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Original Research Article

Plasmodium falciparum, Schistosoma mansoni and Amebiasis Co-Infections: Synergetic and Antagonistic Effect on Anemia in Cameroonian School Children

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ABSTRACT

School children aged 3-15 years were enrolled in a cross-sectional study in Yoro village, of the Central region of Cameroon. Stool samples were examined for *Schistosoma mansoni* eggs using Kato Katz technique. The formol ether technique was used to identify protozoan cysts. Blood samples were examined for malaria parasites and hemoglobin concentrations using the Giemsa stain and Haemoque methods, respectively.

Out of 254 children examined, 161 (63.4%) were infected with any parasites (*P. falciparum*, *S. mansoni*, *E. histolytica* and *E. coli*) among whom, 120 (74.5%) had single infection while 41 (25.5%) had multiple infections. *P. falciparum* parasitaemia was significantly higher in males compared to females (P<0.05). *P. falciparum* infections occurred significantly more frequently as single species infection (P<0.05).Co-infections of other parasites among *P. falciparum* infected children was 28.3%.Children with *E. coli* infection were significantly infected with *S. mansoni* (P<0.05).Malaria parasite densities increased with increasing infection intensity of *S. mansoni* and with increasing number of other co-infecting amoeba species.

Anemia prevalence was 70.1%. The mean Hb levels decreased significantly with increasing infection intensities for *P. falciparum* and *S. mansoni* infections (P < 0.05).Anemia prevalence of at least 50.0% was observed for combinations involving *E. coli* with significantly high values recorded for *P. falciparum* +*E. coli*, *S. mansoni* +*E. coli* and *P. falciparum* + *S. mansoni* +*E. coli*+ *E. histolytica* (P<0,05).

These results demonstrate that polyparasitism involving malaria, schistosomiasis and amebiasis is prevalent in infected individuals of Yoro village, thus calls for integrated disease control interventions.

Keywords: *Malaria, Schistosomiasis, amebiasis, co-infections, anemia, Cameroon.*

INTRODUCTION

Malaria, schistosomiasis and intestinal parasites are the most important parasitic infections in Sub-Saharan Africa, where a significant proportion of the populations including school children are exposed to these infections. They are particularly more prevalent in rural communities and are closely associated with poverty (Brooker *et al*., 2007). [1] Understanding the complexities of the pathogen-host landscape in settings

endemic for multiple human parasites is essential for mitigating morbidities. Identifying interspecies associations could advance intervention by targeting efforts that have the most prevention and treatment benefits (Sousa-Figueiredo *et al*., 2012). [2]

In Cameroon, infections due to malaria, schistosomiasis and intestinal parasites (helminthes and protozoans) account for a large proportion of the burden of disease, with far-reaching effects on the nation's health and economy. Malaria is responsible for 57% of admissions in hospital, 45% of deaths in health units and 42% of morbidity. In children less than 5 years, 41% of deaths are due to malaria (MINSANTE, 2007, 2012). [3-4] The infection rate of schistosomiasis stands at more than 5 millions people at risk of infection, with 2 millions people currently infected and more than 10.000 cases declared each year. For intestinal parasites, more than 10 million persons are infected (PNLSHI, 2005) [5] with transmission trend varying in different localities (Tchuem Tchuente *et al*., 2012). [6]

Despite extensive research on these individual infections or some combinations of these infections (malaria and soil transmitted helminthes, schistosomiasis and soil transmitted helminthes or malaria and intestinal parasites) (Ebako*et al*., 2010; Richardson *et al*., 2011; Makoge *et al*., 2012; Nkengazong *et al*., 2014, 2015), little is known about the distributions, causes and effects of co-infections involving malaria, schistosomiasis and intestinal parasites. [7-11]

The purpose of this study was to investigate co-infection with malaria, schistosomiasis, and amebiasis in a population of children in the central region exposed to these diseases. It was specifically aimed to:

i) Determine the prevalence and intensity of different parasite species, and infestation rate of different parasite associations in relation to anemia;
ii) Compare malaria and *S. mansoni* parasite densities in co-infected children with those not co-infected for increasing levels of infestation.
iii) Study the possible effect of *Entamoeba histolytica* and *E. coli* on malaria and schistosome infections.

Results from this study could advance intervention by targeting efforts that have the most prevention and treatment benefits for these infections in endemic areas of Cameroon.

MATERIALS AND METHODS

Study area

The present study was conducted in Yoro village (04°32.626'N, 11°09.496 E) in Bokito town located in Mbam and Inoubou Division of Center region. This village was selected because of previous records that showed it to be endemic for parasitic infections (Moyou *et al*., 2003). [12] Two streams cross this village: Assaga, which passes in Yoro center of about 100 m from Government school and homes of inhabitants; Gindigueldje, which flows towards other villages (Bougnangagne and Bongando). The population density of these villages is made up of about 1000 inhabitants and the villagers practice mostly farm work, fishing and hunting. Despite the presence of forages in the village, the human populations still carry out their daily activities (fishing, swimming, laundry etc) in streams. This village is characterized by almost total absence of toilets that make the inhabitants to defecate in the bush or in the streams. Garbage piles on which children play on, are also found around the school premises.

Study subjects

The study was conducted during the months of October to February 2015. Out of the 295 school children contacted, 254 (135 boys: 53.1% and 158 girls:

46.9%) participated in the survey. The sampled population was between the ages of 3-5 years, 6-10 years and 11-15 years.

Subject Consent

Administrative authorities (Chief of health District, school Directors and traditional Leaders) were informed about the project and they gave their verbal consent for the study to be undertaken. A written informed consent that met the standards of the National Ethical Commission was obtained from the pupils or the guardians of the young children that accepted to participate in the study.

Parasitological study

Following registration, two stool samples were collected from each participant in 50 ml screw-cap vials. In one of the srew-cap vials was added 10% formol to conserve the parasitic forms of the parasites. The samples were transported to the Parasitology laboratory (Nkomo) of the Medical Research Centre (IMPM, Yaounde) and examined following the protocol used by Nkengazong *et al*. (2015). [11] The eggs were counted under a light microscope at 10X magnification and their number expressed in eggs per gram of stool (epg),while the cysts of protozoan (Amoeba species) were observed at a magnification of 40X. Intensity of schistosome infection was evaluated according to Kinung'h *et al*. (2014). [13]

Blood samples were collected from a finger prick to prepare thick and thin blood smears. Thin smears (only) were fixed with methanol. Thick and thin smears were stained with 10% Giemsa solution and examined under a light microscope. A blood smear was considered negative if parasites were not detected after examining 100 oil-immersion fields of the thick smear. When malaria parasites were detected in a blood smear, the parasite density was determined on the base of the number of parasites per 200 leukocytes and then converted to 8,000 WBC/µl of blood, while parasitaemia was classified as mild, moderate and heavy infection according to Onyido *et al*. (2015). [14]

Quantitative measurement of total hemoglobin in fresh whole blood followed the protocol used by Nkengazong *et al*. (2015). [11]

Data Analysis

The Chi-square test was used to compare the prevalence of parasitic infections and anemia in relation to sex and age groups while one – way ANOVA or Kruskal-Wallis tests were used to compare the parasite intensity in relation to sex, age groups, and different parasites combinations. The Kruskal-Wallis test was used when the conditions of parametric ANOVA were not fulfilled. The level of statistical significance was at 95% ($P < 0.05$).

RESULTS

Parasite prevalence and infection intensities

Out of the 254 children included in the analysis, 161 (63.4%) were infected with at least one of the parasites *P. falciparum, S. mansoni, E. histolytica* and *E. coli. S. mansoni* infections were generally light to moderate with only 13 children (22.4%) being heavily infected (eggs per gram ≥ 400). *P. falciparum* infections ranged from mild to heavy infection with high values observed for mild infection (40.7%) followed by heavy infection (37.2%). *P. falciparum* infection (44.5%) was the most prevalent, followed by *S. mansoni* (22.8%), *E. coli* (20.1%) and *E. histolytica* (4.7%). Parasitaemia for malaria parasite ranged from 40- 20000 trophozoites/µl of blood, while the egg count of 24-5160 eggs per gram (epg) of stool was recorded for *S. mansoni* (Table 1). *P. falciparum* parasitaemia was significantly higher in males compared to females ($P<0.05$). Children of age group 6-10 years had high prevalence of the different parasites species same as high parasite intensities (*P. falciparum* and *S.*

mansoni) with the exception being for *E. coli* where the highest prevalence was observed in children aged 11-15 years. This difference was however not significant (P>0.05).

Prevalence of co-infections

Out of the 161 infected children, 120 (74.5%) had single infection, while 41 (25.5%) harbored more than one parasite species. Overall, *P. falciparum* infections occurred significantly more frequently as single species infection (48.4%; P<0.05) compared to other parasite species, while the occurrence of multiple species infection was more observable for combinations involving *S. mansoni* (23.6%) and *E. coli* (21.7%). *E. histolytica* occurred only as multiple species infection (Fig. 1).

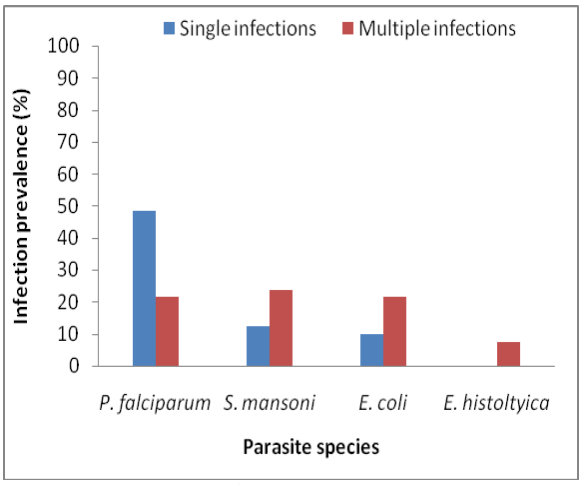


Figure 1. Occurrence of parasites species as single and multiple infections

Associations between parasite infections

Co-infection of *S. mansoni*, *E. coli* and *E. histolytica* among *P. falciparum* infected children was 28.3% (32/113). The most common parasite combinations were *P. falciparum* + *S. mansoni* (14.2%), *P. falciparum* + *E. coli* (5.3%), *P. falciparum*+ *S. mansoni* + *E. coli* + *E. histolytica* (1.8%). Co-infections of *E. coli* and *E. Histolytica* equally occurred among *S. mansoni* infected children with the most common parasite combinations being *S. mansoni* + *E. coli* (12.1%), *S. mansoni* + *E. coli* + *E. histolytica* (5.2%). *E. histolytica* occurred together in 11.8% of *E. coli* infected children. Children with *E. coli* infection were more likely to be infected with *S. mansoni* (P<0.05) compared to children who were not infected with *E. coli.* The prevalence of malaria and other parasite co-infection among the different age groups were 9.1% (1-5years), 31.4% (6-10 years) and 66.6% (11-15 years). However, the difference was not significant.

Association between malaria parasite density and intestinal infections

Malaria parasite densities tended to increase with increasing infection intensity of *S. mansoni.* The mean density (386 trophozoites/µl of blood) of *P. falciparum* in children moderately infected with *S. mansoni* was lower than those not infected, while in children heavily infected, this value (1845.7 trophozoites/µl of blood) was higher than those uninfected with *S. mansoni*. No significant difference was observed in the two cases. Malaria parasite density decreased or increased with increasing number of co-infecting intestinal parasites species. The mean malaria parasite parasitaemia for children without any intestinal infection (malaria only) (n = 78) was higher compared to those recorded for children co-infected by *P. falciparum* + *S. mansoni* and *P. falciparum* + *E. coli*. Also, high mean malaria parasitaemia were observed in children co-infected by *P. Falciparum* + *E. histolytica* and *P. falciparum* + *S. mansoni* + *E. coli* + *E. histolytica*. Children infected with *S. mansoni* only (n=13) had higher infection intensity compared to those co-infected with other parasites species (Table 1). No significant difference was observed for any of the cases.

Table 1.*P. falciparum* and *S. mansoni* density in different parasite associations

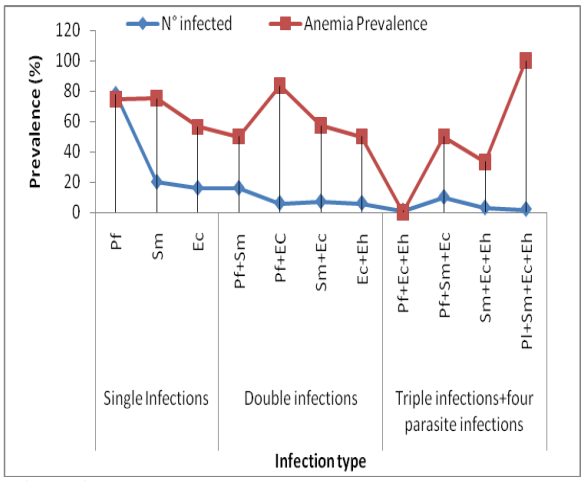
Parasite species	P. f only (n=78)	P.f+S.m (n=16)	P.f+E.c (n=6)	P.f +E.h (n=1)	P.f+S.m+E.c+E.h (n=10)
Mean parasitaemia	1021.02±2741.5	390±354.3	213±190.4	3040±0.0	1368±2335.2
Parasite species	S.m only (n=13)	S.m+P.f (n=9)	S.m+E.c (n=6)	S.m+P.f+E.c+E.h (n=2)	----------
Mean density	775.4±1567.2	200±218	136±195.4	240±169.7	……

P.f: *P. falciparum*; S.m: *S. mansoni*; E.c: *E. coli*; E.h: *E. histolytica*

Prevalence of anemia associated with infection status

Out of the 254 children included in the study, 178 (70.1%) were anemic. Of this number, 5 (2.8%), 160 (89.9%) and13 (7.3%) had light, moderate and severe anemia respectively. Overall mean hemoglobin (Hb) concentration was 8.9±2.6 g/dl. For single infection, high values of anemic infected children were recorded for *S. mansoni* (75.0%) followed by *P. falciparum* (74.4%) in almost equal proportion of mean Hb values (7.1±1.1 and 7.4 ± 0.9g/dl respectively).

When double infections, triple infections and four parasite infections are considered, with the exception of *P. falciparum* + *E. coli* + *E. histolytica* and *S. mansoni* + *E. coli* + *E. histolytica*, anemia prevalence of at least 50.0% was observed for combinations involving *E. coli* with significantly high values recorded for *P. falciparum* + *E. coli*, *S. mansoni* + *E. coli* and *P. falciparum* + *S. mansoni* + *E. coli* + *E. histolytica* (P <0.05). For *P. falciparum* and *S. mansoni* infections, mean Hb levels decreased significantly with increasing infection intensities (P<0.05). Children singly infected had low Hb values and anemia prevalence ranging between 56.0% and 75.0%, while children who were infected with more than one parasite species tended to have lower mean hemoglobin levels and anemia prevalence ranging from 33.3% to 100.0%. The highest prevalence of anemia (100.0%) was observed in children co-infected with four parasites species *P. falciparum*+ *S. mansoni* + *E. coli* + *E. histolytica* (Fig. 2). There was no significant difference in the prevalence of anemia and mean hemoglobin levels between uninfected children and those infected with one or more parasites.



P.f: *P. falciparum*; S.m: *S. mansoni*; E.c: *E. coli*; E.h: *E. histolytica*

Figure 1. Anemia prevalence in different parasites infection associations

DISCUSSION

Malaria, schistosomiasis and intestinal parasites are a major public health problem particularly in school children in Cameroon where their occurrence as multiple species infections is known to be the norm, causing enormous morbidities in infected children.

Understanding the epidemiology of these infections among infected children and their associated morbidities (lower hemoglobin levels and anemia etc) is vital as findings may support design appropriate disease control strategies. Results revealed from this study showed that malaria, schistosomiasis and amebiasis infections are prevalent in school children of Yoro village and co-infections of these parasites were common. These findings are supported by other studies conducted in other localities of Cameroon (Richardson *et al*., 2011; Nkengazong *et al*., 2014) and in other countries (Midzi*et al*., 2008; Mazigo *et al*., 2011; Kinung'h *et al*., 2014). [8,10,13,15,16] The most prevalent parasite species in the studied population were *P. falciparum*, *S. mansoni*, and *E.*

coli with *E. histolytica* being the least prevalent.

The presence of *S. mansoni* is in accordance with previous studies in the area and is related to the occurrence of its snail intermediate hosts with their ecological preferences (Moyou*et al*., 2003). [12] Children of 6-10 years had significantly high egg load of *S. mansoni*. This is in line with the declarations of PNLSHI (2005) which situated the most vulnerable age group of schistosomiasis between 6-14 years. [5] The significant occurrence of *P. falciparum* compared to other parasites could be related to the period that this study was conducted. It has clearly been demonstrated that, the biting activities of female anopheles mosquitoes occur all year round (Songue *et al*., 2013) and the high malaria transmission period is found in September and November (Briand *et al*.,2005). [17-18] This observation could explain the high prevalence of malaria obtained, since the study was conducted towards the end of the rainy season, when numerous standing water points are observable that constitute suitable breeding areas for the mosquito vectors. The significant high parasitaemia of *P. falciparum* observed in males compared to females has equally been reported in other studies (Briand *et al*., 2005). [18] Socio-economic and behavioral factors could act as shared risk factors for exposure of males to high frequency of mosquitos' bites than females. In malaria endemic areas, parasite intensity decreases with increasing age due to development of anti-malarial specific immunity (Warrel *et al*., 2002; Kinung'h *et al*., 2014). [13,19] Our result is contrary to this observation, as children of age 6-10 years had significantly high malaria parasite prevalence and high intensity compared to those below 5 years and above 10 years. According to the recommendations of the National Control Program of Malaria (MINSANTE, 2010), implementation of control strategies against malaria infection is targeted on children below 5 years and pregnant women, which could however lead to a displacement of anti-malarial specific immunity in children. [3] Also, immunity develops with age, thereby reducing the chances of contracting the disease. This could explain the low prevalence and parasite parasitaemia observed in other age groups.

Similar to the present work, previous studies have demonstrated that most parasitic infections do not occur only singly but as co-infections (Fleming *et al*., 2006; Brooker *et al*., 2006; Steinman *et al*., 2008; Nkengazong *et al*., 2010, Nkengazong et al., 2014), since a variety of environmental and host factors may influence the epidemiological and geographical patterns of infections and diseases (Mwangi*et al*., 2006). [10,20-23,24] The coexistence of malaria, schistosomiasis and amebiasis could be due to the availability of breeding sites for the intermediate host (fresh water snails), malaria vectors (*Anopheles* mosquitoes) and inadequate hygienic conditions (lack of toilets and good water sources) in the study area.

This study showed to some extend, a negative association between *S. mansoni* infections and malaria parasite intensity. Many other investigations support our findings (lower *P. falciparum* intensities in children harboring light and moderate schistosome infections vis-a`-vis those not co-infected (Briand *et al*., 2005; Lyke *et al*., 2005; Kinung'h *et al*., 2014). [13,18,25] This could be due to cross reactivity between anti-*P. falciparum* antibodies and anti-schistosomal antibodies as earlier reported for *S. mansoni* and *P. falciparum* specific antibodies (Pierrot *et al*., 2006; Helmby, 2007). [26-27] However, the high mean value of *P. falciparum* parasitaemia in single infection than multiple infections and in children heavily infected by *S. mansoni* compared to uninfected ones could equally highlight the importance of host factors in determining parasite loads,

and also suggest a synergetic relationship. This falls in line with the observation of Lia *et al.* (2012) who found a positive association between the intensities of *P. falciparum* and *S. haematobium* among co-infected children. [28] It has been demonstrated that the immune responses induced in humans during malaria and schistosomiasis infection is complex and not well known, and the nature of immune responses may vary according to the stage and intensity of infections (Briand *et al*., 2005). [18] This could to a global extend, explain the non-linear relationship we observed between malaria parasite densities and *S. mansoni* intensities. Till present, studies involving the co-existence of malaria, schistosomiasis and amebiasis in infected individuals in Cameroon are still rare. However, previous results (Nkengazong *et al*., 2014) in accordance to the results of this study showed that, co-existence of *P. falciparum* and *Schistosoma* species with *E. histolytica* or *E. coli* could lead to an antagonistic or a synergetic relationship, consequently being at the origin of much morbidity in infected children. [10]

Anemia was found to be a serious public health problem among our study population with high prevalence (70.1%) compared to previous results obtained in the same area and those obtained in other localities of Cameroon (Richardson *et al*., 2011; Nkengazong *et al*., 2014, 2015). [8,10,11] This observation may reflect a changing pattern in the distribution of parasite infections (prevalence and infection intensity) in different study areas. Similar results have been obtained in two different studies conducted in the same area (Kinung'h *et al*., 2014). [13] Children infected by *E. histolytica* were systematically infected by *E. coli*. This could explain the significantly high values of anemia observed in children with multiple infections involving *E. coli* and other parasites species. Previous studies have showed multi factorial aetiology of anemia with parasitic infections including malaria, schistosomiasis and amebiasis contributing important role (Friedman *et al*., 2005; Koukounari *et al*., 2008; Soares Magalhães *et al*., 2013). [29-31] Also, the low mean Hb values obtained in children with single infections compared to some cases of multiple infections indicate that, in addition to the known effect of single parasite species on anemia, multiple parasite infections can interact to enhance the risk of anemia. This observation is in line with the studies carried out in North-western Tanzania (Kinung'h *et al*., 2014). [13] Majority of anemia cases in the current study were moderate. Only 13 (7.3%) had severe anemia, probably due to the fact that majority of infections were light or moderate. This could indicate that the severity of anemia is dependent on infection intensity. This observation is in agreement with previous findings (Ajanga *et al*., 2006; Koukounari *et al*., 2008; Kinung'h *et al*., 2014). [13,30,32] Limitations of the current study in elucidating associations between malaria and other intestinal parasites co-infections include the lack of information on household, socioeconomic status and environmental factors which have been shown to influence occurrence of co-infections by other studies (Brooker *et al*., 2012), in association with other causes of anemia such as malnutrition (Miguel *et al*., 2015). [33-34]

Overall, results of this study have demonstrated that malaria, schistosomiasis and amebiasis infections are prevalent in school children of our study area and that polyparasitism is also very common. These findings also suggest that concurrent *P. falciparum, S. mansoni* and amoeba infections increase the risk of lower Hb levels and anemia which in turn calls for integrated disease control interventions. The associations between malaria and other parasitic infections detected in this study were not conclusive and hence needs further investigation.

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