

Original Research Article

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Prevalence of *Entamoeba histolytica* and *Giardia lamblia* infection in a Rural Area of Haryana, India

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ABSTRACT

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An intestinal parasitic infestation is a major public health problem in India. These two important parasites of the gastrointestinal tract lead to gastroenteritis in human population. The present prospective study is carried out to check the prevalence of these two parasites in patients with gastrointestinal symptoms at MM Institute of Medical Sciences and Research Mullana, Ambala. A total of 656 stool samples of HIV negative patients with gastrointestinal symptoms from OPD and wards are included in this study. Stool specimens obtained from all participants were examined for the presence of intestinal parasite cysts, eggs, trophozoites and larvae by saline wet mount and Iodine mount. Out of 656 stool specimens *Entamoeba histolytica* was found in 80(12.2%) patients whereas *Giardia lamblia* was isolated in 6.7% cases. The *Giardia lamblia* was more prevalent in < 15 years and *Entamoeba histolytica* was more prevalent in > 15 years. The seasonal variation of *Entamoeba histolytica* and *Giardia lamblia* was also seen with more prevalence in hot and moist season being water borne disease($p < 0.05$). Improvement of safe drinking water supply and sanitation facilities could significantly reduce the burden of these parasitic diseases.

Introduction

An intestinal parasitic infestation is major public health problem in the world. It is estimated that 3.5 billion people are involved globally and 450 millions are suffering as a result of these infections, majority being children (WHO, 2000). The most important protozoan parasites are the *Entamoeba histolytica* and *Giardia lamblia*. *Giardia lamblia* (syn *G. intestinalis*, *G. duodenalis*) is one of the most common intestinal parasites in the world (WHO, 2004).

Giardiasis results in different intestinal symptoms including diarrhoea, steatorrhea, abdominal cramps, bloating, flatulence, pale greasy and malodorous stools, and weight loss; nausea or vomiting may also occur. Active infection leads to lactose intolerance which lasts for several months after clearance of the parasite (Ponce-Macotella *et al.*, 2005). *Entamoeba histolytica* is another important parasite of the human gut which causes amoebiasis. It is the infection of human intestinal and extra-intestinal organs.

The amoebic infection is considered as the third most common cause of death among parasitic diseases (Benenson AS {ed}2011). The problem is more in tropical countries because of the humid climate, poor social economic status, improper water supply, insanitary disposal conditions and unhygienic environment (Rai *et al.*, 2002; Rajeswari *et al.*, 1994). The geographic variation is seen because of differing socioeconomic and environmental conditions. The problem is more in rural areas as compared to urban areas (Sayyari *et al.*, 2005). Poly parasitism is also reported in some areas (Estevez *et al.*, 1983; Sharma *et al.*, 2004).

There are reports with varying isolation rates of various parasites, *Entamoeba histolytica* and *Giardia lamblia* commonly being isolated (Sayyari *et al.*, 2005; Sharma *et al.*, 2004) amongst protozoa. Other parasites reported are *Cryptosporidium* species, *Balantidium coli*, *Trichuris trichura*, *Ascaris lumbricoides*, *Strongyloides stercoralis*, *Enterobius vermicularis*, *Hymenolepis nana* (Kaur *et al.*, 2002; Awasthi and Pande, 1997; Kang *et al.*, 1998). Infestation rate varies in different age group (Sayyari *et al.*, 2005; Sharma *et al.*, 2004). *Giardia lamblia* is frequently reported in younger age group and *Entamoeba histolytica* is reported more in elderly people (Roche and Benito, 1999). The seasonal variations are very common with more infestations reported in hot and rainy season (Vargas *et al.*, 2004). Both parasites have a low infectious dose, spread through feces and contaminated water. They have similar clinical presentations and have simple life cycles having a resistant, infectious cyst form and a fragile, disease-causing trophozoite, which are the diagnostic stages of these parasites (Tanyuksel *et al.*, 2001). Keeping in view the above facts this study is designed to access the prevalence of *Giardia lamblia*

and *E. histolytica* in the rural area surrounding Mullana.

Materials and Methods

Sample Collection

The present prospective study was conducted in the Department of Microbiology at MM. Institute of Medical Sciences and Research Mullana, Ambala. A total of 656 stool samples from HIV negative patients with gastrointestinal symptoms, irrespective of age, from OPD and wards were included in the study. A single stool sample was collected from each patient before the institutional treatment in the universal plastic container without contamination with urine, water or disinfectant and immediately transported to laboratory. Informed consent was obtained from the patient or relatives. Blood samples from the patients were also taken for knowing HIV status using standard procedures. The patient's Performa was filled regarding age, sex, place, symptoms, antibiotic treatment etc. The study was reviewed and approved by the ethical committee of the institute.

Processing of Specimens and Examination for Parasites

Stool samples were examined macroscopically for the presence of parasitic forms, mucus and blood. Microscopic examination of stool was done under low and high power in unstained preparation for typical parasitic movement and stained preparations (Iodine staining) were used for nuclear details. (Proctor EM, 1991). The negative samples were further examined by formal ether concentration technique (Petri and Singh, 1999). Half tea-spoonful of stool sample was transferred to 10 ml of 10% formalin in shell vial and allowed to fix for

30 minutes and then filtered through two gauge pieces into 15 ml centrifuge tube. Normal saline was added to within ½ inch of tube and centrifuged for 10 minutes at 500 g. Blood samples from the patients were tested by HIV Tridot method to know the HIV status of the subjects.

Data Analysis

Chi-square and paired t test and ANOVA tests were used to study significant associations or differences. For seasonal variation the study population was divided into two groups that is May to August and rest of the year.

Results and Discussion

During the study period a total of 656 stool specimens were examined. *Entamoeba histolytica* was identified in 12.2% and *Giardia* in 6.7% of the patients. According to the age the statistical analysis showed that there were significant relation between Age group and infectivity rate of *Entamoeba histolytica* and *Giardia lamblia* at ($P \leq 0.05$). The *Entamoeba histolytica* was more prevalent in older age group with statistically significant. The *Giardia lamblia* was more prevalent in younger age group with statistically significant in < 15 years of age ($p < 0.05$). (Table 1 & 2) The Prevalence of *Entamoeba histolytica* and *Giardia*

lamblia was more in males as compared to females but it was not statistically significant ($p > 0.05$). (Table 3 & 4) The result showed that the high prevalence of *Giardia lamblia* and *Entamoeba histolytica* was recorded in May to August, while the lower prevalence was recorded in October and November to April. (Table 5). The prevalence of *Entamoeba histolytica* and *Giardia lamblia* as single and mixed infection in stool samples is shown in Table 6.

The prevalence rates of *Entamoeba histolytica* and *Giardia lamblia* exhibit wide variation between geographic areas, different age groups and even seasonal variations are also known. *Entamoeba histolytica* was more prevalent in >15 age group (18.5%) as compared to children (8.61%) with significant statistical difference. Similar findings have been reported by other workers (Roche and Benito, 1999). *Giardia lamblia* was prevalent in younger age group and there was a statistically significant decline in prevalence of *Giardia lamblia* in >15 age group There are also reports of higher prevalence of *Giardia lamblia* in younger age group (Roche and Benito, 1999). The lower prevalence in adults shows that adults develop immunity after repeated infections in young age as suggested by others (Kang et al., 2003).

Table.1 Prevalence of *Entamoeba histolytica* and *Giardia lamblia* in different age groups

Parasites	Number of positive samples (%)				Total
	Age group				
	0-5	5-10	10-15	>15	
<i>Giardia lamblia</i>	18(6.5)	10(10.6)	8(16.6)	8(3.4)	44(6.7)
<i>Entamoeba histolytica</i>	22(7.9)	10(10.6)	4(8.3)	44(18.5)	80(12.2)
Total patients	276	94	48	238	656

Table.2 Comparison of prevalence in different age groups

	Age (years)	Positive samples	Total samples	χ^2	p value
<i>Giardia lamblia</i>	<15	36(8.61%)	418	6.665	<0.01
	>15	8(3.36%)	238		
<i>Entamoeba histolytica</i>	<15	36(8.61%)	418	13.82	<0.001
	>15	44(18.48%)	238		

Table.3 Prevalence of *Entamoeba histolytica* among males and females

Gender	Positive for <i>Entamoeba histolytica</i>	Total samples	χ^2	p value
Male	56 (12.2 %)	456	0.01	>0.05
Female	24 (12.0 %)	200		

Table.4 Prevalence of *Giardia lamblia* among males and females

Gender	Positive for <i>Giardia lamblia</i>	Total samples	χ^2	p value
Male	36	456	3.37	>0.05
Female	8	200		

Table.5 Seasonal variation of parasite with maximum positive cases in May-August

Month	Total samples	Positive for <i>Entamoeba</i>	Positive for <i>Giardia</i>
Jan	48	2	4
Feb	20	0	2
Mar	10	0	2
Apr	32	2	0
May	76	10	6
June	56	16	6
July	70	6	2
Aug	142	18	12
Sep	78	4	2
Oct	58	14	4
Nov	36	2	2
Dec	30	6	2
Total	656	80	44

Table.6 The prevalence of *Entamoeba histolytica* and *Giardia lamblia* as single and mixed infection in stool samples

Parasites	Positive	Single infection	Mixed infection(Two or more organisms)
<i>Giardia lamblia</i>	44	32	12(27%)
<i>Entamoeba histolytica</i>	80	51	29(36%)

This may be also due to the reason that the older age group persons have better personal hygiene habits than younger children. *Entamoeba histolytica* and *Giardia lamblia* were present as mixed infection in twenty seven and thirty six percent of case(Table-6) which shows that the various parasites have their common source of infection that is contaminated water and poor sanitation. Other workers have also reported the mixed infestation in their studies attributing to similar factors (Estevez *et al.*, 1983; Sharma *et al.*, 2004). In the present study the prevalence of *Entamoeba histolytica* as well as *Giardia lamblia* was higher in males than in females though it was not statistically significant. Other studies have also reported the higher incidence of these parasites in males (Shenoy *et al.*, 1998). The reason behind this may be that the males have more outdoor activities as compared to females in rural areas with the result they are more exposed to unhygienic environmental conditions as studied by other workers (Sayyari *et al.*, 2005).

The seasonal variation was observed in present study, the finding that incidence of both *Entamoeba histolytica* and *Giardia lamblia* increased from May to August ($p < 0.05$), which are hot and humid season followed by decrease in winter season. This may be due to reason that diarrhoeal diseases are more in this season due to more contamination of drinking water by various means (Natividad *et al.*, 2008)

In conclusion, in present study we can conclude that parasitic infestation is an important cause of gastro intestinal problems in all ages with *Giardia lamblia* particularly in younger age group and *Entamoeba histolytica* in older age group. Seasonal variations show that there is need of better hygiene and safe drinking water especially in hot and humid season to prevent transmission of these infections.

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