Original Research Article

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

Surinder Kumar¹* and Varsha A. Singh²

¹BPS GMC For Women Khanpur Kalan, Sonepat, India
²MM Institute of Medical Sciences and Research, Mullana, Ambala, Haryana, India

*Corresponding author

**ABSTRACT**

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.

**Keywords**

*Entamoeba histolytica*, *Giardia lamblia*, Prevalence, intestinal parasite cysts, eggs, trophozoites.

**Article Info**

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**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-Macotela *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≤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 (%) | Age group | Total |
|--------------------|--------------------------------|-----------|-------|
|                    |                                | 0-5       | 5-10  | 10-15 | >15  |       |       |
| *Giardia lamblia*  |                                | 18(6.5)   | 10(10.6)| 8(16.6)| 8(3.4)| 44(6.7)|       |
| *Entamoeba histolytica* |                            | 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 | $\chi^2$ | p value |
|------------------|-------------|------------------|---------------|----------|---------|
| *Giardia lamblia*| <15         | 36 (8.61%)       | 418           | 6.665    | <0.01   |
|                  | >15         | 8 (3.36%)        | 238           |          |         |
| *Entamoeba histolytica* | <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 *Entamoeba histolytica* | Total samples | $\chi^2$ | 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 *Giardia lamblia* | Total samples | $\chi^2$ | 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 Entamoeba | Positive for Giardia |
|-------|---------------|------------------------|----------------------|
| 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) |
|------------------|----------|------------------|-----------------------------------------|
| *Giardia lamblia*| 44       | 32               | 12 (27%)                                |
| *Entamoeba histolytica* | 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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