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COMPARISON OF DIRECT MICROSCOPY AND *IN VITRO* CULTURE FOR THE DETECTION OF *BLASTOCYSTIS HOMINIS* AMONG THE SLUM CHILDREN OF DHAKA CITY

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Abstract

Multiple stool samples were collected from 2980 children of Mirpur slum area. The direct microscopy and xenic *in vitro* culture (XIVC) for 48 hours were done for each of the sample. The prevalence of *Blastocystis hominis* was found 5.1% in direct microscopy and the highest prevalence was observed in May, 2013 among the children of 9 - 11 years. In culture method, 10.93% children were found positive for the organism. Most affected age group was 9 - 11 years and prevalence was highest in August. In culture, morphological cyst forms were most commonly observed. Data analysis showed that there was a significant association between age group and infection ratio. By comparing the direct microscopy with *in vitro* culture, it was seen that culture was the most sensitive and reliable diagnostic method for the identification of *B. hominis.*

Introduction

Blastocystis hominis is one of the most commonly observed intestinal parasites in human. It is an anaerobic protist that inhabits the gastrointestinal tract of humans and many groups of animals(1). It is a single-celled enteric protozoan that has a world-wide distribution. *Blastocystis hominis* is the most common parasite isolated from human stool samples in developing and developed countries(2). Prevalence is low in countries such as Japan (0.5 to 1%)(3) and Singapore (3.3%)(4) and high in developing nations including Argentina (27.2%)(5), Brazil (40.9%), Cuba (38.5%) and Egypt (33.3%)(6) and Indonesia (60%)(7). Certain subtypes of *Blastocystis* may cause symptomatic infection, or may pose a risk only when combined with other types of infection. In some cases, *Blastocystis* simply resides in the digestive tract without causing harm. For proper study of prevalence, epidemiology and pathogenic potentiality of *Blastocystis*, a reliable and practical diagnosis is of paramount importance. Techniques currently in use for the diagnosis include microscopy, molecular detection, and xenic *in vitro* culture(8).

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Direct microscopy is usually done with stained specimens. Stool culture may be the most sensitive method available for *Blastocystis*[9]. The xenic or monoxenic laboratory cultures of *Blastocystis* isolates, which are the cultures of *Blastocystis* cells grown in association with non-standardized or single known species of microorganisms, respectively, can be maintained by following Jones[10] or Boeck and Drbohlav's inspissated egg[11] medium. The axenic cultures of *Blastocystis* isolates are important for molecular and biochemical studies. Axenization can be achieved by the addition of antibiotic cocktails to eliminate contaminating bacteria and yeasts, and a variety of antibiotic mixtures have been described, with various levels of success[12]. In the present paper two methods - direct microscopy and *in vitro* culture were used in the detection of *Blastocystis hominis* among the slum children of Dhaka city.

Materials and Methods

The study site is located in a slum of 14.22 sq.km at Bauniabadh area of Mirpur, Dhaka. The slum has a population of about half a million in an area of 14.22 km. About 20% of the inhabitants of the slum have a monthly income of only US$ 62, about 30% of mothers never attended school, and only 3% obtained secondary school education. Most of the people are day laborers, garment workers, and transport workers. About 72% mothers always wash their hands with soap after helping the child defecate and 6.6% never wash their hands with soap. The diarrheal infection rate for Mirpur is 4.69 episodes per child per year[13]. The study site is a representative of a typical urban slum of Dhaka city in terms of demographic collection of samples.

The multiple stool samples of the patients with irritable bowel syndrome (IBS) and inflammatory bowel disease (IBD) were collected from the slum area from January to December, 2013. All the laboratory works were done in the Parasitology laboratory of ICDDR,B, Dhaka. A total of 2980 stool samples consisting of 1334 males and 1646 female children aged between zero and 12 years, was collected. The stool samples were collected in a clean, leak proof, transparent container. No antiseptic was used. The stool samples were not contaminated with urine. Microscopic examination was done under an Olympus light microscope (model number BX 41). The liquid stool samples were taken on a slide with the aid of a pipette and examined under the microscope; in case of solid stool, the sample were diluted into normal saline and taken on a slide and examined under the microscope. The microscopic examination was performed with unpreserved specimens. A drop of mixture of feces was taken on a slide and covered by a cover slip to examine parasite under the microscope.

For xenic culture approximately 10 mg rice starch, 0.12 ml erythromycin solution and sufficient Bleacher Report Medium (BRM) were taken into a bottle containing a sterile agar slope. Nearly 50 mg of feces were discarded leaving only starch and feces. Overlay was replaced with sufficient BRM, diluted at the ratio 1 : 4 with the phthalate

solution to cover slope. Erythromycin solution (0.06 ml), bacto-peptone solution and additional starch were added to the phthalate solution. After the incubation of 24 hours, a drop of the mixture of feces and starch was removed from the bottom of the slope, placed on a slide and examined under an optical microscope (Olympus BX 41) for the presence of parasites.

Results and Discussion

The presence of *Blastocystis* was detected in 152 (5.1%) out of 2980 stool samples (1334 males and 1646 females) (Table 1). On analysis, 6.07% male and 4.93 % female were found positive (Table 1). On the other hand, the prevalence of the parasite was 10.93%, when these samples were cultured *in vitro* (Table 1). All of the samples were collected from the asymptomatic children who were either *B. hominis* positive or negative but did not show any symptoms of diarrhea.

Table 1. Prevalence of *Blastocystis hominis* positive samples detected in microscopy and culture method (n = 2980).

	Microscopically positive			Culture positive		
	Total no. of samples	Single occurrence of parasites	Multiple occurrence of parasites	Total no. of samples	Single occurrence of parasites	Multiple occurrence of parasites
No. of samples	152	152	0	328	130	198
Percentage	5.1	5.1	0	10.93	4.3	6.6

Most of the infected males represented the 9 - 12 years age group and in the female, the highest prevalence of the pathogen was observed in 6 - 8 age group in the microscopy study (Fig. 1). The result was more or less similar to the culture method in which 9 - 12 years male age group was the most prevalent group for *B. hominis* infection (Table 2).

Regarding the month wise distribution of the parasite, the highest prevalence was found in May (11. 49%) and the lowest in October (2.83%) when microscopy was done. On the contrary, 27.32% prevalence was observed in August which was the highest prevalence of the parasite (Fig. 2).

Morphological forms of *Blastocystis hominis* observed in culture study: In the total samples of 2980, 328 were found positive for *B. hominis* in culture study. Cyst forms were found in 227 samples. Other morphological forms, such as amoeboid and granular were 2.91 and 0.3%, respectively. Vacuolar forms were found rare (0.1%) (Table 3).

Positive for both cyst and granular form were 32%, whereas 12% for both cyst and amoeboid forms and 7% for both amoeboid and granular forms. Only four samples were positive for all the morphological forms (Fig. 3).

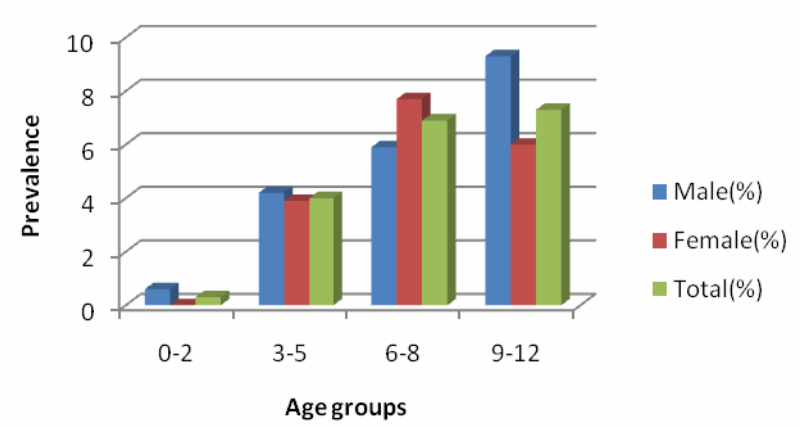

Fig. 1 . Prevalence of *Blastocystis hominis* in different sexes and age groups in microscopy.

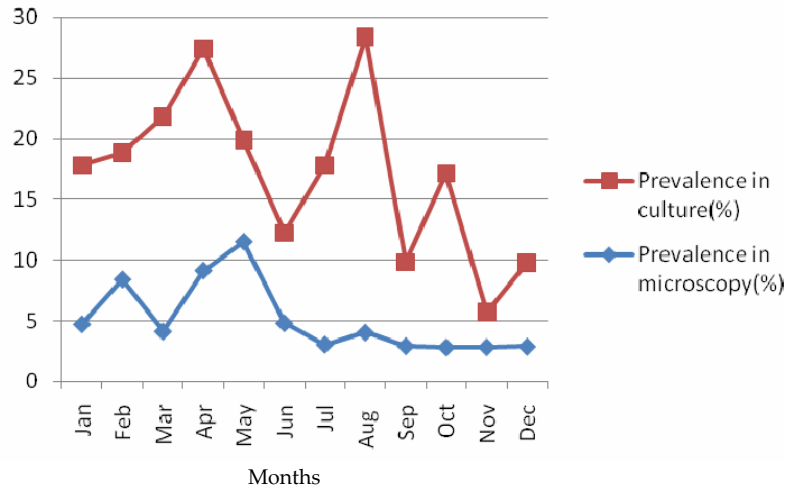

Fig. 2. Monthwise prevalence of *B. hominis* infectoin in the year 2013.

Different types of protozoan organisms were observed in the stool samples by the microscopy test along with the 152 (5.1%) samples of *B. hominis*. Using permanent stain the following parasites were observed: in the samples 90 (3%) children had infection with *E. histolytica,* 31 (1%) with *I. butschli,* 2 (0.06%) *Trichomonas*, 184 (6.17%) with *Giardia* sp., 52 (1.74%) with *E. coli*, 225 (7.5%), with *A. lumbricoides* and 92 (3.1%) with *T. trichiura* and 1 (0.00%) with *H. nana,* respectively (Table 4).

Zierdt[14] examined the stool specimens of irritable bowel syndrome (IBS) patients for *B. hominis* and found 23% prevalence of the parasite by direct microscopy and 32% prevalence by culture method which he suggested that stool culture was more sensitive than microscopy. Similar observational trend i.e. high prevalence in the culture method

than the microscopic method was observed in the present study in which the prevalence of *B. hominis* was 5.1%, by direct microscopy, whereas by culture study the prevalence was 10.93% (Table 1).

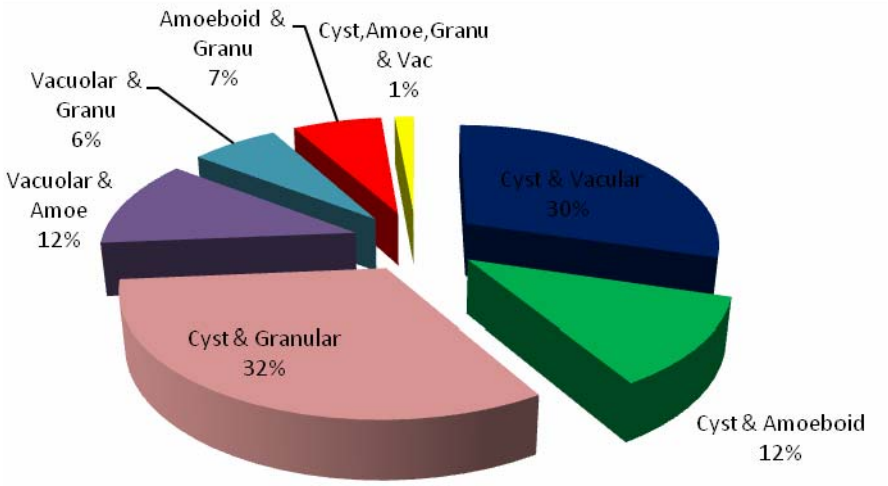

Fig. 3. Percentage of morphological forms observed in culture method.

Table 2. Perevalence of *Blastocystis hominis in vitro* culture of the stool of different age groups and sex of the children of a slum area of Dhaka city from January to December, 2013.

Age (year)	Male			Female			Total		
	No. of examined samples	Cul (+)	Preva-lence (%)	No. of examined samples	Cul(+)	Preva-lence (%)	No. of examined samples	Cul (+)	Preva-lence (%)
0-2	166	7	4.21	216	8	3.7	382	15	3.92
3-5	528	52	9.84	537	64	11.91	1065	116	10.92
6-8	393	60	15.26	492	47	9.55	885	107	12.09
9-12	247	62	25.01	401	28	6.98	648	90	13.88
Total	1334	181	13.56	1646	147	8.93	2980	328	11

Cul (+) = Culture positive.

Cook *et al*.[15] investigated in the Palajunoj Valley of Guatemala where 5,705 viable stool samples were screened for infection with gastrointestinal parasites; they reported that the prevalence of infection for specific parasites were: *A. lumbricoides* - *17.7%, E. histolytica* - 16.1% and *B. hominis* - 2.8%. In the present microscopy study the prevalence of *A. lumbricoides* was *7.5%, E. histolytica-3%* and, *B. hominis*, 5.1% (Tables 1 and 4). In another study conducted in Spain in 1992, the infections of *B. hominis* were found the highest between 10 and 14 years of aged children. In the present study, 6 - 8 years age

group was more prevalent group for *B. hominis* by using both the microscopy and the culture method.

Table 3. Prevalence of *Blastocystis hominis* morphological forms found *in vitro* culture.

Total sample tested	Culture positive sample	Cyst form	Amoeboid form	Granular form	Vacuolar form
2980	328	227	3	9	87
		7.68%	0.1%	0.3%	2.91%

Table 4. Parasites found in conjunction with *Blastocystis* by using permanent stain (n = 2980).

Different parasites	No. of infected sample	Prevalence (%) (n = 2980)
Entamoeba histolytica	90	3.0
I. butschli	31	1.0
Trichomonas sp.	2	0.06
Giardia sp.	184	6.17
E. coli	52	1.74
A. lumbricoides	225	7.5
T. trichiura	92	3.1
H. nana	4	0.001

Most commonly found morphological forms of *B. hominis* was cyst form. Among 2980 samples, cyst forms were found in 227 samples (7.68%) (Table 3). Stenzvold *et al.*[8] investigated fecal samples by using the native - Lugol - trichorome and Kinyoun acid-fast staining method after sedimentation in fecal concentration tubes[16]. One or more parasites were detected in 1510 (8.50%) of the patients. *B. hominis* was the most frequently seen parasite. Distribution of various morphological forms were much like that of vacuolar forms - 67.49%, granular forms- 14.78%, both vacuolar and granular forms- 17.73%. It is contradictory with the present study where 2.91% vacuolar forms and 0.1% granular forms were reported. Both vacuolar and granular forms were 0.06% among the total samples (Table 3).

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