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Falciparum malaria, helminth infection, and anaemia in asymptomatic pupils in four villages in Cameroon.

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ABSTRACT

About half the world's population is at risk for malaria, which causes more than half a million deaths annually. Malaria remains the number one killer of children in sub-Saharan Africa, where 90% of the deaths occur. Associated usually with malaria are infections caused by helminth parasites which have an impact on the health of humans. A major consequence of these parasites is anaemia. The objective of this study was to determine prevalence of malaria parasites, infection by soil transmitted helminthes and anaemia in asymptomatic pupils in four primary schools in Mbonge sub-division, Cameroon. One school, in each of Marumba I, Marumba II, BaiManya, and Pete Bakundu villages were included; 484 pupils participated. Blood, stool, axial temperature and demographic data were obtained for each participant. To determine parasitaemia and anaemia, blood was collected by finger pricking. Stool samples were collected to determine presence of intestinal parasites. Data analyses included the calculation of prevalence, and comparisons were made using the Pearson Chi-Square test and ANOVA. Results showed that prevalence of malaria parasites was 50.7%, soil transmitted helminthes was 22.3%, and co-infection between malaria and helminthes was 22.6%. Anaemia (haemoglobin values <11g/dl) was prevalent in 57.6% of pupils. Anaemia was more common in younger children (83.3%) than older ones (62%). Results show a high prevalence of malaria parasites and anemia in asymptomatic pupils in the villages studied and emphasise the need for routine screening and treatment to prevent malaria, helminth infection and anemia in asymptomatic children.

Key words: malaria in children, anaemia, helminthes, co-infection.

INTRODUCTION

Having over half a million deaths to its credit annually, malaria -of which approximately half of the world's population is at risk- remains single handedly the number one killer of young children in sub-Saharan Africa where 90% of the deaths occur.[1]. Although the World Health Organization reports that malaria deaths have been reduced by 33%in the WHO African region, a child still dies every minute as a result of malaria [2]. This disease commonly found in the tropics and transmitted by parasites of the genus *Plasmodium* is a major public health problem with a consequent high morbidity and mortality. In Cameroon, the most common species found is *Plasmodium falciparum* which is also the most dangerous [2]. Statistics in Cameroon are a cause for concern. Malaria, the primary reason for consultation in health facilities, accounts for 57 % of all admissions in hospitals and 45 %of all deaths in health units and 42% of morbidity [3]. Another infection closely associated with malaria in endemicity and morbidity in

Cameroon is helminth infection. The four most common STHs are roundworm *(Ascarislumbricoides*affecting 1.221billion), whipworm *(Trichuristrichuria* affecting 795 million) and the anthropophilic hookworms *(Necatoramericanus*and *Ancylostomaduodenale*both affecting 740million) [4] Although they rarely cause death, these infections have an impact on physical and mental growth in childhood, nutritional status, cognitive development and lifelong health of humans [5,6,7] They are also very common in children with school children reported to bear the heaviest burden of the disease [8]. Usually, a major consequence of infections by these parasites is anaemia, a condition of lower than normal levels of healthy red blood cells and their oxygen carrying component called haemoglobin. Children with hemoglobin level <11g/dl are considered to be anaemic [9]. Anaemia seriously affects the growth of children. In the presence of polyparasitism, it effects are even more severe. Studies have shown that children having anaemia as well as other parasitic infections are a number of times more likely to be stunted and underweight than those who do not [10]. This study is an innovative one for this area, and is important because it seeks to investigate the health status for these asymptomatic pupils in relation to anaemia, malaria and helminth parasites so as to give evidence-based propositions for timely interventions.

MATERIALS AND METHODS

Study area:

Ethical clearance was obtained from the Ethical Committee of the Institute of Medical Research and Medicinal Plant Studies (IMPM) of the Ministry of Scientific Research and Innovation, Cameroon. This study was carried out in four villages: Marumba I, Marumba II, BaiManya, and Pete Bakundu villages all situated in Mbonge sub-division of the South-west province of Cameroon. Marumba I and II are respectively 22.2 and 23.7km from Kumba a large town with about 144413 inhabitants.Pete is about 25.2km from Kumba. BaiManya having coordinates latitude 431’42.996’’N and longitude 918’1.008E is about 3km from Marumba II. Pete, Marumba I and Marumba II are found in Oroko land which covers about two-thirds of the landmass of Meme and Ndian divisions. In this area, peasant farming is the major occupation of the inhabitants. Among all the villages, only Pete had a public tap as an additional source of water, while in the rest of the villages, water for everyday use is obtained from streams and wells.

Study Population:

This study comprised of 484 asymptomatic pupils from four primary schools in the four villages mentioned above. Informed consent was obtained from the guardians and parents of the pupils as well as the headmaster of each school. The age range of the pupils was from 3 years to 16 years. All pupils whose parents accepted their participation were included in the study after informed consent. Prior to collection of blood and stool samples, axial temperature (to establish or infirm fever) was obtained from all the children as well as the demographic data of each child. These were: age, weight, height, middle upper arm circumference (MUAC), and head circumference. The height was obtained using seca 225 statometer while the MUAC was obtained with seca measuring tape 201.

Parasite detection and quantification:

In order to establish parasitaemia, blood was collected from finger pricking. The middle left finger was pricked (unless the child had a wound on that finger) and blood was deposited on the slide for thin and thick blood films to be made. A slide with both thin and thick blood films was air-dried and then transported to the laboratory where they were fixed and stained with Giemsa. Microscopy was used for identification and quantification of malaria parasites. Malaria parasites were counted against 200 leucocytes and expressed as number of parasites per µl of blood with the assumption that there was an average leucocyte count of per µl of blood.

Detection of hemoglobin level:

A drop of blood was put on strips of URIT-12 haemoglobinometer for quantitative measurement of total haemoglobin in fresh whole blood. Pupils with values less than 11g/dl were considered to be anemic in this study [9].

Helminth parasites detection and quantification:

Stool samples were collected in plastic containers with a scoop. Clear instructions were given to the pupils so that they could properly collect the stool samples. The youngest ones were assisted in the collection. The samples were then kept cool in a flask with icepacks and transported to the laboratory. Collected faecal samples were stored in the refrigerator at +4◦C. The Kato-katz technic was used for the identification (based on their morphology) and quantification of the parasites and these parasites were expressed as number of eggs per gram of faeces. The

41.7mm plastic template was used for measuring stool before smearing. The slides were prepared according to WHO guidelines [11]. In addition to the Kato-Katz technique, the formol-ether concentration technique as described by Cheesbrough[12] was used for the concentration of cysts, ova and larvae in the samples.

Statistical Analysis:
Data analyses included the calculation of prevalence of the different infections. The SPSS software was used to make comparisons and determine the relationships between the parasitic infections and sex, age-groups, schools or villages. The Pearson Chi-Square test and ANOVA were performed to find out relationships between anaemia and helminthes infection and also anaemia and parasitaemia. The one-way ANOVA was also used to compare the parasite load in relation to sex, age-groups. P values <0.05 and p<0.1 were considered significant.

RESULTS

The study population of 484 children consisted of 238 (49.2%) males and 246 females (50.8%) as shown in **Table I**.

Table I: **Distribution of the pupils who participated in the study by sex and by village**

Village	Sex			
	Male		Female	
	No. of pupils	No. %	No. of pupils	No. %
BaiManya	46	43.4	60	56.6
Marumba1	18	46.2	21	53.8
Marumba2	76	51.4	72	48.6
Pete_Bakundu	98	51.3	93	48.7
Total	**238**	**49.2**	**246**	**50.8**

The mean age for the study was 9.4± 2.1years while the mean temperature was 37.4± 0.4 degrees, the mean weight was 28 ± 8kg and the mean height was 127.1cm ± 13 cm. The mean haemoglobin level for the study population was 10.7± 1.4g/dl. According to the WHO definition of anaemia as heamoglobin(Hb) levels less than or equal to 11g/dl of blood, this study showed that 57.6% of the primary school pupils were anaemic. There were two cases of severe anaemia(Hb level < 5.4) and two with Hb level <6.5. The highest level of anemia was seen in the younger children than the older ones. 83.3% of the under 6 age group were anaemic while 62% in the 6-10 age group and 47.6% in the group with children above 10. This difference was significant (P=0.02). When considered according to sex, 59.6% of the girls were anaemic as opposed to 55% of boys. There was no significant relationship between malaria parasiteamia and anemia or between helminth infection and anemia.**Table II**shows the prevalence of anemia in the different villages.

Table II: Blood Haemoglobin levels in the villages

Village	Haemoglobin			
	< 11(Anaemia)		> 11	
	No. pupils	No. %	No. pupils	No. %
BaiManya	65	61.3	41	38.7
Marumba1	32	82.1	7	17.9
Marumba2	78	52.7	70	47.3
Pete_Bakundu	105	54.4	88	45.6
Total	**280**	**57.6**	**206**	**42.4**

The prevalence of malaria parasites in the study was 50.7%. Parasitaemia was higher in males than in females (52.3 to 49.0%). This difference was not significant. However, there was a significant relationship between parasitaemia and age (p<0.1, p=0.88). **Table III** shows the prevalence of parasitaemia in the different villages.

The mean trophozoite count in the positive samples was 1593 parasites per mm3 of blood.

The prevalence of intestinal helminthes was 22.3%. Using the Kato-katz technique, the mean egg count with *Ascarislumbricoides* as the highest parasite was 975±171eggs/gram of faeces. Mostly two species of intestinal helminthes were recovered from the stool samples and these were *Ascarislumbricoides*(12%) and *Trichuristrichuria*(10.8%). Two methods were used to analyse the stool samples. With the kato-katz technic, the results are as shown above. With the formol-ether concentration technic, the helminth prevalence was 25.7%.

Bacteria such as *Entamoeba coli* and *E. histolytica* were also seen in the samples when the concentration technique was used. Ages 6-10 carried 54% and 68% of the infection with malaria parasites and intestinal helminthes respectively. There was a significant difference of infection among the different age groups(p=0.091).

The results of this study also showed that some children were harbouring infections from both the malaria as well as the intestinal parasites. This co-infection of malaria and helminthes stood at 22.6%. Also, 22.7% of children had malaria and helminth parasites as well as had anemia.

Table III: Age Distribution ofParasitaemiain the villages

Village	Age group	Parasitaemia PN			
		Negative		Positive	
		No. pupils	No. %	No. pupils	No. %
BaiManya	6 to 10	37	52.9	33	47.1
	> 10	26	72.2	10	27.8
Marumba1	< 6	2	66.7	1	33.3
	6 to 10	10	37.0	17	63.0
	> 10	1	11.1	8	88.9
Marumba2	6 to 10	36	38.7	57	61.3
	> 10	31	60.8	20	39.2
Pete_Bakundu	< 6	4	44.4	5	55.6
	6 to 10	57	48.7	60	51.3
	> 10	34	50.0	34	50.0
Total	**< 6**	**6**	**50.0**	**6**	**50.0**
	6 to 10	**140**	**45.6**	**167**	**54.4**
	> 10	**92**	**56.1**	**72**	**43.9**

DISCUSSION

Malaria: This study is innovative in being the first reported for this region in Cameroon. The results indicate a very high prevalence of malaria (50.7%) in asymptomatic pupils in these villages. There was a significant association between malaria parasite presence and age. The prevalence value obtained is higher than that reported in asymptomatic pupils in Bolifamba in Cameroon[13]in children in Tiko, Limbe and Idenau[14] all areas in the South-west region of Cameroon. It is also higher than reports in Kajola in Nigeria[15]. Such prevalence is evidence of hyper endemic nature of the study area. Houses, in the four villages in which this study was carried out, are mostly surrounded by bushes, ponds and slow-moving streams which are suitable breeding areas for the mosquito vectors and would explain such prevalence. Furthermore, previous studies neighbouring regions showed the biting activities of mosquitoes to be all year round with peaks during the rainy season [14]. This study was carried out from the beginning of the rainy season and would also explain the high prevalence. The significant association of malaria parasitaemia with age as revealed in this study could be explained by the fact that with age comes immunity and thus a reduced chance of succumbing to disease. This corresponds to other studies in Bolifamba, also found in the South-west region of Cameroon[13]. Asymptomatic parasitaemia in these pupils indicate the degree of immunity that has developed. So far malaria control relies mostly on prompt diagnosis and treatment. This study suggests intermittent preventive treatment especially in school could be the way to go given this number of asymptomatic cases.

Anaemia

The results of his study showed anaemia to be significantly associated to age (p=0.02).The prevalence of anaemia was 57.6%.This is also unusually high, and will have consequences on learning and development in these children if it is not addressed. Vulnerability to anaemia is usually seen in pupils or children of school going age and this is rendered more severe by the carrying of other parasitic diseases such as malaria or helminth infections [16]. In 6-10year old age-group especially, we report multiple infections in the children as well as lowest levels of hemoglobin in blood. The presence malaria parasites as well as helminth infection promises severe consequences of anaemia. Studies carried out in in Kenya and Tanzania showed similar high prevalence [9,16]. Other studies in Cameroon and Kenya reported a lower prevalence of anaemia, 30% and 13.5% respectively [13, 17]. Many factors can be attributed to causing anaemia and these include a combination of poor nutrition, helminth infections, infectious diseases and haemoglobinopathies[9,10]. In endemic countries, malaria is the usual identified cause of anaemia [9]. However, nutrition has been reported to be an even greater risk factor for anemia than malaria [18]. This would explain the results we have since given that the association between malaria infection and the presence of anaemia though present was not significant indicating that another factor needs to be considered. It is therefore evident that nutritional tendencies in these villages have a role to play in promoting anaemia. From interviews of the children and teachers of the schools in which this study was carried out, it was gathered that vegetables were not a preferred choice of food. In order to make a worthwhile management of anaemia in this region, proper nutrition advice should be included in the school curriculum.

Helminths

Studies have shown that most helminth infections occur in developing countries with the greatest burden being on children [24] where it has been linked to malnutrition, and iron-deficiency anaemia. In terms of infection rates, this study has shown that when considered according to the different age groups, the age group of 6-10 harboured the majority of helminth infections too. The benefits of treating school-aged children for intestinal parasites have been documented repeatedly and convincingly [19, 20, 21].In this study, helminth infection was present in 22.3% if the pupils. The parasite *Necatoramericanus*(hookworm) though very common elsewhere[13] was rare probably due to the time it took from collection to laboratory transportation and analyses. It could also be that hookworm is rare in this area as has been reported previously by another study in Marumba II which also did not find hookworms in the region [22]. In this previous study too, a much higher percentage of the helminths *Ascarislumbricoides* and *Trichuristrichuria* was reported than in our study. This is probably because in this particular village, the pupils of the school had received treatment for worms prior to our study. Low hookworm prevelences were also recorded in Bolifamba, Cameroon and in this study, the absence was associated with the soil type in the area [13]. Two methods were used for the identification and quantification of the helminth parasites. Results with the formol ether concentration technic showed a higher percentage of helminth infection than with the Kato-katz technic indicating the former technique to be more sensitive than the latter.

There was no significant relationship between parasitaemia and helminth status. This is in contrast with observations made in other studies in Nigeria [15] where a positive and statistically significant relationship was found between geohelminths and malaria parasite infection.

Co-infections: Diseases such as malaria and helminth infections are usually most prominent in the poorest and most economically disadvantaged people of the society. Across Africa, malaria parasites and helminths occur in the same regions[23]. School age children also bear the highest burden of these diseases. Most times these infections do not occur singly but in a multiple way leading to even severe consequences. Brooker*et al*[23] reported that helminth infections can increase susceptibility to malarial infection. In our study, co-infection between malaria and helminthes stood at 26.1%. It was also found that 31.8% of the children had anaemia in addition to infection with both malaria parasites and helminths.These results are higher than those obtained in Osogbo [15], Nigeria and also those obtained in Buea in Cameroon[13] and underline the urgency of measures to be taken to combine malaria control with helminth control.

CONCLUSION

It can be concluded that there is prevalence of malaria parasites, anaemia and helminth infection in Marumba I, Marumba II, BaiManya and Pete-Bakundu villages in Mbonge Sub-division in the Southwest Province of Cameroon. This burden is highest in children of school age. We recommend that routine screening and treatment of malaria parasites, antihelminthictreaments, and micro-nutrient supplementation be done in schools and in the community. Also, social education programmes targeting parents and inhabitants of communities to increase awareness of the consequences of these infections and anemia should be initiated as well as proper hygiene practices. The importance of such interventions based on evidence, will encourage the parent to be a partner in ensuring the well-being of the children.

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