Blastocystis: To Treat or Not to Treat…

Christina M. Coyle,[1,4] Julie Varughese,[1] Louis M. Weiss,[1,2,3] and Herbert B. Tanowitz[1,2,4]

[1]Department of Medicine, Division of Infectious Diseases, [2]Department of Pathology, Division of Parasitology and Tropical Medicine, and [3]Global Health Center, Albert Einstein College of Medicine, and [4]Diagnostic Parasitology Laboratory and Parasitology Clinic, Jacobi Medical Center, Bronx, New York

Parasites in the genus *Blastocystis* comprise several subtypes (genotypes) and have a worldwide distribution. In some surveys, these are the most common parasites found in human stool specimens. An emerging literature suggests that the pathogenicity of *Blastocystis* is related to specific subtypes and parasite burden, although even individuals with small numbers of cysts may be symptomatic. Some data suggest an association between infection with *Blastocystis* and irritable bowel syndrome. However, there are few clinical studies demonstrating a direct relationship between the presence of this parasite and disease, few animal models to explore this relationship, and no consensus as to appropriate treatment. We recommend that asymptomatic individuals with few cysts not be treated. However, those who have gastrointestinal or dermatologic signs and symptoms and many cysts in stool specimens may require treatment. Metronidazole is the drug of choice. Additional studies are required to determine pathogenicity and appropriate therapy.

Members of the genus *Blastocystis* are ubiquitous parasites with a worldwide distribution that are transmitted via the fecal-oral route. Nine subtypes (genotypes) of *Blastocystis* are described on the basis of small-subunit ribosomal RNA gene analysis, and nonhuman primates, mammals, and birds seem to be the major reservoir hosts for most subtypes [1]. Because of this vast diversity, it has been suggested that the human parasite should no longer be referred to as *Blastocystis hominis* but instead should be called *Blastocystis* spp. or *Blastocystis* spp. subtype *n* (where *n* is the subtype number according to the Stensvold classification) [2]. In this article, we refer to this organism as *Blastocystis* to avoid confusion.

In many epidemiologic surveys, *Blastocystis* is the most frequently isolated parasite, with a higher prevalence in underdeveloped countries [3]. This may be attributed to poor hygiene, exposure to animals, and consumption of contaminated food or water. *Blastocystis* is the most common parasite identified in stool samples in many institutions in the United States. It is more common than *Giardia lamblia* and *Dientamoeba fragilis*. Within regions in the same country, the prevalence can vary widely [3].

The taxonomy of *Blastocystis* remained elusive for many years. It had been classified as flagellate, vegetable, yeast, or fungus, but in 1991, Zierdt et al [4] classified it as a protist on the basis of its morphologic features. With the advent of molecular data (on small-subunit ribosomal DNA and other genes), these organisms are now thought to belong to the stramenopiles, a branch of the Chromalveolata [5, 6]. This group includes both unicellular and multicellular protists, such as brown algae, diatoms, chrysophytes, water molds, and slime nets [7]. *Blastocystis* from humans and animals can be divided into at least 12 species, of which several are found in humans [8].

This classification of *Blastocystis* into several species on the basis of molecular data may explain the variations in symptoms and the response to treatment reported in *Blastocystis* infections. Humans can have zoonotic infections due to *Blastocystis* that are found in primates, pigs, cattle, and birds, as well as infection with the *Blastocystis* subtype 3 isolate, which is found only in humans [9]. Prevalence studies indicate that subtypes 1 and 3 predominate in human infection and suggest that

Received 25 July 2011; accepted 27 September 2011; electronically published 10 November 2011.

Correspondence: Herbert B. Tanowitz, MD, Department of Pathology, Albert Einstein College of Medicine, 1300 Morris Park Ave, Rm 504, Forchheimer Bldg, Bronx, New York, 10461 (herbert.tanowitz@einstein.yu.edu).

Clinical Infectious Diseases 2012;54(1):105–10

DOI: 10.1093/cid/cir810

subtype variation is constant and does not vary much between populations [10, 11]. Other genotypes have been isolated in other prevalence studies, but in decreasing frequency; these are subtypes 2, 4, 6, 7, 8, and 9 [2]. Most individuals seem to harbor a particular subtype, but combinations have also been reported. Pathogenesis may be associated with particular subtypes or species. Because subtypes of *Blastocystis* cannot be distinguished morphologically, molecular techniques are required for epidemiologic, clinical, and therapeutic studies to understand the dynamics of this infection.

CLINICAL SYMPTOMS AND EPIDEMIOLOGY

Some have suggested that certain populations may be more susceptible to *Blastocystis* infections. This parasite was the most common parasite found in Indonesian patients with human immunodeficiency virus infection and AIDS who had chronic diarrhea before receipt of antiretroviral therapy, but this study lacked a control group [12]. Similarly, *Blastocystis* was one of the parasites most frequently isolated from patients with cancer in a series from Turkey [13]. Similar to their susceptibility to other intestinal protozoa, children in underdeveloped countries have a higher incidence of infection [14]. Those who are in close contact with animals also seem to be at increased risk of acquisition of *Blastocystis*, reinforcing the notion that this is probably a zoonotic infection [2]. A few studies found that expatriates with traveler's diarrhea had a high prevalence of *Blastocystis*, whereas some studies found that 25%–75% of those with *Blastocystis* have a history of recent foreign travel [15–19]; however, there was no association between *Blastocystis* and traveler's diarrhea in a prospective case-control study performed in Nepal [16, 20].

There has been debate in the literature concerning the question of the pathogenicity of *Blastocystis*. Some studies suggest an association between the parasite and disease, but others do not [3, 18, 21]. The limitations of these studies have included adequate sample size, diagnostic methods details, case definitions, duration of organism carriage, and an absence of culture studies to rule out other causes [22]. Many of these studies have lacked data on the specific concentration of organisms and the *Blastocystis* subtype. Recently, a 29-kDa parasite protein and a parasite-associated protease have received attention as potential markers of pathogenicity [23, 24]. There is a lack of reliable animal models for this infection that would allow adequate and detailed investigation of its pathogenesis [25].

It has been suggested that finding >5 parasites per high-power field (40× objective) or, less commonly, by oil immersion (100× objective) is associated with the presence of gastrointestinal disease [3]. Many studies were done in hospitals located in developing countries where the risk of infectious diarrhea is high. A causal relationship has never been established for *Blastocystis* and diarrhea, and no reliable animal model exists, so Koch's postulates have not been demonstrated for this organism. Interestingly, in some studies the clinical presentations have been shown to be subtype dependent, adding to the confusion and possible underestimation of the organism's importance in the literature [26]. Recent studies have focused on correlating disease pathogenicity with subtypes irrespective of parasite density, but the results have been discrepant [26–29].

A variety of signs and symptoms, ranging from intestinal symptoms to cutaneous disorders, have been attributed to *Blastocystis* infection [30, 31]. The most common intestinal symptoms described are diarrhea and abdominal pain [2, 3, 22]. Nonspecific symptoms such as nausea, anorexia, abdominal pain, bloating, flatulence, and acute or chronic diarrhea, have also been reported [2, 3, 26, 32]. Diarrhea may be mild and self-limited or chronic, with reports of acute gastroenteritis [2, 3]. *Blastocystis* does not seem to be invasive, despite 2 case reports describing its recovery in deep tissue. In both of these cases, there were coexisting conditions predisposing to disruption of the gut barrier that probably led to coinfection with *Blastocystis* [33, 34]. In addition, there are no reports of *Blastocystis*-associated dysentery or endoscopic evidence of invasion [2, 35, 36]. Allergic cutaneous lesions, particularly urticaria, have been associated with this organism. In these reports, the resolution of dermatologic signs and symptoms were reported after treatment and eradication of the parasite from the stool [37–39]. It has been suggested that cutaneous manifestations in the setting of *Blastocystis* carriage are probably immune mediated, although the mechanism is unclear [2].

Irritable bowel syndrome (IBS) is defined as a functional group of bowel disorders in which abdominal pain is associated with defecation or alterations in bowel habits in the absence of an organic cause [40]. An association between *Blastocystis* and IBS has been suggested in the recent literature [2, 22, 36, 41–43]; however, in some studies, *Blastocystis* was detected more frequently in patients with IBS than in a control group [27, 32, 35], whereas in other studies there was no association [18, 44]. The pathophysiology of IBS remains elusive, and there are probably several factors causing the constellation of signs and symptoms associated with this clinical entity. These include altered gut reactivity (colonic and/or small-bowel motility) in response to luminal or psychological stimuli, visceral afferent hypersensitivity, a hypersensitive gut with enhanced visceral perception and pain [45], and chronic immune activation [46, 47]. It has been suggested that low-grade inflammation due to ongoing immune activation caused by carriage or infection with *Blastocystis* providing persistent antigenic exposure could play a role in IBS [45]. For example, one study found levels of immunoglobulin G2 directed against *Blastocystis* in IBS patients when compared with asymptomatic controls [48]. Other researchers have speculated that the increased incidence of *Blastocystis* is not a cause of IBS but is rather an indicator of intestinal dysfunction [18].

Thus, current studies do not suggest a clear role for *Blastocystis* as an etiologic agent of IBS, and controlled trials demonstrating a resolution of symptoms in *Blastocystis*-infected IBS patients with eradication of the organisms are needed [2].

DIAGNOSIS

Blastocystis is a polymorphic organism, and 4 major morphologic forms—vacuolar, cyst, granular, and amoeboid—can be seen in stool and axenic cultures. *Blastocystis* is detected using standard clinical parasitologic techniques that are used to detect other enteric parasites, including direct smears stained with trichrome (Figure 1) [49]; various concentration techniques, such as formol-ether (ethyl acetate) concentration technique [32]; and in vitro culture [50] Culture techniques are, most likely, more sensitive than direct smears. Amplification of *Blastocystis*-specific DNA by polymerase chain reaction directly from stool has been reported and permits identification of the *Blastocystis* subtypes [2, 9]. In comparative studies of diagnosis, polymerase chain reaction–based methods coupled with short-term axenic in vitro culture had the highest diagnostic utility in characterizing stool specimens [1, 32]. As with other enteric pathogens, the examination of multiple stool specimens increases the diagnostic yield. *Blastocystis* infection results in a serologic response, which can be detected by enzyme-linked immunosorbent assay or other assays; however, serologic testing is not currently used for diagnosis of this infection [51]. Fecal immunoassays are under development but have not been commercialized. Remarkably, many laboratories do not report *Blastocystis* because of the long-held view by some in the medical community that it is always nonpathogenic.

TREATMENT

Treatment of *Blastocystis* infection remains a complicated issue. Because there is still a great deal of debate about the true pathogenicity of *Blastocystis*, there is still much debate about the need for treatment. Further knowledge about the genotyping and subtyping of *Blastocystis* and the impact that various subtypes may have on pathogenicity and antimicrobial efficacy is still being sought [22]. In a symptomatic patient, isolation of cysts in stool specimens should trigger a thorough evaluation for other causes of the patient's gastrointestinal tract complaints, given the possibility for coinfection with other pathogens. Patients with *Blastocystis* isolated in the stool can be coinfected with other pathogens, such as *G. lamblia, Entamoeba histolytica*, and *D. fragilis* [52]. It is quite possible that patients who respond to treatment for *Blastocystis* with metronidazole or trimethoprim-sulfamethoxazole (TMP-SMX) may actually have clinical improvement owing to treatment of a secondary pathogen.

To date, a number of antimicrobial agents have been used for treatment of *Blastocystis* infection (Table 1); however, randomized, controlled trials are limited. Metronidazole is considered first-line treatment, but the success of eradicating *Blastocystis* with this drug has been reported to be anywhere from 0% to 100% [53]. There have been 2 published placebo-controlled studies, and both concluded that resolution of symptoms was associated with eradication of the organism. Nigro et al [54] conducted a placebo-controlled trial evaluating 76 patients with *Blastocystis* infection, of whom 88% treated with metronidazole had resolution of clinical symptoms, compared with only 14% in the placebo group, 1 month after treatment. Microbiologic resolution was found in 80% of metronidazole-treated patients, compared with only 3% of the placebo group. In addition, when patients were evaluated 6 months after initiation of treatment, 30 (75%) of 40 in the metronidazole-treated group were still asymptomatic, compared with 12 (33%) of 36 in the placebo group. It is not clear whether any of these patients harbored resistant subtypes or were reinfected [54]. In a study by Mogaddham et al [55], 28 of 104 *Blastocystis*-infected patients were classified as having severe infection. Twelve of these patients were treated with metronidazole, with infection eradicated in only 4. It is possible that metronidazole is effective for certain patients but does not provide complete eradication, particularly in those with severe infection, or that patients who did not respond may have been infected with resistant subtypes.

TMP-SMX has been used as a second-line agent in patients who may not be able to tolerate or do not respond to treatment with metronidazole. Ok et al [56] examined 38 children and 15 adults with symptomatic infection with *Blastocystis* (stool specimens were negative for other parasitic or bacterial infections) treated with TMP-SMX for 7 days. *Blastocystis* was eradicated from stool in 36 (94.7%) of 38 children and in 14 (93.3%) of 15

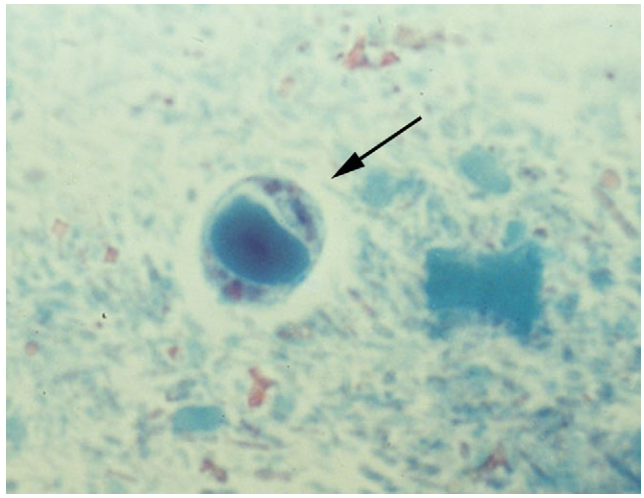

Figure 1. Trichrome stain showing a cyst of *Blastocystis* in stool. From the collection of Herman Zaiman, a collection of pictorial parasites. American Society of Tropical Medicine and Hygiene.

Table 1. Antimicrobials Reported as Useful in the Treatment of *Blastocystis* Infection

Drug	Dose
Metronidazole	
Adult dose	750 milligrams thrice daily for 10 days; or 500 milligrams thrice daily for 10 days; or 1.5 grams daily for 7 days
Pediatric dose	15 mg/kg twice daily for 10 days
TMP-SMX	
Adult dose	2 double strength tablets daily for 7 days (320 milligrams TMP: 1600 milligrams SMX)
Pediatric dose	6 mg/kg TMP daily for 7 days
Nitazoxanide	
Adult dose	500 milligrams twice daily for 3 days
Pediatric dose	100–200 milligrams twice daily for 3 days
Paromomycin	25 mg/kg thrice daily for 10 days; 500 milligrams thrice daily for 7 days
Iodoquinol	650 milligrams thrice daily for 10–20 days
Ketoconazole	200 milligrams daily for 14 days
Tinidazole	
Adult dose	2 grams daily for 5 days
Pediatric dose (<40 kg body weight)	50 mg/kg/day for 5 days
Saccharomyces boulardii	250 milligrams twice daily for 10 days

Given the variable agreement regarding the pathogenicity of *Blastocystis*, there is no consensus as to which patients, if any, should undergo treatment for *Blastocystis* infection.

Abbreviations: kg, kilograms; SMX, sulfamethoxazole; TMP, trimethoprim.

adults. Clinical symptoms resolved in 39 (73.6%) and improved in 10 (18.9%) of 53 patients evaluated.

Similar outcomes were observed in a study using nitazoxanide, but this study did not follow patients over a long period, so the effect may have been short-lived. Although these studies provide the best evidence to date for the pathogenic potential of this parasite, both studies used broad-spectrum antiparasitic agents, and thus the response to treatment could be attributed to the clearance of another possible enteric pathogen [2].

Additional agents, such as iodoquinol, tinidazole, nitazoxanide, emetine, pentamidine, iodochlorhydroxyquin, and furazolidone, have been used and have shown variable efficacy in eradicating *Blastocystis* infection [55, 57]. One of the difficulties in assessing therapeutic efficacy is the tremendous variability in posttreatment follow-up of patients treated for *Blastocystis* infection [22]. Posttreatment microbiologic analysis showing *Blastocystis*-positive stools may not reflect treatment failure or resistance but could represent reinfection. Patients may also have a secondary process that responded initially to treatment with subsequent treatment failure.

There have been several studies examining the use of alternative agents in the treatment of *Blastocystis* infection. For example, Yakoob et al [58] studied the in vitro efficacy of garlic and other dietary herbs, compared with that of metronidazole, in the treatment of *Blastocystis* infection in both control subjects and patients with IBS. The authors evaluated the efficacy of garlic and metronidazole at concentrations of 0.01 and 0.1 mg/mL in suppressing the growth of *Blastocystis*. They found that garlic and metronidazole were equally effective at both concentrations. The isolates of *Blastocystis* were not as sensitive to the other herbs tested, which included ginger, black pepper, and white cumin.

Saccharomyces boulardii (a probiotic) has also been studied for *Blastocystis* treatment [59]. In a study by Dinleyici et al [59], children with a 2-week history of gastrointestinal symptoms and isolation of *Blastocystis* from the stool were randomized to treatment with *Saccharomyces*, metronidazole, or placebo for 10 days. Patients were evaluated for clinical and microbiologic cure at days 15 and 30 after initiation of treatment. Clinical cure was found in 77.7% of the *Saccharomyces* group and 66.6% of the metronidazole group at 15 days, compared with 40% in the placebo group. Persistence of cysts in stool was noted in 20% of the metronidazole group and 27.8% of the *Saccharomyces* group, compared with 73.4% of the placebo group. Thirty days after initiation of treatment, clinical cure was found in 94.4% of subjects in the *Saccharomyces* group, compared with 73.3% of those in the metronidazole group. Resolution of cysts in the stool was found in 94.4% in the *Saccharomyces* group and 93.3% in the metronidazole group, and the difference was not statistically significant ($P = .43$) [59].

Given the variable agreement regarding the pathogenicity of *Blastocystis*, there is no consensus as to which patients should undergo treatment for *Blastocystis* infection. In the asymptomatic individual, treatment is not necessarily indicated. Isolation of *Blastocystis* in stool from a symptomatic individual should lead to a thorough investigation for other causes of the gastrointestinal complaints. It is reasonable to initiate a trial of antimicrobial therapy in patients who have persistent diarrhea or who have undergone an extensive work-up without any other pathogen or gastrointestinal source identified. Reasonable first-line agents include metronidazole or TMP-SMX. Additional randomized, controlled trials are needed to better assess the therapeutic efficacy of the additional antiparasitic drugs and their use in this infection.

The relation of *Blastocystis* to human disease remains unclear, and many of the drugs used in the treatment of *Blastocystis* infection have significant side effects. A minimum of 3 stool examinations separated in time and performed in a reliable parasitology laboratory constitute an adequate examination. If the individual is asymptomatic, we do not recommend specific antiparasitic therapy. However, we understand that a counterargument to this recommendation may be made, because most experts recommend treatment of asymptomatic individuals who pass cysts, such as those with *E. histolytica* and *G. lamblia*

infection. If the individual patient indeed has gastrointestinal signs and symptoms and has a significant number of cysts in the stool (ie, >5 cysts per high-power field), this patient may be a candidate for therapy. In those cases, other potential causes need to be ruled out. Thus, these patients may require additional stool specimens for parasite analysis, as well as cultures for bacterial pathogens. We have observed patients with *Blastocystis* in the stool who have *G. lamblia* or *E. histolytica* detected at subsequent examinations. Some of these patients may require endoscopy and imaging studies to rule out entities such as inflammatory bowel disease and IBS. Those with *Blastocystis* in the stool who have an associated skin eruption should be considered for treatment in the absence of other causes, but this recommendation is based on scant data. When treatment is given, we usually use metronidazole as first-line treatment, based on the available clinical trial data. If metronidazole is not effective, we usually use either TMP-SMX or nitazoxanide as second-line treatment.

Note

Potential conflicts of interest. All authors: No reported conflicts.

All authors have submitted the ICMJE Form for Disclosure of Potential Conflicts of Interest. Conflicts that the editors consider relevant to the content of the manuscript have been disclosed.

References

1. Stensvold CR, Arendrup MC, Jespersgaard C, Molbak K, Nielsen HV. Detecting *Blastocystis* using parasitologic and DNA-based methods: a comparative study. Diagn Microbiol Infect Dis **2007**; 59:303–7.
2. Tan KS, Mirza H, Teo JD, Wu B, Macary PA. Current views on the clinical relevance of *Blastocystis* spp. Curr Infect Dis Rep **2010**; 12:28–35.
3. Tan KS. New insights on classification, identification, and clinical relevance of *Blastocystis* spp. Clin Microbiol Rev **2008**; 21:639–65.
4. Zierdt CH. *Blastocystis hominis*–past and future. Clin Microbiol Rev **1991**; 4:61–79.
5. Silberman JD, Sogin ML, Leipe DD, Clark CG. Human parasite finds taxonomic home. Nature **1996**; 380:398.
6. Arisue N, Hashimoto T, Yoshikawa H, et al. Phylogenetic position of *Blastocystis hominis* and of stramenopiles inferred from multiple molecular sequence data. J Eukaryot Microbiol **2002**; 49:42–53.
7. Patterson DJ. The evolution of protozoa. Mem Inst Oswaldo Cruz **1988**; 83(Suppl 1):580–600.
8. Noel C, Peyronnet C, Gerbod D, et al. Phylogenetic analysis of *Blastocystis* isolates from different hosts based on the comparison of small-subunit rRNA gene sequences. Mol Biochem Parasitol **2003**; 126:119–23.
9. Santin M, Gomez-Munoz MT, Solano-Aguilar G, Fayer R. Development of a new PCR protocol to detect and subtype *Blastocystis* spp. from humans and animals. Parasitol Res **2011**; 109:205–12.
10. Stensvold CR, Alfellani MA, Norskov-Lauritsen S, et al. Subtype distribution of *Blastocystis* isolates from synanthropic and zoo animals and identification of a new subtype. Int J Parasitol **2009**; 39:473–9.
11. Yoshikawa H, Wu Z, Kimata I, et al. Polymerase chain reaction-based genotype classification among human *Blastocystis hominis* populations isolated from different countries. Parasitol Res **2004**; 92:22–9.
12. Kurniawan A, Karyadi T, Dwintasari SW, et al. Intestinal parasitic infections in HIV/AIDS patients presenting with diarrhoea in Jakarta, Indonesia. Trans R Soc Trop Med Hyg **2009**; 103:892–8.
13. Tasova Y, Sahin B, Koltas S, Paydas S. Clinical significance and frequency of *Blastocystis hominis* in Turkish patients with hematological malignancy. Acta Med Okayama **2000**; 54:133–6.
14. Londono AL, Mejia S, Gomez-Marin JE. [Prevalence and risk factors associated with intestinal parasitism in preschool children from the urban area of Calarca, Colombia]. Rev Salud Publica (Bogota) **2009**; 11:72–81.
15. Taylor DN, Houston R, Shlim DR, Bhaibulaya M, Ungar BL, Echeverria P. Etiology of diarrhea among travelers and foreign residents in Nepal. JAMA **1988**; 260:1245–8.
16. Babcock D, Houston R, Kumaki D, Shlim D. *Blastocystis hominis* in Kathmandu, Nepal. N Engl J Med **1985**; 313:1419.
17. Keystone JS. *Blastocystis hominis* and traveler's diarrhea. Clin Infect Dis **1995**; 21:102–3.
18. Udkow MP, Markell EK. *Blastocystis hominis*: prevalence in asymptomatic versus symptomatic hosts. J Infect Dis **1993**; 168:242–4.
19. Grossman I, Weiss LM, Simon D, Tanowitz HB, Wittner M. *Blastocystis hominis* in hospital employees. Am J Gastroenterol **1992**; 87:729–32.
20. Shlim DR, Hoge CW, Rajah R, Rabold JG, Echeverria P. Is *Blastocystis hominis* a cause of diarrhea in travelers? A prospective controlled study in Nepal. Clin Infect Dis **1995**; 21:97–101.
21. Clark CG. Extensive genetic diversity in *Blastocystis hominis*. Mol Biochem Parasitol **1997**; 87:79–83.
22. Stensvold CR, Nielsen HV, Molbak K, Smith HV. Pursuing the clinical significance of *Blastocystis*–diagnostic limitations. Trends Parasitol **2009**; 25:23–9.
23. Abou Gamra MM, Elwakil HS, El Deeb HK, Khalifa KE, Abd Elhafiz HE. The potential use of 29 kDa protein as a marker of pathogenicity and diagnosis of symptomatic infections with *Blastocystis hominis*. Parasitol Res **2011**; 108:1139–46.
24. Abdel-Hameed DM, Hassanin OM. Protease activity of *Blastocystis hominis* subtype3 in symptomatic and asymptomatic patients. Parasitol Res **2011**; 109:321–7.
25. Elwakil HS, Hewedi IH. Pathogenic potential of *Blastocystis hominis* in laboratory mice. Parasitol Res **2010**; 107:685–9.
26. Stensvold CR, Lewis HC, Hammerum AM, et al. *Blastocystis*: unravelling potential risk factors and clinical significance of a common but neglected parasite. Epidemiol Infect **2009**; 137:1655–63.
27. Dominguez-Marquez MV, Guna R, Munoz C, Gomez-Munoz MT, Borras R. High prevalence of subtype 4 among isolates of *Blastocystis hominis* from symptomatic patients of a health district of Valencia (Spain). Parasitol Res **2009**; 105:949–55.
28. Eroglu F, Genc A, Elgun G, Koltas IS. Identification of *Blastocystis hominis* isolates from asymptomatic and symptomatic patients by PCR. Parasitol Res **2009**; 105:1589–92.
29. Yakoob J, Jafri W, Beg MA, et al. Irritable bowel syndrome: is it associated with genotypes of *Blastocystis hominis*. Parasitol Res **2010**; 106:1033–8.
30. Zuel-Fakkar NM, Abdel Hameed DM, Hassanin OM. Study of *Blastocystis hominis* isolates in urticaria: a case-control study. Clin Exp Dermatol **2011**. doi: 10.1111/j.1365-2230.2011.04127.
31. Hameed DM, Hassanin OM, Zuel-Fakkar NM. Association of *Blastocystis hominis* genetic subtypes with urticaria. Parasitol Res **2011**; 108:553–60.
32. Suresh K, Smith H. Comparison of methods for detecting *Blastocystis hominis*. Eur J Clin Microbiol Infect Dis **2004**; 23:509–11.
33. Hu KC, Lin CC, Wang TE, Liu CY, Chen MJ, Chang WH. Amoebic liver abscess or is it? Gut **2008**; 57:627, 683.
34. Patino WD, Cavuoti D, Banerjee SK, Swartz K, Ashfaq R, Gokaslan T. Cytologic diagnosis of *Blastocystis hominis* in peritoneal fluid: a case report. Acta Cytol **2008**; 52:718–20.
35. Yakoob J, Jafri W, Jafri N, et al. Irritable bowel syndrome: in search of an etiology: role of *Blastocystis hominis*. Am J Trop Med Hyg **2004**; 70:383–5.
36. Chen TL, Chan CC, Chen HP, et al. Clinical characteristics and endoscopic findings associated with *Blastocystis hominis* in healthy adults. Am J Trop Med Hyg **2003**; 69:213–16.
37. Valsecchi R, Leghissa P, Greco V. Cutaneous lesions in *Blastocystis hominis* infection. Acta Derm Venereol **2004**; 84:322–3.
38. Kick G, Rueff F, Przybilla B. Palmoplantar pruritus subsiding after *Blastocystis hominis* eradication. Acta Derm Venereol **2002**; 82:60.
39. Katsarou-Katsari A, Vassalos CM, Tzanetou K, Spanakos G, Papadopoulou C, Vakalis N. Acute urticaria associated with

amoeboid forms of *Blastocystis* sp. subtype 3. Acta Derm Venereol **2008**; 88:80–1.
40. Brandt LJ, Bjorkman D, Fennerty MB, et al. Systematic review on the management of irritable bowel syndrome in North America. Am J Gastroenterol **2002**; 97:S7–26.
41. Boorom KF, Smith H, Nimri L, et al. Oh my aching gut: irritable bowel syndrome, *Blastocystis*, and asymptomatic infection. Parasit Vectors **2008**; 1:40.
42. Giacometti A, Cirioni O, Fiorentini A, Fortuna M, Scalise G. Irritable bowel syndrome in patients with *Blastocystis hominis* infection. Eur J Clin Microbiol Infect Dis **1999**; 18:436–9.
43. Yakoob J, Jafri W, Beg MA, et al. *Blastocystis hominis* and *Dientamoeba fragilis* in patients fulfilling irritable bowel syndrome criteria. Parasitol Res **2010**; 107:679–84.
44. Tungtrongchitr A, Manatsathit S, Kositchaiwat C, et al. *Blastocystis hominis* infection in irritable bowel syndrome patients. Southeast Asian J Trop Med Public Health **2004**; 35:705–10.
45. Stark D, van Hal S, Marriott D, Ellis J, Harkness J. Irritable bowel syndrome: a review on the role of intestinal protozoa and the importance of their detection and diagnosis. Int J Parasitol **2007**; 37: 11–20.
46. Liebregts T, Adam B, Bredack C, et al. Immune activation in patients with irritable bowel syndrome. Gastroenterology **2007**; 132: 913–20.
47. Ohlsson B, Sjoberg K, Alm R, Fredrikson GN. Patients with irritable bowel syndrome and dysmotility express antibodies against gonadotropin-releasing hormone in serum. Neurogastroenterol Motil **2011**. 23: 1000–e459. doi 10.1111/j.1365-2982.2011June 30.
48. Hussain R, Jaferi W, Zuberi S, et al. Significantly increased IgG2 subclass antibody levels to *Blastocystis hominis* in patients with irritable bowel syndrome. Am J Trop Med Hyg **1997**; 56:301–6.
49. Kukoschke KG, Necker A, Müller HE. Detection of *Blastocystis hominis* by direct microscopy and culture. Eur J Clin Microbiol Infect Dis **1990**; 9:305–7.
50. Zman V, Khan KZ. A comparison of direct microscopy with culture for the diagnosis of *Blastocystis hominis*. Southeast Asian J Trop Med Public Health **1994**; 25:792–3.
51. Zierdt CH, Nagy B. Antibody response to *Blastocystis hominis*. Ann Int Med **1993**; 118:985–6.
52. Markell EK, Udkow MP. B*lastocystis hominis*: pathogen or fellow traveler? Am J Trop Med Hyg **1986**; 35:1023–6.
53. Stensvold CR, Smith HV, Nagel R, Olsen KE, Traub RJ. Eradication of *Blastocystis* carriage with antimicrobials: reality or delusion? J Clin Gastroenterol **2010**; 44:85–90.
54. Nigro L, Larocca L, Massarelli L, et al. A placebo-controlled treatment trial of *Blastocystis hominis* infection with metronidazole. J Travel Med **2003**; 10:128–30.
55. Moghaddam DD, Ghadirian E, Azami M. *Blastocystis hominis* and the evaluation of efficacy of metronidazole and trimethoprim/sulfamethoxazole. Parasitol Res **2005**; 96:273–5.
56. Ok UZ, Girginkardesler N, Balcioglu C, Ertan P, Pirildar T, Kilimcioglu AA. Effect of trimethoprim-sulfamethaxazole in *Blastocystis hominis* infection. Am J Gastroenterol **1999**; 94:3245–7.
57. Sohail MR, Fischer PR. *Blastocystis hominis* and travelers. Travel Med Infect Dis **2005**; 3:33–8.
58. Yakoob J, Abbas Z, Beg MA, et al. In vitro sensitivity of *Blastocystis hominis* to garlic, ginger, white cumin, and black pepper used in diet. Parasitol Res **2011**; 109:379–85.
59. Dinleyici EC, Eren M, Dogan N, Reyhanioglu S, Yargic ZA, Vandenplas Y. Clinical efficacy of *Saccharomyces boulardii* or metronidazole in symptomatic children with *Blastocystis hominis* infection. Parasitol Res **2011**; 108:541–5.