Original article

Prevalence of malaria and intestinal parasites coinfections and their effects on hemoglobin levels among school-aged children in Bebuatsuan clan, Obudu, Cross River State, Nigeria

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Abstract

Malaria and intestinal helminths coexist with negative consequences on health of children. This work was to investigate the prevalence and the impact of these infections on hemoglobin levels of children in Bebuatsuan clan, Obudu. Blood films and stool samples from 285 school-aged children were examined using standard parasitological methods for malaria infection and intestinal parasites while packed cell volume and hemoglobin estimation were determined using the standard methods of Dacie and Lewis, (2015). Of the 285 subjects examined, 117(41.1%) harbored malaria parasites only, 75(26.3%) harbored intestinal parasites and 36(12.6%) were co-infected with both malaria and intestinal parasites. The mean hemoglobin levels of the control group (12.97$\pm$2.1) was significantly higher than that of the test group (11.13$\pm$6.1) (p=0.000). Malaria, intestinal parasites infection(s) and coinfection were highest in children aged 8 to 11years 43.3%, 36.7% and 16.7%, respectively. Malaria and intestinal parasites infections were higher in males (47.16%, 28.3%) than in females (33.3%, 23.81%) while co-infection was higher in females (14.28%) than in males (11.32%). Subjects with body weight of 42-56kg had the highest malaria infection (100%), intestinal parasitosis (33.3%) and co-infection (33.3%). *Ascaris lumbricoides* had highest prevalence among intestinal parasites 42(28.88%). This work has demonstration a strong influence of malaria and intestinal parasitosis on the blood levels of school-aged children.

Key words: Coinfections, Hemoglobin, Intestinal parasites, Malaria, School aged children

Malaria affects approximately 3.4 billion people worldwide, 1.2 billion are at high risk; and 198 million cases of symptomatic malaria have been reported in 2013[1]. Malaria occurs mostly in tropical and sub tropical regions particularly in sub Saharan Africa and south East Asia[2]. The six highest burden countries in the WHO African region (in order of estimated number of cases) are: Nigeria, Democratic Republic of Congo, United Republic of Tanzania, Uganda, Mozambique and Cote d`ivoire[2]. School-age children have attracted relatively little attention as a group in need of special measures to protect them against malaria[3]. The severity, duration and eventual outcome of malaria episodes in children may depend on the absence or presence of concurrent infections. When these diseases occur concurrently with malaria, they have the ability to interfere with the immune responses to malaria and may initiate some of the life threatening metabolic and pathophysiological disorders observed in childhood malaria. Among parasitic infections, malaria and intestinal helminths coexist widely with micronutrient deficiencies and contribute importantly to anemia and a cycle of retarded growth and development. Studies from human populations conducted in Africa revealed that helminthic infection can have a negative effect on host response to malaria, including increased susceptibility to plasmodium infection and increased severity of disease[4]. Children tend to be more active in the infected environment and rarely employ good sanitary behavior. As a result, since they are the primary sufferers, they may become essential vectors for the reintroduction of the pathogens to the local environment, hence maintaining transmission. This infestation leads to nutritional deficiencies and impaired physical development which have negative consequences on cognitive function and learning ability[5]. The aim of this study was to determine the current prevalence of malaria parasites and intestinal parasites among children in the study area, to determine the factors that predispose these children to this co-infection and to determine the impact of malaria and intestinal parasite co-infection on the children involved in the study.

Materials and methods

Study area

The study area was Bebuatsuan clan which is a small rural settlement in the Ipong ward of Obudu Local Government Area, Cross River State. The Bebuatsuan people belong to the Ekoi tribe and speak the Bette dialect. Bebuatsuan has eight (8) villages within which there are two primary schools, one secondary school, one community health center and one daily market. The clan has a population of 2143 people[6]. The people are predominantly farmers with yams being the major cash crop together with groundnuts, cassava, rice, beans and vegetables. Their source of water is from a number of streams, however, during the dry seasons the water table may fall below the level of the stream's bed causing it to dry out. Because of this, there have been several borehole projects resulting in six completed functional boreholes within the various villages.

Study design

This was a cross sectional survey.

Study population

Blood and Stool samples were collected from 285 children selected by simple random sampling method. The inclusion criteria included children;

- Who were aged 4-15 years

- Whose parents or guardians gave informed consent

- Who met the criteria above and were residents in the study area

The exclusion criteria included children;

- Who were undergoing antimalarial drug therapy or who were on medication for parasitic infections during the period of sample collection

- Residing outside the study area.

- Whose parents or guardians refused to give consent

Ethical consideration

Ethical clearance for this research was sought and obtained from the Cross River State Ministry of Health. Informed consent form was signed after explanation to the parents/ guardians of the subjects on the advantages of the research.

Questionnaire administration

Structured questionnaires were administered with the help of the subjects' parents to obtain relevant data.

Sample collection

Two milliliters of blood was collected from each subject for the diagnosis of malaria infection and the remaining was added into Ethylene Diamine Tetra-acetic Acid (EDTA) bottle in the concentra-

tion of 2mg/ml of blood for packed cell volume and hemoglobin estimation. Parents were provided with clean, dry, leak-proof universal containers for stool sample collection. They were then instructed on how to collect the specimen in the morning from their children without contaminating their hands or the sample. The specimen containers were collected from them early in the morning and taken to the laboratory for processing.

Laboratory methods

Malaria infection was diagnosed by Giemsa staining technique. Packed cell volume (PCV) was determined using microhematocrit method while hemoglobin (Hb) estimation carried out using Cyanmethemoglobin method as described by Dacie and Lewis[8]. Mean cell hemoglobin concentration (MCHC) was calculated by dividing hemoglobin value in g/dl by packed cell volume values in % and multiplying by 100. It was expressed in percentage (%).

Diagnosis for intestinal parasites was done using direct wet mount microscopic examinations and formol ether concentration technique as described by Cheesbrough, (2006).

Data analysis

Data analysis was done using the statistical package for social sciences (IBM SPSS version 20.0, International Business Machines Corporation, Armonk, New York, USA).

Descriptive results were presented as frequencies and percentages. PCV, Hb and MCHC were described as Mean ± Standard error of mean using Microsoft Excel 2013. Chi-square (X^2) was used to study the relationship of infection with various demographic factors. A probability value $p < 0.05$ was considered statistically significant.

Results

Of the 285 subjects enrolled in the study, 117(41.1%) harbored malaria parasites only, 75(26.3%) harbored intestinal parasites and 36 (12.6%) were co-infected with both malaria and intestinal parasites. Table 1 shows the distribution of parasitic infections amongst subjects examined according to age group. The mean hemoglobin levels of the control group (12.97±2.1) was significantly higher than that of the test group (11.13±6.1) (x^2= 62.5 df(2), p=0.000). Children aged 8-11 years had the highest infection rate of 43.3% for malaria parasites, 36.7% for Intestinal

parasites and also had the highest level of co-infection (16.7%) while subjects aged 4-7 years were least infected with malaria parasites (39.13%) and subjects 12-15 years were least infected by Intestinal parasites (15.8%) and also had the lowest level of co-infection (10.53%). The difference in infection among the age groups was statistically significant (x^2= 0.595, df(2), p<0.001). Table 2 shows the distribution of parasitic infections amongst subjects examined according to gender. Malaria and intestinal parasites infections was higher in males 47.16% and 28.30% than in females 33.33% and 23.81% respectively while co-infection was higher in females (14.28%) than in males (11.32%). The difference in the distribution of parasitic infections amongst the subjects examined according to gender was statistically significant (x^2= 1.650, (df=1), p=0.438). Uninfected male subjects had higher hemoglobin (13.37±3.0g/dl) than infected male subjects 10.32± 0.43g/dl. Similarly uninfected female subjects had higher hemoglobin (12.57± 1.8g/dl) than infected female (10.53± 0.59g/dl). The difference was statistically significant (x^2 = 24.5 df(1), p=0.000). Table 3 shows the distribution of parasitic infections amongst subjects according to body weight. Subjects with body weight of 42-56 kg had the highest malaria infection (100%), intestinal parasitosis (33.3%) and Co-infection (33.3%) while subjects with body weight 57-71 kg had the least malaria infection (0%), intestinal parasitosis (0%) and co-infection (0%). The difference in the distribution of parasitic infections according to body weight was statistical significant (x^2= 2.116, df=3, p=0.714).

Figure 1 shows the distribution of intestinal parasites among subjects examined. Males were more infected with *Ascaris lumbricoides* (16.98%) than the females (11.9%). The difference was statistically significant (X^2 = 54.6 df (1) P = 0.001). *Ascaris lumbricoides* 42(28.88%) recorded the highest intestinal parasite detected while *Strongyloides stercoralis* recorded the lowest prevalence 6(3.77%). Fig. 2 shows the relationship between packed cell volume (PCV) and parasitic infections among subject examined. Subjects with PCV ranges between 20-25% had the highest intestinal parasite infection and also co-infection (33.3%). Subjects with PCV 38 to 43% had the highest prevalence of malaria parasite infection (42.1%) while those with PCV range 20-25% had the lowest prevalence of malaria infection (33.3%). The difference was not statistically significant (x^2= 7.994, df= 3, p=0.239).

Table 1: Distribution of parasitic infections amongst subjects according to age

| Age group (years) | No. Examined | Test (Infected subjects) | | | | | | Control (Uninfected subjects) | | | |
		No. with malaria parasites	No. with intestinal parasites	No.(%) with co-infection	PCV (%)	Hb (g/dl)	MCHC (%)	No. (%) without infection	PCV (%)	Hb (g/dl)	MCHC (%)
4-7	138	54(39.13)	33(23.91)	15(10.87)	31.50±0.74*	10.13±0.56	32.10±0.71	67(48.55)	36.4±3.2	12.13±2.0	33.3±4.5
8-11	90	39(43.33)	33(36.67)	15(16.67)	34.47±0.67	10.81±0.53	31.88±0.62	34(37.78)	38.6±5.1	12.87±1.8	33.3±3.8
12-15	57	24(42.11)	9(15.80)	6(10.53)	37.50±0.45[a]	12.45±0.38	33.26±0.46	28(49.12)	41.7±4.0	13.9±0.9	33.3±2.1
Total	285	117(41.1)	75(26.3)	36(12.63)	34.49±2.0	11.13±6.1	32.41±3.0	130(45.61)	38.9±1.0	12.97±2.1	33.3±5.6
		P<0.05			P<0.05	P>0.05	P>0.05				

Data is presented as mean±standard error of mean; [a]Significantly higher than*

Table 2: Distribution of parasitic infections amongst subjects according to gender

| Gender | No. Examined | Test (Infected subjects) | | | | | | Control (Uninfected subjects) | | | |
		No. with malaria parasites	No. with intestinal Parasites	No.(%) with co-infection	PCV (%)	Hb (g/dl)	MCHC(%)	No. (%) without infection	PCV (%)	Hb (g/dl)	MCHC (%)
Male	159	75(47.16)	48(28.30)	18(11.32)	33.23±0.59	10.32±0.43	31.03±0.48	60(37.74)	40.1±2.4	13.37±3.0	33.4±3.6
Female	126	42(33.33)	27(23.81)	18(14.28)	34.16±0.75	10.53±0.59	30.71±0.71	70(55.50)	37.7±0.4	12.57±1.8	33.4±2.7
Total	285	117(41.1)	75(26.30)	36(12.63)	34.49±2.0	11.13±6.1	32.41±3.0	130(45.61)	38.9±1.0	12.97±2.1	33.3±5.6
		P<0.05			P>0.05	P>0.05	P>0.05				

Data is presented as mean±standard error of mean

Table 3: Distribution of parasitic infections amongst subjects according to body weight

| Weight (kg) | No. Examined | Test (Infected subjects) | | | | | | Control (Uninfected subjects) | | | |
		No. with malaria parasites	No. with intestinal Parasites	No.(%) with co-infection	PCV (%)	Hb (g/dl)	MCHC(%)	No. (%) without infection	PCV (%)	Hb (g/dl)	MCHC (%)
12-26	183	78(42.6)	51(27.9)	21(11.47)	31.80±0.57*	10.35±0.61	32.24±0.53	78(42.62)	36.40±3.1	12.13±1.3	33.3±3.6
27-41	90	30(33.3)	21(23.3)	12(13.3)	34.40±0.67	10.83±0.53	31.88±0.62	49(54.44)	38.30±5.6	12.77±1.1	33.4±4.3
42-56	9	9(100)	3(33.3)	3(33.3)	38.66±0.85[a]	12.52±0.81	32.37±0.52	0(0.0)	0.0	0.0	33.3±5.8
57-71	3	0(0)	0(0)	0(0)				3(100)	39.6±8.1	13.2±1.6	
Total	285	117(41.1)	75(26.3)	36(12.63)	34.49±2.0	11.13±6.1	32.41±3.0	130(45.61)	38.9±1.0	12.97±2.1	33.3±5.6
		P<0.05			P<0.05	P<0.05	P>0.05				

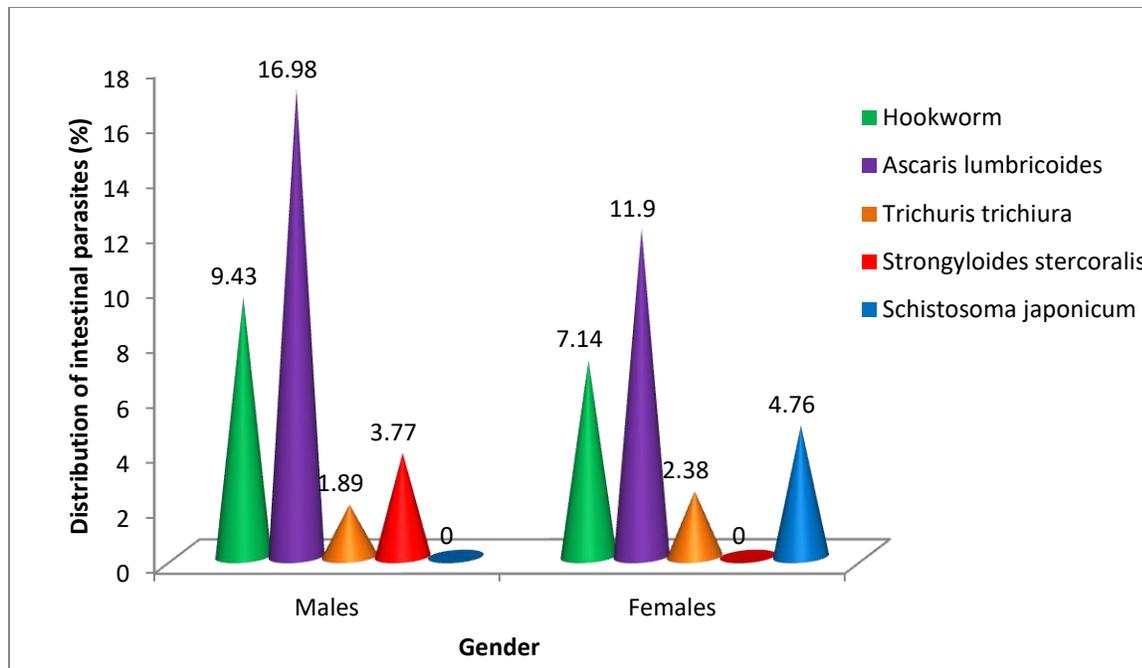Data is presented as mean±standard error of mean; [a]Significantly higher than*

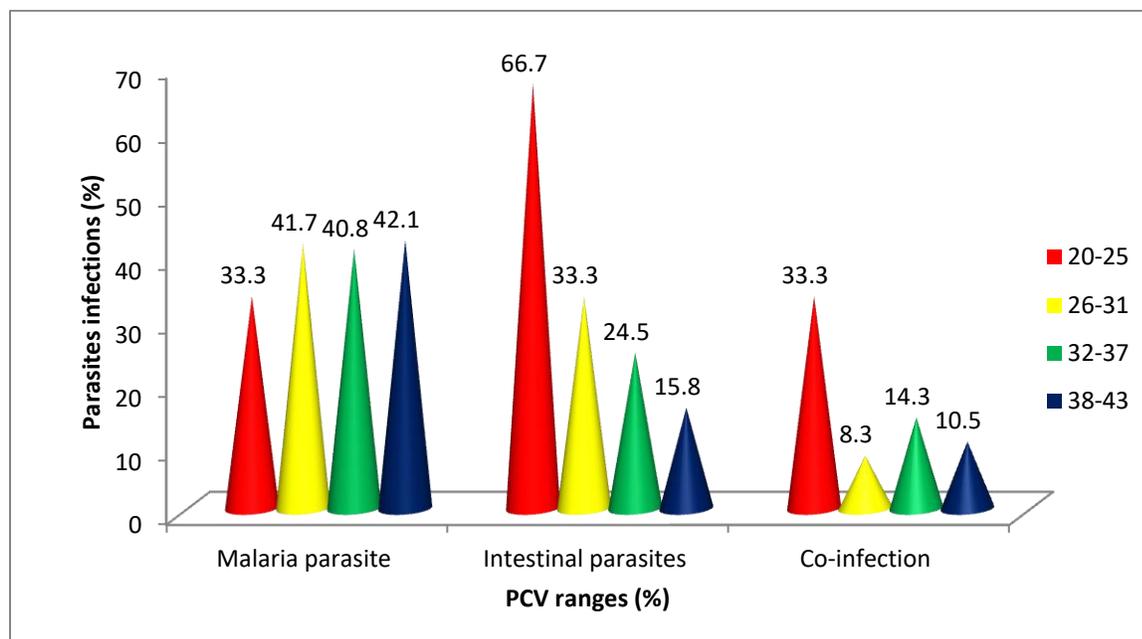**Fig 1.** Distribution of intestinal parasites among subjects examined

Fig 2. Relationship between packed cell volume and parasitic infections in subjects examined

Discussion

This study was to investigate the impact of intestinal parasitosis and malaria parasites co-infection in school-aged children and their influence on hemoglobin. The mean Hb levels of the control group (12.97±2.1) was significantly higher than that of the test group (11.13±6.1) (p=0.000). Investigation of intestinal parasitosis showed that Hookworm, *Ascaris lumbricoides, Trichuris trichiura, Strongyloides stercoralis* and *Schistosoma*

japonicum were the most common parasite in the study population. However, the incidence of parasitic infections was higher in females and children aged 8 – 11years. Intestinal parasites infestation are the most common infestations among school aged children. The infestation leads to nutritional deficiencies and impaired physical development which have negative consequences on cognitive function and learning ability[5]. The prevalence of intestinal parasites could probably be related to the

high occurrence of unhygienic habits amongst children living in rural communities. According to Agbolade et al[9], these unhygienic conditions have been known to increase the prevalence of intestinal parasites in some rural areas.

The highest prevalence of intestinal parasites (36.67%) was found in children between 8 – 11 years. However this age group was found to have lower malaria parasite densities. A similar study with an asymptomatic population in Osogbo, Southwest Nigeria, reported 20.9% prevalence of co-infection[10], which is high compared to the finding in this study. Studies from Ghana (16.6%) and Ethiopia (55.7%) were higher in this regard[11,12], which suggests that the pattern of coinfections may vary not only within Nigeria but even within sub-Saharan Africa.

Yatich et al[13] in Ghana and Basavaraju et al[14] in Jos, Nigeria reported high hookworm and malaria co-infection, supposedly due to the wider thermal tolerance of hookworm eggs compared to those of *Ascaris lumbricoides* and *Trichuris trichiura*. In this study however, a higher association between malarial parasites and *Ascaris lumbricoides* was observed, possibly because it is the most prevalent helminth amongst children. This finding is in agreement to that of Dada-Adegbola et al[10] in Ibadan, Nigeria which also reported a similar association as well as multiple parasitic infections with *Ascaris lumbricoides* and hookworm.

It can be concluded from this study that there are high levels of intestinal parasitosis, malaria infection and co-infection in school aged children within the rural areas and that children between the ages 8 to 11 years have the highest percentage of this co-infection. The results suggest that the occurrence of *Ascaris lumbricoides* infection and malaria are strongly associated. The mechanism behind this association is not clearly understood but could be that Th2 profile-associated immunoglobulin E production could down-modulate Th1 antimalarial immune responses, hence increase the risk of malaria[15]. The study showed a significant effect of the co-infection on the weight of the subjects although the impact on packed cell volume was minimal which is in contrast to results from similar studies in other areas that suggest a strong relationship between the co-infection and anaemia[10,14]. Although the PCV range of 20 – 25% and also among female was found to also have the highest parasitosis and co-infection. We also observed low Hb value among amongst with weight 12 – 41kg, age group 4 – 11years and in gender category. The results observed in the PCV and Hb indicates

that some of the patient had anemia. From the result observed, PCV and Hb test should be requested alongside the stool microscopy to ascertain patient's health status. This study reported that anemia can always be common in children with *Plasmodium falciparum* malaria and other parasitosis infection, which is capable of causing mortality and morbidity.

Today there is a renewed interest in the potential of the education sector to address the impact of malaria on school-age children. Health promotion activities and teaching within schools to encourage good hygiene practices and awareness of malaria prevention methods, such as the use of insecticide treated nets (ITN), will help prevent malaria, support and maintain healthy gains. The sanitary conditions of people in rural areas should be regarded as an issue of public concern. Though several effective malaria control interventions currently exist, including residual house spraying, insecticide-impregnated materials (particularly insecticide treated bed nets), and antimalarial drugs, it is important for healthcare planners to carry out health education to parents and provide insecticide treated bed nets during enlightenment health programmes that will involve periodic deworming. The delivery of malaria chemoprophylaxis to school children through schools was widespread in Africa during the 1950s and 1960s and resulted in significant reductions in parasitemia and rates of anemia and clinical malaria attacks. However, regular chemoprophylaxis in malaria-endemic countries has proven to be unsustainable, largely because of problems in drug distribution and financing and the concerns about potential emergence of drug resistance[16]. Increasing political and financial support for these projects, as well as renewed emphasis on population-based anthelmintic treatment will improve the combined control of this co-infection. In addition, school-based treatment delivery programs offer several cost advantages because of the use of the existing school infrastructure and the fact that schoolchildren are accessible through schools. Thus, school-based deworming programmes may provide a possible entry point for combined delivery of IPT and antihelmintics, or at least a way of sharing resources to do so, with limited increase in operational costs. To effectively decrease the morbidity and mortality as a result of diseases, populations should be provided with the access to water from home instead from outside. Therefore in addition to the installation of standpipes, water supplies and sanitation should be provided within houses.

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