

Review

Mysterious chronic urticaria caused by *Blastocystis* spp.?

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Introduction

Species of the genus *Blastocystis* are unusual, unicellular, enteric protozoan parasites present in humans throughout the world. In the healthy population, prevalences of this parasite have been reported at 30–50% in developing countries and 1.5–10.0% in developed countries.¹ The prevalence of *Blastocystis* spp. infestation is higher than that of other intestinal protozoan parasites such as those of the genera *Giardia*, *Entamoeba*, and *Cryptosporidium*.² In immunocompromised people such as those with human immunodeficiency virus (HIV) infection, prevalences have been found to approach 38% in developed countries,³ and an association with diarrhea is suggested. The nutritional status of an individual may be one of the most important risk factors for co-infection.⁴ Children and aged people are highly susceptible to *Blastocystis* infection,^{5,6} whereas research showed that people aged 30–50 years are mostly infected.⁷

The pathogenic potential of *Blastocystis* spp. is still controversial. For many years, it has been suggested that *Blastocystis* spp. are commensal organisms of the human intestine. Recent epidemiologic data demonstrate the

Abstract

Species of the genus *Blastocystis*, which are single-cell, intestinal protozoan parasites of humans and animals, remain mysterious, with unclear clinical and epidemiologic significance. In recent years, many researchers have suggested a possible connection between *Blastocystis* spp. infection and chronic urticaria. In the present article, we review the literature and discuss the possible associations between the clinical symptomatology and pathogenicity of this organism in terms of its subtypes, morphologic forms, genetic diversity, and interactions with other intestinal microbiota.

association of *Blastocystis* spp. with a variety of disorders, including diarrhea, abdominal pain, fatigue, constipation, flatulence, and irritable bowel syndrome (IBS),¹ as well as with extraintestinal manifestations such as skin rashes or urticaria.^{8,9} However, *Blastocystis* spp. have been found in both patients with gastrointestinal symptoms and asymptomatic individuals.¹⁰ The life cycle of *Blastocystis* spp. and its pathogenic aspects remains unclear. Among the major different morphologic forms described to date, namely vacuolar, granular, amoeboid, and cystic, the cyst acts as the infective stage and the amoeboid form plays a more active role in the development of clinical manifestations.⁹ However, little is known about the pathophysiologic mechanism involved in the induction of urticaria.¹¹ The concept of intestinal protozoa as causative agents for cutaneous symptoms is interesting, especially with reference to the elicitation of immunoglobulin E (IgE) allergic responses mediated by parasite molecules.¹²

In this article, we discuss the direct and indirect evidence for a role of *Blastocystis* spp. in causing urticaria, and examine the host microbiota and the subtypes, genetic diversity, and morphologic forms of the parasite.

Classification, life cycle, and pathogenicity of *Blastocystis* spp.

Originally the parasite was considered as an innocuous yeast until evidence collated in the 1970s showed that species of the genus *Blastocystis* were actually protozoa.⁹ Recently, the parasite has been placed within an informal group of organisms, the Stramenopiles, based on molecular studies.¹³ Irikov *et al.*¹⁴ proposed a revision of the current five-kingdom classification and the placing of this organism in a separate sixth kingdom named Chromista. The taxonomy of the various species is still an unresolved challenge. Until recently it was based on the host from which the species was isolated (*Blastocystis hominis* from humans, *Blastocystis ratti* from rats). Modern phylogenetic studies notice human–animal and animal–human transmission and therefore propose to summarize the group as *Blastocystis* species.¹⁵

The life cycle and transmission of the parasite are still under vigorous investigation. Two types of reproduction have been described: asexual reproduction by binary fission, and sexual reproduction by autogamy to form primary cysts.⁴ The cysts are the only transmissible forms of *Blastocystis* spp. and are transmitted by a fecal–oral route.¹⁶ After invasion in intestinal lumen, the cysts develop first into the vacuolar form and then into the multivacuolar or amoeboid form before they reach precyst status and then become either thick-walled or thin-walled cysts that can infect other individuals. *Blastocystis* spp. are strict anaerobes and common inhabitants of the oxygen-poor environment of the gastrointestinal tract in humans and other mammals. The organism's cell contains mitochondrion-related organelles (MROs) such as mitochondrion-like organelles (MLOs), which function like H₂-producing mitochondria or hydrogenosomes.¹⁷ *Blastocystis* MROs contain genomes that have evolved from mitochondrion under oxygen-restricted conditions. The commensal or pathogenic nature of the infection has been subject to controversy for decades. Many case reports, as well as epidemiologic and microbiologic studies, support a pathogenic role of *Blastocystis* spp. in causing intestinal inflammation and symptoms of urticaria,^{1,8} and a higher number of reports describe asymptomatic infections by *Blastocystis* spp.¹⁸ *Blastocystis* spp. are most commonly associated with diarrhea in immunocompromised hosts such as persons infected with HIV and also in homosexuals, travelers, children in day care, animal handlers, people with intellectual disabilities, and people who are under high levels of stress.¹⁹

Blastocystis spp. are speculated to represent a direct or indirect cause of gastrointestinal symptoms including IBS.²⁰ The proposed mechanism in IBS involves a low-grade inflammation caused by ongoing immune activation

induced by *Blastocystis*, which provides a persistent antigenic exposure.²¹ Hussain *et al.*²² showed that IgG antibody levels to *Blastocystis* in patients with IBS were significantly higher than those in asymptomatic controls. In addition to humans, *Blastocystis* spp. also exist in other mammals, such as dogs, cats, pigs, rats, and other primates.^{23,24} A recent study conducted in the Pacific northwest of the USA showed that carriage of *Blastocystis* was not associated with increased risk for gastrointestinal signs in shelter-resident or client-owned dogs and cats.²⁵

Urticaria as symptom of parasite infection

Urticaria represents a heterogeneous group of diseases associated with a large variety of underlying causes. Its clinical presentation is highly variable and can be elicited by a great diversity of factors.²⁶ Parasitic diseases have long been considered as potential causes of urticaria.²⁷ *Blastocystis* spp., *Giardia lamblia*, and some other parasites are reported to be associated with urticaria in a few case reports.^{11,28} Clinical investigations of the link between parasitosis and skin allergic symptoms are very limited, despite the fact that some parasites are among the most potent inducers of IgE production.²⁹ Recent reports suggest that interaction between parasites and host immunity is more complex than previously understood, especially with reference to *Trichuris muris*, *Strongyloides* spp., *Echinococcus* spp., and *Anisakis simplex*.³⁰ Mouse studies on the immune response to *G. lamblia* and *Giardia muris* showed that *Giardia* spp. can express different types of variant surface protein.³¹ Each parasite is able to induce a different mechanism of immune reaction according to its life cycle. Parasites can efficiently manipulate host behavior and their immediate life cycle can also change according to the behavior of the infected host.^{32,33}

In comparison with only 1.1% of healthy controls, 38.8% of patients with chronic urticaria of unknown etiology were found to have protozoan infestations of *Entamoeba coli*, *Blastocystis* spp., or *Giardia* spp.³⁴ There was a four-fold increase in total serum IgE levels in urticaria patients with protozoa in comparison with those without protozoa. A recent retrospective study reported that 11.25% of enrolled patients, mainly women, exhibited skin manifestations associated with *Blastocystis* spp.¹ However, by contrast with helminth infestations, the association between chronic urticaria and protozoa appears to be less clear. In endemic areas where raw or marinated fish is eaten frequently, *A. simplex* hypersensitivity is a common cause of chronic urticaria.³⁵ In a prospective study of 92 children with chronic urticaria aged 4–15 years, 5.4% were found to have parasitic infections, including those of *Blastocystis* spp., *Dientamoeba fragilis*, and *G. lamblia*.³⁶

The possible role of *Blastocystis* spp. in the etiopathogenesis of urticaria

Asymptomatic carriage is a common form of intestinal parasitic infestation worldwide.³⁷ Findings in one study demonstrated that 33 of 54 (61.1%) urticaria patients (chronic and acute urticaria) were positive for *Blastocystis*, whereas only 8.0% of the control group were infested.¹¹ Of these 33 *Blastocystis*-positive patients, 21 had intestinal symptoms (abdominal pain, diarrhea, constipation) and 12 were asymptomatic. After two administrations of treatment with metronidazole, all patients became free of symptoms.¹¹ In another study, which investigated the association of *Blastocystis* with urticaria by determining the genotypic subtypes of parasites in an Egyptian population, the parasite was detected in a significantly higher number of people in the patient group than in the control group.³⁸ A similar study from Saudi Arabia showed that 13.7% of stool samples from patients with chronic urticaria, atopic dermatitis, or pruritus of unknown origin were infected by *Blastocystis* spp. in the absence of other known allergic causes of urticaria.³⁹ Nonetheless, there are some single case reports describing the presence of *Blastocystis* spp. and symptoms of urticaria in which the causal association remains to be determined.^{8,12,37}

In a pathogenic pathway similar to the food-dependent, exercise-induced anaphylactic reaction, it is proposed that aspirin or nonsteroidal anti-inflammatory drugs (NSAIDs) may act as cofactors to *Blastocystis* spp. in causing the urticarial reaction. Other authors have hypothesized an IgE-mediated allergic response, in which parasite antigens induce activation of specific clones of Th2 lymphocytes with production of specific cytokines such as interleukin (IL)-3, IL-4, IL-5, and IL-13, leading to antibody switch and IgE production. An increase in circulating eosinophils, which is often found in patients with parasitic infection, can be another factor related to allergic reaction.^{12,40} Yan *et al.*⁴¹ speculated that ST3 of *Blastocystis* spp. may contribute to the pathogenic potential only when the amoeboid form is present. It has been suggested that the amoeboid form may adhere better to the epithelial cells lining the gut, affecting gut immune homeostasis and resulting in immune responses against antigens on the surface of parasites, with consequent recruitment of inflammatory cells.⁴² Histamine-releasing factors could then be released by immigrant cells, activating mast cells, and basophils, and lead to short-lived urticaria.⁸ Furthermore, the parasite antigen can induce activation of specific clones of Th2 lymphocytes to release cytokines, as well as IgE production and mast cell activation.¹¹ The significant oxidative stress associated with *Blastocystis* spp. infection is currently considered to be a predisposing factor in the pathogenesis of chronic urti-

caria. The ability of the vacuolar form of *Blastocystis* spp. to invade the gut wall may facilitate the absorption of other antigens.⁴³ A recent *in vitro* study demonstrated that *Blastocystis* antigens can induce abundant expression of proinflammatory cytokines in mouse macrophages, including IL-1 β , IL-6, and tumor necrosis factor- α (TNF- α), with activation of ERK, JNK, and p38, the three major groups of mammalian mitogen-activated protein (MAP) kinases that play essential roles in the expression of proinflammatory cytokines. The serine proteases produced by *Blastocystis* were found to play an important role in the induction of ERK activation and proinflammatory cytokine expression by macrophages (Fig. 1).⁴⁴

Influence of the morphologic forms of *Blastocystis* on urticaria induction

Four different major morphologic forms of *Blastocystis* spp. have been described in the literature: the vacuolar, granular, amoeboid, and cystic forms.^{15,45} Other vegetative forms identified include the avacuolar and multivacuolar forms.¹⁵ Certain morphotypes have been found to play a more active role in the development of clinical manifestations in patients infected with *Blastocystis*.⁹ Irregular and polymorphic amoeboid forms with multiple extended pseudopodia were observed predominantly in symptomatic patients.^{9,46} This parasite form is strongly adherent and considered to be associated with higher pathogenicity.²⁰ The amoeboid form was found in 20 of 21 (95.2%) symptomatic urticaria patients, whereas no amoeboid form was found in a control group with *Blastocystis* infection.¹¹ The detection of numerous amoeboid forms in patients with severe urticaria and diarrhea indicates their greater virulence.^{15,46} The demonstration of more than five specimens of *Blastocystis* per high-power field ($\times 400$) in stool samples was found to be associated with certain allergic cutaneous diseases, including urticaria.^{8,21} Whether or not the rarely reported irregular amoeboid form is clinically of pathogenic importance has been the subject of some controversy.^{8,11,46}

Genetic diversity of *Blastocystis* in connection with urticaria

Techniques using small subunit rDNA (SSU rDNA) analysis have detected at least 17 subtypes (STs) of *Blastocystis* which colonize a wide range of hosts including humans, other mammals, birds, reptiles, and insects. In animals, the parasites occur asymptotically or rarely cause gastrointestinal symptoms.⁴⁷ Some STs exhibit host specificity with variable geographic distribution.⁴⁸ There are high prevalences of ST1 and ST2 in North and South America, ST1 and ST3 in Australia, Europe, and Southeast Asia,

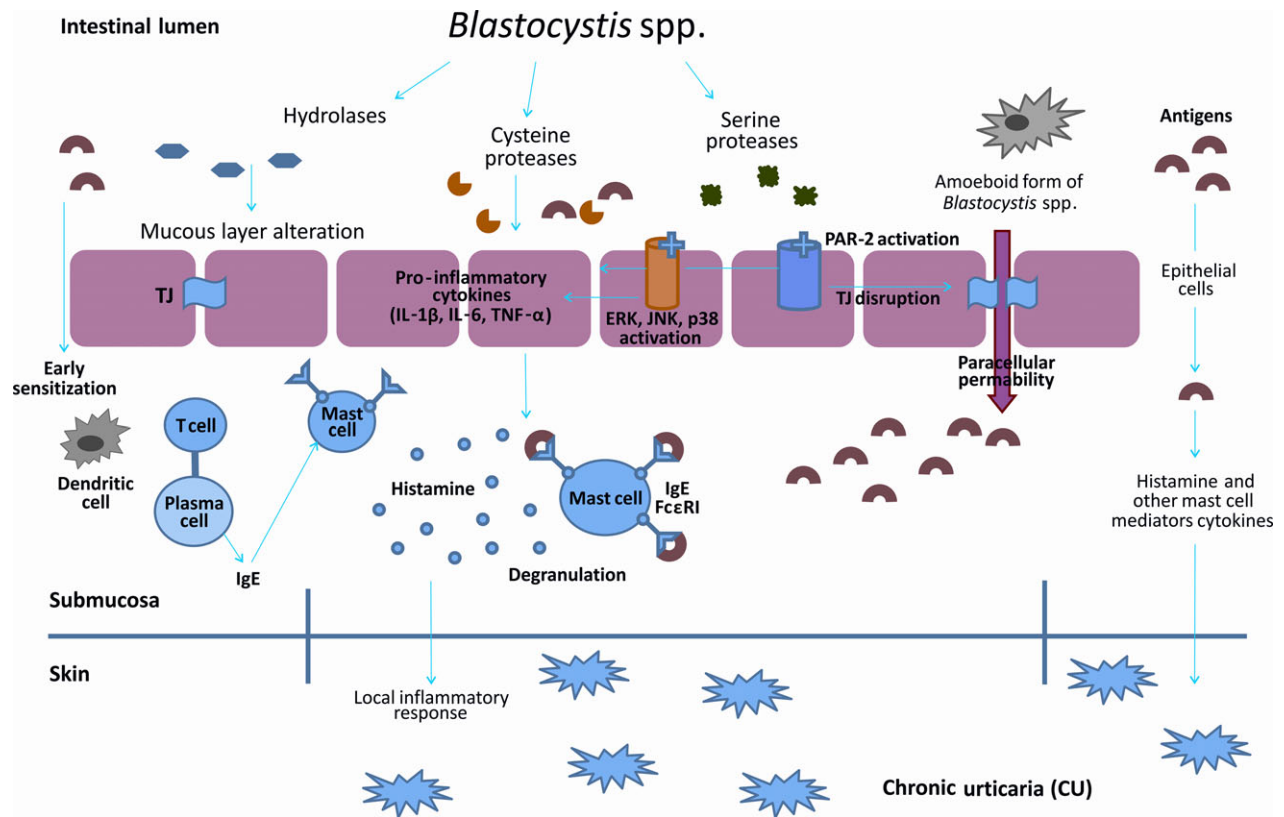


Figure 1 Hypothetical model of the pathogenic roles of *Blastocystis* spp. ST2, ST3, ST4, ST7 in chronic urticaria

and ST4 in Europe. Humans are colonized mainly by ST1–4, but, depending on the region and country in question, infections by ST5–9 are also observed.^{48,49} To date ST10–17 have not been found in humans.^{49,50} Gender differences in prevalences of *Blastocystis* subtypes have been reported. In Sweden, for instance, ST3 was found to be more common in males than in females, and in females ST4 was almost as common as ST3. However, the difference in the relative frequency of ST4 between men and women was statistically insignificant.⁵¹

In the recent literature, researchers have debated the correlation between distinct *Blastocystis* subtypes and their pathogenic potentials. In 1997, Clark was the first to suggest that different subtypes with different pathologic potentials may exist.⁵² Consequently in 2001, Kanada *et al.*⁵³ indicated that STs 1, 2, and 4 may be responsible for gastrointestinal symptoms. In 2012, Poirier *et al.*⁵⁴ suggested ST7 is correlated with IBS. Puthia *et al.*⁵⁵ have shown that in rat epithelial cells ST4 can induce apoptosis in a contact-independent manner to increase epithelial permeability. ST7 is likely to use hydrolases to attack host tissue for its nutrient supply.⁵⁴ In 2006, Yan *et al.*⁴¹ demonstrated only ST1 in a group of symptomatic patients, a finding that was confirmed in

2013 by El Safadi *et al.*,⁵⁶ who demonstrated that ST1 was associated with an elevated pathogenicity. The pathogenicity of ST4 was also hypothesized by Stensvold *et al.*⁵⁷ in a short report in 2011. Explanations for pathogenicity may include intra-subtype differences in *Blastocystis* protease activity.⁵⁸ The cysteine proteases produced by ST4 and ST7 were revealed to be able to cleave human IgA *in vitro*,⁵⁹ a capacity that was suggested to represent a mechanism for parasite survival and colonization in gut, and for immune evasion, as well as virulence and cell cycle regulation.⁶⁰ Enzymes can also modulate inflammatory IL-8 production⁵⁵ and are able to increase the permeability of intestinal epithelial cells.⁶¹

By contrast, several studies showed no distinct differences in STs between isolates from symptomatic and asymptomatic groups of individuals with gastrointestinal disorders.⁶² In addition to nonspecific gastrointestinal symptoms, associations between the parasite subtypes and certain cutaneous disorders have been observed,^{11,37,38,63} such as the presence of *Blastocystis* spp. ST2,³⁷ ST3 (especially the amoeboid form), and ST4 in patients with acute or chronic urticaria.^{8,11,12,38,64} To date, little is known about why certain subtypes cause urticaria only in certain groups of patients. Probable explanations may

include intra-subtype differences in protease activity,⁵⁸ variations in intestinal microbiota among individual hosts, and the symbiotic role of viruses associated with *Blastocystis*,⁶⁵ which can interact to mediate host colonization and *Blastocystis* virulence. In this sense, the presence of gut microbiota seems to be essential for the pathogenic expression of enteric protozoa such as *Blastocystis* spp., *Entamoeba histolytica*, and different species of *Eimeria* and *Giardia*. The hypothesis of protozoan axenization by some bacteria has been proposed. There is evidence that interactions of *E. histolytica* of low pathogenicity with a variety of Gram-negative bacteria, mainly *Escherichia coli* or *Shigella dysenteriae* strains, may be responsible for the increase in amoebic virulence.⁶⁶ Oxidative stress may be another reason why certain *Blastocystis* subtypes are more virulent. *Blastocystis* infection has been reported to correlate with significant oxidative bursts, leading to oxidative stress.⁶⁷ Some subtypes may induce higher concentrations of oxidative stress and precipitate skin reactions such as urticaria. It would be highly interesting to examine and compare the pathogenic subtypes of *Blastocystis* spp. in patients with chronic urticaria from different countries and populations. A recent study showed that live *Blastocystis* spp. parasites and whole-cell lysate alone did not activate toll-like receptors (TLRs) in the human TLR reporter monocytic cell line (THP1-Blue), whereas live ST4-WR1 parasites inhibited the lipopolysaccharide (LPS)-mediated NF- κ B activation. By contrast, whole-cell lysates of ST7-B and ST4-WR1 induced pleiotropic modulation of ligand-specific TLR-2 and TLR-4 activation, with an augmenting effect of ST7-B on LPS-mediated NF- κ B activation.⁶⁸ A higher caspase-like activity of *Blastocystis* spp. ST3 was found in isolates from individuals with gastrointestinal symptoms.⁶⁹

Treatment

As a result of the controversy over the potential pathogenicity of *Blastocystis* spp. and the self-limiting nature of most symptoms, the treatment of this disease is equivocal.⁷⁰ It has also been proposed that the different STs of *Blastocystis* have varying susceptibility to antimicrobial drugs. Treatment with metronidazole at 250–750 mg three times per day for 10 days is the most frequently prescribed regimen. There have been reports of treatment failure or resistance to metronidazole, especially in the cyst form. In these cases, a combination of metronidazole with other drugs, such as paromomycin or trimethoprim-sulfamethoxazole, is required. In a case report on the simultaneous occurrence of gastrointestinal illness and generalized chronic urticaria possibly linked with *Blastocystis* spp. ST2 infection, the eradication of the parasite and permanent resolution of clinical symptoms were finally achieved

with the combined use of metronidazole and paromomycin.³⁷ No standard therapy for the eradication of *Blastocystis* has yet been established, and well-controlled clinical studies demonstrating the benefit of eradication in infected patients with chronic urticaria are so far lacking.

Conclusions

Although *Blastocystis* spp. were identified over a century ago, their physiologic role and clinical significance remain enigmatic. Genomic studies have identified various putative genes responsible for the physiology, virulence, and intra-subtype genetic diversity of the organism. Further characterization of the microbial and viral communities associated with specific *Blastocystis* genotypes is important to understanding their role in mediating host colonization and in modulating the severity of disease.

Blastocystis infection has been controversially discussed as a causative factor in urticaria of unknown etiology. There is some evidence to show that the amoeboid morphologic form and genetic subtypes ST2, ST3, and ST4 may be more closely associated with chronic urticaria. Further studies that compare patients with chronic urticaria with control groups from the general population in different regions for different pathogenic subtypes, concomitant viral or bacterial infections, and how cofactors such as the intake of certain medications or nutrition influence pathogenicity, are required. Well-designed interventional studies that observe alterations in urticarial activity before and after the effective treatment of patients infected with *Blastocystis* spp. may help to clarify the pathogenic role of the protozoa.

Open challenges for researchers may include ascertaining the correlation between morphology and molecular biology, the interplay and homeostasis with other intestinal microorganisms among the intestinal microbiota, and the host-parasite immune interaction, and resolving questions of potential endosymbiosis in pathogenicity. Human *Blastocystis* infestation can serve as a good model for the study of human-parasite relations and interactions.

Questions (answers provided after references)

- 1 Where can *Blastocystis* spp. parasites be found?
 - a) In the blood
 - b) Under the skin
 - c) In the gastrointestinal tract
 - d) (b) and (c)
- 2 The invasive stage of *Blastocystis* spp. for humans is:
 - a) The vacuolar form
 - b) A thick-walled cyst
 - c) The amoeboid form
 - d) The granular form

3 Humans are colonized mostly by *Blastocystis* spp. subtypes:

- a) ST₁ - ST₄
- b) ST₅ - ST₉
- c) ST₁₀ - ST₁₃
- d) ST₁₄ - ST₁₇

4 How many subtypes of *Blastocystis* spp. have been detected so far?

- a) 9
- b) 14
- c) 17
- d) 21

5 The most common subtype identified worldwide is:

- a) ST₁
- b) ST₂
- c) ST₃
- d) ST₄

6 Which morphologic form of certain subtypes (e.g. ST₃) of *Blastocystis* spp. is most likely to be connected with urticaria symptoms?

- a) The vacuolar form
- b) The amoeboid form
- c) The multivacuolar form
- d) The cyst

7 *Blastocystis* spp. are anaerobes containing mitochondrion-related organelles (MROs) such as:

- a) Mitochondrion-like organelles (MLOs)
- b) H₂-producing mitochondrion
- c) Hydrogenosomes
- d) Mitosomes

8 The pathogenesis of *Blastocystis* spp. in inducing urticaria may include:

- a) Aspirin or NSAIDs may act as cofactors with *Blastocystis* spp.
- b) Oxidative stress associated with *Blastocystis* spp.
- c) Parasite antigens induce the activation of specific clones of Th₂ lymphocytes with the production of specific cytokines
- d) All of the above

9 Which enzymes produced by *Blastocystis* were found to play an important role in the induction of ERK activation?:

- a) hydrolases
- b) cysteine proteases
- c) oxydases
- d) serine proteases

10 The prevalence of *Blastocystis* spp. in immunocompromised people such as HIV-infected individuals is estimated to be:

- a) 30–50% in developing countries
- b) 1.5–10.0% in developed countries
- c) 30–38% in developed countries
- d) None of the above

References

- 1 Bálint A, Dóczi I, Bereczki L, *et al.* Do not forget the stool examination! Cutaneous and gastrointestinal manifestations of *Blastocystis* sp. infection. *Parasitol Res* 2014; 113: 1585–1590.
- 2 Wawrzyniak I, Poirier P, Viscogliosi E, *et al.* *Blastocystis*, an unrecognized parasite: an overview of pathogenesis and diagnosis. *Ther Adv Infect Dis* 2013; 1: 167–178.
- 3 Albrecht H, Stellbrink HJ, Koperski K, *et al.* *Blastocystis hominis* in human immunodeficiency virus-related diarrhea. *Scand J Gastroenterol* 1995; 30: 909–914.
- 4 Basak S, Rajurkar MN, Mallick SK. Detection of *Blastocystis hominis*: a controversial human pathogen. *Parasitol Res* 2014; 113: 261–265.
- 5 Tan KSW, Mirza H, Teo JDW, *et al.* Current views on the clinical relevance of *Blastocystis* spp. *Curr Infect Dis Rep* 2010; 12: 28–35.
- 6 El Safadi D, Gaayeb L, Meloni D, *et al.* Children of Senegal River Basin show the highest prevalence of *Blastocystis* sp. ever observed worldwide. *BMC Infect Dis* 2014; 14: 164–174.
- 7 Lu CTL, Sung YJ. Epidemiology of *Blastocystis hominis* and other intestinal parasites among the immigrant population in northeastern Taiwan by routine physical examination for resistance approval. *J Microbiol Immunol Infect* 2009; 42: 505–509.
- 8 Katsarou-Katsari A, Vassalos CM, Tzanetou K, *et al.* Acute urticaria associated with amoeboid forms of *Blastocystis* sp. subtype 3. *Acta Derm Venereol* 2007; 88: 80–81.
- 9 Arutchelvan R, Suresh KG. Amoebic forms of *Blastocystis* spp. – evidence for a pathogenic role. *Parasit Vectors* 2013; 6: 295–303.
- 10 Tan KS, Singh M, Yap EH. Recent advances in *Blastocystis hominis* research: hot spots in terra incognita. *Int J Parasitol* 2002; 32: 789–804.
- 11 Hameed DM, Hussanin OM, Zuel-Fakar NM. Association of *Blastocystis hominis* genetic subtypes with urticaria. *Parasitol Res* 2011; 108: 553–560.
- 12 Pasqui AL, Savini E, Saletti E, *et al.* Chronic urticaria and *Blastocystis hominis* infection. A case report. *Eur Rev Med Pharmacol Sci* 2004; 8: 117–120.
- 13 Silberman JD, Sogin ML, Leipe DD, *et al.* Human parasite finds taxonomic home. *Nature* 1996; 380: 398.
- 14 Irikov OA, Antokhin AI, Romanov YA. Study of the dynamics of *Blastocystis hominis* reproduction *in vitro*. *Bull Exp Biol Med* 2009; 148: 99–102.
- 15 Parija SC, Jeremiah SS. *Blastocystis*: taxonomy, biology and virulence. *Trop Parasitol* 2013; 3: 17–25.
- 16 Yoshikawa H, Yoshida K, Nakajima A, *et al.* Fecal–oral transmission of the cyst form of *Blastocystis hominis* in rats. *Parasitol Res* 2004; 94: 391–396.
- 17 Makiuchi T, Nozaki T. Highly divergent mitochondrion-related organelles in anaerobic parasitic protozoa. *Biochimie* 2014; 100: 3–17.

- 18 Raś-Noryńska M, Białkowska J, Sokół R, *et al.* Parasitological stool examination from children without the typical symptoms of parasitic disease. *Przegl Epidemiol* 2011; 65: 599–603.
- 19 Chandramathi S, Suresh K, Sivanandam S, *et al.* Stress exacerbates infectivity and pathogenicity of *Blastocystis hominis*: *in vitro* and *in vivo* evidences. *PLoS One* 2014; 9: e94567.
- 20 Scanlan PD. *Blastocystis*: past pitfalls and future perspectives. *Trends Parasitol* 2012; 28: 327–334.
- 21 Coyle C, Varughese J, Weiss LM, *et al.* *Blastocystis*: to treat or not to treat. *Clin Infect Dis* 2012; 54: 105–110.
- 22 Hussain R, Jaferi W, Zuberi S, *et al.* Significantly increased IgG2 subclass antibody levels to *Blastocystis hominis* to patients with irritable bowel syndrome. *Am J Trop Med Hyg* 1997; 56: 301–306.
- 23 Maesano G, Capasso M, Ianniello D, *et al.* Parasitic infections detected by FLOTAC in zoo mammals from Warsaw, Poland. *Acta Parasitol* 2014; 59: 343–353.
- 24 Ramírez JD, Sánchez LV, Bautista DC, *et al.* *Blastocystis* subtypes detected in humans and animals from Columbia. *Infect Genet Evol* 2014; 22: 223–228.
- 25 Ruaux CG, Stang BV. Prevalence of *Blastocystis* in shelter-resident and client-owned companion animals in the US Pacific Northwest. *PLoS One* 2014; 9: e107496.
- 26 Zuberbier T, Asero R, Bindslev-Jensen C, *et al.* EAACI/GA²LEN/EDF/WAO guideline: definition, classification and diagnosis of urticaria. *Allergy* 2009; 64: 1417–1426.
- 27 De Gentile L, Grandiere-Perez L, Chabasse D. Urticaria and parasites. *Allerg Immunol (Paris)* 1999; 31: 288–291.
- 28 Nenoff P, Domula E, Willing U, *et al.* *Giardia lamblia* – cause of urticaria and pruritus or accidental association? *Hautarzt* 2006; 57: 518–520.
- 29 Lynch NR, Hagel IA, Palenque ME, *et al.* Relationship between helminthic infection and IgE response in atopic and nonatopic children in a tropical environment. *J Allergy Clin Immunol* 1998; 101: 217–221.
- 30 Bakiri AH, Mingomataj EÇ. Urticaria as symptom of parasite migration through the biological barriers. *Open Allergy J* 2011; 4: 1–7.
- 31 Von Allmen N, Bienz M, Hemphill A, *et al.* Experimental infections of neonatal mice with cysts of *Giardia lamblia* clone GS/M-83-H7 are associated with an antigenic reset of the parasite. *Infect Immun* 2004; 72: 4763–4771.
- 32 Sánchez-Monsálvez I, de Armas-Serrá C, Bernardina W, *et al.* Altered autonomic control in rat intestine due to both infection with *Anisakis simplex* and incubation with the parasites crude extract. *Dig Dis Sci* 2003; 48: 234–252.
- 33 Dobson AP. The population biology of parasite-induced changes in host behavior. *Q Rev Biol* 1988; 63: 139–165.
- 34 Farthing MJG. Immune response-mediated pathology in human intestinal parasitic infection. *Parasite Immunol* 2003; 25: 247–257.
- 35 Ventura MT, Napolitano S, Menga R, *et al.* *Anisakis simplex* hypersensitivity is associated with chronic urticaria in endemic areas. *Int Arch Allergy Immunol* 2013; 160: 297–300.
- 36 Chansakulporn S, Pongpreuksa S, Sangacharoenkit P, *et al.* The natural history of chronic urticaria in childhood: a prospective study. *J Am Acad Dermatol* 2014; 71: 663–668.
- 37 Vogelberg C, Stensvold CR, Monecke S, *et al.* *Blastocystis* sp. subtype 2 detection during recurrence of gastrointestinal and urticarial symptoms. *Parasitol Int* 2010; 59: 469–471.
- 38 Zuel-Fakkar NM, Abdel Hameed DM, Hassanint OM. Study of *Blastocystis hominis* isolates in urticaria: a case–control study. *Clin Exp Dermatol* 2011; 38: 908–910.
- 39 Zagloul DA, Khodari YA, Othman RAM, *et al.* Prevalence of intestinal parasites and bacteria among food handlers in a tertiary care hospital. *Niger Med J* 2011; 52: 266–270.
- 40 Demirci M, Yildirim M, Aridogan BC, *et al.* Tissue parasites in patients with chronic urticaria. *J Dermatol* 2003; 30: 777–811.
- 41 Yan Y, Su S, Lai R, *et al.* Genetic variability of *Blastocystis hominis* isolates in China. *Parasitol Res* 2006; 99: 597–601.
- 42 Valsecchi R, Leghissa P, Greco V. Cutaneous lesions in *Blastocystis hominis* infection. *Acta Derm Venereol* 2004; 84: 322–323.
- 43 Raho G, Cassano N, D'Argento V, *et al.* Over-expression of Mn-superoxide dismutase as a marker of oxidative stress in lesional skin of chronic idiopathic urticaria. *Clin Exp Dermatol* 2003; 28: 318–320.
- 44 Lim MX, Png CW, Tay CY, *et al.* Differential regulation of proinflammatory cytokine expression by mitogen-activated protein kinases in macrophages in response to intestinal parasite infection. *Infect Immun* 2014; 82: 4789–4801.
- 45 Clark CG, van der Giezen M, Afellani MA, *et al.* Recent development in *Blastocystis* research. *Adv Parasitol* 2013; 82: 1–32.
- 46 Tan TC, Suresh KG. Predominance of amoeboid forms of *Blastocystis hominis* in isolates from symptomatic patients. *Parasitol Res* 2006; 98: 189–193.
- 47 Alfellani MA, Taner-Mulla D, Jacob AS, *et al.* Genetic diversity of *Blastocystis* in livestock and zoo animals. *Protist* 2013; 164: 497–509.
- 48 Alfellani MA, Stensvold CR, Vidal-Lapiedra A, *et al.* Variable geographic distribution of *Blastocystis* subtypes and its potential implications. *Acta Trop* 2013; 116: 11–18.
- 49 Stensvold CR, Suresh GK, Tan KS, *et al.* Terminology for *Blastocystis* subtypes – a consensus. *Trends Parasitol* 2007; 23: 93–96.
- 50 Parkar U, Traub RJ, Vitali S, *et al.* Molecular characterization of *Blastocystis* isolates from zoo animals and their animal-keepers. *Vet Parasitol* 2010; 169: 8–17.

- 51 Forsell J, Granlund M, Stensvold CR, *et al.* Subtype analysis of *Blastocystis* isolates in Swedish patients. *Eur J Clin Microbiol Infect Dis* 2012; 31: 1689–1696.
- 52 Clark CG. Extensive genetic diversity in *Blastocystis hominis*. *Mol Biochem Parasitol* 1997; 87: 79–83.
- 53 Kaneda Y, Horiki N, Cheng XJ, *et al.* Ribodemes of *Blastocystis hominis* isolated in Japan. *Am J Trop Med Hyg* 2001; 65: 393–396.
- 54 Poirier P, Wawrzyniak I, Vivarès CP, *et al.* New insights into *Blastocystis* spp.: a potential link with the irritable bowel syndrome. *PLoS Pathog* 2012; 8: e1002545.
- 55 Puthia MK, Lu J, Tan KSW. *Blastocystis ratti* contains cysteine proteases that mediate interleukin-8 response from human intestinal epithelial cells in an NF- κ B-dependent manner. *Eukaryot Cell* 2008; 7: 435–443.
- 56 El Safadi D, Meloni D, Poirier P, *et al.* Molecular epidemiology of *Blastocystis* in Lebanon and correlation between subtype 1 and gastrointestinal symptoms. *Am J Trop Med Hyg* 2013; 88: 1203–1206.
- 57 Stensvold CR, Christiansen DB, Olsen KE, *et al.* *Blastocystis* sp. subtype 4 is common in Danish *Blastocystis*-positive patients presenting with acute diarrhea. *Am J Trop Med Hyg* 2011; 84: 883–885.
- 58 Abdel-Hameed DM, Hassanin OM. Protease activity of *Blastocystis hominis* subtype 3 in symptomatic and asymptomatic patients. *Parasitol Res* 2011; 109: 321–327.
- 59 Mirza H, Tan KSW. *Blastocystis* exhibits inter- and intra-subtype variation in cysteine protease activity. *Parasitol Res* 2009; 104: 355–361.
- 60 Puthia MK, Vaithilingam A, Lu J, *et al.* Degradation of human secretory immunoglobulin A by *Blastocystis*. *Parasitol Res* 2005; 97: 386–389.
- 61 Wawrzyniak I, Texier C, Poirier P, *et al.* Characterization of two cysteine proteases secreted by *Blastocystis* ST7, a human intestinal parasite. *Parasitol Int* 2012; 61: 437–442.
- 62 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: 22–29.
- 63 Verma R, Delfanian K. *Blastocystis hominis* associated acute urticaria. *Am J Med Sci* 2013; 346: 80–81.
- 64 Vassalos CM, Spanakos G, Vassalou E, *et al.* Differences in clinical significance and morphologic features of *Blastocystis* sp. subtype 3. *Am J Clin Pathol* 2010; 133: 251–258.
- 65 Teow WL, Ho LC, Ng GC, *et al.* Virus-like particles in a *Blastocystis* species from the sea snake *Lapemi hardwickii*. *Int J Parasitol* 1992; 22: 1029–1032.
- 66 Berrilli F, Di Cave D, Cavallero S, *et al.* Interactions between parasites and microbial communities in the human gut. *Front Cell Infect Microbiol* 2012; 2: 141.
- 67 Chandramathi S, Suresh K, Shuba S, *et al.* High levels of oxidative stress in rats infected with *Blastocystis hominis*. *Parasitology* 2010; 137: 605–611.
- 68 Teo JD, MacAry PA, Tan KSW. Pleiotropic effects of *Blastocystis* spp. subtypes 4 and 7 on ligand-specific toll-like receptor signaling and NF- κ B activation in a human monocyte cell line. *PLoS One* 2014; 9: e89036.
- 69 Balakrishnan DD, Kumar SG. Higher caspase-like activity in symptomatic isolates of *Blastocystis* spp. *Parasit Vectors* 2014; 7: 219.
- 70 Roberts T, Stark D, Harkness J, *et al.* Update on the pathogenic potential and treatment options for *Blastocystis* sp. *Gut Pathog* 2014; 28: 6–17.

Answer key

1. c
2. b
3. a
4. c
5. c
6. b
7. a
8. d
9. d
10. c