International Journal of TROPICAL DISEASE & Health

Volume 45, Issue 7, Page 214-225, 2024; Article no.IJTDH.118035
ISSN: 2278–1005, NLM ID: 101632866

Prevalence and Associated Risk Factors of Intestinal Amoebiasis among Asymptomatic School Children in the Ho Municipality, South-eastern Ghana

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Authors' contributions

This work was carried out in collaboration among all authors. Authors CYD, PBT-Q and PFA-K designed the study. Authors CYD, VD, CYA, PBT-Q and PFA-K participated in data collection, laboratory and statistical analyses of the data. Authors CYD, EA, RA, FCNK, PBT-Q and PFA-K drafted and revised the manuscript. Authors CYD, NTKDD, IA, PBT-Q and PFA-K jointly developed the structure and arguments for the manuscript and made critical revision of the manuscript. All authors read and approved the final manuscript.

Article Information

DOI: https://doi.org/10.9734/ijtdh/2024/v45i71575

Open Peer Review History:
This journal follows the Advanced Open Peer Review policy. Identity of the Reviewers, Editor(s) and additional Reviewers, peer review comments, different versions of the manuscript, comments of the editors, etc are available here: https://www.sdiarticle5.com/review-history/118035

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Cite as: *Dumevi, Christopher Yaw, Victoria Dumevi, Fleischer C. N. Kotey, Emmanuel Afutu, Robert Aryee, Charles Yao Agede, Nicholas T.K.D Dayie, Irene Ayi, Patience B. Tetteh-Quarcoo, and Patrick F. Ayeh-Kumi. 2024. "Prevalence and Associated Risk Factors of Intestinal Amoebiasis Among Asymptomatic School Children in the Ho Municipality, South-Eastern Ghana". International Journal of TROPICAL DISEASE & Health 45 (7):214-25. https://doi.org/10.9734/ijtdh/2024/v45i71575.*

Original Research Article

Received: 15/04/2024
Accepted: 17/06/2024
Published: 01/08/2024

ABSTRACT

Background: Intestinal amoebiasis is a common cause of gastroenteritis in children, with the *Entamoeba* complex consisting of genetically diverse but morphologically identical species. Amoebic infections are generally subclinical and may be acute or chronic, leading to high morbidity or mortality, especially among children.

Objective: This study assessed the prevalence rate and associated factors of intestinal amoebiasis and other enteric parasitosis among asymptomatic school children within the Ho Municipality of the Volta Region in Southeastern Ghana.

Methodology: This was a cross-sectional study, involving 302 primary school children (aged 4–15 years) in 6 socio-economic settings within the municipality. Single fresh stool specimens were collected from the children and examined microscopically using direct wet mount, iodine preparation and formal ethyl-acetate concentration technique. Structured questionnaire was used to determine demographics and risk factors associated with *Entamoeba* complex infection among the school children.

Results and Discussion: A total of 106 (35.1%) children had gastrointestinal parasitic infection. Of these, seventy-two (23.8%) had *Entamoeba* complex infection with the highest infection (30.4%) recorded among 8–9-year-olds. Children from Peri-Urban communities were more infected with *Entamoeba* complex (32.4%, 33/102) compared to Urban (24.8%, 25/101) and Rural (14.1%, 14/99) respectively. Other parasites detected included; *Giardia lamblia* 21(7.0%), Hookworm 11(2.3%), *Trichuris trichuira* 1(0.3%) and Ascaris lumbricoides 1(0.3%). Factors associated with Intestinal amoebiasis were source of food, fingernails biting/thumbs sucking habits and mother's level of education.

Conclusion: Intestinal parasitic infections is prevalent in the study area and we recommend personal and environmental hygiene practices as well as health education for effective elimination of amoebic and other enteric parasitic infections.

Keywords: Entamoeba complex infections; school children; helminthes; Giardia lamblia.

ABBREVIATIONS

E/P : Evangelical Presbyterian
R/C : Roman Catholic
SSNIT : Social Security and National Insurance Trust
UNICEF : United Nations Children's Education Fund
KVIP : Kumasi Ventilated Improved Pit
WC : Water closet

1. INTRODUCTION

Amoebiasis is a type of gastroenteritis caused by the parasite *Entamoeba histolytica,* commonly occurring among immigrant populations, travelers, immunocompromised individuals, those residing in mental care homes, prisoners, children in day care centres and their families, and sexually active homosexuals [1-6]. Besides *E. histolytica*, about seven (7) [7,8] other amoebic organisms- *Entamoeba dispar, Entamoeba coli, Entamoeba moshkovskii, Entamoeba polecki*, *Entamoeba hartmanni*, *Endolimax nana* and *Iodamoeba bütschlii* have been identified [9-12]. That notwithstanding, *Entamoeba polecki, Iodamoeba butschlii*, and additional amoebic organism, *Dientamoeba fragilis*, are occasionally implicated in human diarrhoea cases [13,14]. Moreover, *E. dispar, E. moshkovskii,* and the predominantly pathogenic amoeba - *E. histolytica* together constitute what has been known as the *Entamoeba* complex, as they are morphologically identical to each other, albeit genetically diverse [10,12,13]. However, of these three, only *E. histolytica* is known to be invasive [10,14].

Amoebiasis is largely transmitted through food or water contaminated with human faeces containing the hardy infective amoebic cyst [15,16]. Hence, improper sanitation and poor personal hygiene practices, which permeate developing countries, are major risk factors of amoebic infection, explaining the disproportionately higher amoebiasis burden in these regions [17-21]. The Sustainable Development Goal (SDG) 6 of the United Nations therefore emphasizes the provision of water, sanitation and hygiene facilities as a means of reducing the burden of amoebiasis [22,23,7,8]. Sadly, although more than 90% of the world's population has access to sources of drinking water, poor water quality remains a major challenge, particularly in sub-Saharan Africa [21,23]. This has frustrated efforts targeted at reducing the high morbidity and mortality associated with the *Entamoeba* complex and other enteric parasites [20,24].

Global prevalence of *Entamoeba* complex infection shows that infected asymptomatic individuals make up about 90% of the total infected persons, whereas the remaining are symptomatic [25,26]. Out of the 10% symptomatic individuals, 90% develop intestinal amoebic colitis while the remaining develop extra-intestinal amoebiasis [27]. This accounts for about 50-100 million reported cases of amoebic colitis or liver abscesses yearly resulting in almost 100,000 deaths [28]. Amoebiasis is next to malaria in relation to mortality from protozoan infection [27,28]. Clinical presentations of amoebic infection include pain in the abdomen, diarrhoea, dysenteric stools, bloody or mucoid stools, ameboma, toxic mega colon, colonic perforations, peritonitis, amoebic cecities and appendicitis, cutaneous amoebiasis, rectovaginal amoebic cutis, hemorrhage among other complications [29]. Other clinical manifestations may involve extra-intestinal organs such as the liver. The most common clinical presentation of invasive amoebic infection is the potentially fatal amoebic liver abscess which affects organs such as the kidney, heart, brain, genitourinary tract and pleura pulmonary [30]. Generally, there is a poor understanding of amoebic infections in sub-Saharan Africa [31]. Some studies documented the distribution and frequency of amoebiasis among children in Ghana but there is a paucity of information on amoebic infections among school children in the Ho Municipality in Southeastern Ghana. Therefore, this study aimed at determining the prevalence and associated risk factors of intestinal amoebiasis among asymptomatic school children within the Ho Municipality.

2. MATERIALS AND METHODS

2.1 Study Area

This study was undertaken within the Ho Municipality, located in the central part of the Volta Region, which lies within the South-Eastern part of Ghana. It is bounded by the Republic of Togo on the east, Adaklu, Agortime-Ziope Districts to the south, and Ho West District to the west and Hohoe Municipality to the north. The municipality has Ho as its capital, occupying a total land mass of 2,361km^2 [32]. The estimated population of Ho municipality is 180,420, consisting of 84,843 males and 95,577 females, according to the 2021 Ghana Population and Housing Census report. Approximately 125,914 people, made up of 59,254 males and 66,660 females, live in the urban areas of the Municipality. However, 54,506 people reside in the rural areas of the municipality, of whom 25,589 and 28,917 are males and females, respectively [33].

2.2 Study Design, Population and Sampling

This was a cross-sectional study that involved 302 children aged 4-15 years sampled from six primary schools in the municipality from October 2016 to March, 2017. Stratified random sampling was used to select six out of the 16 primary schools within the municipality. The selected schools were further categorized as "Rural", "Peri-Urban" and "Urban" based on the socio-economic settings of their location. Simple random sampling technique was used to select two (2) primary schools from each category. From each category of two (2) schools, 10 pupils each were selected from Primary 1 – Primary 6 using simple random sampling technique. The selected schools included; Rural (Klefe Achatime and Klefe Demete E/P Primary Schools), Peri-Urban (Sokode Gbogame M/A and Sokode Gbogame R/C Primary Schools), and Urban (Ho Bankoe E/P and Ho SSNIT Flats Presby Primary Schools) all within the Ho Municipality of the Volta Region.

In each class, pupils were made to pick cards on which was written "Yes" or "No" from a box. All pupils who picked "Yes" were given a special code of identification and were included in the study whereas those who picked "No" were excluded. This was replicated for the other categories and the study subjects were further grouped for analysis into ages 4-5, 6-7, 8-9, 10-11, 12-13 and 14-15 respectively.

Standardized questionnaires were used to collect demographic and socio-economic data on the children and their parents or legal guardians in English and the local (EWE) language. Briefly, the sex, age, socio-economic settings, source of food, drinking water, fingernails biting /thumb sucking habit, type of toilet facility at home were obtained from the children as well as the educational level of their mothers were obtained. The researcher contacted parents or legal guardians of children who were enrolled in the study directly to get information that their wards were unable to provide.

Written Informed Consent was obtained from parents or legal guardians of the school children and assent forms were duly signed by the children before they were enrolled in the study. Interviews were conducted in the local language (Ewe) for pupils who could not communicate effectively in the English language. All interviewers were trained to avoid interviewer-bias. The minimum sample size was 255, calculated using the Cochran formula at 95% confidence interval (CI), with 5% margin of error with 21% prevalence of *Entamoeba* complex infection in the Ho Municipality of the Volta Region [34,35].

2.3 Stool Specimen Collection

A clean wide-mouthed leak-proof stool containers, screw-capped fitted with scoop and clearly labeled with unique respective specimen number, date and location was provided to each child who had consented to be part of the study. Each child was carefully instructed on how to collect the specimen (to circumvent urine contamination and to fill about a third of the container with fresh stool specimen) in the privacy of the school's toilet facility. Thorough hand washing with soap under running water was ensured to prevent possible infection and re-infection. Single fresh stool specimens were collected from the children each school day, and were immediately transported for laboratory investigation. Twenty (20) stool specimens were collected per school per day for laboratory investigation. That was replicated in all selected schools. Children were assisted to collect the stool specimens where necessary.

2.4 Laboratory Investigation

The consistency of the stool was observed and recorded as formed, semi-formed, loose, mucoid, slimy or watery. Evidence of parasitic infection was based on the identification of cysts, trophozoites, ova, larvae or adult worms from direct saline wet mount [36,37], iodine preparation and formol-ethyl acetate sedimentation technique [37-39]. Low power 10X and high power 40X objective lenses were used to examine the stool samples for the identification of enteric parasites as described elsewhere [36]. In all, three microscopists examined the slides. Two independently, observed the slides and a third was called on to resolve any discordant results. A high power 40X objective was used to observe the morphological features of any motile organism (protozoan trophozoites) or larvae detected [37].

2.5 Data Processing and Analysis

Data from interviews and parasitic investigations were entered into Microsoft Excel 2016, and exported into Statistical Products and Services Solutions (SPSS, version 26) for statistical analyses. The data were summarized by determining the frequency distribution of amoebic infection among the sample population. The correlation of amoebic infection between males and females, age groups and risk factors were determined using chi-square test analysis and odds ratios at a 95% confidence interval. A *p*-value of <0.05 was considered statistically significant.

3. RESULTS

A total of 302 Primary School children aged 4 to 15 years (Mean, 50.3; SD,34.1) in six (6) different communities participated in the study. Of these, 153 (50.7%) were males, while the remaining 149 (49.3%) were females indicating an evenly distributed study population in terms of gender. Children (31.0%), who regularly consumed food bought from the street or school canteen, 25.5% who bit their fingernails, 18.2% who practised open defecation and 23.3% who used pipe-borne water as their main source of water supply, were all infected with *Entamoeba* complex.

3.1 Parasitological Profiles of School Children

Of the 302 stool specimens examined, 106 (35.1%) of them had gastrointestinal parasitic organisms. The overall prevalence of *Entamoeba* complex infection was 72 (23.8%). Four other parasites were identified: *Giardia* *lamblia* 21(7.0%), Hookworm 11(2.3%), *Trichuris trichuira* 1(0.3%) and *Ascaris lumbricoides* 1(0.3%). Of those infected, 94 (31.1%) had protozoan infections, while the remaining 12 (4.0%) had helminth infections. One-hundred and four (34.4%) of those had mixed infections, while the remaining 2 (0.7%) had single infection. Mixed infections involving a combination of protozoan organisms (*Entamoeba* complex and *Giardia lamblia)* were higher (93:30.8%) than co-infections involving *Entamoeba* complex and helminths (83:27.5%). No co-infection involving helminthic parasites was detected. The prevalence of parasitic infections in the various socio-economic settings was as follows: Urban (25: 24.8%), Peri-Urban (33: 32.4%) and Rural (14: 14.1%) (Fig. 1).

3.2 Parasitic Infection among Different Age Groups

Overall, *Entamoeba* complex infection peaked in children aged 8-9 years (30.4%) compared to other age groups (Table 1). However, there was no significant association between *Entamoeba* complex infection and age of the participants (p = 0.071) (Table 1).

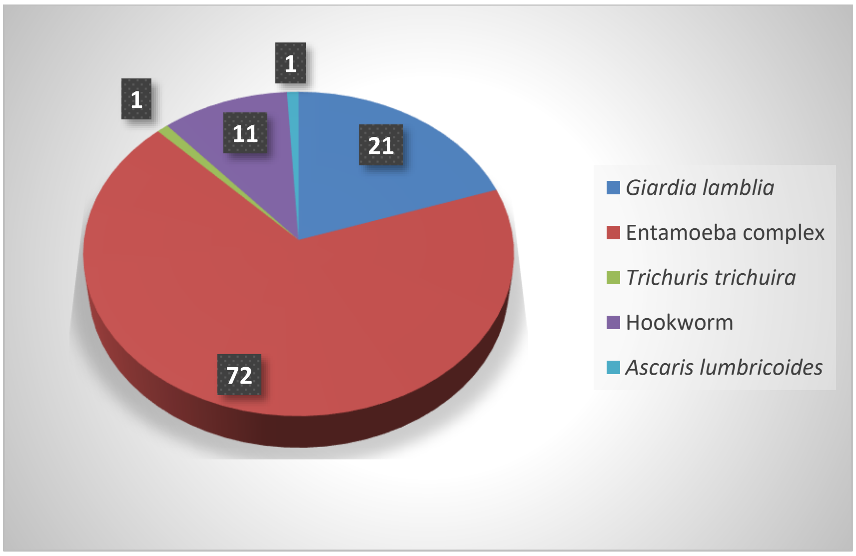

Fig. 1. Frequency of gastrointestinal parasites detected by microscopy

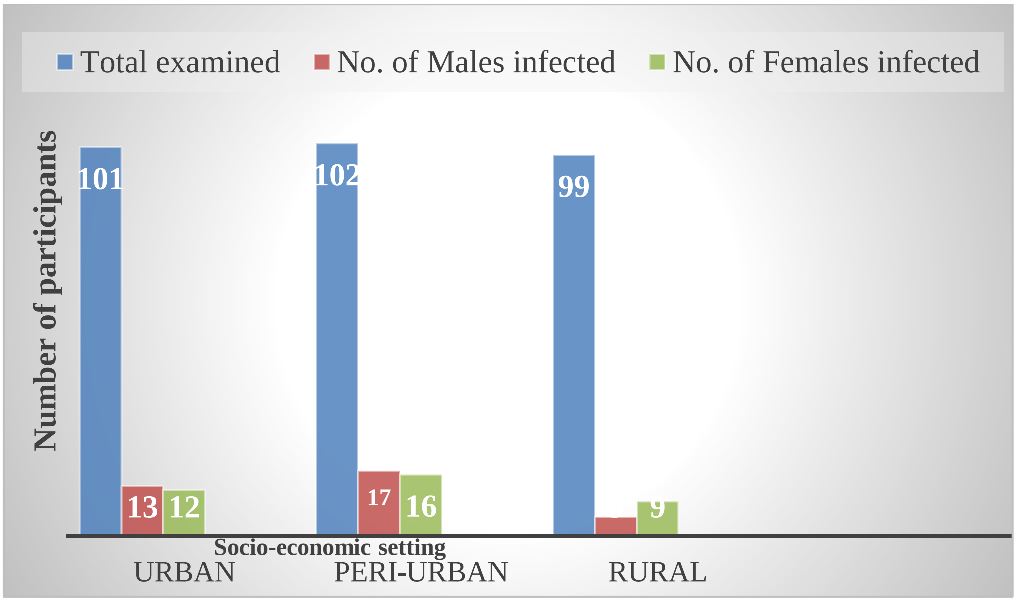

Fig. 2. *Entamoeba* complex Infection in different socio-economic settings

Table 1. *Entamoeba* complex infection in different age groups

Age group (years)	Stool examined	No. infected	X^2	*p*-value	OR	95% CI
4-5	4	1(25)				0.182-2.568
6-7	59	10 (16.9)				1.013-1.292
8-9	69	21(30.4)				1.226-1.499
10-11	79	15(19.0)	15.770	0.071	0.264	1.114-1.380
12-13	80	23(28.8)				1.116-1.359
14-15	11	2(18.2)				1.413-2.041

Table 2. Association of *Entamoeba* complex infection and source of food among the children

Variable (Source of food)	Examined (n)	Infected (n) %	X^2	*p* value	OR	95% CI
Home	126	18(14.3)				0.356-0.418
Street/School canteen	174	54(31.0)	6.249	0.002	0.576	0.469-0.558
Friends	2	0(0.0)				
Total	**302**	**72(23.8)**				

Table 3. Association of *Entamoeba* complex infection and fingernails biting/thumbs sucking behaviour among the children

Variable (Biting of fingernails)	Examined (n)	Infected (n) %	X^2	*p* value	OR	95% CI
Yes	98	25(25.5)				0.375-0.483
No	168	38(22.6)	5.972	0.022	0.556	0.449-0.532
Sometimes	36	9(25.0)				0.352-0.457
Total	**302**	**72(23.8)**				

Table 4. *Entamoeba* complex infection and other related risk factors

Variable (Mother's level of education)	Examined (n)	Infected (n) %	X^2	*p* value	OR	95% CI
Basic	222	28(12.6)				0.414-0.481
Secondary	24	23(95.8)				0.377-0.501
Tertiary	18	17(94.4)	2.776	0.007	0.735	0.272-0.421
No Formal education	38	4(10.5)				0.488-0.717
Total	**302**	**72(23.8)**				
Variable (Toilet facility at home)	**Examined (n)**	**Infected (n) %**	**X^2**	***p* value**	**OR**	**95% CI**
KVIP	37	14(37.8)				1.062-1.452
Water Closet	97	21(21.6)	4.937	0.389	0.397	1.252-1.480
Pit latrine	48	15(31.3)				1.050-1.366
Open defecation	120	22(18.3)				1.122-1.328
Total	**302**	**72(23.8)**				
Variable (Source of drinking water)	**Examined (n)**	**Infected (n) %**	**X^2**	***p* value**	**OR**	**95% CI**
Pipe-borne	283	66(23.3)				0.434-0.495
Well/Bore hole	2	2(100)	3.562	0.07	0.953	0.250-0.433
Stream/River	1	0(0.0)				-2.189-3.1805
Sachet	16	4(25.0)				-0.185-1.085
Total	**302**	**72(23.8)**				

3.3 *Entamoeba* Complex Infection in Different Socio-economic Settings

Children in Peri-Urban areas (32.4%) had more parasitic infections compared to those in the Urban (24.8%) and Rural areas (14.1%) respectively (Fig. 2).

3.4 Parasitic Infection and Associated Risk Factors

Thirty-one percent of children who purchased food from the school canteen or the street had *Entamoeba* complex infection compared to those who went home during break periods to eat (Table 2). There was significant association (p = 0.002) between source of food and *Entamoeba* complex infection among the school children (Table 2). In this study, children who either bite their fingernails or suck their fingers or thumb regularly (25.5%) had *Entamoeba* complex infection. Statistically, there was significant association (p = 0.022) between the age of participants and fingernails biting or thumb sucking behaviour among the children (Table 3). Highest rate of *Entamoeba* complex infection was recorded among children whose mothers had secondary education (95.8%) (Table 4). The Association between *Entamoeba* complex infection and mother's educational level was statistically significant (p = 0.007). *Entamoeba* complex infection (37.8%) was reported in children who used KVIP although there was no significant association (p = 0.389) between *Entamoeba* complex infection and the type of toilet facility used by the children (Table 4).

4. DISCUSSION

Entamoeba complex infection is a common parasitic infection among children in developing nations including Ghana, due to poor personal hygiene practices, sanitation and unsafe water [40-42]. In the present study, *Entamoeba* complex was the most common intestinal protozoan parasite identified (23.8%) among the asymptomatic children sampled in the Ho Municipality of the Volta Region of Ghana. The high prevalence of *Entamoeba* complex infection in the study area could be due to the poor personal and environmental hygiene practices in the study area [41]. Similar studies conducted by Simon-Oke and Ogunleye [43] recorded a high prevalence of (67.6%) 188/278 among primary school children in Akure, Nigeria, whereas Walana *et al.,* [44] reported a low prevalence of (0.21%) 5/2400 among primary school children in Kumasi, Ghana. Also, Verweij et *al.,* [34] reported a prevalence of (39.8%) 98/246 in the Bawku Municipality of the Upper East Region of Ghana. The asymptomatic children become carriers (cyst excreters) and could serve as points of transmission and infection to other uninfected children [45,46].

The sex of the study participants was not statistically associated with *Entamoeba* complex infection. The *Entamoeba* complex infection was slightly higher in females (24.8%) compared to males (22.9%). Similar study conducted in North East India by Nath et al., [47] revealed that females had higher *Entamoeba* complex infection (15.2%) compared to males (11.5%). Although studies have linked gender-specific roles to *Entamoeba* complex infection, the present study has not established that [47,48].

In the present study, there was a reported high incidence of *Entamoeba* complex infection 31% (54/174) among children who either buy food from the street or school canteen compared to 14.3% (18/126) who obtained their food from home. The poor personal hygiene of most food vendors, who may be naïve asymptomatic carriers poses a serious health risk to consumers especially children who patronize their foods. Many of such food vendors do not go for routine medical check-ups to ascertain by public health standards if they are medically fit to sell food to the general public especially children. This is corroborated by Ayeh-Kumi *et al.,* [41] who suggested that food vendors in Accra, Ghana, were possible sources of intestinal parasitic infection including *Entamoeba* complex as a result of lack of safe water, improper personal and environmental hygiene.

The study has associated fingernail biting or thumb sucking behaviours of some children with *Entamoeba* complex infection. Nearly a third of the study participants either bite their fingernails or suck their thumbs with associated health risks. This assertion is confirmed by the findings that *Entamoeba* complex cyst can survive days to weeks in moist external environment with the capability of causing infection [49]. Sofiana *et al.,* [50] also observed that fingernails biting was a possible source of *Entamoeba* complex infection since the viable cyst can survive under fingernails for up to 45 minutes. This negative habit could be stopped when parents, legal guardians, and teachers help educate the children and regularly inspect and trim the fingernails of these children.

It is assumed that the level of education of mothers will have a positive impact on the health of their children [51,52]. However, the findings of this study proved otherwise. High incidence of *Entamoeba* complex infection was reported in children whose mothers had secondary (95.8%) and tertiary education (94.4%) compared to those without any formal education (10.5%). Most educated mothers are career women who by the demands of their jobs may not have time to provide home-cooked food for their wards each school day. They may resort to buying food for their wards from the street or giving money to their wards to buy food from school canteen which might be a source of *Entamoeba* complex infection.

Lack of hygienic toilet facilities in the study area was a major risk factor in *Entamoeba* complex infection. *Entamoeba* complex infection was higher in children from the Peri-urban (33%) and Urban (25%) compared to the Rural (14%) areas. This is due to lack of adequate toilet facilities in the peri-urban and urban areas compared to the rural areas. In these socio-economic settings, nearly a third of the school children practised open defecation or defecate in disposable plastic bags and throw away (“fly away”) into a nearby bush or open space. Others also due to poverty could not pay to use the public toilet facilities in the community. The study also revealed that some children defecate in holes they dug in their backyards with simple implements such as hoes or cutlasses and covered with soil. That behaviour was particularly prevalent in peri-urban and rural areas. These holes may be uncovered by free-range-domestic poultry birds due to their shallow nature, or washed into homes or nearby water bodies by run-offs posing a public health risk [22,53].

The level of infection recorded in children who used water closets (WC) may be attributable to the unavailability of water to flush the excreta, wash hands after defecation, or the general insanitary conditions which was conducive for *Entamoeba* complex transmission. Again, contaminated water closet flash handles, or seats pose a greater health risk to users. The present study agrees with the findings of Nath *et al*., [47] which suggested that those who used unhygienic toilet facilities had higher *Entamoeba* complex infection than those who used hygienic toilets. Therefore, proper and hygienic sanitary facilities including toilets are critical to ensuring the health of children against *Entamoeba* complex infection [22,41,43,53].

Safe water is a critical resource for guaranteed health and wellbeing of children. Unsafe water predisposes children to a higher risk of gastroenteritis including amoebiasis. Furthermore, unsafe water and poor sanitation may lead to infections and disease conditions that could negatively impact the physical, cognitive and academic development of children [41,50]. The availability and access to safe drinking water and proper sanitation are human rights, and safe water and adequate sanitation are critical for the development and well-being of humans [50,52,53].

The present study revealed that *Entamoeba* complex infection was highest (100%) among children who regularly depended on well or bore hole as against sachet (25%) and pipe borne water (23.3%) respectively. The findings of this study agree with the assertion that, water from wells or springs consumed had higher *Entamoeba* complex infection [54-58] compared to other sources.

The present study also revealed the presence of other parasites such as, *Giardia lamblia*, *Trichuris trichuira,* Hookworm and *Ascaris lumbricoides.* Even though the Ho Municipal Health Directorate in collaboration with Municipal Education Directorate conducted mass administration of Metronidazole and Albendazole to primary school children in December 2015 prior to this study, it is unclear why the prevalence of gastrointestinal parasites (35.1%) and *Entamoeba* complex infection (23.8%) remain high among the study population.

5. CONCLUSION

This study reported a high prevalence of gastrointestinal parasites among the study participants with *Entamoeba* complex infection being the most common. More females were infected compared to males and children between 8-9 years had the highest rate of infection compared to other age groups. Source of food, fingernail biting or fingers/thumbs sucking habits, level of mothers’ education, and socio-economic settings were related risks factors associated with *Entamoeba* complex infection. We recommend a much wider molecular study to differentiate between species or zymodemes of *Entamoeba* complex in the Ho

Municipality. The local authorities and other relevant stake holders should intensify their efforts at providing safe water, sanitation and hygiene facilities (WASH) in areas where they are lacking. Also, public education on the prevention of parasitic infections in the municipality should be intensified.

6. LIMITATIONS OF THIS STUDY

Firstly, the study was conducted between October 2016 to March, 2017 and a lot might have changed since then. Secondly, the use of microscopy as the main diagnostic tool, which is the gold standard in resource limited-settings including Ghana could have affected the overall prevalence of the parasites detected.

DISCLAIMER (ARTIFICIAL INTELLIGENCE)

Authors hereby declare that NO generative AI technologies such as Large Language Models (ChatGPT, COPILOT, etc) and text-to-image generators have been used during writing or editing of manuscripts.

DATA AVAILABILITY

All data supporting the reported results have been included in the paper.

CONSENT AND ETHICAL APPROVAL

Written Informed Consent was obtained from parents or legal guardians of the school children following an explanation of the objective of the study to them. Further, assent forms were duly signed by the children before they were enrolled in the study. Laboratory examination of stool specimens was performed free of charge. Study participants who were positive for intestinal parasites were linked to nearby health centres for treatment. The study was conducted in accordance with the Helsinki Declaration (1964). This study was reviewed and approved by the Ethical and Protocol Review Committee of the College of Health Sciences, University of Ghana, Accra, Ghana (Protocol ID no: CHS-Et/M.1- P 4.11/2016–2017). The Institutional Review Board of the Volta Regional Hospital (now Ho Teaching Hospital), as well as the Ho Municipal Directorate of Education also approved this study.

ACKNOWLEDGEMENTS

The authors wish to thank the parents or legal guardians of children who participated in the study. Our gratitude also goes to Ho Municipal Directorate of Education, Headteachers and Staff of the selected schools. We would also like to thank the Management and Staff of the Parasitology Unit of the Volta Regional Hospital (now Ho Teaching Hospital) for their support.

COMPETING INTERESTS

Authors have declared that they have no known competing financial interests OR non-financial interests OR personal relationships that could have appeared to influence the work reported in this paper.

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