

DETECTION OF *ENTAMOEBIA HISTOLYTICA*, *GIARDIA LAMBLIA* AND *CRYPTOSPOIDIUM SP.* INFECTION AMONG DIARRHEAL PATIENTS

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Abstract: Tri-combo ELISA tests were carried out to determine the prevalence of protozoan parasites (*Entamoeba histolytica*, *Giardia lamblia* and *Cryptosporidium* spp) in 1712 stool samples of diarrhoeal patients. Among the samples tested, 118 (6.89%) were found positive. The age group 6-14 years were recorded to be more susceptible (14.81%) and prevalence gradually decreased as the age group increased. Individual Antigen Detection Tests were done on the positive samples and 1 sample was noted to carry *E. histolytica* (0.06%), 92 (5.37%) samples with *G. lamblia* and 22 (1.29%) samples with *Cryptosporidium* spp. Maximum prevalence was recorded in summer (8.47%) and rainy seasons (8.14%) and highest infections (10.46%) were found in the month of September. The prevalence of *G. lamblia* was higher in male (5.37%) whereas, the prevalence of *Cryptosporidium* spp. was higher in female (1.64%).

Key words: Tri-combo ELISA, *Entamoeba histolytica*, *Giardia lamblia*, *Cryptosporidium sp.*

INTRODUCTION

Diarrheal diseases are extremely common in the developed and developing worlds and are major causes of morbidity and mortality, affecting millions of individuals each year (Guerrant *et al.*, 1990, Mitchell *et al.*, 2008). Diarrhea is a major public health problem worldwide, especially in children. One in ten child deaths result globally from diarrheal disease before their fifth birthday, resulting in about 800 000 fatalities worldwide annually, most occurring in sub-Saharan Africa and south Asia (Islam *et al.*, 1983, Liu, *et al.*, 2010). In Bangladesh, 1 in 30 children die of diarrhea or dysentery by his or her fifth birthday (Haque *et al.* 2003) and one third of the total child death occur is due to diarrhea (Victoria *et al.* 1993). Amoebiasis is an intestinal infection that may or may not be symptomatic. When symptoms are present it is generally known as invasive Amoebiasis. *G. lamblia*, is the most frequently isolated intestinal protozoa in the world (Khanum *et al.* 2009, Mhlanga *et al.*, 1992, Chowdhury *et al.*, 1991).

It is a common cause of diarrhea and morbidity, especially in children. Cryptosporidiosis is a diarrheal disease caused by microscopic parasites of the genus *Cryptosporidium* (Natividad *et al.*, 2008). Once an animal or person is infected, the parasite lives in the intestine and passes through the stool. The parasite is protected by an outer shell (Keusch *et al.*, 1995, Tanyuksel *et al.*, 2001) that allows it to survive outside the body for long periods of time and makes it very resistant to chlorine- based disinfectants (Juraneck, 1995).

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The geographical, ecological and socio-economical variations such as, poverty, illiteracy, lack of knowledge of personal hygiene, improper sanitation, contaminated drinking water are the major factors which are responsible for varied rates of prevalence of amoebiasis, giardiasis and cryptosporidiosis (Warhurst and Green, 1989). Control of this organism can prove difficult due to its resistance to standard disinfection methods (e.g., chlorination of water sources) (Dillingham *et al.*, 2002). Lack of rapid and cost-effective diagnostic tools is a major challenge to the surveillance of disease caused by these three pathogens.

Weinke *et al.*,(1989) reported the prevalence and clinical importance of *Entamoeba histolytica* in two high-risk groups: travelers returning from the tropics and male homosexuals. For a long time, it was known that people who were infected with *E. histolytica* never developed symptoms and spontaneously clear the infection, i.e. those who were shedding *E. histolytica* cysts in their stools did not show symptoms of the disease (Ackers 2002).

Members of the genus *Cryptosporidium* infect epithelial surfaces, especially those along the gut, and can be found in a wide range of vertebrates, including humans. A second species, *C. parvum* infects the small intestine of an unusually wide range of mammals, including humans, and is the zoonotic species responsible for approximately one-half of human cryptosporidiosis (Huang and White, 2006). A relatively newly discovered species, *C. hominis* is responsible for the other one-half of human infections. This species is morphologically identical to *C. parvum*, but is more difficult to study due to lack of animal models (Natividad *et al.* 2008).

Different diagnostic methods are being utilized to detect the intestinal protozoan parasites but there is a need of rapid and cost-effective diagnostic screening methods for a developing country like Bangladesh. The general objective of the study are to determine the prevalence of the common protozoan diarrheal parasites (*E. histolytica*, *G. lamblia*, and *Cryptosporidium* spp.) in the hospital samples of diarrheal patients of icddr,b and to develop a simple low cost technique for identifying these parasites.

MATERIAL AND METHODS

This study was conducted in the Parasitology laboratory, of International Centre for Diarrheal Disease Research, Bangladesh (icddr,b) at Dhaka. Stoolsamples from 1712 diarrhoeal patients were collected and the study was conducted from November 2011 to October 2012.

Tri-combo ELISA: The Tri-combo ELISA is an enzyme immunoassay for the qualitative detection of *Giardia lamblia*, *Cryptosporidium* spp. and *Entamoeba histolytica* antigen in human fecal specimens. The test is indicated for use as a screen for fecal specimens from patients with diarrhea or dysentery to aid in the diagnosis of gastro intestinal infection resulting from giardiasis,

cryptosporidiosis and amebiasis. The Tri-combo ELISA test serves as a screen to rule out negative specimens and reduce the number of specimens that require additional follow-up testing.

Principle: The Tri-combo ELISA test uses monoclonal and polyclonal antibodies to cell- surface antigens of *Giardia lamblia*, *Cryptosporidium* spp. and *Entamoeba histolytica*. The micro assay plate in the kit contains immobilized monoclonal antibodies against the antigens and the Conjugates consist of polyclonal against the antigens .In the assay, an aliquot of a diluted fecal specimen is transferred to a micro assay well. The immobilized monoclonal antibodies bind the *Giardia*, *Cryptosporidium* and *Entamoeba histolytica* antigens if they are present. Upon addition of Conjugate, it binds to the antigen-antibody complex. The cut-off value of these ELISA was ≥ 0.150 at 450 nm wavelengths and ≥ 0.090 at 450/630 nm wavelengths.

A positive test result in the Tri-combo ELISA test indicates that the person is shedding detectable amount of *Giardia*, *Cryptosporidium* or *E. histolytica* antigen. The incidence of *Giardia*, *Cryptosporidium* or *E. histolytica* infection varies significantly between population and geographic regions.

RESULTS AND DISCUSSION

A total of 1712 stool samples were examined and among them 118 were found positive for three protozoan parasites (*E. histolytica*, *G. lamblia*, *Cryptosporidium* spp.). And among the parasites, the percentages of infection for *E. histolytica*, *G. lamblia* and *Cryptosporidium* spp. were 0.88, 80.7 and 9.29 respectively. The Prevalence of the parasites was 6.89%. Where 74 were male and 44 were female patients. The difference between sexes was statistically non significant, because $p > 0.05$ ($p = 0.68$). Klein in 2000 also stated that males are more susceptible to these parasites. It can be concluded that males become more infected compared to females due to their food habit, which are usually taken outside the home and become affected by water born diseases.

The patients were from 0 to above 40 years old. At the age of 0-5 years the prevalence was 6.83%, in case of 6-14 years the prevalence was 14.81%, in case of 15-40 years the prevalence was 7.25% and in case of 40 above years the prevalence was 3.69% (Table 01). The difference between age groups was statistically significant, because $p < 0.05$. ($p = 0.008$)

The differences in prevalence of infestation were noticeable between different months of the study period (November 2011 to October 2012). The lowest prevalence of infection found in the month of January (0.71%) and the highest prevalence found in the month of September (10.46) (Table 02).

Table 1. Tri-combo ELISA screening of protozoan parasites in the stool samples of male and female diarrheal patients of different age-groups

Age groups (year)	Male			Female		
	No. of tested samples	Positive samples	Prevalence (%)	No. of tested samples	Positive samples	Prevalence (%)
0-5	530	37	6.98	310	21	6.58
6-14	51	7	13.73	30	5	16.67
15-40	318	23	7.23	220	16	7.27
40+	144	7	4.86	100	2	2
Total	1043	74	7.09	669	44	6.57

The prevalence of the protozoan parasites was higher in summer (8.47%) and in rainy season (8.14%) and lowest in winter (3.12%). It was also observed that, in all the seasons the prevalence was higher in male than female (Table 03).

Table 2: Tri-combo screening of the stool samples from diarrheal patients (both sexes) for the identification of protozoan parasites

Sampling months	Sex of patients Male			Sex of patients Female		
	No. of patients	Positive cases	Prevalence (%)	No. of patients	positive cases	Prevalence (%)
November,2011	70	3	4.29	49	2	4.08
December, 2011	78	3	3.58	41	1	2.44
January, 2012	89	0	0	61	1	1.64
February, 2012	56	3	5.36	47	2	4.26
March,12	141	13	9.22	84	2	2.38
April,12	146	10	6.85	124	15	12.9
May,12	132	13	9.85	75	7	9.33
June,12	87	8	9.19	49	3	6.12
July,12	80	4	5	45	3	6.66
August,12	51	4	7.84	25	2	8
September,12	58	5	8.62	28	4	14.29
October,12	65	8	12.31	41	2	4.88
Total	1043	74	7.09	669	44	6.57

Table 3: Seasonal variation in the prevalence of *Entamoeba histolytica*, *Giardia lamblia*, *Cryptosporidium* spp. in diarrheal patients

Rainy season (July-October)			Winter (November-February)			Summer (March-June)		
Total number of patients	Number of infected patients	Prevalence (%)	Total number of patients	Number of infected patients	Prevalence (%)	Total number of patients	Number of infected patients	Prevalence (%)
254	21	8.27%	283	9	3.18%	506	44	8.69%
139	11	7.91%	198	6	3.03%	332	27	8.13%
393	32	8.14%	481	15	3.12%	838	71	8.47%

The difference between different seasons were statistically significant, because $p < 0.05$. ($p = 0.001$)

According to Haque *et al.* (2012), out of 618 samples from three international sites of the world, 169 samples were positive by Tri-combo ELISA and most of the samples were positive for *Giardia* spp.

Similar results have been reported by Sharma *et al.* (2004) and Hamze *et al.* (2004) that prevalence of *E. histolytica* for females was higher than males. According to Khanum *et al.* (1998) the prevalence of *G. lamblia* in Bangladesh ranges from 4-21%. Khanum *et al.* (2000) gave a corollary that sex is not related to *G. lamblia* infection, which is actually goes with the present observation. Sayyari *et al.*, (2005), observed that *E. histolytica* infection was more prevalent in younger age groups. The more susceptible age group was 6-14 years old and as the age progressed, the prevalence kept falling. According to Hossain *et al.*, (1983), the prevalence of giardiasis was higher among the children between the ages of 5-15 years. Hossain *et al.*, (1983) also suggested that the prevalence of Giardiasis declined with the increasing age, which supports the finding of the present study. At that age children are school going and they usually take food and water from outside which is the main cause of being affected by these diarrhea causing agent. Nevine *et al.*, (2012) reported that *Cryptosporidium* infection is one of the important causes of diarrhea in children below 5 years of age. Walker *et al.*, (2012) Estimated high rate of diarrhea mortality among young children in low and middle income countries

Tri-combo ELISA offers a simple, highly sensitive and specific method of screening fecal specimens to identify protozoan parasites, parasites, eliminating the need for expensive microscopy methods.

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