

PREVALENCE OF DIFFERENT PROTOZOAN PARASITES IN PATIENTS VISITING AT ICDDR'B HOSPITAL, DHAKA

SHAHELA ALAM, HAMIDA KHANUM¹, RIMI FARHANA ZAMAN
AND RASHIDUL HAQUE²

Department of Zoology, University of Dhaka, Dhaka-1000, Bangladesh
*²ICDDR'B(International Centre for Diarrhoeal Disease Research,
Bangladesh) Mohakhali, Dhaka*

Abstract

Gastrointestinal protozoan parasites are important cause of diarrhoeal illness in Bangladesh. The present study was conducted to observe the prevalence of different human gastrointestinal parasites in patients visiting a Hospital in Dhaka. Several classical techniques were employed to diagnose the causal agents which include *E. histolytica*, *G. intestinalis*, *Cryptosporidium sp.* and *A. lumbricoides* etc. A total of 540 samples from outdoor patients of ICDDR'B Hospital at Dhaka was examined where different protozoan parasites including *E. histolytica* (1.11%), *G. intestinalis* (0.37%), and *Cryptosporidium sp.* (4.44%) were detected. Several diagnostic tools were employed that include ELISA, acid fast staining and trichrome staining techniques. Age and sex-specific susceptibility and seasonal incidence pattern were also assessed.

Key words: ICDDR'B, Hospital, Gastro intestinal parasites, Outdoor patients

Introduction

Diarrhoeal diseases are one of the leading causes of mortality and morbidity worldwide including Bangladesh. Dhaka is the capital of the country that has highly densed population. There are many slums with poor sanitation and lack of access to drinking water making the slum-dwellers more vulnerable to different water-borne diarrhoeal illnesses. The elderly people and children are the most susceptible group as thought to have reduced immunity to different pathogens causing diarrhoea.

Since long time, diarrhoeal diseases were considered as a leading public health problem, particularly in children in Bangladesh (Lima and Guirrant 1992). Early studies in rural Bangladesh also indicated persistent diarrhoea in children as a concern for public health (Huttly *et al.* 1989). A separate recent study also indicated that *E. histolytica*, *C. hominis*, *C. parvum* and *G. lamblia* assemblage A infections are important causes of diarrhoeal illness in Bangladesh population. The prospective case-control study was performed which involved a total of 3,646 case patients and 2,575 control subjects with asymptomatic infection (Haque *et al.* 2009). Cryptosporidiosis has long been considered as an important pathogen causing diarrhoea in Bangladesh (Shahid *et al.* 1987). The very

¹ Corresponding author: Email: hamida_khanum@yahoo.com

first report of cryptosporidiosis in Bangladesh indicates possible zoonotic transmission as reported from calves, animal handlers and associated family members at a dairy farm in Savar (near the capital city, Dhaka) (Rahman *et al.* 1985). A prospective study on the urban slum in capital city, Dhaka reported that malnutrition significantly increases the risk of cryptosporidiosis along with some enteropathogen (Mondal *et al.* 2009). In another study, association of enteric protozoan-associated diarrhoeal illness with that of the nutritional status and growth of preschool children in Bangladesh was investigated. (Mondal *et al.* 2006). Several other investigators have reported different aspects of prevalence of *Cryptosporidium* and their harmful effects in the country (Haque *et al.* 2007, Stroup *et al.* 2006 and Khan *et al.* 2004).

Stool examination for intestinal protozoan parasites and helminths is one of the most frequently performed examinations in parasitological laboratories. Most of protozoan parasites usually excrete through stool in both cyst and vegetative stages. The present study was undertaken to find out the prevalence of protozoan parasites of gastrointestinal tract in patients with diarrhoeal illness at a Dhaka hospital while optimising suitable methods for their identification to aid disease diagnosis.

Materials and Methods

The present study was carried out during May 2006 to April 2007 and several classical laboratory techniques were used to detect the protozoan cysts or trophozoites. The stool samples were collected from patients visiting the ICDDR, Dhaka Hospital with complains of diarrhoeal illness. Patient visiting the clinic were supplied with clean sterilized vials for stool collection and all relevant data were recorded. Upon submission, the stool samples were carried to the Parasitology Laboratory, Laboratory Sciences Division (LSD) at the International Centre for Diarrhoeal Disease Research, Bangladesh (ICDDR) Dhaka for microscopic examination. Stool samples were preserved in 10% formal saline and also stored specimens frozen (-20°C) which could not be performed within 72 hours of collection. The stool samples were examined for trophozooid, cysts of protozoan parasites using microscopy and additional diagnostic procedures were followed.

For direct smear analysis, a minute portion of the stool was diluted with normal saline (0.9%) on a slide and later covered with a cover slip. Later the smear was examined under a binocular microscope in different objectives (X10 and X40). As a complementary approach, several classical staining techniques were used during this study. These include trichrome stain and modified acid-fast stain as described elsewhere. In addition ELISA tests were also performed during this study.

Results and Discussion

One of the aims of the present study was determining the prevalence of protozoan cysts or trophozoites from gastrointestinal tract such as *Entamoeba histolytica*, *Giardia*

intestinalis, *Cryptosporidium* sp. among the hospital samples. A total of 540 stool samples was examined and analysed to understand the overall prevalence of different protozoa. The findings revealed that *Cryptosporidium* sp. was prevalent in most stool specimens (4.44%) compared to *E. histolytica* (1.11%) and *G. intestinalis* (0.37%) as shown in Fig. 1.

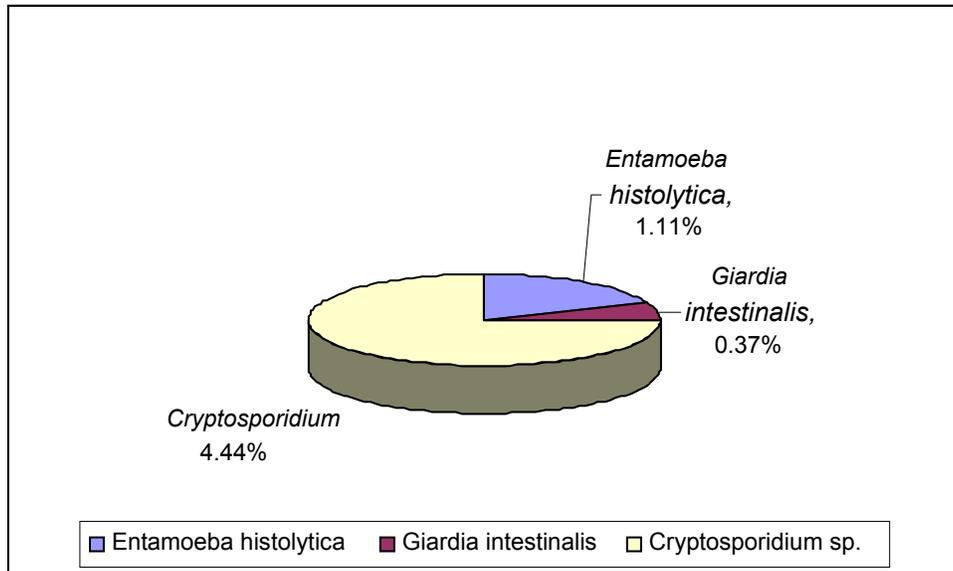


Fig. 1. Percent prevalence of gastrointestinal parasites among patients of ICDDR B hospital.

The sex-specific susceptibility was also assessed during this study which showed that male patients were preferred victim compared to their female counterparts (data not shown). However the age-specific data analysis showed that 21–25 years age group was more vulnerable to *E. histolytica* infection and >35 years age group was more prone to suffer from giardiasis (Figs. 2 and 3). A different scenario was found in cryptosporidiosis, which was found in maximum number among patients of age group 26-30 years and lowest in patients of 11-15 years age group (Fig. 4).

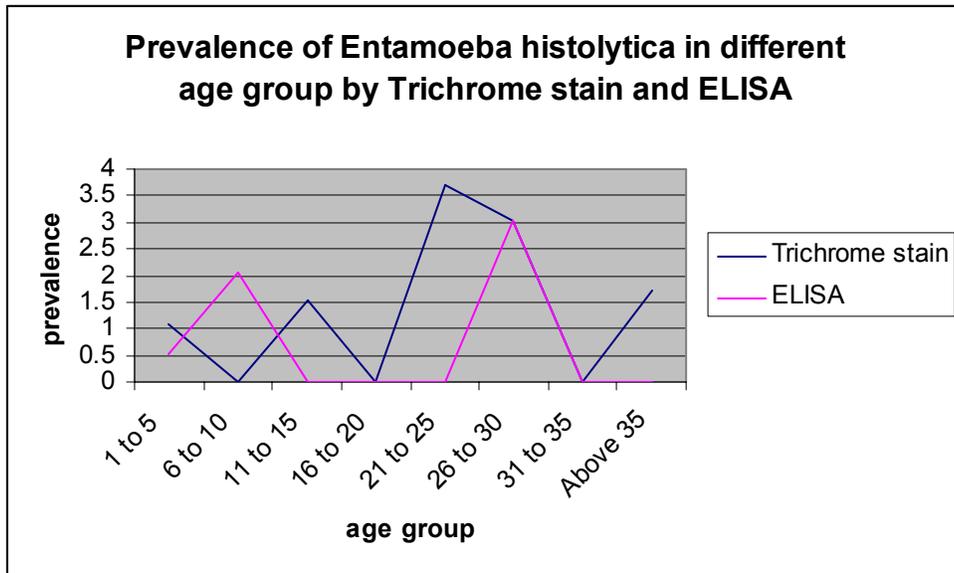


Fig. 2. Prevalence of *E. histolytica* according to different age groups.

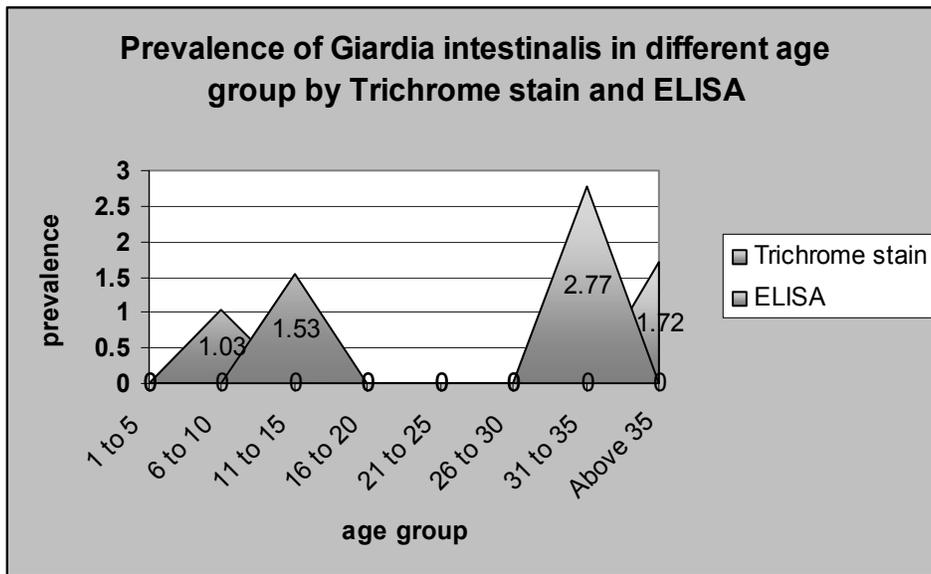


Fig. 3. Prevalence of *G. intestinalis* according to different age groups.

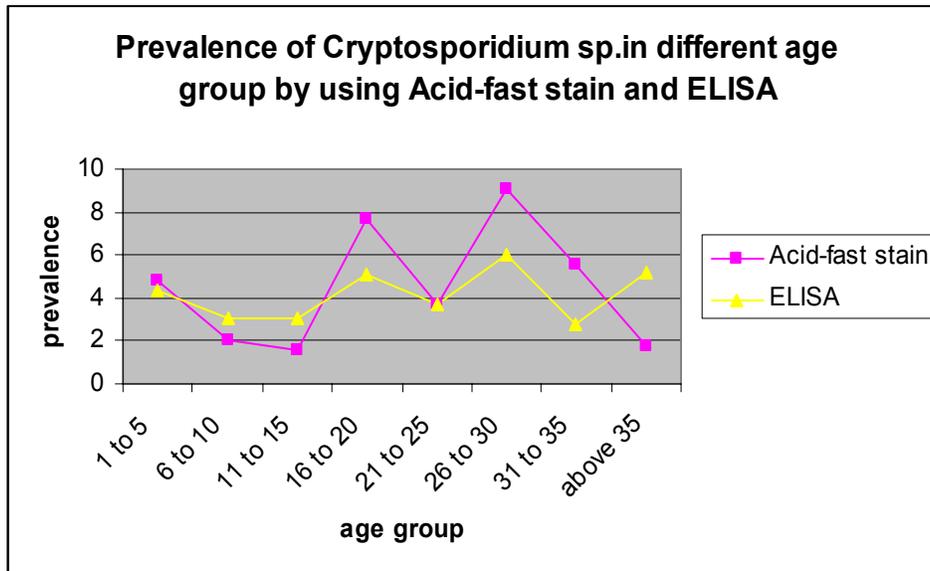


Fig. 4. Prevalence of *Cryptosporidium* sp. according to different age groups.

During the study, seasonal variations in prevalence of protozoan parasites among hospital patients were also investigated. The case records were used to organize the positive cases according to the month of the year. The analysis revealed that June was the most important month of the year when maximum number (11.1%) of cases were found or reported. On the contrary, lowest (2.04%) number of cases were recorded in January which is usually winter season in Bangladesh (Fig.5).

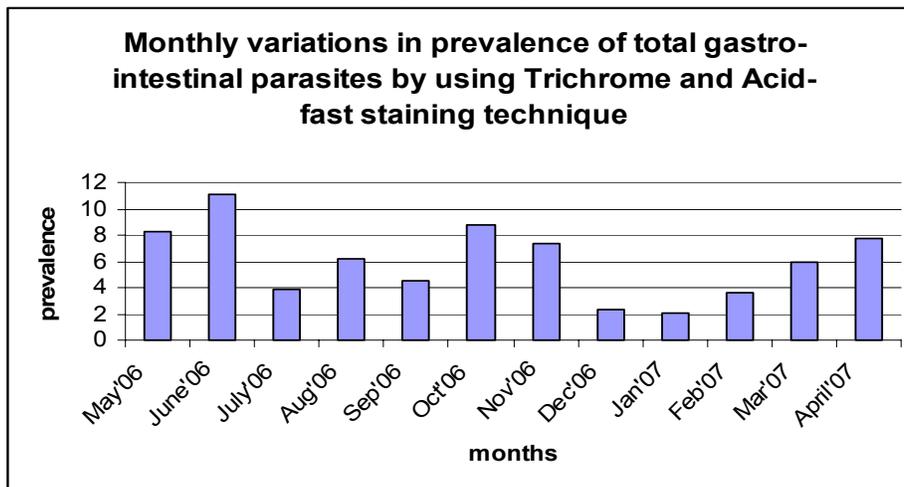


Fig. 5. Monthly variations in prevalence of total gastrointestinal parasites.

Protozoa are among the most important pathogens that can cause infections in immuno-compromised hosts. Over recent decades, parasitic protozoa have been recognized as having great potential to cause waterborne and food borne disease. *Entamoeba histolytica*, *G. intestinalis* and *Cryptosporidium* spp. are not only three of the most important and common diarrhoea-causing parasitic protozoa, but they often have similar clinical presentations.

Comparatively few studies have been directed to investigate the prevalence and incidence of gastrointestinal protozoan infection in human in Bangladesh. Some of the recent reports have indicated cryptosporidiosis most important among different diarrhoea causing organisms. Surveillance study (over three years of time) from the same hospital recorded 3.5% (n=1949) cases contributed by the same pathogen (Bhattacharya *et al.* 1997). Another year-long study from the same hospital identified 3% (n=1382) incidence of *Cryptosporidium* oocysts in the diarrhoeal stool samples (Rahman *et al.* 1990). During this present study we have recorded 4.44% (n=540) cases of cryptosporidiosis which is somewhat similar with previous reports. Another important observation is the high incidence of positive cases during summer which could be due to increased drinking water intake by the people giving rise to more water-borne outbreaks. Likewise during winter season, the incidence of diarrhoeal illness is reduced and we have recorded least number of cases in winter during this study.

Age and sex-specific vulnerability is an important issue for prevalence of diarrhoeal illness and various factors can contribute to this fact. They may include different biochemical factors like hormones, enzymes and specific proteins or other genetic or immunologic factors along with food habit, culture etc. Further study can highlight these different attributes which can increase our understanding of the transmission of these protozoan pathogens and their further control strategies.

In conclusion, diagnosis of protozoan parasitic cases heavily rely on suitable approach and use of modern molecular tools like PCR, Real time PCR can be more sensitive for accurate identification of pathogens causing diarrhoea in any clinical setting. During present study we have found ELISA approach more sensitive (data not shown) and further optimization of classical staining techniques can be an added advantage to diagnose protozoan parasites efficiently.

References

- Bhattacharya M. K., T. Teka, A. S. Faruque and G. J. Fuchs 1997. "*Cryptosporidium* infection in children in urban Bangladesh." *J Trop Pediatr.* **43**(5) : 282-286.
- Haque R, S. Roy, A. Siddique, U. Mondal, S. M. Rahman, D. Mondal, E. Houpt and W. A. Petri Jr. 2007. Multiplex real-time PCR assay for detection of *Entamoeba histolytica*, *Giardia intestinalis*, and *Cryptosporidium* spp. *Am. J. Trop. Med. Hyg.* **76**(4) : 713-717.
- Haque R., D. Mondal, A. Karim, I. H. Molla, A. Rahim, A. S. Faruque, N. Ahmad, B. D. Kirkpatrick, E. Houpt, C. Snider and W. A. Petri Jr. 2009. Prospective case-control study

- of the association between common enteric protozoal parasites and diarrhoea in Bangladesh. *Clin. Infect. Dis.* **48**(9) : 1191-1197.
- Huttly S. R., B. A. Hoque, K. M. Aziz, K. Z. Hasan, M. Y. Patwary, M. M. Rahaman and R. G. Feachem. 1989. Persistent diarrhoea in a rural area of Bangladesh: a community-based longitudinal study. *Int. J. Epidemiol.* **18** : 964-969.
- Khan, W. A., K. A. Rogers, M. M. Karim, S. Ahmed, P. L. Hibberd, S. B. Calderwood, E. T. Ryan and H. D. Ward. 2004. Cryptosporidiosis among Bangladeshi children with diarrhoea: a prospective, matched, case-control study of clinical features, epidemiology and systemic antibody responses. *Am. J. Trop. Med. Hyg.* **71**(4) : 412-419.
- Lima, A. A. and R. L. Guerrant. 1992. Persistent diarrhoea in children: epidemiology, risk factors, pathophysiology, nutritional impact, and management. *Epidemiol. Rev.* **14** : 222-242.
- Mondal, D., W. A. Petri Jr., R. B. Sack, B. D. Kirkpatrick, R. Haque. 2006. *Entamoeba histolytica*-associated diarrhoeal illness is negatively associated with the growth of preschool children: evidence from a prospective study. *Trans. R. Soc. Trop. Med. Hyg.* **100**(11) : 1032-1038.
- Mondal D., R. Haque, R. B. Sack, B. D. Kirkpatrick and W. A. Petri Jr. 2009. Attribution of malnutrition to cause-specific diarrhoeal illness: evidence from a prospective study of preschool children in Mirpur, Dhaka, Bangladesh. *Am J Trop Med Hyg.* **80**(5): 824-826.
- Rahman A. S., S. C. Sanyal, K. A. Al-Mahmud and A. Sobhan. 1985. *Cryptosporidium* diarrhoea in calves & their handlers in Bangladesh. *Indian. J. Med. Res.* **82** : 510-516.
- Rahman M., N. S. Shahid, H. Rahman, D. A. Sack, N. Rahman and S. Hossain. 1990. Cryptosporidiosis: a cause of diarrhoea in Bangladesh. *Am. J. Trop. Med. Hyg.* **42**(2) : 127-130.
- Shahid N.S., A. S. Rahman and S. C. Sanyal. 1987. *Cryptosporidium* as a pathogen for diarrhoea in Bangladesh. *Trop. Geogr. Med.* **39**(3) : 265-270.
- Stroup S.E., S. Roy, J. Mchele, V. Maro, S. Ntabaguzi, A. Siddique, G. Kang, R. L. Guerrant, B. D. Kirkpatrick, R. Fayer, J. Herbein, H. Ward, R. Haque and E. R. Houpt. 2006. Real-time PCR detection and speciation of *Cryptosporidium* infection using Scorpion probes. *J. Med. Microbiol.* **55** (Pt 9) : 1217-1222.

(Received revised manuscript on 22 May 2013)