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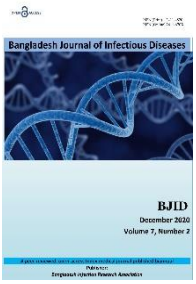

Giardiasis among Under Five Children Living in Tea Plantation Colony at Sylhet District of Bangladesh

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Abstract

Background: Giardia lamblia infection is still frequently encountered especially in children living in crowded and unhygienic conditions leading to various public health problems. **Objective:** The purpose of the study was to detect giardiasis among under five children living in tea plantation colony. **Methodology:** This cross-sectional observational study was conducted in the Department of Microbiology, Sylhet MAG Osmani Medical College, Sylhet during the period from January 2019 to December 2019. For this purpose children were selected according to inclusion and exclusion criteria irrespective of sex. Four tea garden from Sylhet district namely Malnicherra, Lackatoorah, Tarapur and Burjan were selected randomly for sample collection. Stool samples were collected in sterile, disposable, plastic containers with proper labeling without any preservatives. Cyst of *Giardia* was determined in stool through microscopic examination. *Giardia* antigen in stool was detected using ELISA kits. **Results:** Out of 120 cases, *Giardia* cyst was found positive in 18(15.0%) cases by microscopy. *Giardia* antigen was found positive in 23(19.17%) cases by ELISA. ELISA showed sensitivity of 94.13% and specificity of 95.0% when compared with microscopy. *Giardia* infection was highest in male children (20.0%) than female children. The infection rate was higher (24%) among age group of 36 to 59 months. **Conclusion:** Giardiasis constitutes a major concern in symptomatic children as well as in asymptomatic children because it causes various public health problem. [*Bangladesh Journal of Infectious Diseases, December 2022;9(2):53-58*]

Keywords: Giardiasis; under five children; unhygienic condition; diarrhea

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Introduction

Giardiasis is the most common human entero-pathogenic protozoan disease that manifests as diarrhoea[1]. Diarrhoea is a major public health problem worldwide, especially in children. One in ten child deaths occur globally from diarrhoeal diseases in under five children. This resulting in about 800,000 fatalities worldwide annually, mostly occurring in Sub-Saharan Africa and South Asia[2]. It is the second most common cause of under-five child death after pneumonia. One in 30 under five

children die of diarrhoea in Bangladesh[3]. Diarrhoea is caused by many infectious organisms including bacteria like *Escherichia coli*, *Vibrio* species; viruses like *Rotavirus, Norovirus* and parasites like *Giardia*, *Entamoeba*, Cryptosporidium[4]. *Giardia* is one of the important cause of parasitic diarrhoeal diseases. It accounts for about 8.0% diarrhoeal diseases in Bangladesh[5]. It is prevalent in under five children due to weak immune system and lack of personal hygiene[6-7]. Giardiasis is defined by the CDC as the detection of *Giardia* organisms, antigen or DNA in stool, intestinal fluid, biopsy specimens or other biological sample[8]. *Giardia lamblia* is a unicellular flagellated protozoan parasite, the causative organism of giardiasis. It is also known as *Giardia intestinalis* or *Giardia doudenalis*[6,9]. It is one of the oldest eukaryotic organism and also most frequently isolated intestinal protozoa in the word[10]. It was first seen by Dutch Microscopist Sir Antonie Van Leeuwenhoek in 1681 while examining his own stool[9]. Mature cyst is the infective form for man. Infective dose is as few as 10 cysts[11-12]. It is transmitted through faeco-oral route via contaminated foods and drinks. It may also spread from person to person and animal to person via direct contact[7]. Risk factors include defecation in open place that contaminates environment such as foods, drinks etc., travelling in developing countries, changing diapers, daycare center, summer camp, eating foods without proper cooking, drinking contaminated water, immunocompromised patient, malnourished patient, parents of infected children, swimmers who swallow contaminated recreational water and persons having a dog[7,12].

Giardia has variant specific surface proteins (VSPs), which help it to survive by protecting against the action of intestinal proteases. Trophozoites are closely associated with intestinal mucosa but do not invade. This close association with mucosa may directly affect the brush border and its enzyme system, by disrupting it during attachment by the sucking disk. Other receptor-ligand adherence mechanism is also important. The close adherence of *Giardia* without invasion and affected brush border may stimulate an inflammatory cytokine response. This can result in secretion of fluid and electrolytes and damage to enterocytes. No classic enterotoxin or cytotoxin is produced by this parasite. Another mechanism for diarrhoea in giardiasis is deconjugation of bile salts by overgrowth of *Giardia* leading to malabsorption[11,13]. Giardiasis has a wide spectrum of clinical expression such as steatorrhoea, acute enterocolitis, chronic enteritis, malabsorption syndrome, weight loss, anaemia, allergic manifestations, chronic cholecystopathy[8,9]. Although most infections are asymptomatic, prolonged diarrhoea with malnutrition and growth failure in infancy can also occur[14]. It is also associated with lower serum zinc, iron, vitamin B12 and vitamin A leading to significant growth and developmental delay even in asymptomatic children[15]. It is a serious public health issue in developing countries because it can cause iron-deficiency anaemia, malnutrition, growth retardation, other physical and mental health disorders especially in children[16]. Most clinicians and health workers do not consider parasitic infections as life threatening condition. Most of the time they go unnoticed or misdiagnosed these diseases[17]. As a result, not much attention is given to treating these diseases. As peoples are unaware about this disease, WHO considered it as an important neglected tropical disease[18].

Giardiasis can be diagnosed through identification of cysts, trophozoites and *Giardia*-specific antigen in faecal samples. Several tests are available for diagnosis but there is need of a rapid, accurate and easy diagnostic method for developing countries like Bangladesh. Comparison of faecal diagnostic method is difficult due to the lack of a true gold standard reference method[19]. The ovum and parasite (O & P) method is the currently accepted gold standard method despite its inferior sensitivity compared to enzyme-linked immunosorbent assay (ELISA), particularly for single faecal sample[20]. Intensity of infection (cyst/gram of faeces) can be determined through light microscopy by direct (routine) and cyst concentration technique. Alternatively, the antigen can be detected by ELISA may offer highly sensitive and specific method. Antigen can also be detected in stool sample by RDT (Rapid antigen detection test). Nucleic acid-based techniques such as PCR, RFLP and specific DNA probe for *Giardia* are available now[21]. Although the nucleic acid based techniques are highly sensitive and specific but not available in all setting and also costly. Therefore, this study was designed to compare between different diagnostic methods and also to evaluate efficacy of ELISA as a sensitive, rapid and easy diagnostic tool in diagnosis of giardiasis among under five children.

Methodology

Study Settings and Population: This cross-sectional observational study was carried out in the department of Microbiology, Sylhet MAG Osmani Medical College, Sylhet from 1st January 2019 to 31st December 2019. All under five children were living in tea plantation colony in Sylhet Sador, Sylhet who fulfilled the eligibility criteria. Children

who took anti-protozoal drugs within last two months were excluded. After explaining the purpose of the study, informed written consent was taken from parents or legal guardians of the children through proper authority

Sample Collection Procedure: The present study was carried out at four tea plantation colony (randomly selected) namely Malnicherra Tea Estate, Lackatoorah, Tea Estate, Burjan Tea Estate and Tarapur Tea Estate located in Sylhet Sador upazilla, Sylhet, Bangladesh. Stool samples were collected from children in dry, clean, wide neck, disposable plastic container with proper labeling without any preservatives and were transported to the 41 microbiology laboratory in the Department of Microbiology, Sylhet MAG Osmani Medical College, Sylhet as soon as possible.

Laboratory Procedure: Macroscopic examination was done for color, consistency, presence of mucus, blood or worms in stool samples. Then microscopic examination of stool was done to detect of cyst & trophozoite of *Giardia lamblia*. Microscopic examination was done to detect cyst & trophozoite of *Giardia lamblia* in saline preparation, iodine preparation and formol-ether concentration technique After microscopic examination, samples were stored at -20 ^{0}c until ELISA was done. Detection of *Giardia* by ELISA: Monoclonal antibodies against giardin protein of *Giardia lamblia* used in a sandwich type method. The assay was carried out according to the manufacturer's instruction.

Statistical Analysis: Statistical analyses was performed with SPSS software, versions 22.0 (IBM SPSS Statistics for Windows, Version 22.0. Armonk, NY: IBM Corp.). Continuous data that were normally distributed were summarized in terms of the mean, standard deviation, median, minimum, maximum and number of observations. Categorical or discrete data were summarized in terms of frequency counts and percentages. When values are missing, the denominator was stated. Chi-square test was used for comparison of categorical variables. Every effort was made to obtain missing data. A two-sided P value of less than 0.05 was considered to indicate statistical significance.

Ethical Clearance: All procedures of the present study were carried out in accordance with the principles for human investigations (i.e., Helsinki Declaration) and also with the ethical guidelines of the Institutional research ethics. Formal ethics approval was granted by the IRB of Sylhet Osmani Medical College. Participants in the study were informed about the procedure and purpose of the study and confidentiality of information provided. All participants consented willingly to be a part of the study during the data collection periods. All data were collected anonymously and analyzed using the coding system. Prior to beginning of this study, approval of the research protocol was obtained from the Ethical Review Committee of Sylhet MAG Osmani Medical College, Sylhet [Memo No/SOMC/2019/69]. Ethical standard was set by following the BMRC guideline.

Results

In this study prevalence of giardiasis was highest 12(24.0%) among 37 to 59 months of age group children followed by lowest 2(15.4%) among 0 to 12 months of age group children (Table 1).

Table 1: Prevalence of Microscopic Confirmed Giardiasis among under Five Children based on Age Group (n=120)

Age Group	Microscopy	
	Positive	**Negative**
0 to 12 Months	2(15.4%)	11(84.6%)
13 to 36 Months	4(7.0%)	53(93.0%)
37 to 59 Months	12(24.0%)	38(76.0%)
Total	**18(15.0%)**	**102(85.0%)**

Chi-square test was performed to measure the level of significance; P value=0.049

Prevalence of giardiasis (ELISA positive) was highest 15(30.0%) among 37 to 59 months of age group children followed by lowest 2 (15.39%) among 0 t o12 months of age group children (Table 2).

Table 2: Prevalence of ELISA Confirmed Giardiasis among under Five Children based on Age Group (n=120)

Age Group	ELISA	
	Positive	**Negative**
0 to 12 Months	2(15.4%)	11(84.6%)
13 to 36 Months	6(10.5%)	51(89.5%)
37 to 59 Months	15(30.0%)	35(70.0%)
Total	23(19.2%)	97(80.8%)

Chi-square test was performed to measure the level of significance; P value=0.036

Giardia cyst was found positive in 18(15.0%) and negative in 102(85.0%) children by microscopy and Giardia stool antigen was found positive in

23(19.2%) and negative in 97(80.8%) children by ELISA (Table 3).

Table 3: Distribution of Study Population according to Microscopic Finding and Stool Antigen Test by ELISA

Diagnostic Test	Positive	Negative
Microscopy	18(15.0%)	102(85.0%)
ELISA	23(19.2%)	97(80.8%)

About 17 cases were positive both in microscopy and in ELISA and 96 cases were negative both in microscopy and ELISA. However, 6 cases were false positive as 6 cases were positive by ELISA but found negative in microscopy. Again 1 case was false negative as this 1 case was negative by ELISA but found positive in microscopy (Table 4).

Table 4: Comparison of Study Population Observed by Microscopy and ELISA

Diagnostic Test ELISA	Microscopy		Total
	Positive	Negative	
Positive	17	6	23
Negative	1	96	97
Total	**18**	**102**	**120**

Discussion

In the present study, the highest prevalence of giardiasis was found in children of 37-59 months of age group (15.39%). The is in agreement with the results of a study done in Bangladesh, where most of the infected children (8.7%) were found in this age group[22]. Hussain[23] and Inabo et al[1] also reported the same. This may have several explanations, first, the age dependent decline rate may be related to anti- *Giardia* immunity and secondly, the acquisition better hygienic practice which make the avoidance of this infection easier. In this age group children play in very close contact and habit of putting everything in mouth, thus allow easy transmission[22-23]. In this study, highest infection rate was found among male (20.0%) children. The results of higher prevalence in case of male was agreed with those of Suman et al[22]. Another two study[24-25] were having the opinion that males are involved in more activities and are more in contact with environmental condition than females.

In current study microscopic examination of stool showed *Giardia* cysts were present in 15.0% children and absent in 85.0% children. This result was supported by an Iranian study where cysts were present in 13.93% cases and absent in 86.07% cases[6]. This result was also supported by an Ethiopian study where cysts were present in 19.6% and absent in 80.4% cases[26]. Hegazy et al[27] reported the presence of cysts in 14.8% stool samples in Egyptian children. The prevalence of giardiasis in the present study is consistent with a previous study (18.8%) from India[28]. Mane et al[29] showed similar rate (12.20%) of infection in asymptomatic under five children conducted in North Maharashtra, India. This study is also supported by a Bangladeshi study[3] where the prevalence rate of giardiasis was 11.0% in children living in urban slum areas.

In this study stool antigen was positive in 23(19.17%) children and negative in 97(80.83%) children. This result was supported by the study of Nigam et al[30] where *Giardia* stool antigen test by ELISA was positive in 19.5% children and negative in 80.5% cases. This result has been also supported by Haque et al[31] where the prevalence rate of giardiasis was 17.1% in asymptomatic patient by ELISA. In this regards Ahmed et al[24] reported that the prevalence was 76.1% in asymptomatic children and 38.9% in symptomatic children by ELISA. This difference may be due to sample size and diagnostic techniques.

Present study showed that ELISA was 94.1% sensitive and 95.0% specific for diagnosis of giardiasis compared to microscopy. The result is comparable with Ahmed et al[24] who found that ELISA shows 88.9% sensitivity and 90.91% specificity. The result was also comparable with Aldeen et al[32] who found that ELISA shows 91.0% sensitivity and 98.0% specificity. The current study highlights the high sensitivity and specificity of ELISA technique that are not commonly used in most diagnostic laboratories provide for diagnosis of *Giardia* infection. As microscopically examination has some disadvantage time consuming and sensitivity could be affected by intermittent excretion of cyst, ELISA is more preferable for rapid diagnosis of large size sample.

Conclusion

Based on the results of this study we concluded that *Giardia* infection rate is higher and a significant public health problem in tea plantation colony. Most of the children carry *Giardia* cyst in asymptomatic state. We, therefore, favour treating asymptomatic cyst passers so as to get rid of a potential source of infection, who consistently contaminate their communities with cysts. Thorough epidemiological survey to identify silent

giardiasis cases using highly sensitive tools is necessary. In this regard we suggest that ELISA is the best method. Though ELISA is expensive method, it definitely gives more accurate result on sensitivity and specificity. Regular practicing in personal hygiene and good sanitation along with proper health education should be continuously given to parents and children for prevention of parasitic infection. The benefits of these actions will be the control of human giardiasis and a reduction in public health problems**.**

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None

Conflict of Interest
We declare that we have no conflict of interest.

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Contribution to authors:
Md. Nahidul Islam and Moynul Haque designed the overall study; Md. Nahidul Islam was responsible for data collection; Md. Nahidul Islam and Premananda Das involved in data cleaning; Md. Nahidul Islam, Premananda Dasand Anwarul Haque conducted data analysis and interpretation; Md. Nahidul Islam and Tahani Momotaz drafted the first manuscript; Md. Nahidul Islam, Moynul Haque, Sharmina Aftab, Anwarul Haque and Premananda Das revised the manuscript; All authors read and approved the final manuscript.

Data Availability
Any questions regarding the availability of the study's supporti ng data should be addressed to the corresponding author, who can provide it upon justifiable request.

Ethics Approval and Consent to Participate
Ethical approval for the study was obtained from the Institutional Review Board. As this was a cross sectional observational study the written informed consent was not obtained from all study participants. All methods were performed in accordance with the relevant guidelines and regulations.

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