PREVALENCE OF INTESTINAL AND MALARIA PARASITIC INFECTIONS AMONG SCHOOL AGE CHILDREN IN A RURAL COMMUNITY (NKWOT NKO) IN AKWA IBOM STATE, NIGERIA

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ABSTRACT

Aims: Nigeria is among the countries with a high rate of intestinal and malaria parasite co-infections. In view of this, it is important to investigate the prevalence and pattern of co-distribution of these parasitic diseases among children living in developing countries especially in the rural communities. This study was undertaken to determine the prevalence of intestinal and malaria parasitic infections in pupils of a private school.

Study design: Samples were collected without prejudice from school children from 4-14years old, for laboratory examination of intestinal and malaria parasite co-infection at the Christian Entrepreneurship and Leadership Academy, Nkwot Nko, a rural community in Ikono Local Government Area of Akwa Ibom State, Nigeria. Using a cross-sectional approach, the prevalence and burden of intestinal and malaria parasite were estimated for both populations.

Methodology: Finger-prick blood and fresh stool samples were collected for malaria parasites detection and intestinal parasites eggs. Stool samples were examined using formol-ether concentration method and Giemsa staining technique was employed for malaria parasite detection. A structured questionnaire was administered for parents/guardians to obtain socio-demographic information.

Result: The prevalence of malaria parasite in the study was 42.6%; 6.4% for *Ascaris lumbricoides;* 0.98% for *Entamoeba coli;* 4.4% for *Entamoeba histolytic;*, 10.3% for Hookworm; 2.5% for *Strongyloides stercoralis. For* co-infection of malaria and intestinal parasites was 14.2%. Males had the highest prevalence rate of Plasmodium falciparum, 43.9% compared to females, 41.5%. Non-availability of toilet facilities and potable drinking water also contributed to the transmission of parasites. Household water sources such as well (49.2%), pond (22.1%), rain (11.1%), public borehole (33.3%) were potential routes of spread of these parasites.

Conclusion: This study showed a high prevalence of intestinal and malaria parasites among pupils in this rural community. This finding calls for greater attention to the effective control of these parasitic infections. This action will thus contain their negative health impact on the school age children.

Keywords: malaria parasite, pupils, intestinal parasites, Akwa Ibom State

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INTRODUCTION

Intestinal and malaria parasitic infections are among the most common infections worldwide [1]. It is widely recognized that school children carry the heaviest burden due to intestinal helminthes and malaria parasitic infections. The parasitic infections present enormous health problem and over 75% of the global clinical cases due to these co-infections are concentrated in Africa [7]. The World Health Organization (WHO) estimated that over 500 million children are infected with intestinal helminthiasis [30]. In sub-Saharan Africa alone, there are 41 million hookworm infected school aged children [10], while in Nigeria, the occurrence of human intestinal parasites is also high in children [10]. The most ubiquitous of intestinal parasitic infections belong to a number of key species: *Ascaris lumbricoides*, *Trichuris trichiura*, *Strongyloides stercoralis*, *Schistosoma mansoni*, *Entamoeba histolytica* and hookworms (*Necator americanus* and *Ancylostoma duodenale*). In the last two decades, the emergence of drug resistant *Plasmodium* has increased in frequency and intensity, mostly in developing countries. It is by far, the world's most important tropical parasitic disease and kills more people than any other communicable disease except tuberculosis [30].

In 1880, Charles Laveran was the first physician to identify *Plasmodium* as the etiologic agent of malaria. The disease is transmitted to humans by the bite of an infected female Anopheles mosquito [16]. Malaria in man is caused by four species of *Plasmodium*; *Plasmodium vivax*, *P. ovale*, *P. malariae*, and *P. falciparum* [29]. Recently another species of *Plasmodium* has been included among this list making it five [5]. *Plasmodium knowlesi* has been detected to be the fifth malaria parasite that can cause malaria in human beings. The parasite is known to commonly infect macaque monkeys. The infection is highly prevalent in South East Asia. It has morphological similarities to *P. malariae* and *P. falciparum* [5]. Among the four species, *P. falciparum* is the most prevalent and is responsible for over 50% morbidity and 98% mortality in the vulnerable groups (under-fives, pregnant women and the aged), and transmission is throughout the year[11]. Intestinal and malaria parasite burdens are prevalent throughout the sub-Saharan Africa and over a quarter of school-aged children in this region appears to be at risk of co-infection and thus enhanced risk of clinical disease. However, the distribution of intestinal and malaria parasites is similar, but the large-scale distribution of parasitic disease such as intestinal helminth and malaria parasites is governed by environmental factors, principally temperature and humidity [14]. It has therefore been stressed that helminth infections can therefore ameliorate or exacerbate malaria severity. In a survey carried out in Western Nigeria, co-infection of 48.6% was recorded for intestinal helminthes and *falciparum* malaria in urban areas, while 83% was recorded for intestinal helminth alone in the rural areas [21]. The interaction of intestinal and malaria infections, indeed has increased severity of anaemia observed in school children. Also, frequent exposure due to these infections may result in impairment of physical and intellectual development in children [24].

Intestinal helminthes infected individuals are more likely to develop clinical *P. falciparum* malaria than helminth-free individuals [22]. Children co-infected with these parasites have reduced learning and school achievements and have increased susceptibility to other infections [20]. Studies have revealed that intestinal parasitic infections are the commonest cause of chronic infection in humans, mostly children in developing countries [15], while the sub-Saharan Africa region has the greatest number of morbidity and mortality due to malaria [5]. In Nigeria, the risk of co-infection is on the increase especially in rural areas, though occasionally recorded in big towns due to poor environmental and personal hygiene, poor nutrition, overcrowding and climatic conditions that favour the development and survival of these parasites transmission [3]. Malaria and intestinal parasites do co-infect hosts and it has been revealed from studies that the level of urbanization greatly affects the frequency and transmission dynamics of both infections [25]. Both infections are parasitic diseases that cause high rates of morbidity and have similar geographical distributions in the tropics and sub-tropics, where climatic conditions, poverty and in-sanitary practices favour their high prevalence. Hookworms are the most pathogenic because of their propensity to feed on blood resulting in anaemia. These infections are associated with decreased child growth, low plasma vitamin A, loss of weight, chronic blood loss, iron deficiency anaemia, diarrhoea, stunted growth and impaired cognitive functions in children [24]. These soil transmitted helminthes with schistosomiasis are responsible for 40% worldwide morbidity from all tropical infections, excluding malaria and the majority of those infected are children [13].

There are certain predisposing factors which may render children vulnerable to intestinal parasites. Factors such as dirty habits of playing or handling of infested soils, unhygienic toilet practices, eating contaminated or discarded food items, drinking of impure water, low socio-economic state, poor sanitation coupled with low literacy rates of parents [27]. Intestinal parasitic infection also results from the ingestion of infective cysts in faecally contaminated food, water or hands. Another source of infection could be from consumption of undercooked meat or by penetration of the skin e.g. Hookworm [28].

In some communities in sub-Saharan Africa, malaria is the cause of children missing school or crops not harvested. For families, malaria means the death and disability of young children and increasing risks in pregnancy for both mother and baby. The sad news is that many African communities simply accept malaria as part of everyday life; trapped in a vicious cycle in which malaria is both the cause and consequence of grinding poverty [2]. Due to the high prevalence of intestinal parasitic infections and malaria parasite burden in school aged children in developing countries including Nigeria. This study was prompted by the need for more information about intestinal and malaria parasite burdens in different parts of Akwa Ibom State, Nigeria.

MATERIALS AND METHODS

Study Area

The assessment of intestinal and malaria parasite co-infection of school children in Nkwot Nko, Ikono L.G.A, was carried out at the Laboratory of the Nigerian Christian Mission Hospital, Nkwot Nko. Nkwot Nko is a rural settlement in Ikono L.G.A of Akwa Ibom state and is located in the South-East of Nigeria. Ikono L.G.A is bounded on the North by Ini L.G.A, South by Abak L.G.A and Uyo LGA, East by Itu L.G.A and West by Ikot Ekpene

LGA. It has a land mass of 407.16 sq. km^2, and a population of 131,904 (NPC, 2006). Ikono L.G.A falls within the tropical zone characterized by rainy season from April to October and dry season from November to March. The majority of the inhabitants are farmers. Ikono lies between latitude $50^0$41N and longitude $70^0$59E (N.P.C 2006).

Collection of Samples

Samples for the project were collected from school children in (Christian Entrepreneurship and Leadership Academy), Nkwot Nko – Ikono L.G.A, Akwa Ibom State for a period of one month. A total of 204 pupils was examined and screened for intestinal and malaria parasite infections. Before samples were collected demographic data of the pupils such as sex, age, weight, and name of subject was recorded. Participants were required to provide blood samples for making malaria blood films and stool samples for intestinal parasites investigation.

Blood samples were collected by finger prick from each of the pupil. The left thumb finger of each pupil was cleaned using a swab moistened with 70% alcohol and allowed to dry. Thereafter, it was pricked using a sterile lancet. The finger was gently squeezed to obtain a small drop of blood which was smeared onto a slide to make thick blood smear. The slide was labelled with the pupil's serial number. The thick film was then prepared, air-dried, Giemsa stained and observed microscopically using x100 objective oil immersion for the detection of malaria parasitaemia.

EXAMINATION OF BLOOD SAMPLE

Giemsa staining procedure for malaria parasite

Thick/Thin Blood Films Preparation

A small drop of blood was placed on a clean microscopic slide at the centre of the slide and a large drop of blood about 15mm to the edge, to make thick and thin films on the slide. Immediately, the thin film is spread using a smooth edge slide spreader and without delay the large drop of blood is also spread to make a thick smear. The thick smear will cover evenly an area of about 15 x 15mm. Then the slide will be labelled with the pupil's serial number. The blood is allowed to air dry with the slide in a horizontal position on a staining rack and placed in a safe place to avoid contaminants or flies. A small drop of methanol is added to the thin film, making sure the alcohol does not touch the thick film as this will prevent lysis of red cells and make the thin film unreadable. The thin film is fixed for 1-2 minutes, while the thick film is stained with an already prepared Giemsa stain for about 30-40 minutes. The slide was washed by placing the film in buffered water for 3 minutes and air dried in a vertical position. The film was then examined under oil immersion lens for detection of malaria parasites [16].

Collection of Stool Sample

For stool samples, children were given a clean plastic container for stool collection and adequately instructed on how to get a little portion of their stool into the clean and dried plastic container. The samples were collected through the assistance of the class teachers and taken to the Laboratory for the intestinal parasites to be examined by direct smear using normal saline with Lugol's iodine solution and formol-ether concentration technique.

Examination of Stool Sample

Macroscopical examination was conducted, and then a small amount of faecal sample about 2mg was collected and placed on a clean microscope slide by the use of an applicator stick. To it was added a saline solution to emulsify the specimen, so as to enhance the clarity of the cysts which may be observed. A cover slip was then placed on the preparation, avoiding the introduction of air bubbles and viewed with the light microscope using x10 and x40 objectives respectively for egg/ova, of the parasite. Diagnosis was based on the identification of helminth ova and protozoan cyst in the sample during microscopic analysis.

Formaldehyde-Ether Concentration Method

To approximately 1g of faeces, 10ml of 10% formalin was added and stirred until a cloudy suspension is formed. Guaze was fitted into the funnel and the funnel placed on top of the centrifuge tube. The feacal suspension was placed through the filter into the centrifuge tube until the 7ml mark was discarded with a lumpy residue. Then 3ml of ether was added and well-mixed for one minute before centrifuging for 3 minutes. After centrifuging, there were four layers in the tube. The first was that of ether, followed by the debris, formalin solution layer and the layer containing the eggs and cysts of parasites. The fatty debris at the interface was then loosed with applicator stick and the supernatant was quickly poured off by inverting the tube. The small deposit at the bottom of the centrifuge was shaken before poured on to a slide and examined [16].

Data Analysis

All statistical analyses were performed using SPSS version 20. The prevalence of malaria and helminths were compared using Chi-square test. The threshold for statistical significance was at $p<0.05$.

RESULTS

A total of 204 questionnaires were administered, out of which 165 were returned. From these questionnaires, 91(44.6%) of the respondents had primary school certificates while 6(2.9%) had no formal education.The prevalence of malaria parasite in the study was (42.6%) while that of intestinal parasites was (24.6%). five species of intestinal parasites were recovered from the stool samples and these were *Ascaris lumbricoides* (6.4%), hookworm (10.3%), *E.coli* (0.98%), *E. Histolytica* (4.4%) and *Strongyloides stercoralis* (2.5%). Ages 9-13 years old had the highest prevalence (50.7%) of malaria and were more infected with hookworms recording the highest prevalecne of 12.7%. this was also true of the prevalence of co-parasites infection (16.9%)/(Table 1).

On Table 2, males had a higher prevalence (43.9%) of malaria compared to their female counterparts (41.5%) but the difference was statistically not significant ($P>0.05$). Females tend to have a higher prevalence with intestinal parasites such as *E. histolytica* (4.7%), and Hookworm (10.4%) compared to their male counterparts however, the difference was statistically not significant ($p>0.05$). Males had higher infection (7.1%) of *Ascaris lumbricoides* than females (5.7%) however, the difference was statistically not significant ($p>0.05$).

For the co-infection of both malaria with any of the intestinal helminths, males were more co-infected than females except for co-infection of malaria parasite and *Ascaris lumbricoides* where the reverse was observed (Table 3). The differences in prevalence of coinfection of malaria parasites and Intestinal helminth between sexes show no statistical significant difference (p>0.05).

Table 1: Prevalence of malaria parasites and intestinal parasite by gender

Age (Yr.)	Subject No.	*Plasmodium falciparum* No. (%)	*Ascaris lumbricoides* No. (%)	*Entamoeba coli* No. (%)	*Entamoeba histolytica* No. (%)	*Hookworm* No. (%)	*Strongyloides stercoralis* No. (%)	MP+ IN No. (%)
4—8	133	51 (38.3)	9 (6.8)	1 (0.8)	4 (3.0)	12 (9.0)	3 (2.3)	17 (12.8)
9—13	71	36 (50.7)	4 (5.6)	1 (0.8)	5 (7.0)	9 (12.7)	2 (1.5)	12 (16.9)
Total	204	87 (42.6)	13 (6.4)	2 (0.98)	9 (4.4)	21 (10.3)	5 (2.5)	29 (14.2)

MP=Malaria parasite, IN=Intestinal parasite

Table 2: The prevalence of malaria parasite and intestinal parasites by gender

Sex	Subject No.	*Plasmodium falciparum* N o. (%)	*Ascaris lumbricoides* No. (%)	*Entamoeba coli* No. (%)	*Entamoeba histolytica* No. (%)	Hookworm No. (%)	*Strongyloides stercoralis* No. (%)
Males	98	43 (43.9)	7 (7.1)	2 (2.0)	4 (4.1)	10 (10.2)	4 (4.1)
Females	106	44 (41.5)	6 (5.7)	0	5 (4.7)	11 (10.4)	1 (0.9)
Total	204	87	13 (6.4)	2 (1.0)	9 (4.4)	21(10.3)	5 (0.5)
p-value		0.73	0.67	0.14	0.83	0.97	0.15

Table 3: Co-infection of malaria and intestinal parasites by gender

Sex	Subject	MP+ *A. lumbri-coides*	MP+*E. coli*	MP+*E. histolytica*	MP+Hook-worm	MP+*S. Stercoralis*
	No.	No. (%)	No. (%)	No. (%)	No. (%)	No. (%)
Males	98	3 (23.1)	1 (50.0)	3 (33.3)	7 (33.3)	2 (40.0)
Females	104	5 (38.5)	0	2 (22.2)	5 (23.8)	1 (20.0)
Total	204	8 (61.5)	1 (50.0)	5 (55.6)	12 (57.1)	3 (60.0)
p-value		0.36		0.29	0.25	0.28

Table 4: Association between intestinal parasites and malaria parasite infection among the study subjects

Intestinal Parasites	Malaria Parasite Absent (N=117)	Malaria Parasite Present(N=87)			
	No. (%)	No. (%)	RR	CI	P-value
Intestinal Parasite	22 (10.8)	29 (14.2)	1.44	1.03– 2.02	0.018
A. lumbricoides	5 (4.3)	8 (9.2)	1.49	0.76 – 3.06	0.15
E. histolytica	4 (3.4)	5 (5.7)	1.30	0.62 –2.73	0.42
E. coli	1 (0.9)	1 (1.1)	1.15	0.29 – 4.62	0.83
Hookworm	9 (7.7)	12 (13.8)	1.47	0.83 – 2.29	0.16
S. stercoralis	2 (1.7)	3 (3.4)	1.44	0.49 – 4.26	0.43

RR **=** Risk RatioCI = Co-efficient interval

Out of the 87(42.6%) children that were positive for malaria, 29(33.0%) were co-infected with intestinal parasites and the difference was statistically significant ($p < 0.05$). Children who were infected with intestinal parasites were (RR=1.44) times more likely to be co-infected with malaria parasite as compared with children having intestinal parasites infection. On Table 5, children whose parents used pit toilet had the highest prevalence, 45.2%, 6.4%, 1.3%, 5.7%, and 11.5% for Malaria, *A. lumbricoides*, *E. coli*, *E histolytica*, and Hookworms respectively.

The prevalence of *E. coli*, and *S. stercoralis* were highest in children whose source of water was public borehole. Those that used rain water had the highest prevalent of *A. lumbricoides* 31 (11.1%).

Table 5: Prevalence of malaria and intestinal parasites in relation to the type of toilet facility used in the households

Toilets	Malaria parasites	*A. lumbricoides*	*E. coli*	*E. histolytica*	*Hookworm*	*S. stercoralis*
	No.%	No.%	No.%	No.%	No.%	*No. %*
Pit	91(45.2)	25(6.4)	2(1.3)	9(5.7)	30(11.5)	3(1.9)
Bush	1(33.3)	0(0)	0(0)	0(0)	0(0)	0(0)
Water closet	1(200)	0(0)	0(0)	0(0)	0(0)	2(40.0)
Others	0(0)	0(0)	0(0)	0(0)	1(20.0)	0(0)
TOTAL	93(44.0)	25(6.6)	2(1.2)	9(5.4)	31(11.4)	5(3.0)

DISCUSSION

In this present study, the 42.6% overall prevalence of malaria parasite infection agrees with the 48.7% reported in the middle belt area of Nigeria by Saanuwan and Abdul [31], and 50.7% reported [23] in South West of Cameroon. The prevalence value obtained in this result is higher than that reported in asymptomatic pupils in Bolfamba in Cameroon [23, 31]. It is also higher than reports in Kajola, Nigeria [5]. The difference in prevalence could be attributed to timing, seasonal difference, environmental conditions and other geographical factors in the study area. This result suggests that rural environment offers adequate conditions for breeding mosquitoes and also socio-economic status of parents in this area also contributes to the transmission of malaria [18].

Table 6: Prevalence of malaria and intestinal parasites in relation to the type of water source

H/H Water Source	Malaria Parasite	*A. lumbricoides*	*E. coli*	*E. histolytica*	Hookworm	*S. stercoralis*
	No.%	**No.%**	**No.%**	**No.%**	**No.%**	**No.%**
Well	31(49.2)	12(4.8)	0(0)	4(6.3)	6(9.5)	4(3.2)
Pond	5(55.6)	0(0)	1(11.1)	0(0)	2(22.1)	0(0)
Rain	0(0)	31(11.1)	0(0)	0(0)	1(11.1)	0(0)
Public Borehole	0(0)	0(0)	0(0)	0(0)	0(0)	0(0)
Private Borehole	18(54.2)	1(4.2)	0(0)	5(12.5)	1(4.2)	1(4.2)
Public Pipe-Bore	20(42.6)	5(10.6)	1(2.1)	2(4.3)	7(14.9)	2(2.1)
Others	1(16.7)	0(0)	0(0)	0(0)	2(33.3)	0(0)
TOTAL	77(43.9)	49(6.1)	2(1.2)	11(5.5)	19(11.6)	7(2.4)

This study was carried out at the beginning of rainy season, and this explains the high prevalence recorded in the case of intestinal parasites, out of the five (5) species recovered from this study, three (3) of the parasites were soil-transmitted helminthes while the other two (2) were protozoan parasites. The most prevalent among these helminthes was hookworm (10.3%), followed by *Ascaris lumbricoides* (6.4%) and *Entameoba histolytica* (3.0%) [4].The prevalence of these helminthes in the study area is in agreement with other findings [4]. The prevalence of *A. lumbricoides* was not in agreement with 54.8% prevalence of *A. lumbricoides* infection reported by Akingbade *et al.* [17] in Abeokuta, Ogun State, Nigeria. The prevalence of *E. histolytica(* 0.6%), in a previous study did not agree with that of this study. This might have been that, the subjects contacted the infection through ingesting contaminated food and water through incessant and regular play on infected soils. Furthermore, the ingestion could have been acquired by eating food items sold by hawkers who are carriers of the parasites (infected food handlers) and outside who sell food/things within the school premises [4]. This result did not agree with a previous study carried out by Adefioye *et al*., [17] in Osun State, Nigeria, where the most prevalent among helminthes was *A. lumbricoides* (36.2%), followed by hookworm infection (10.5%). The highest prevalence and intensity of *A. lumbricoides* recorded in a previous study suggest a high level of unhygienic practices and the habit of defecating indiscriminately in open places among school children which eventually contaminate the environment [8]. Therefore, intestinal helminthiasis caused by hookworm and *A. lumbricoides* is a common disease among rural population in Nigeria. The findings that hookworm has the highest prevalence rate among the age group (9-13) years in this area may be due to the unhygienic habit of children walking barefoot to school and farmlands, while the lower age group (4-8) years old might have contacted the infection throughintense activities such as playing with soil contaminated with faeces. However, the prevalence rate of intestinal helminthes was not statistically significant ($p > 0.05$).

Numerically, males were more co-infected than females, however, more males were examined in this study, except for the co-infecion of malaria parasite and *Ascaris lumbricoides*, where the reverse was observed. Therefore, the prevalence rate was decreasing with increasing age group possibly due to the change in attitude, habits and more awareness regarding personal hygiene among the older school children. The overall prevalence of intestinal helminthes (25.0%) in this study show a decline in trend and this is not in agreement with a previous study carried out by Ezeaguna *et al*. [18] in Anambra State, where the intestinal helminth was 48.08%. Also, this is in contrast with the observations made by Ojurongbe *et al*. [5], where a positive and statistically significant relationship was found between intestinal helminth parasite infections. Intestinal parasites are reported to have deleterious effect on school children [8]. The predominance of *A. lumbricoides* (9.6%) in a previous study [12] is in line with the findings from this study (6.4%).

Hadidjaja *et al*. [26] observed that the presence of *A. lumbricoides* in school children is associated with nutritional status and cognitive development with a consequence of under-developed skills and learning ability. The threat of malaria continues for at least the first five years of life before most children living in endemic regions develop immunity sufficient to suppress severe pathogenesis [9, 13]. In the last two decades malaria epidemics have increased in frequency and intensity in most sub-Saharan African populations due to the emergence of drug resistance Plasmodium [6]. Many asymptomatic infections go undetected and untreated while causing little or no clinical manifestation. Therefore, the extent of asymptomatic parasitaemia is inversely related to a population’s susceptibility to clinical disease [20]. Males had a slightly higher prevalence (43.9%) of malaria compared to their female counterparts (41.5%). This is in agreement with the report of Ogunrin [3,6], which observed that the high level of parasitaemia is not dependent on sex. Therefore, the difference in males and females infected with malaria parasite was statistically significant ($p<0.02$).

In this study the co-infection of these parasites was 14.2% and the co-infection by gender was low. This is probably because in this village, the pupils had received treatment for worms prior to the study. This is in contrast with the observations made by Ojurongbe *et al*. [5], where a positive and statistically significant relationship was found between intestinal helminth and malaria parasite infection. The decline in the trend of co-infection with these parasites in this study was not in conformity with previous study [9]. It was also found that 42.6% of the children had malaria in addition to co-infection with intestinal helminth (33%). This result is higher than those obtained in Osogbo, Nigeria [5], and also those obtained in Cameroon [6]. Therefore, the difference was statistically significant ($p<0.05$). The low monthly income earned by parents of the children in the study area could have implications on level of expenditure on malaria and intestinal parasite preventive measures by the member of the households. The result implies that majority of the parents cannot afford good health care and this has negative effects on the health status of the children.

CONCLUSION

Intestinal and malaria parasitic infections are endemic among school children in Nkwot Nko, Ikono Local Government Area, Akwa Ibom State, Nigeria. It is evident that malaria parasitaemia is more widespread among children in the lower age groups. This development should be checked to avert increased incidence to malaria attacks. Frequent exposures to these infections may result in the impairment of physical and intellectual development in children who are the future of the nation.

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COMPETING INTEREST

"Authors have declared that no competing interest exist"

AUTHOR'S CONTRIBUTIONS

Topic, research design, input, literature review, materials and methods, results and discussion were done by the first author. The second, third and the fourth authors did the data collection, data analysis and produced the first draft of the paper.

All authors read and approved the final manuscript.

ETHICAL CONSIDERATION

Ethical clearance for the work was obtained from the Mission School Authority and Parents Teachers Association of the school. The objectives and potential benefits of the study was made known to the Headmistress and the teachers of the schools before giving out consent forms to the pupils for their parents/guardians' attention. Questionnaire, aimed at obtaining personal data and information on residence, gender, education, occupation of parents, toilet facility and water supply was administered to the parents/guardians. The study and methodology of the survey were explained, and arrangements made with the teachers of the school on when to collect the samples.

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