

Prevalence of intestinal protozoan infestation among primary school children in Urban and peri-urban communities in Kumasi, Ghana

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Abstract: Introduction- Intestinal protozoan infections continue to remain a global public health challenge, particularly in developing countries. Children are greatly prone to these infections via the ingestion of food, water or soil contaminated with the infective stage of these parasites. Objectives- This study focused on establishing the prevalence of intestinal protozoan infection among primary school children aged 5 to 12 years from six communities in the Kumasi metropolis of Ghana. Methods- A total of 2400 children were randomly selected sampled. Stool samples collected from the children were analyzed using the formol-ether concentration technique. The modified Ziehl-Neelsen staining technique was employed in the identification of *Cryptosporidium parvum*. Results- The overall prevalence of intestinal protozoan identified among the studied school children was 42.9%. Prevalence was significantly higher in males than females with rates of 51.0% (604/1162) and 30.8% (381/1238) respectively. The highest protozoan infestation was *Giardia lamblia* with a prevalence of 16.8% (195/1162) and 7.8 % (97/1238) in males and females respectively. Three pathogenic strains (*Giardia lamblia*, *Entamoeba histolytica/dispar* and *Cryptosporidium parvum*) and four non-pathogenic species (*Entamoeba coli*, *Endolimax nana*, *Chilomastix mesnili* and *Iodamoeba butschlii*) were identified in the study. *Giardia lamblia* and *Cryptosporidium parvum* recorded prevalence rates of 12.2% and 8.5% respectively. Prevalence was however proportional to age with respect to *Endolimax nana* and *Iodamoeba butschlii* infections. Conclusion- There is relatively high prevalence of intestinal protozoan infection among the studied children. This obviously suggests that there are possible household, school based and behaviour oriented risk factors which predispose the children to these parasites.

Keywords: Intestinal Protozoan, Parasites, Pathogenic

1. Introduction

Intestinal protozoan infections are of public health importance globally, particularly among children of school going age and the immunocompromised [1, 2]. Protozoan parasites contribute immensely to the burden of intestinal parasitic infections among children [3, 4]. Poor hygiene coupled with children voracious eating habits particularly puts them at greater risk of acquiring these infections [5, 7]. Transmission is by feco-oral route and most infections tend

to be asymptomatic. However, in few cases, common symptoms include vomiting, abdominal discomfort and dysentery [7]. The consequences of intestinal parasites among children particularly include malnutrition, poor physical and mental development, and cognitive and behavioural deficiencies [8, 9].

The global burden of intestinal protozoan infestation is still huge even though there have been tremendous achievements

in the reduction of their prevalence. About 50 million people are currently living with *Entamoeba histolytica* while close to 3 million others are infected with *Giardia lamblia* [10, 11]. Prevalence of *Cryptosporidium parvum* ranges between 2-50% globally [12, 13]. Epidemiological investigations conducted in some communities in Ghana have revealed that prevalence of intestinal protozoan infestations ranges between 0.1-89.0% [14, 15].

Globally, interventions are being put in place to control the morbidity and mortality due to these protozoan infections [16, 17]. Some of these interventions target school going children in the context of school-based health intervention [18]. It is believed that children of school going age forms the majority of the at risk population, and are therefore capable of maintaining the transmission of the infection in the community, particularly among their peers [19-21]. Knowing the prevalence and the associated risk factors in such group will be critical to informing policy interventions. This study therefore focused on investigating the prevalence of intestinal protozoan infections among primary school children in six selected communities consisting of urban, urban-poor and peri-urban settlements in the Kumasi metropolis of Ghana.

2. Methodology

2.1. Study Design and Study Area

Between the months of January to September 2011, cross-sectional school-based study was conducted in the Kumasi Metropolitan area of Ghana. In all, six communities were selected from the peri-urban (Kentinkrono, Gyinyase and Kyirapatre), urban-poor (Ayigya and Aboabo) and urban (Manhyia) areas of the Metropolis. Kumasi is the second largest city in Ghana located in the transitional forest zone of West Africa and is about 270 km north of the national capital, Accra. The city is located between latitude 6.35°N–6.40°N and longitude 1.30°W–1.35°W. The city is densely populated with an estimated population of 2,035,064 living in some 254 square kilometre area [22]. Average annual rain fall is 1400 mm with two distinct rainy seasons.

2.2. Study Population

The study population comprises of primary school children between the ages 5 to 12 years living in the six selected communities in the Kumasi metropolis. From each community, one primary school was selected by the lottery method. The schools selected included Ayigya M/A primary school, Kentinkrono M/A primary school, Aboabo M/A primary school, Afia Kobi Serwaa Ampem M/A primary school, Gyinyase M/A primary school and Kyirapatre R/C primary school.

2.3. Sampling

A simple random sampling technique was used to select the participants. This was achieved by selecting pupils aged

five to twelve years. Pupils were allowed to randomly pick cards inscribed 'Yes' or 'No'. Those who picked 'Yes' were included in the study. The exclusive criteria covered pupil below 5 years and those above 12 years of age, as well as pupils who were sick at the time of the study. A labeled clean plastic container was given to each participant. The container bears the participant's identity number, the age and sex. The teachers in the various schools helped to educate the children on how to collect the stool sample properly.

2.4. Processing and Microscopic Analysis

A fresh stool sample was obtained from each participant. These fresh stool samples were transported to the Diagnostic Microbiology Laboratory of the Department of Clinical Microbiology, Kwame Nkrumah University of Science and Technology, Kumasi, for analysis. After a gross examination of the sample characteristics, a direct wet faecal smear was prepared from each of the fresh samples by emulsifying about 2 mg of the stool sample on a clean 26x76 mm glass slide in a drop of Lugol's iodine. The preparation was covered with a 22x22 mm cover slip and observed using low power (x10) and high power (x40) objectives for the identification of protozoan trophozoites and cyst. A smear was also made from the fresh stool samples on 26x76 mm glass slide and stained with Modified Ziehl-Neelsen acid-fast stain as described by Garcia [23] for the identification of the oocysts of *Cryptosporidium parvum* using the x40 and x100 objectives. Formol-ether concentration method as described by Cheesbrough [24] was performed on each of the stool samples. The sediment obtained was stained with Lugol's iodine and was mixed thoroughly. A drop of the iodine stained sediment was placed on a clean 26x76 mm glass slide and covered with a 22x22 mm cover slip. The protozoan cysts were identified using x40 objective lens. Morphological features used in the identification of the parasites microscopically was aided by pictures and colour atlases provided by Cheesbrough, Washington *et al.*, and CDC [24-26].

2.5. Statistical Analysis

The data obtained from the 2400 primary school children who participated in the study were entered into Microsoft excel 2007 version and validated for double entry errors. The data were then exported to SPSS 16.0 version statistical package. The software package was used to determine the frequency distribution of the studied children in the various communities with respect to the intestinal protozoan infection.

2.6. Ethical Clearance

Ethical approval for the study was obtained from the Ethics and Research committee of the Komfo Anokye Teaching Hospital. Permission to conduct the study in the schools was sought from the primary school head teachers and the parents through written consent.

3. Results

3.1. Gender Distribution of Intestinal Protozoan among the Studied Children

The prevalence rate of intestinal protozoan was significantly higher in males than females. Out of the 1162 males enrolled, 604 (51.0%) were infected while 30.8% (381/1238) females were infected with intestinal protozoan. The highest protozoan infection was *Giardia lamblia* with the prevalence of 16.8% (195/1162) and 7.8% (97/1238) in males and females respectively. The lowest infection was *Entamoeba histolytica/dispar*, 0.3% (4/1162) and 0.1% (1/1238) in males and females respectively (Table 1).

3.2. Intestinal Protozoan Identified by Microscopy

The stool specimens analyzed revealed seven species of intestinal protozoan. Three were pathogenic (*Giardia lamblia*, *Entamoeba histolytica/dispar* and *Cryptosporidium parvum*) and four were non-pathogenic species (*Entamoeba coli*, *Endolimax nana*, *Chilomastix mesnili* and *Iodamoeba butschlii*). The overall prevalence of intestinal protozoan

identified among the studied school children was 42.9%. The studied children had 292/2400 (12.2%) *Giardia lamblia*, 5/2400 (0.2%) had *Entamoeba histolytica*, 204/2400 (8.5%) had *Cryptosporidium parvum*, 248/2400 (10.3%) had *Entamoeba coli*, 169/2400 (7.0%) had *Endolimax nana*, 76/2400 (3.2%) had *Chilomastix mesnili*, and 34/2400 (1.5%) had *Iodamoeba butschlii* (Table 2).

3.3. Age Distribution of Intestinal Protozoan Infections among the Studied Children in the Six Communities

There were variations in intestinal protozoan infections with respect to age in the studied children. Children of 5-6 years age group recorded highest prevalence of pathogenic intestinal protozoan infection while children within the age group 11-12 years recorded the lowest. Among the pathogenic intestinal protozoans, it was observed that prevalence decreases with advancement in age. Prevalence was however proportional to age with *Endolimax nana* and *Iodamoeba butschlii* infections. Varied pattern of prevalence was exhibited by *Entamoeba coli* and *Chilomastix mesnili* (Figure 1).

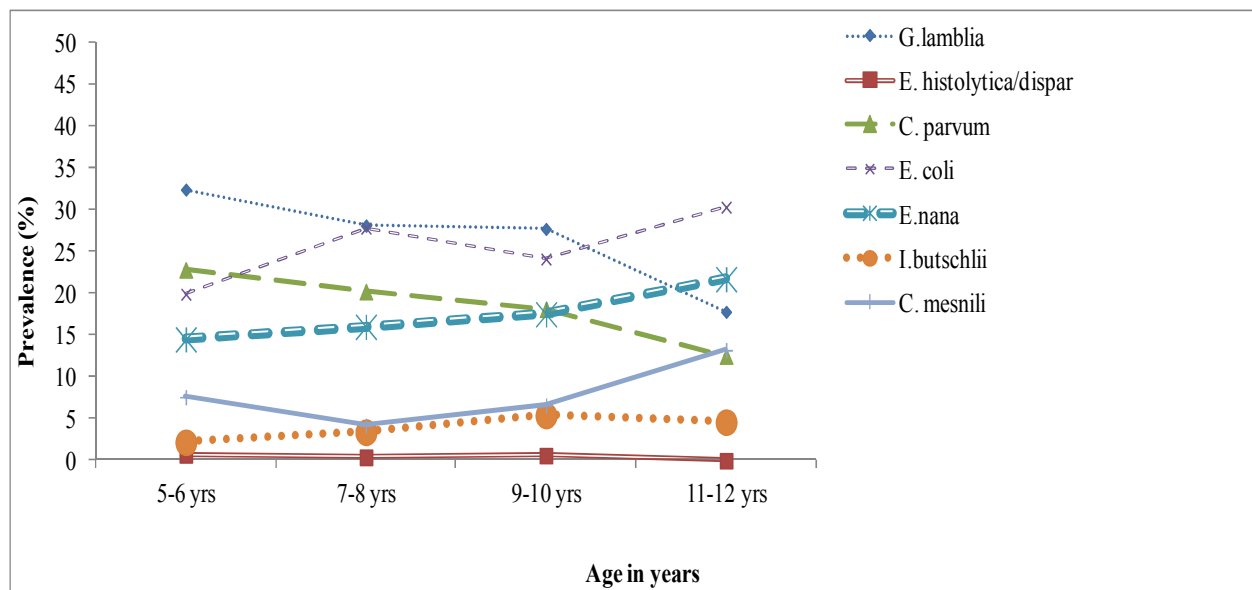
Table 1. The overall distribution of intestinal protozoan infections by sex.

Intestinal Parasite	Number infected (%)		P-value
	Males = 1162	Female = 1238	
<i>G. lamblia</i>	195(16.8)	97(7.8)	0.001
<i>E. histolytica/dispar</i>	4(0.3)	1(0.1)	0.150*
<i>C. parvum</i>	129(11.1)	75(6.1)	0.001
<i>E. coli</i>	149(12.8)	99(8.0)	0.010
<i>E. nana</i>	105(9.0)	64(5.2)	0.001
<i>I. butschlii</i>	18(1.5)	17(1.4)	0.220*
<i>C. mesnili</i>	48(4.1)	28(3.1)	0.060*
Total	648 (55.8%)	381(30.8)	0.001

Key: *Non-significant P-value, *E. coli*= *Entamoeba coli*, *G. lamblia*= *Giardia lamblia*, *E. histolytica/dispar*= *Entamoeba histolytica/dispar*, *C. parvum*= *Cryptosporidium parvum*, *E. nana*= *Endolimax nana*, *I. butschlii* = *Iodamoeba butschlii* and *C. mesnili*= *Chilomastix mesnili*.

Table 2. Intestinal Protozoan identified microscopically in the stool samples of the primary school children from the six communities.

Communities	Ayigya	Kentinkrono	Aboabo	Manhyia	Gyinyase	Kyirapatre	Total
Intestinal Parasites	N=400 n(%)	N=400 n(%)	N=400 n(%)	N=400 n(%)	N=400 n(%)	N=400 n(%)	N=2400 n(%)
<i>Giardia lamblia</i>	45(11.3)	72(18.0)	37(9.3)	20(5.0)	62(15.5)	56(14.0)	292(12.2)
<i>Entamoeba histolytica/dispar</i>	1(0.3)	3(0.8)	1(0.3)	0(0.0)	0(0.0)	0(0.0)	5(0.2)
<i>Cryptosporidium parvum</i>	30(7.5)	50(12.5)	25(6.3)	16(4.0)	38(9.5)	45(11.3)	204(8.5)
<i>Entamoeba coli</i>	32(8.0)	55(13.8)	32(8.0)	26(6.5)	45(11.2)	58(14.0)	248(10.3)
<i>Endolimax nana</i>	20(5.0)	35(8.8)	25(6.3)	18(4.5)	30(7.5)	41(10.3)	169(7.0)
<i>Iodamoeba butschlii</i>	5(1.3)	10(2.5)	5(1.3)	3(0.8)	5(1.3)	7(1.8)	35(1.5)
<i>Chilomastix mesnili</i>	10(2.5)	20(5.0)	10(2.5)	2(0.5)	14(3.5)	20(5.0)	76(3.2)
Total	143(35.8)	245(61.3)	135(33.8)	85(21.3)	194(48.5)	227(56.8)	1029(42.9)



Key: *E. coli*= *Entamoeba coli*, *G. lamblia*= *Giardia lamblia*, *E. histolytica/dispar*=*Entamoeba histolytica/dispar*, *C. parvum*=*Cryptosporidium parvum*, *E. nana*= *Endolimax nana*, *I. butschlii* =*Iodamoeba butschlii* and *C. mesnili*=*Chilomastix mesnili*.

Figure 1. Age distribution of Intestinal Protozoan infections among the studied children.

4. Discussion

This school based cross-sectional study aimed at estimating the prevalence of intestinal protozoan among primary school children aged five years to twelve years in the Kumasi metropolis. A total of 2400 primary school children from six communities were studied. The communities were selected to represent urban, urban-poor and peri-urban distributions of the metropolis.

Our study revealed that the overall prevalence of intestinal protozoan among the studied group was 42.9% (Table 2). Such high prevalence has been reported by a number of studies conducted in similar populations [20, 27]. In addition, higher prevalence of intestinal protozoan has been recorded in most rural settings [28, 29]. However, the prevalence recorded in the present study suggests that intestinal protozoan infection is not only limited to rural folks [30]. Hopefully, the current prevalence could be higher if three consecutive stool samples were taken from each participant.

Intestinal protozoan infection was significantly common among male children (55.8%) than female children (30.8%) (Table 1). Most scholars have attributed this skewness to socio-cultural and behavioural differences between males and females children. Males obtained higher prevalence of intestinal parasitic infection because they get more freedom than females whose leisure hours are strictly controlled and restricted hence are less exposed to parasitic infections [31]. In addition, the highly aggressive and explorative behaviour of the boys consequently make them more prone to infection and re-infection than girls.

Seven intestinal protozoans were identified in the study: three pathogenic species and the rest were non-pathogenic

(Table 2). The commonest intestinal pathogenic protozoan associated with the children was *Giardia lamblia*, followed by *Cryptosporidium parvum* while *Entamoeba histolytica/dispar* was the least (Table 1). Some studies have reported similar finding [32-33]. The effect of persistent *Giardia lamblia* infection could be devastating particularly among children as it results in mal-absorption of fats from the gut. *Cryptosporidium parvum*, which is commonly associated with immunocompromised individuals [34, 35] was found to be relatively high in the children. *Cryptosporidium* and *Giardia* are potential epizoonotic [36, 37] particularly in typical African settings where livestock and domestic animals have been integrated into the community [38]. It is therefore possible that most of the children are living in such communities which predispose them to these parasites. *Entamoeba histolytica/dispar* has been established to be associated with diarrhoea, dysentery and rarely liver abscess [39]. The low prevalence of the parasite observed possibly suggests that morbidity due to *Entamoeba histolytica/dispar* is slim in the studied children. The non-pathogenic protozoan seen in the study obviously confirms the fact that these children are frequently exposed to contaminated food and water, and that the possibility of acquiring other infectious agents either than parasites is high.

A comparison of the children from the primary schools in the six communities: Ayigya (urban poor), Kentinkrono (peri-urban), Aboabo (urban poor), Manhyia (urban), Gyinyase (peri-urban) and Kyirapatre (peri-urban), revealed that intestinal protozoan infection is relatively high in the peri-urban communities followed by the urban poor. The urban community had the least prevalence. Our finding confirms the fact that protozoan parasites are endemic in rural areas [5] as the peri-urban belts and the urban poor zones of the metropolis somehow depict rural communities.

A prevalence rate of 21.3% recorded in the urban community is still on the high side, though low if compared with similar communities [30].

Stratifying our results into age groups, we observed that the prevalence of the infection generally decreases with increasing age. However, children in advanced age groups (9-10 years and 11-12 years) seem not to follow the pattern. Children in age group 5-6 years had the highest prevalence followed by those in age group 7-8 years (Figure 1). According to a study conducted by Heresi *et al.* [40], children acquire some immunity following initial infections in early life which results in some protection in later life, hence the high infection rate observed in this study among younger ages. Interestingly, the study could not explain why the prevalence of two of the non-pathogenic protozoans, *Endolimax nana* and *Iodamoeba butschlii*, seem to increase proportionally with age.

5. Conclusion

The study revealed that the present prevalence of intestinal protozoan infection among primary school children aged 5 to 12 years in the Kumasi Metropolis is 42.9%. *Giardia lamblia* was the commonest pathogenic protozoan in the studied children followed by *Cryptosporidium parvum*. *Entamoeba histolytica/dispar* recorded the least prevalence. Among the non-pathogenic intestinal protozoan, *Entamoeba coli* archived the highest prevalence followed by *Endolimax nana*. The least recorded non-pathogenic intestinal protozoan was *Iodamoeba butschlii*. Generally, most of the agents identified in the study exhibited decreasing prevalence with increasing age. However, two of the non-pathogenic intestinal protozoans, *Endolimax nana* and *Iodamoeba butschlii* seem to have prevalence rates proportional to age.

Competing Interest

The authors declare none.

Authors' Contribution

The study was jointly conceived by all authors. Sample and data collection were done by PT and WW. Laboratory analysis was carried out by SCKT, PT and WW. Data entry and analysis was done by JBZ and WW. WW developed the first manuscript. All authors made significant contributions to the development of the paper.

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