health

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District as reported by Anguh et al. [39]. The prevalence of falciparum malaria and anaemia were 44.3% and 38.0% respectively, demonstrating the severity of malaria in children in the health district. The prevalence of *S. haematobium* infection among children in this study was 16.1%. This prevalence reflects exposure to infection among children due to dependence on rivers as their main source of water for domestic activities and playing. The level of infection in the present study is similar to result (16.16%) obtained in the three health areas (Holforth, Tiko Town and Likomba) from a previous study by Esum et al. [40]. However, it is below the level (31.5%) reported in three health areas (Likomba, Holforth and Mutengene) in the same health district by Green et al. [41]. This discrepancy could result from the fact that this previous study used a larger sample size (n=1029) with participants from 5 years of age and above, compared to our study with a smaller sample size (n=397) having age limit for participants (from 5 to 15 years). Moreover, higher prevalence (38%) of urogenital schistosomiasis among children (5-20 years) has been reported in Likomba, Tiko by Anguh et al. [39]. The differences in the prevalence may be due to the surface area sampled because this study sampled one health area (Likomba) meanwhile the present study sampled three health areas (Likomba, Holforth and Tiko town). A higher prevalence of 41.1% was also seen by Njunda et al. [42] in a study conducted in Magba Sub-division of Cameroon, this difference in prevalence could be due to the use of a more sensitive urine filtration diagnostic method and also because this study was performed in a rural area as opposed to the current study done in a semi-urban setting. As noted by Njiokou et al. [43], urbanization leads to the creation of modern water points which limits the frequency of human water contact. For transmission of schistosomiasis to take place, the schistosomes require an avenue where it is in direct contact with the human host. Children living in Tiko get in contact with infection during activities such as laundry, water fetching and playing. In addition to domestic activities, swimming/bathing in the streams poses a greater risk of infection among children in this area. Health education to instruct children and parents to make less water contact and the implication of voiding their bladder in water bodies is paramount. These behavioural changes could reduce the risk of *S. haematobium* infection among children. Older children are less likely to be engaged in water contact behaviours compared to younger children. Similarly, reports from other rural settings endemic for schistosomiasis failed to identify any socioeconomic variables that are strongly associated with schistosomiasis prevalence. The present study had a zero prevalence of *Schistosoma mansoni* in the Tiko Health District. Malaria is common among children in this study area with a prevalence of 44.3%. This prevalence is in line with a study conducted by Teh et al. [44] along the slopes of Mt Cameroon (41.7%) and by Lehman et al. [45] in school children from the Littoral Region. Lower prevalence has been reported in the Mount Cameroon area [46, 47] compared to the overall prevalence of malaria in this study. This difference may be due to the hot climate in Tiko which favours the breeding of the malaria parasites vector. The prevalence of anaemia (38.0%) in the present study is slightly higher than the 36.4% reported in a study conducted in school-aged children in Jindut, Nigeria by Dakul et al. [48]. There was a significant difference between the Hb concentrations of children with concurrent infection and mono-infection of malaria or schistosomiasis. This finding is contrary to those reported by Deribew et al. [2] and Dakul et al. [48] conducted in children in Ethiopia and Nigeria respectively. Furthermore, the anaemia prevalence was significantly higher in individuals who were co-infected. Marked anemia was seen in participants with high malaria parasitaemia (3.3%). Malaria and schistosomiasis co-exist in Sub-Saharan African countries. In the present study, the overall prevalence of co-infection with schistosomiasis and malaria was 7.8%. This prevalence was lower compared to a recent study carried out in Southern Ethiopia, 67% [49]. The risk of co-infection in this study was associated with the visits to the stream and health area. The present study also showed that there was no association between schistosomiasis and the malaria parasitaemia load. This is contrary to a study which reported that children lightly infected with *S. haematobium* had lower *P. falciparum* densities than those not infected suggesting a negative interaction

between the parasites [50]. In an earlier study conducted in Mali, children co-infected with schistosomiasis and heavy intensity of schistosomiasis were at risk of developing severe malaria [51]. Despite the reported association between these two parasites discussed above, Briand et al. [50] were confident that the association observed was not ecologically determined because even if malaria and schistosomiasis are water-associated diseases, their modes of transmission (place and time) are different, suggesting an independent risk of contamination and concluding that there is a negative interaction between the two parasites. Parasitic co-infection and interaction phenomena are complex. These phenomena require further studies to understand the mechanisms and public health implications between malaria and *Schistosoma* parasites. Community therapy programs which usually focus on single parasitic disease, such as control of schistosomiasis in school age children should be modified since several diseases may co-exist in the same individuals and affect the severity of each other. It could be worthwhile to implement integrated control programs that deliver multiple treatments against several parasitic infections simultaneously. In co-infected individuals, such programs could avoid possible deleterious effects of a single-targeted treatment on other diseases. The present study analyzed only one urine and stool sample per participant considering that the egg release rate of schistosomes is generally irregular. Thus, the prevalence of schistosomiasis may have been underestimated considering that their egg release is irregular.

5. CONCLUSION

The prevalence of urinary schistosomiasis, *falciparum* malaria and co-infection were 16.1%, 44.3% and 7.8% respectively. There was an association between malaria parasite density and co-infection with *Schistosoma haematobium*. The prevalence and the severity of anaemia were higher in participants co-infected with *falciparum* malaria and urinary schistosomiasis but the difference was not significant.

CONSENT

The objectives and the plan of action of the study were explained to the heads of each community and the parents of the children. Written assent was gotten from parents or legal guardians of children before their inclusion in the study. Participation in the study was voluntary. The children were told that they will experience minor pain at the site of blood collection.

ETHICAL APPROVAL

Ethical clearance for the study was obtained from the Institutional Review Board of the Faculty of Health Sciences, University of Buea. Administrative clearances were obtained from the Head of the Tiko Health District and the Divisional Officer of Tiko.

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REFERENCES

1. Akufongwe, P.F., Dakul, D.A., Michael, P.D., Dajagat, P.D., and Arabs, W.L. Urinary Schistosomiasis in rural communities of some Local government Areas in Plateau State Nigeria: A preliminary Parasitological and Malacological survey. *J. Helminthol.* 1996; 70(1):3-6.
2. Deribew, K., Tekeste, Z., and Petros, B. Urinary Schistosomiasis and Malaria Associated anemia in Ethiopia. *Asian Pac J Trop Biomed.* 2013; 3(4):307-310.
3. Elenwo, E.J.O., and Elenwo, A.C. Assessment of Morbidity in Malaria and Urinary schistosomiasis using some specific indicators. *J. Nat. Sci. Res.* 2014; 4(1):7-13.
4. WHO. "Malaria Fact sheet N^o 94". World Health Organization; 2015
5. WHO. World malaria report 2012 summary. World Health Organization; 2013
6. WHO. World Malaria Report. World Health Organization; 2011
7. Richard-Fabian, S., and Elena, S. Malaria in children. *Mediterr J Hematol Infect Dis.* 2012; 4(1): e2012073.
8. Schumacher. R.F., Spinelli, E. Malaria in children. *Mediterr J Hematol Infect Dis.* 2012; 4(1):e2012073.
9. WHO. Schistosomiasis: Control and Preventive Chemotherapy. World Health Organization; 2014
10. Steinmann, Keiser, J., Bos, R., Tanner, M., and Utzinger J. Schistosomiasis and water resources development: systematic review, meta-analysis, and estimates of people at risk. *Lancet Infectious Disease.* 2006; 6(7):411-25.
11. WHO. Schistosomiasis: A major public health problem. World Health Organization; 2015
12. Sama, T. M., Oyono, E., and Retard, R. C. High Risk 4. Behaviours and schistosomiasis Infection in Kumba, South West Province, Cameroon. *Int. J. Environ. Res. Public Health.* 2007; 4:101-105.
13. Keusch, G. T., Migasena, P. Biological implications of polyparasitism. *Rev. infect. dis.* 1982; 4: 880–882.
14. Sangweme, D. T., Midzi, N., Zinyowera-Mutapuri, S., Mdluza, T., Diener-West, M., and Kumar, N. Impact of schistosome infection on Plasmodium falciparum malarimetric indices and immune correlates in school age children in Burma Valley, Zimbabwe. *PLOS Negl. Trop. Dis.* 2010; 4: e882.
15. Doumbo, S, Tran, T.M., Sangala, J., Li, S., Doumtabe, D., Younoussou K. et al. Co-infection of Long-Term Carriers of *Plasmodium falciparum* with *Schistosoma haematobium* Enhances Protection from Febrile Malaria: A Prospective Cohort Study in Mali. *PLoS Negl Trop Dis.* 2014; 8(9): e3154.
16. Kealey, A., and Smith, R. J. Neglected tropical diseases: infection, modelling and control. *J Health Care Poor Underserved.* 2010; 21, 53–69.
17. Semenya, A.A., Sullivan, J.S., Barnwell, J.W., and Secor, W.E. *Schistosomamansoni* Infection impairs antimalaria treatment and immune responses of rhesus macaques infected with mosquito-borne *Plasmodium coatneyi*. *Infection Immunology.* 2012; 80(11): 3821–3827.
18. Hotez, P.J., Molyneux, D.H., Fenwick, A., Ottesen, E., and Ehrlich, S.S. Incorporating a rapid-impact package for neglected tropical diseases with programs for HIV/AIDS, tuberculosis, and malaria. *PLoS Med.* 2006; 3, e102.
19. Yapi, R.B., HRurimann, E., Hougbedji, C.A., Ndri, P.B., and SiluKe, K. D. Infection and co-infection with helminthes and plasmodium among school children in Côte d'Ivoire: results from a national cross-sectional survey. *PLOS Negl. Trop. Dis.* 2014; 8(6): e2913.
20. Helmbly, H., Kullberg, M., and Troye-Blomberg, M. Altered immune responses in mice with concomitant *Schistosomamansoni* and *Plasmodium chabaudi* infections. *Infectious Immunology.* 1998; 66: 5167–5174.
21. Yoshida, A., Maruyama, H., Kumagai, T., Amano, T., Kobayashi, F., Zhang, M., et al. *Schistosomamansoni* infection cancels the susceptibility to *Plasmodium chabaudi* through induction of type 1 immune responses in AJmice. *Int. Immunol.* 2000; 12: 1117–1125.

22. Abdel-Wahab, M. F., Powers, K. G., Mahmoud, S. S., and Good, W.C. Suppression of schistosome granuloma formation by malaria in mice. *Am. J. Trop. Med. Hyg.* 1974; 23:915-918
23. Nacher, M., Gay, F., Singhasivanon, P., Krudsood, S., Treeprasertsuk, S., Mazier, D., Vouldoukis, I. et al. *Ascaris lumbricoides* infection is associated with protection from cerebral malaria. *Parasite Immunol.* 2000; 22: 107–113.
24. Murray, M. J., Murray, A. B., Murray, M. B., and Murray, C. J. Parotid enlargement, forehead edema, and suppression of malaria as nutritional consequences of ascariasis. *Am. J. Clin. Nutr.* 1977; 30: 2117–2121.
25. Nacher, M., Singhasivanon, P., Yimsamran, S., Manibunyong, W., Thanyavanich, N., Wuthisen, R., and Looareesuwan, S. Intestinal helminth infections are associated with increased incidence of *Plasmodium falciparum* malaria in Thailand. *J. Parasitol.* 2002; 88(1):55–58.
26. Le-Hesran, J. Y., Akiana J, Ndiaye, H.M., Dia, M., Senghor, P., and Konate, L. Severe malaria attack is associated with high prevalence of *Ascaris Lumbricoides* infection among children in rural Senegal. *Trans. R. Soc. Trop. Med. Hyg.* 2004; 98: 397–399.
27. Salgame, P., Yap, G.S., and Gause, W.C. Effect of helminth-induced immunity on infections with microbial pathogens. *National Immunology*, 2013; 14:1118–1126
28. Hartgers, F. C., Obeng, B. B., and Kruize, Y. C. Responses to malarial antigens are altered in helminth-infected children. *J. Infect. Dis.* 2009; 199: 1528–35.
29. Kazeem, O. O. and Robert, S. On the Co-infection of malaria and schistosomiasis. 2016.
30. Brutus, L., Watier, L., Hanitraso, V., Razanatsio, H., and Cot, M. Confirmation of the protective effect of *Ascaris lumbricoides* on *Plasmodium falciparum* infection: results of a randomized trial in Madagascar. *Am. J. Trop. Med. Hyg.* 2007; 77:1091–5.
31. Sokhna, C., Le-Hesran, J. Y., Mbaye, P. A., Jean, A., Pape, C., Mamadou, D., Abdoulaye, L., and Pierre, D. Increase of malaria attacks among children presenting concomitant infection by *Schistosoma mansoni* in Senegal. *Malar. J.* 2004; 3:43.
32. Spiegel, A., Tall, A., Raphenon, G., Trape, J. F., and Druilhe, P. Increased frequency of malaria attacks in subjects co-infected by intestinal worms and *Plasmodium falciparum* malaria. *Trans. R. Soc. Trop. Med. Hyg.* 2003; 97:198–199.
33. Wilson, S., Vennervald, B. J., and Kadzo, H. Hepatosplenomegaly in Kenyan schoolchildren: exacerbation by concurrent chronic exposure to malaria and *Schistosoma mansoni* infection. *Trop. Med. Int. Health.* 2007; 12:1442–1449.
34. Kimbi, J. A. K., Dillys, M. E., Gemain, T. S., and Akum, E. A.D. Coinfection with *Schistosoma haematobium* and *Plasmodium falciparum* and Anaemia Severity among Pregnant Women in Munyenge, Mount Cameroon Area: A Cross-Sectional Study. *Parasitol.* 2017.
35. Sumbele, I. U. N., Gladys B. N., and Helen K. K. Soil-transmitted helminths and plasmodium falciparum malaria among individuals living in different agroecosystems in two rural communities in the mount Cameroon area: a cross-sectional study. *Infect. Dis. Poverty.* 2017; 6:67
36. Kish, L. *Survey Sampling.* New York: John Wiley and Sons, Inc; 1965.
37. WHO. *Basic Malaria Microscopy: Part 1. Second edition. Learner's Guide;* 2010.
38. Cheesbrough, M. *District Laboratory Practice in Tropical Countries.* USA: Cambridge University Press; 2009.
39. Anguh, E., Simon, A., Emmanuel, N., Bimabam, J., and Vicky, N. Presence of an unmapped focus for urogenital schistosomiasis in the Tiko health district, implications for control. *Int. J. Trop. Dis. Health.* 2018; 32(2): 1-8.
40. Esum, E.M., Junior, E.E., Dlonga, H.M., Patrick, I.V., and Nicholas T. Prevalence, Infection intensity and Risk factors of Schistosomiasis and soil Transmitted Heminthiasis among School Aged Children in Tiko Health District, South West Cameroon: A Community Based-Cross sectional Study. *Int. J. Trop. Dis. Health.* 2020; 14(7): 12-29

41. Green, A.E., Anchang-Kimbi, J.K., Wepnje, G.B., Ndassi, V.D. and Kimbi, H.K. Distribution and Factors Associated with Urogenital Schistosomiasis in the Tiko Health District, a Semi-Urban Setting, South West Region, Cameroon. *Infect. Dis. Poverty.* 2021; 10, Article No. 49.
42. Njunda, A. L., Emmanuel, N. N., Jules, C. N. A., Lucien, H., and Tebit N. E. Prevalence and factors associated with urogenital schistosomiasis among primary school children in barrage, Magba sub-division of Cameroon. *BMC Public Health*, 2017; 17:618.
43. Njiokou, F., Tsemo, L. C.Y., Kuete, T., and Ekobo, A. S. Dynamics of intestinal schistosomiasis in Cameroon: evolution of transmission in the mixed zone of nkolmebanga. *Lekie. Medicine tropical*, 2004; 64:351-4
44. Teh, R. N., Ule, N. S., Derrick, N. M., Samuel, T. V. O., and Helen, K. K. Malaria parasitaemia, anaemia and malnutrition in children less than 15 years residing in different altitudes along the slopes of mount Cameroon: prevalence, intensity and risks factors. *BMC malaria journal*, 2018; 17:336.
45. Lehman, L. G., Foko, L. P. K., Tonga, C., Nyabeyeu, H., Eboumbou, E. C., and Nono, L. K. (2018). Epidemiology of malaria using LED fluorescence microscopy among school children in Douala Cameroon. *Int. J. Trop. Dis. Health*, 2018; 29:1-13.
46. Apinjoh, T. O., Anchang-Kimbi, J. K., Mugri, R. M., Tangoh, D. A., Nyingchu, R. V. and Chi, H.F. The effects of ITNs in rural and semi urban communities in the southwest region of Cameroon. *Plos one*.2015; 10:e01116
47. Nyasa, R. B., Zofou, D., Kimbi, H. K., Kum, K. M., Ngu, R. C., and Titanji, V. P. The current status of malaria epidemiology in Bolifamlba, atypical Cameroonian rainforest zone: an assessment of intervention strategies and seasonal variations. *BMC Public health*.2015; 15:1105.
48. Dakul, D.A., Akindigh, T.M, Dogonyaro, B. J., Abba, O. J., Tangtur, K. T., and Sambo, N. *Plasmodium falciparum* and *Schistosoma haematobium* co-infection in school aged children in Jinduut, Shendam Local government area of plateau state, north central Nigeria. *Proceedings of Academics World 8th international conference*, Dubai. 2015
49. Mulu, A., Legesse, M., Erko, B., Belyhun, Y., Nugussie, D., and Shimelis, T. Epidemiological and clinical correlates of malaria-helminth co-infections in southern Ethiopia. *Malar. J.* 2013; 12:227.
50. Briand, V., Watier, L., Hesran, J. V. L., Garcia, A., and Cot, M. Co-infection with *Plasmodium falciparum* and *Schistosoma haematobium*: protective effect of schistosomiasis on malaria in Senegalese children. *Am. J. Trop. Med. Hyg.* 2005; 72(6):702–7.
51. Lyke, K. E., Dicko, A., Dabo, A., Sangare, L., Kone, A., Coulibaly, D., Guindo, A., and Traore, K. Association of *Schistosoma haematobium* infection with protection against acute *Plasmodium falciparum* malaria in Malian children. *Am. J. Trop. Med.* 2005; 73(6): 1124-1130.