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Intestinal protozoa and intestinal helminthic infections in displacement camps in Sierra Leone.

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Summary

Displacement and refugee camps provide ideal grounds for the transmission of parasites and increase the risk of acute respiratory infections, diarhoea diseases, and intestinal parasitic infection. *Cryptosporidium parvum, Giardia lamblia. Entomoeba histolytica, Ascaris lumbricoides*, hookworm infection; *Schistosoma haematobium, S. mansoni and Strongyloides stercoralis* are important cosmopolitan intestinal parasites that are common among children, the immunocompromised and displaced populations. Five hundred and eighty one residents from 5 Internally Displaced Persons (IDPs) Camps voluntarily participated in the study by providing stool and urine samples for analysis. The stool specimens were used for the detection of *Cryptosporidium* specific and *Giardia* specific antigens by the DMSO modified Acid-Fast and Trichrome-PLUS stain for *C. parvum* and *G. lamblia* and *E. histoyltica* respectively. Stool specimens for the demonstration of helminth eggs and larvae were prepared by the modified Kato technique. One hundred and seventy eight (31%) of the 581 camp residents that submited samples were children below 10 years of age and were selected because they were screened for various forms of malnutrition. However, the data on *C. parvum* and *G. lamblia* were included in the analysis for all parasites. More children were positive for *G. lamblia* (29%) than for *C. parvum* (10%) and 5% had double infection with both parasites. The antigen positive rate decreased with age for *C. parvum and G. lamblia* infections. Adult samples were also examined for the *C. parvum, G. lamblia, E. histolytica, A. lumbricoides*, hookworms, *S. haematobium, S. mansoni and S. stercoralis*. The prevalence of hookworm was highest at Parade Ground Camp (50%) and hookworm had the highest pevalence rate of 18% among the 581 IDP residents followed by *S. mansoni* (16.7%) and. *A. lumbricoides* (15%). The overall prevalence of *E. histolytica* among the study population was 9.0%. The results of this study indicate that intestinal protozoan and helminth parasites are highly prevalent among camp residents in Sierra Leone with five (5) different helminth parasites demonstrated in the stool specimens of residents in the five IDP camps.

Keywords: *Displacement camps, intestinal parasites, Sierra Leone.*

Résumé

Le déplacement et les camps des réfugiés demeurent des lieux de transmission des parasites et des risques d'infections respiratoire acute. les diarrhées et d'infection des variétés parasites intestinales. Ces parasites sont d'importante infection intestinales cosmopolitaine commun parmi les enfants immunodéficitaires et déplacés. Cinq cent quatre vingt un réfugiés résidents de cinq centres de concentrations participaient volontairement dans cette étude et leurs échantillons d'urine et des selles analysés. Les 2chantillons des selles étaient analysés pour détecter des antigènes cryptosporidum et de Giardia par la solution du DMSO modifiée et de trichrome plus pour le *C. parvum, G.lamblia et E. Histolytica* respectivement. Les échantillons des

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selles étaient utilisés pour évaluer les œufs des verres et des larves étaient préparé à l'aide de la technique modifiée de Kato. Cent soixante dix huit (31%) des 581 réfugiés avaient moins de 10 ans et étaient sélectionnés à titre de leur condition de malnutrition. Plusieurs des enfants étaient positif au *G.lambia* (29%) qu'au *C. parvum* 10% ; et 5% avait d'infection mixe. Les taux d'antigène positif réduisaient avec l'age pour les infections du *C.parvum et Glambia*. Les échantillons des adultes étaient analysés. La prévalence des amibes était plus élevée dans ces camps (50%) et d'un taux de 18% des 518 réfugiés résidents, suivi de 16.7% *S. Mansoni* et 15% *A. lumbicoide*. La prévalence totale de *E Hystolytique* était de 9.0% dans cette population. Ces résultats de cette étude indiquent que les protozoaires intestinaux et les amibes sont plus fréquents dans les camps de concentration des réfugiés en Sierra Leone.

Introduction

The main feature of armed conflicts is of course violence or the threat of violence and in these conflicts; large numbers of civilian population are displaced and find themselves in temporary camps without sanitary facilities. The other effects of conflict on public health are mediated through a wider complex of circumstances [1]. Furthermore, since the end of the Cold War, there has been a dramatic increase in civil conflicts resulting in over 100 million refugees and internally displaced civilians globally [2]. The public health impact of these conflicts has been immense, leading to high rates of communicable diseases, increased prevalence of acute malnutrition, and excess mortality rates [3]. The relationship between diseases and population movements is neither simple nor, necessarily one-directional because migration facilitates the transmission of diseases in a number of ways, including, directly-by disseminating the causative agents, or indirectly-by changing the environment and thereby increasing vector population [1, 2, 3].

The civil war in Sierra Leone has generated over 200,000 internally displaced persons (IDPs) and majority of these IDPs lived in overcrowded camps with gross unhygienic conditions and suffering [4]. In addition, this civil war, which began in 1991, has claimed the lives of over 100,000 people and displaced roughly 2.5 million others that lived outside the country. Similarly, the effects of the war forced these people to move frequently from camp to camp until they arrived in the camps in and around Freetown that were safe from further attacks by the rebels.

Giardiasis, a disease caused by the protozoan parasite *Giardia lamblia* is a world recognized intestinal disease. The parasite is transmitted by cysts in contaminated water or food or by person-to-person contact [5]. The standard diagnosis has been the microscopic demonstration of cysts in the feces, which yields many false negatives due to high variability in cyst excretion. New methods that detect infection even when few parasites are present are now available on the market. These immunodiagnostic or *Giardia* Specific Antigen (GSA 65) tests are rapid, sensitive, and specific, and typically require only one stool specimen [6]. Duodenal juice microscopy for the diagnosis of giardiasis has proven effective in the past [7].

Giardia has been implicated in a number of epidemics [7, 8], and the endemicity in the world is well recognized [10, 11, 12, 13, 14]. Prevalence rates are reportedly higher in children [15], and homosexual males [16]. The prevalence rates are even higher in developing nations [17, 18, 19], due to poor hygiene and lack of proper sanitary practices.

Cryptosporidium parvum is a zoonotic intestinal pathogen that is widespread in the environment and infects cattle, piglets, reptiles, and other mammals [19, 20] although humans and cattle are considered the primary sources of oocysts [22]. Cryptosporidiosis has been recently recognized as an important cause of diarrhoeal illness in both immunocompetent and immunocompromised persons in many areas of the world [15, 17, 23]. Cryptosporidiosis cases have increased over the years ever since the first case was reported in 1976, notably in immunocompromised patients [16, 24, 25], in normal individuals during waterborne disease outbreaks [8, 26, 27, 28], among children in day care settings [15], and among individuals in developing countries [16, 17].

The household transmission of *Cryptosporidium parvum* infection is thought to be inversely related to age, which is most likely, a proxy for hygiene [15]. There are no reports however indicating that *Cryptosporidium parvum* infection is sex related. Good hygiene and good water supplies are especially important among children in any facilities used by the public to prevent a possible outbreak. The major soil transmitted helminths, i. e. ascariasis, hookworm and schistosomiasis account for 40% of the global morbidity from all tropical infections, excluding malaria [38]. The purpose of this study was to investigate the prevalence of *Cryptosporidium parvum*, *Giardia lamblia* and other intestinal parasitic infections among different populations but with emphasis on children living in 5 displacement camps in and around Freetown, Sierra Leone, and to provide the information to the Ministry of Health and Sanitation and NGOs working with the various camps.

Patients and Methods

Study site and population.

The study was conducted in five (5) internally displaced camps in the western area of Sierra Leone. These camps were constructed by the Government of Sierra Leone in collaboration with Non-Governmental Organizations for persons displaced by the civil conflict. The five (5) camps had a total population of 50,426 persons (Table 3), with 581 persons voluntarily consenting to participate in the study.

Stool and urine collection and examination

Each person was given two different coloured containers with lids, one colour for stool and the other for urine collection. The volunteers were also given a scoop each for the sterile transfer of their stool specimens to the stool containers. For the collection of the stool, each was given a large filter paper and told to first put the stool on that filter paper and then carefully transfer a good portion with the scoop in the stool container. A portion of each stool sample was concentrated by high speed centrifugation and two drops of iodine was added to the sediments. The entire sediment was microscopically examined for parasite eggs and larvae. The rest of the stool specimens were treated with 10% formalin and stored at room temperature and were tested within a month of collection, according to the manufacturer's instructions. A portion of each stool specimen was mixed in Proton-Fixed TM Fixative [6]. Proton-Fixed TM Fixative is designed for the collection, transport, and preservation of fecal specimens for the diagnosis of intestinal parasites. It is environmentally safe, one-vial processing fixative and or wet preparations for permanent smear preparations and for concentration techniques. All stool specimens were processed by the modified Kato technique [27], and all helminth eggs and larvae were counted on a prepared template and enumerated according to manufacturer's instructions. The results were expressed as eggs per gram of faeces. Ten milliliters of each urine specimen was concentrated by centrifugation and the entire sediment was microscopically examined for the eggs of *Schistosoma haematobium*, counted, averaged and are expressed as eggs per 10 ml of urine. A portion of the stool specimens was concentrated by high-speed centrifugation and sediments were examined for *S. stercoralis* larvae and portions of the stool were stained with Trichome Plus stain [6] and examined for cysts of *E. histolytica*.

Detection of Cryptosporidium specific and Giardia specific antigens:

An enzyme immunoassay (ProSpecT *Giardia* microplate assay and ProSpecT *Cryptoporidium* microplate assay) was used to detect *Cryptosporidium* and *Giardia* specific antigens respectively according to manufacturer's instructions. Briefly, for *Cryptosporidium and Giardia* assays, two drops of each liquid specimen were added to each well in the microtiter plates as well as to the negative and positive controls wells. The plates were incubated at room temperature (20-25^{o}C) for 60 minutes, washed 3 times with wash buffer, and excess liquid removed by placing plate inverted on paper towels draining the excess liquid wash. Four drops each of (200 μl) of enzyme conjugate was added to each well and the plates incubated further at room temperature for 30 minutes. The plates were washed 5 times and 4 drops (200 μl) of color substrate added to each well and incubated at room temperature for 10 minutes. Then, one drop (50 μl) of stop solution was added to each well, mixed well, and reaction was read on a spectrophotometer at 450 nm. The Optical Density (O. D.) of the negative control was substracted from the positive control and from the test wells before our results were interpreted. The results were interpreted according the manufacturer's instructions. A sample is positive if the O. D. is $>$ 0.050 blanked value (i.e. after the O. D. of the negative control is subtracted) and a negative sample has O. D. of $<$ 0.050 blanked value (i.e. after the O. D. of the negative control is subtracted). The sensitivity of the ProSpect *Crytosporidium* assay was between 92.5-99.2% and specificity was between 95.4–100%. The sensitivity for the ProSpect Giardia assay was between 96–100% and the specificity was between 98-100% [6].

Preparation of smear and staining

In order to identify *Cryptosporidium* and/or *Giardia* microscopically, we used the high-speed Cytospin 2 Centrifuge (Shandon, Pittsburg, PA) to make a smear from each specimen that was ready for staining. The DMSO modified Acid-Fast Stain was used to stain for *Cryptosporidium* according the manufacturer's instructions. The advantage of the concentrated material is to ensure the detection of even small numbers of organisms. Briefly, the air dried slide was dipped into absolute methanol for 10 seconds, placed in reagent A stain solution for 5 min, and rinsed thoroughly with tap water and excess water was drained. The slide was then dipped into in reagent B decolourizer solution for 5-10 seconds, rinsed thoroughly with tap water and excess water was again drained. The slide was then dipped into reagent C counter stain solution for 1-4 minutes, rinsed with tap water, and excess water was drained and allowed to air dry.

For staining of *Giardia*, the Trichrome-*PLUS* TM Stain was used. The air dried slide was fixed in 70% alcohol for a min, drained, dipped 2 to 4 times in distilled water to remove alcohol, and placed in Trchrome-*PLUS* for 3 to 5 min. The slide was rinsed, dipped 2 times in Trichrome-*PLUS* decolourizer, dipped 5 times in 100% denatured alcohol, drain and blotted. Treatment was repeated twice (5 times each time) with 100% denatured alcohol and dipped in Pro-ClearTM until sheeting of alcohol was completely removed. The slide was then mounted with No.1 cover slip and examined under oil for *Giardia* cyst.

Results

Five hundred and eighty one residents from 5 IDP camps participated in the exercise by providing stool and urine samples for analysis. Of the 581 camp residents that submitted samples, 178 (31%) were children below 10 years of age, Table 2 represents the prevalence of *C. parvum and G. lamblia* amongst the 178 children. More children were positive for *G. lamblia* infection (29%) than for *C. parvum* infection (10%). The prevalence of double infection with *C. parvum* and *G. lamblia* was 5% among the 178 children examined (Table 2).

When the total population in the various camps was analyzed, the mean infection rate for *C. parvum* was higher in males than in females in age groups 6-10 and 11-15. However, in 1-5 year age group, infection rate was higher among the female children that their male counterparts (Figure 1), and the difference in the infection rate between male and female children for 1-5 year age group was significant by the student t-test ($P < 0.005$). In addition, none of the female children in 6-10 age group were diagnosed positive for *C. parvum* infection (Figure 1). For the 16 years plus age group, the 2% infection rate for *C. parvum* was all females, with no males positive in this age group (Figure 1).

When the total population was taken and analyzed for *G. lamblia* infection, the mean infection rate was not significantly different among the males (13.5%) compared to their female counterparts (13.75%, Figure 2). But when the data is examined according to age group, females had a higher infection rate than the male children in the 1-5 and 16 plus age groups, but the differences were not significant. For age groups 6-10 and 11-15 years, while the male children had higher infection rate than their female counterparts, the differences were however, not significant (Figure 2).

The prevalence of various intestinal parasites by displacement camp is shown in Table 3. The highest single intestinal parasitic infection occurring in any of these IDP camps was hookworm infection, with a prevalence of 50% at the Parade Ground Camp. In addition, when the overall prevalences were calculated for each parasitic infection, hookworm infection was again the highest (18.1%), followed by *S. mansoni* (16.7%) and *Ascaris lumbricoides* (15.0%) respectively (Table 3). Except for these different individual parasitic prevalence rates, Approved School camp had the highest overall prevalence rates for most of the different infections. For example, Approved School camp had the highest infection rates for *Ascaris lumbricoides* (19.3%), *Entamoeba histolytica* (11%), *S. mansoni* (38%), and for *Strogyloides stercoralis* (9%) (Table 3). In addition, *Strongyloides stercoralis* infection was the least prevalent amongst all the six intestinal parasitic infections in all five IDP camps (Table 3). We found four positive cases of *Trichuris trichiura* and one case of *Taenia* spp in the Parade Ground Camp, but we found no positive cases in the other IDP camps. These results were not included in Table 3 because of the low numbers of positives for *Trichuris trichura* and for *Taenia* spp.

Discussion

The results of this pilot cross sectional study show the negative effects of civil conflicts on the civilian

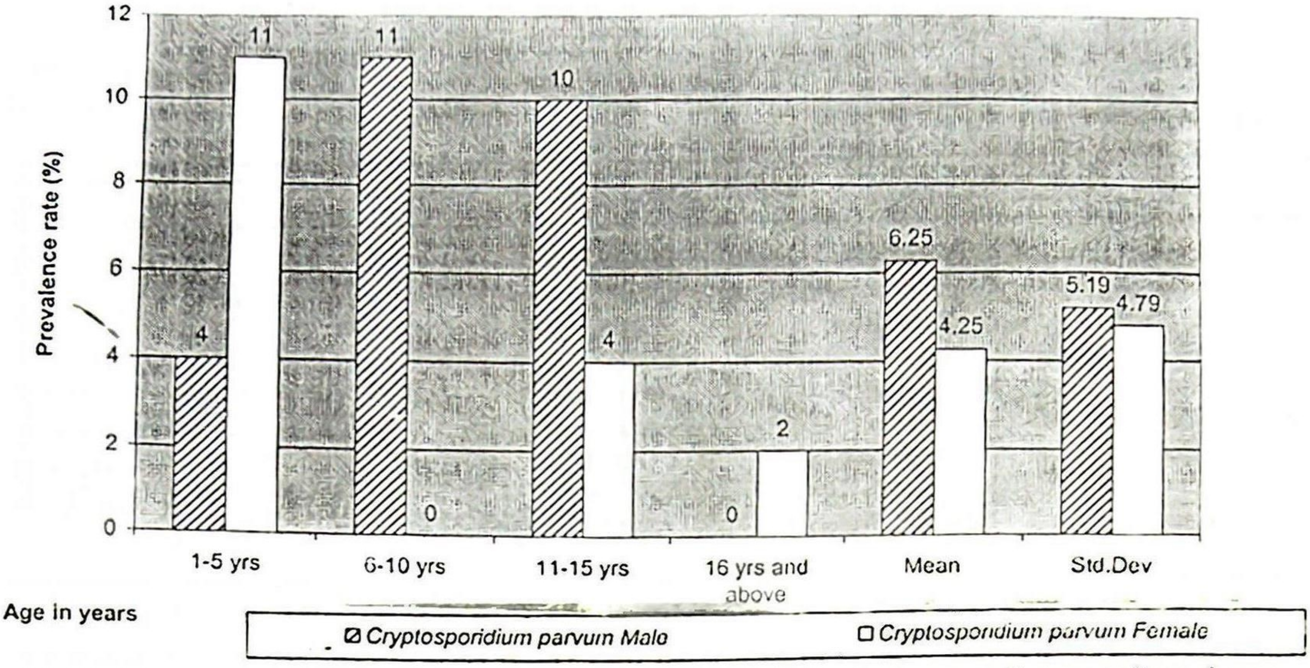

Fig. 1: Prevalence of Cryptosporidium parvum infection among children in displacement Campus in Sierra Leone

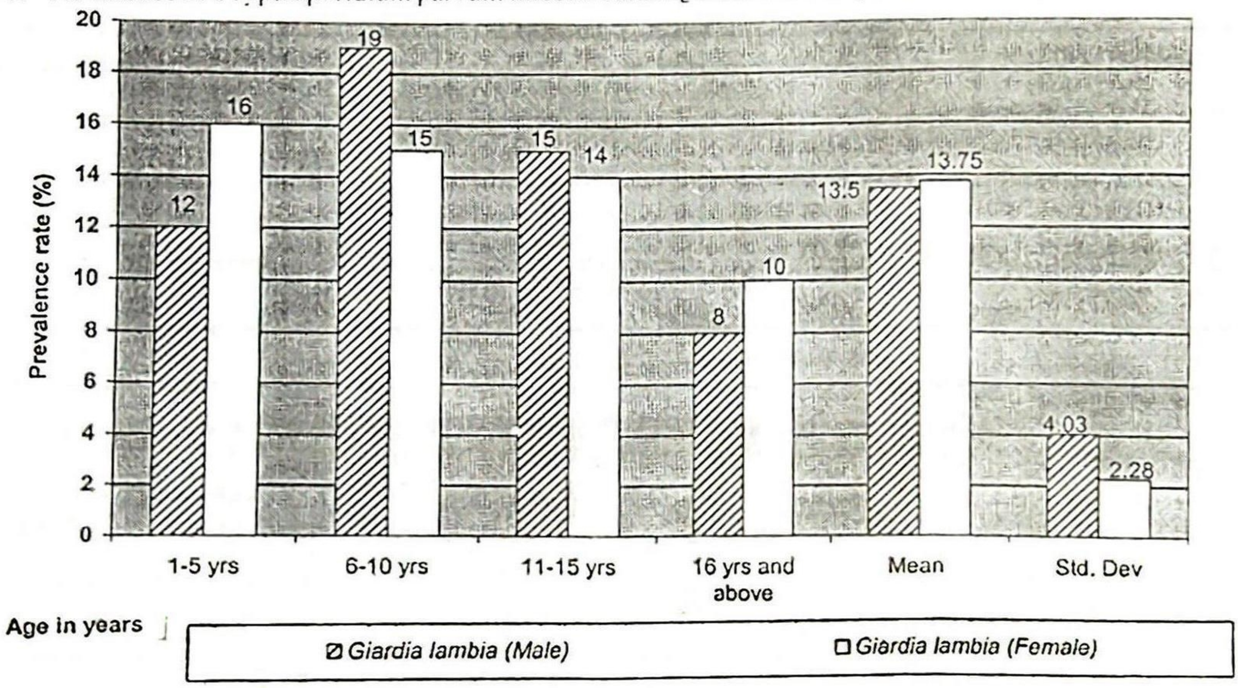

Fig. 2: Prevalence of Giardia lamblia infection among children in displacement camps in Sierra Leone

population in any country, but in this case, the results on internally displaced Sierra Leoneans. The impact of the 10-year rebel war in Sierra Leone has resulted in the forced internal displacement of millions of people in search of stable and peaceful living environments as they moved from one displacement camp to another. The public health impact of these situations has been disastrous, comprising high rates of communicable diseases, elevated prevalence of acute malnutrition, and high mortality rates. Besides the violent deaths, injuries and disabilities, displacement of populations increases the risk for acute respiratory infections, diarrhoeas, epidemic and parasitic diseases [1, 2].

Cryptosporidium parvum and *Giardia lamblia* are important causes of diarrhoea in children and adults in developing and developed countries [17, 18, 19, 30, 31]. While various environmental and parasite factors play key roles in increasing prevalence, epidemics, and pathogenicity, the key factors among displaced persons are mainly due to poor hygiene supported by the lack of proper sanitation. Similarly, over crowding in the camps predispose the residents

Table 1: Internally Displaced Persons (IDPs) Camps in Western Area, Sierra Leone

Name of Camp	Location	Managing Agency/NGO	Camp Population
Amputee Camp	Aberdeen Road	MSF/F	1210(946+264 amptees)
Approved School	Wellington	IIRO	15,000
Bailor Barrie Garage	Bai Bureh Road	SLRCS	1,350
Grafton	Grafton	GOSL	5,000
Mandella Camp	Ross Road	ARD	412
National Workshop	Cline Town	ARD	10,000
Parade Ground	Circular Road	CORD-SL	1,000
Trade Center Camp	Ferry Road, Kissy	EF-SL	1,365
Waterloo Camp	New Site, Waterloo	ADRA	15,000
Total Population in all Camps			50,426

Table 2: Prevalence of *Cryptosporidium parvum* and *Giardia lamblia* in children living in displacement camps in and around Freetown, Sierra Leone.

Cryptosporidium parvum		*Giardia lamblia*		Double Infection	
No. exam.	NO (%) pos.	No. exam.	No (%) pos.	No. exam.	No (%) pos.
178	18 (10.0)	178	51 (29.0)	178	8 (5.0)

Table 3: Prevalence of intestinal helminths among Internal Displaced Persons (IDPs) in seven camps in and around Freetown, Sierra Leone.

IDP Camp		*Ascaris*	*Entamoeba*	*HookwormS. mansoni*	*S. hamatobium*	*S. steroralis*	*lumbricoides* *histolytica* infection
	No.	No(%) pos.	No (%) pos.	No (%) pos.	No (%) pos.	No (%) pos.	No (%) pos.
Approved School	140*	27(19.3)	15 (11.0)	28 (20.0)	53 (38.0)	13 (9.3)	12 (9.0)
Bailor Barrie	110	15 (14.0)	11 (10.0)	9 (8.2)	4 (3.6)	9 (8.2)	5 (5.0)
Mandela Camp	153	25 (16.3)	10 (7.0)	0 (0.0)	10 (7.0)	8 (5.2)	7 (5.0)
Parade Ground	107	7 (7.0)	14 (13.1)	53 (50.0)	20 (19.0)	7 (6.5)	4 (4.0)
Trade Center	71	10 (14.1)	0 (0.0)	15 (21.1)	10 (14.1)	12 (16.9)	0 (0.0)
Total	581	84 (15.0)	50 (9.0)	105 (18.1)	97 (16.7)	49 (8.4)	28 (5.0)

**The number of persons examined in each camp was the same for each parasitic infection, and was not repeated for each infection.*

to many infections, including *C. parvum*, *G. lamblia*, *A. lumbricoides*, hookworm infection, *S. stercoralis* and schsistosomiasis.

The 29% and 10% prevalence rates among children from the different IDP camps for *G. lamblia* and *C. parvum* infections respectively is not surprising since we found a prevalence rate of 4% among primary school children in Njala, southern Sierra Leone [19]. In other studies in Guinea Bissau, Molbak et al. [32] reported a 6% prevalence rate among 1216 diarrhoeic children, with peak prevalence during the rainy season and the highest prevalence among children younger than 18 months old. Striking seasonal distribution was also reported in studies carried out in South Africa [33]. In Burkina Faso and Nigeria, *Cryptosporidium* was a major factor in the development of diarrhoea and dehydration in the paediatric hospital setting and was closely linked to poor hygienic conditions among the **paediatric** population [34, 35].

The prevalence rate of *Cryptosporidium* among the age groups and between males and females was evenly distributed as the infection decreased with an increase in age, indicating that in normal populations, the development of host immunity has a suppressive effect on the infection [24, 28]. Okafor & Okunji [34] reported similar findings in Nigeria; those children between the ages 2 and 15 years were mostly infected with *C. parvum*.

Progressive armed conflicts have increased the number of people forced to leave their homes and live in displacement and/or refugee camps all over the world, but most notably in developing countries. These conflicts have created conditions favourable for the transmission of helminths due to overcrowding, contamination of the soil of refugee and IDP camps by human excreta, and environmental degradation. Helminthic infections have been known to negatively affect school performance, cognitive processes and nutritional status of children living with multiple helminth infections [36].

After examination of the stool and urine samples from the 851 IDP camp residents, *A. lumbricoides, hookworm, S. haematobium, S. mansoni* and *S. stercoralis* helminths were present at different prevalence levels. In addition, we also found intestinal protozoa, *Entamoeba histolytica* but the prevalence was very low. While these infections have insidious effects, affecting more than 2 billion people with lifelong infections, which have forced public health communities to reassess the importance of these infections [37].

We found five different helminth infections among the 581 IDP camp residents in the 5 IDP camps. In addition, we also found intestinal protozoa, *Entamoeba histolytica*. These multiple parasitic infections were probably due to the break down in the sanitary systems in these camps despite the fact that various non-governmental organizations (NGOs) were overseeing the camps in the country. While our results did not show any effect on the nutritional status of the infected versus the uninfected population in IDP camps, however, when these factors are superimposed on the general nutritional status of these IDP camp residents, it shows that multiple parasitic infections in these IDP camps may pose serious public health problems that may have severe consequences for the resident populations in these camps. The significance of these findings may be immense including high rates of communicable diseases, increased prevalence of other diseases and high infant mortality, but largely dependent on the state of the conditions in each IDP camp.

It is recommended that the Ministry of Health and the NGOs in charge of these camps aggressively work together to improve living, nutritional, environmental conditions in the camps. These efforts can begin with a partnership between the health and education sectors, using deworming as an entry point. But in the long term, a more comprehensive approach is required, dealing with poverty, reduction of over crowding, addressing health care and education, improving living conditions, marked improvement in sanitation particularly with regard to the proper disposal of faeces and the provision of safe water supplies.

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