Original article

Significance of *Blastocystis hominis* in patients referred for bacteriological stool culture at EHNRI

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Abstract

Background: The pathogenic potential of the parasite *Blastocystis hominis* is often considered controversial. However, it is now gaining acceptance as a human intestinal parasitic agent showing different clinical symptoms.

Objective: To determine the prevalence and related clinical manifestation of *B. hominis* infection in patients referred for bacteriological stool culture at the Ethiopian Health & Nutrition Research Institute (EHNRI).

Methods: A total of 152 patients referred for bacteriological stool culture to the bacteriology and parasitology labs at EHNRI, were examined for possible infection with *B. hominis*. A single stool sample from each individual was collected and processed for isolation of bacteria by using a standard culture method for enteric bacteria, while direct and formol-ether concentration methods were used for the detection of ova and parasites; and the Modified Ziehl Neelsen method was applied for *Cryptosporidium parvum* and *Isospora belli* and water-ether sedimentation with Uvitex-2B staining method was used for detecting intestinal microsporidia. Clinical information was recorded during stool sample collection.

Results: One *Salmonella* spp., two *Shigella* spp. and one case of *Escherichia coli* were isolated. *Blastocystis hominis* was detected in 71(46.7%) of the 152 patients examined and 51/71(71.8%) of the patients were found to have been infected with *B. hominis* alone. Well known opportunistic intestinal parasites - *Cryptosporidium parvum* 11(7.2%), *Isospora belli* 13 (8.6%) and *Enterocytozoon bieneusi* 2 (1.3 %) - were also recorded. Among the helminths, *Strongyloides stercoralis* 5 (3.3 %) was identified to be the most prevalent. The most common clinical symptoms significantly associated with *B. hominis* were distension, flatulence and anorexia ($P<0.05$). Among the positive cases, four staff members (three males aged 38, 40 and 45 years old and one female aged 42 years old) who were infected with *B. hominis* alone were treated with metronidazole 250 mg, 2 tablets three times a day for 10 days, and responded favourably and all clinical symptoms resolved.

Conclusions: This information is expected to strengthen the newly emerging perception on the pathogenic potential of *B. hominis* infection. It will also create an awareness of laboratory technicians and physicians for proper diagnosis and management of the disease. From this and other related studies conducted elsewhere, it could be concluded that treating B. *hominis* infections where defined symptoms are presented with large numbers of parasites in the stool and in the absence of other cases of the disease is recommendable. [*Ethiop.J.Health Dev.* 2006;21(1):61-67]

Introduction

Until recently, *Blastocystis hominis* has been described as a non-pathogenic human intestinal protozoan parasite with a worldwide distribution. However, controversy surrounds its pathogenic potential (1,2). Different surveys have pointed out that the infection rate can vary from 1.6% in industrialised countries to more than 50% in developing countries (2,3). *Blastocystis hominis* infection is more common in developing countries in the tropical and subtropical regions (4,5).

B. hominis, formerly considered as a yeast, has recently been reported to have protozoan characteristics supporting its classification as an intestinal protozoan parasite (6,7,8). Different diarrhoeal illnesses and other clinical symptoms have been attributed to *B. hominis* infection in subtropical countries (1,2,4,5). Some investigators have reported the problem of *B. hominis* infection in symptomatic and asymtomatic cases (9,10).

The problem of *B. hominis* infection in AIDS patients has been reported (11, 12, 13, 14, 15). Garavelli *et.al*., (13) indicated that patients with full-blown AIDS and who test positive for *Blastocystis hominis* presented with diarrhoea, nausea, flatulence, tenesmus, itching, abdominal pain and peripheral blood eosinophilia. However, unlike other newly emerging opportunistic intestinal parasites (such as *Cryptosporidium parvum, Isospora belli, Enterocytozoon bieneusi* and *Encephalitozoon intestinalis)*, *B. hominis* has not been studied well and recognised as an important opportunistic pathogen in immunocompromised and HIV/AIDS patients. The prevalence of this parasite in developing countries is very high, yet there is no adequate information about its pathogenic potential. According to previous studies done in Ethiopia, Fisseha *et.al* (16), for example, identified a single case of *B. hominis* out of 143 HIV/AIDS patients and Hailemariam *et.al*. (17) detected 5 cases among 234 HIV infected individuals. In addition, *B. hominis* parasite was detected from baboons and vervet monkeys from the Rift Valley area of Ethiopia, depicting its zoonotic nature (18). The zoonotic potential of *B hominis*. infection and speculation on strain variation that resulted in differences of pathogencity of the parasites has been demonstrated by different investigators (19, 20).

Different diagnostic methods are used for the detection of

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Blastocystis hominis infections, such as culturing and staining of the parasites from faecal samples. In addition, the diagnosis of *B. hominis* (abbreviated for Blastocystis throughout the rest of the paper) infection involves iodine stained wet mount direct microscopy for the differentiation of the internal structures. Examining the *in vitro* culture of the parasite is another well-established method (21). However, in most health institutions, especially in developing countries where other well-recognised intestinal parasites are rampant diagnosis, the reporting and treatment of *Blastocystis hominis* infections have been overlooked by the laboratory technicians and physicians (22). Most of the researchers have indicated the following two criteria for considering *Blastocystis hominis* as a pathogen: (i) when present in large numbers (greater than 5 per high power field) and (ii) when diagnosed in the absence of other potential pathogens (2, 9,23).

Morphological heterogeneity is observed in *Blastocystis hominis* parasites, which have significance for the diagnosis of the infection. Among these, the amoeboid form is small in diameter (2.6-7.8μm), irregular in shape and often has extended pseudopodia and possesses one or two nuclei located at the center of the cell (2,8). This form is commonly detected in symptomatic patients with diarrhoea stool samples (2, 24). The vacuolated form measures 5-30μm, and is predominant in faecal specimens. This form has been considered to be the typical cell form and is generally used for the diagnosis of *Blastocystis hominis*. The granular form of *Blastocytis hominis* has an ultrastructure similar to that of the vacuolar form, apart from having morphologically and cytochemically different central vacuoles (1,2,6).

This study is aimed at determining the prevalence of *Blastocystis hominis* and to evaluate its potential clinical significance in the absence of other intestinal pathogens in patients referred for bacteriological stool culture.

Methods

Study subjects and sample collection: A single fresh stool specimen was collected from each of the 152 patients who were referred to the bacteriology laboratory at the EHNRI for bacteriological stool culture examination from March 2003 to May 2004. Clinical information such as abdominal pain, nausea, vomiting, distension, anorexia, flatulence, bowel movement/day, type of diarrhoea, appearance of stool was collected during sample collection. The population included were children, adolescent, adults and the elderly of both sexes. Fresh stool samples were collected in a sterile Petri dish and transported immediately to the bacteriology laboratory for processing. From there, the remaining portion of stool samples was taken to the parasitology laboratory for further analysis for different intestinal parasites.

Parasitological methods: Fresh stool samples were processed by direct wet mount and formol-ether concentration method for the detection of ova and parasites. The slides were observed under light microscope with 100X and 400X magnifications by adding lugol's iodine for the detection of protozoan parasites. *Blastocystis hominis* parasite in faecal specimens varies in shape and diameter (3-20μm). In wet mounts, all appear as refractile organisms with or without a single vacuole, and with four to six 'dots' clustered around the rim or within the body of the organism. The number of *Blastocystis hominis* parasites >5 parasites per high power field (7,9) was recorded.

Detection of coccidian intestinal parasites using the Modified-Ziehl Neelsen method: Oocysts of the parasites were sought by direct smear as well as smears from the sediment by using the formol-ether concentration method. The smears were air dried and then fixed in methanol for 5 minutes followed by staining with carbol fuschin for 30 minutes. After washing in tap water, the slides were decolourised with 1% acid-alcohol for three minutes. After being washed in tap water, the slides were counterstained with 0.5% methylene blue for one minute. Then the slides were observed under oil immersion using a light microscope. Oocysts usually appeared bright pink or dark-red against a blue background. The size of oocyst of *Cryptosporidium parvum* was 4-6 μm while oocyst of *Isospora belli* measures 10-19 μm by 20-30μm. Each slide was observed for 10 minutes to decide whether the slide is positive or negative.

Detection of intestinal microsporidia: Fresh stool samples were processed by a water-ether sedimentation method for staining by the Uvitex-2B method as described previously (25). Briefly, 8ml of distilled water was taken in a 15 ml conical test tube. One gram of fresh stool is added and mixed thoroughly. After sieving with gauze, 3ml of ether was added. The mixture was shaken for one minute and centrifuged at 2000g for 2 minutes. From the sediment, thin smears were prepared on microscope slides and allowed to air dry. The slides were fixed in methanol for 5 minutes followed by staining with Uvitex-2B for ten minutes. After washing in PBS, the slides were counter-stained with 0.5% Evans blue (sigma) for 30 seconds. After washing in PBS, the slides were observed under the fluorescent microscope with a 50-w Mercury high-pressure lamp, excitation filter of 355-425 nm and suppression filter of 460 nm (Leitz Ploemopack Filter Block D, Germany) at the magnification of X1000 for the presence of spores of intestinal microsporidia. Each slide was observed for about 10 minutes to decide whether it was positive or negative.

Isolation of entropathogenic bacteria: All fresh stool specimens were brought to bacteriology laboratory

immediately and tested for *Salmonella* spp., *Shigella spp*., *Vibro cholerae* and diarrheogenic *Escherichia coli* by using a standard culture method. Briefly, fresh stool samples were inoculated on to a MacConkey agar (MA), Salmonella-Shigella agar (SS), Thiosulfate Citrate Bile Sucrose agar (TCBS) and Butzer Campylobacter plates and incubated at 37^0C for 24 hours. The plates were read and identified by experienced technicians. Further biochemical and sensitivity tests were done for positive samples.

Results

Out of the 152 samples requested for bacteriological culture only 4 (2.6%) were found to be positive for bacteria: 1 *Salmonella* spp, 2 S*higella* spp. and 1 case of *Escherichia coli* were isolated. The same samples were analysed for different intestinal parasites. Among these, 71/152 (46.7%) were found to be positive for *Blastocystis hominis* parasites. Infection with *Blastocystis hominis* was considered as positive only when the parasite count is greater than 5 per high power field of the light microscope. This is based on an established rating procedure in various studies conducted by different investigators elsewhere (7,9,23). Opportunistic intestinal parasites, *Cryptosporidium parvum* 11(7.2%), *Isospora belli* 13(8.6%) and *Entero-cytozoon bieneusi* 2 (1.3%) were detected. Among the helminths, *Strongyloides sterocoralis* 5 (3.3%), *Trichuris trichiura* 4(2.6%), *Ascaris lumbricoides* and *Schistosoma mansoni* 1(0.7%) each and Hookworm spp. and *Taenia* spp. each 2 (1.3%) were recorded (Table 1). Pathogenic intestinal protozoan *Entamoeba histolytica/dispar* 9(5.9%) and *Giradia lamblia* 6 (3.9%) were identified. Non-pathogenic intestinal protozoa were also observed. *Entamoeba coli* 15(9.9%), *Endolimax nana* 4 (2.6%), *Iodoamoeba butschilli* and *Chilomastix mesnelli* 1(0.9%) each, and *Sarcocystis* spp. 5 (3.3%) were identified.

Out of 71 *B. hominis* infections, *B. hominis* alone was detected in 51/71 (71.8%) specimens and in 20/71(28.2%) with other intestinal parasites. Concurrent infections with pathogenic and non-pathogenic intestinal parasites were also recorded (Table 2). Among the pathogenic, coccidian parasites *Cryptosporidium parvum* and *Isospora belli* 2 (4%) each were detected. *Entamoeba coli* 5 (10%), *Endolimax nana* 2(4%) and *Iodoamoeba butchilli* 1(2%) were among the non-pathogens, and 2 (4%) of *Strongylodes stercoralis* and 1(2%) of *Trichuris trichuria* were also concurrently detected.

The major clinical manifestations noted with *Blastocystis hominis* alone were distension in 43 (84.3%), flatulence in 41 (80.4%) and anorexia in 43 (86%) and were significantly higher ($P<0.05$) in is group than in those with concurrent infection. Abdominal pain with cramping and vomiting was also recorded. In 31 (60.8%) of the cases, bowel movement per day was 4 times and in 28 (54.8%) the nature of diarrhoea was intermittent (Table 3).

Table 1: **Prevalence of intestinal parasites and bacteria detected in the stool samples referred for bacteriological culture**

Parasite identified	No. positive (%) (N=152)
Protozoa	
Blastocystis hominis	71 (46.7)
Isospora belli	13 (8.6)
Cryptosporidium parvum	11 (7.2)
Enterocytozoon bieneusi	2 (1.3)
Entamoeba histolytica/dispar	9 (5.9)
Giardia lamblia	6 (3.9)
Entamoeba coli	15 (9.9)
Endolimax nana	5 (3.3)
Chilomatix mesnelli	1 (0.7)
Entamoeba hartmanni	1 (0.7)
Iodoamoeba butcheilli	1 (0.7)
Sarcocystis spp.	5 (3.3)
Helmiths	
Ascaris lumbricoides	1 (0.7)
Trichuris trichiura	4 (2.6)
Strongyloides stercoralis	5 (3.3)
Hookworm *spp.*	2 (1.3)
Schistosoma mansoni	1 (0.7)
Taenia spp.	2 (1.3)
Hymenolepis nana	1 (0.7)
Bacteria	
Salmonella spp.	1 (0.7)
Shigella spp.	2 (1.3)
Escherichia coli	1 (0.7)

Table 2: **Concurrent infection with *Blastocystis hominis* with other protozoa (n=20)**

Parasites Identified	No. positive (%)
Cryptosporidium parvum	2 (2.8)
Isospora belli	2 (2.8)
Strongyloides stercoralis	2 (2.8)
Entamoeba coli	5 (7.0)
Entamoeba histolytica/dispar	2 (2.8)
Endolimax nana	3 (4.2)
Giardia lamblia	2 (2.8)
Iodoameaba butchilli	1 (1.4)
Trichuris trichuria	1 (1.4)

The clinical features recorded in those *Blastocystis hominis* infections with other intestinal parasites were abdominal cramps, distension, anorexia and flatulence (Table 3) but with no significant differences among the groups ($P>0.05$). The nature of diarrhoea in the majority of *Blastocystis hominis* with other parasites was intermittent in 12 (60.0%) and loose appearance in 17 (85.0%) of the cases.

The prevalence of intestinal parasites was higher in males than in females (Table 4), but with no significant difference between the sexes ($P>0.05$). The prevalence of intestinal parasites with age dependence range cannot be analysed because the majority of the patients were aggregated in the age range of 20-40 years.

Table 3: **Clinical features of patients infected with *Blastocytis hominis* alone and *Blastocystis hominis* with other intestinal parasites**

Features	*Blastocystis alone* (%) (n=51)	*Blastocystis* with others (%) (n=20)	P-value
Abdominal pain	47 (92.2)	18 (90.0)	
Nausea	26 (51.0)	12 (60.0)	
Vomiting	9 (17.6)	3 (15.0)	
Distension	43 (84.3)*	15 (75.0)	*P=0.008
Anorexia	40 (78.4)*	14 (70.0)	*P=0.046
Flatulence	41 (80.4)*	15 (75.0)	*P=0.013
Fever	16 (31.4)	6 (30.0)	
Tensmus	20 (39.2)	8 (40.0)	
Bowel movement/ day:			
1X	1 (2.0)	0	
2X	6 (11.8)	2 (10.0)	
3X	7 (13.7)	1 (5.0)	
4X	31 (60.8)	15 (75.0)	
≥5X	6 (11.8)	2 (10.0)	
Type of diarrhoea			
Intermittent	28 (54.8)	12 (60.0)	
Chronic	12 (23.8)	8 (40.0)	
Acute	11 (21.6)	0	
Appearance			
Loose	42 (82.4)	17 (85.0)	
Formed	1 (2.0)	0	
Watery	8 (15.7)	3 (15.0)	
Bloody	0	0	
Mucoid	0	0	

* P<0.05

Table 4: **prevalence of intestinal parasite from stool samples referred for stool culture by sex**

Parasite identified	Male (%) (n=93)	Female (%) (n=59)	Total (%) (n=152)
Protozoa			
*Blastocystis hominis**	44 (47.3)	27 (45.8)	71 (46.7)
Isospora belli	9 (9.7)	4 (6.8)	13 (8.6)
Cryptosporidium parvum	5 (5.4)	6 (10.2)	11 (7.2)
Enterocytozoon bieneusi	2 (2.2)	0 (0.0)	2 (1.3)
Entamoeba histolytica/dispar	6 (6.5)	3 (5.1)	9 (5.9)
Giardia lamblia	3 (3.2)	3 (5.1)	6 (3.9)
Entamoeba coli	7 (7.5)	8 (13.6)	15 (9.9)
Endolimax nana	3 (3.2)	3 (3.2)	6 (3.3)
Chilomatix mesnelli	1 (1.1)	0 (0.0)	1 (0.7)
Entamoeba hartmanni	0 (0.0)	1 (1.7)	1 (0.7)
Iodoamoeba butcheilli	1 (1.1)	0 (0.0)	1 (0.7)
Sarcocystis spp.	3 (3.2)	2 (3.4)	5 (3.3)
Helmiths			
Strongyloides stercoralis	3 (3.2)	2 (3.4)	1 (0.7)
Trichuris trichuria	3 (3.2)	1 (1.7)	4 (2.6)
Ascaris lumbricoides	1 (1.1)	0 (0.0)	1 (0.7)
Hookworm *spp.*	2 (2.2)	0 (0)	2 (1.3)
Schistosoma mansoni	0 (0.0)	1 (1.7)	1 (0.7)
Taenia spp.	2 (2.2)	0 (0.0)	2 (1.3)
Hymenolepis nana	0 (0.0)	1 (1.7)	1 (0.7)
Bacteria			
Salmonella spp.	0 (0.0)	1 (1.7)	1 (0.7)
Shigella spp.	0 (0.0)	2 (3.4)	2 (1.3)
Escherichia coli	0 (0.0)	1 (1.7)	1 (0.7)

*P>0.05

Discussion

The question of whether *Blastocystis hominis* is pathogenic or a commensal parasite or perhaps is capable of being pathogenic in specific situations has remained controversial. However, there have been many reports suggesting that *B. hominis* causes illness in some infected individuals (2,3,10,13,19).

According to previous reports from Ethiopia, very few cases of human infection were identified among HIV/AIDS patients (16,17). Even though the immune status of our study subjects was not known, 71/152 (46.8%) of the examined individuals in this study were positive for *B. hominis*, and some of the cases concurrently occurred with other pathogenic and non-pathogenic intestinal parasites. A similar *B. hominis* infection rate was reported by the EHNRI Parasitology Laboratory, 96/135 (71.0%) (unpublished report, 2004) among referred patients. In the present study, patients with *B. hominis* infection presented with abdominal pain with cramp, nausea, distension, anorexia, flatulence and minor symptoms such as fever, vomiting and tenesmus, which is in agreement with the findings of others elsewhere (1, 3, 9, 11, 12). Clinical symptoms like distension, anorexia and flatulence were significantly higher ($P<0.05$) in patients with *B. hominis* infections alone; similar findings were noted by other investigators (1, 2, 3, 26). In the majority of the patients, the nature of diarrhoea was intermittent with frequency of bowel movement which is 4 times a day. The appearance of the stool in the majority of the individuals was loose, and in rare cases it was watery. This is in agreement with the findings of others, who suggested that infection with *B. hominis* is non-specific and includes diarrhoea; sometimes profuse watery without blood or leukocytes, nausea, and abdominal pain (2, 26).

Several case reports suggested that *B. hominis* could infect extraintestinal sites such as infection of synovial fluids which results in joint pain, swellings and arthritis (2,31,32). In the present study, some non-specific symptoms such as gastroenteritis and inflammation of the extremities were noted in patients with *B. hominis* infections alone. In addition, among the study subjects, the cases of four staff members aged 38, 40 and 45 years old, males and 42 years old, female who gave stool samples for bacterial culture was described. Abdominal cramping, distension, and flatulence with intermittent type of diarrhoea were their main problem. Two of them had severe diarrhoea, i.e., bowel motion frequency was greater than 5 times per day. One had anorexia and tenesmus. Similar information was documented by Rolston *et.al.*, (27) in individuals with *B. hominis* infection. Stool culture for bacteria was negative in all of the four cases. Except *B. hominis*, no other pathogenic or non-pathogenic intestinal parasites were detected among the four cases. Three of them had the parasite load greater than 5 per high power field. In fact, their immune status is not known. All of them were treated with metronidazole at the dose of 2x3 tablets of 250 mg for 10 days. A week after treatment, they gave stool sample for check up and no *B. hominis* parasite was found in their stool. They responded favourably to treatment and all symptoms were resolved. This observation has given additional supportive evidence to recognise that *B. hominis* is a potential pathogen of humans in the absence of other pathogenic organisms and when the parasite load is > 5 under high power field (7,9,23). The rest of the positive patients were treated in the respective health institutions that referred them for bacteriological stool culture to the Bacteriology Laboratory of EHNRI.

Meanwhile, co-occurrence of *B. hominis* infection with other well known pathogenic organisms may confuse its pathogenic potential. The clinical symptoms observed may lead to focus on other widely recognised pathogenic ones than *B. hominis* infection (28,29). However, in this study, the number of co-infections with other pathogenic intestinal parasites is very low with no significant differences in clinical manifestations ($P>0.05$). Concurrent infection with non-pathogenic intestinal protozoan parasites such as *Entamoeba coli*, and others may have an indication of similar routes of transmission, mainly faecal-oral contamination of foods and drinking water (1,9). It has been suggested that *B. hominis* is associated with the development of diarrhoea in travellers (4,30). However, some reports indicated that the frequency of concurrent infections with other intestinal pathogens has confused the spectrum of the disease in travellers returning from developing countries infected with different species of intestinal parasites (28, 29,30).

The other interesting findings observed in this study were that the opportunistic intestinal parasites- *Cryptosporidium parvum, Isospora belli* and *Enterocytozoon bieneusi* - were identified in patients complaining of gastrointestinal problems and who were referred only for bacteriological stool culture but not for detection of ova and intestinal parasites including opportunistic ones. Thus, this finding creates an awareness to perform additional type of stool examination for intestinal parasites with emphasis on opportunistic intestinal parasites in patients complaining of abdominal problems and associated illnesses.

Conclusion

The finding of this study is expected to create awareness among laboratory technicians and physicians about the proper diagnosis and management of the disease. From this and other related studies elsewhere, it could be concluded that treating B. *hominis* infections where defined symptoms are present with a large number of parasites in the stool and in the absence of other cases of the disease is recommendable. This study provides further supportive evidence for the pathogenic potential of *B. hominis* infection and its possible role in causing

clinical symptoms in the absence of other intestinal pathogens and when the parasite load is high. In addition, in-depth and extensive clinico-epidemiological studies must be conducted in order to confirm its true status of pathogenicity both in immunocompetent, immunocompromised and HIV/AIDS patients.

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