Blastocystis, an unrecognized parasite: an overview of pathogenesis and diagnosis

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***Abstract*:** *Blastocystis* sp. is among the few enteric parasites with a prevalence that often exceeds 5% in the general population of industrialized countries and can reach 30–60% in developing countries. This parasite is frequently found in people who are immunocompromised (patients with human immunodeficiency virus/acquired immunodeficiency syndrome or cancer) and a higher risk of *Blastocystis* sp. infection has been found in people with close animal contact. Such prevalence in the human population and the zoonotic potential naturally raise questions about the impact of these parasites on public health and has increased interest in this area. Recent *in vitro* and *in vivo* studies have shed new light on the pathogenic power of this parasite, suggesting that *Blastocystis* sp. infection is associated with a variety of gastrointestinal disorders, may play a significant role in irritable bowel syndrome, and may be linked with cutaneous lesions (urticaria). Despite recent significant advances in the knowledge of the extensive genetic diversity of this species, the identification of extracellular proteases as virulence factors and the publication of one isolate genome, many aspects of the biology of *Blastocystis* sp. remain poorly investigated. In this review, we investigate several biological aspects of *Blastocystis* sp. (diversity and epidemiology, diagnosis tools and pathophysiology). These data pave the way for the following challenges concerning *Blastocystis* sp. research: deciphering key biological mechanisms and pathways of this parasite and clarification of its clinical impact in humans.

***Keywords*:** Blastocystis, pathogenesis, diagnosis, subtypes, gut

Blastocystis sp. is an anaerobic intestinal parasite of humans and a wide range of animals [Stenzel and Boreham, 1996; Tan, 2004, 2008]. This parasite belongs to the stramenopiles, a complex and heterogeneous evolutionary assemblage of heterotrophic and photosynthetic protozoa [Silberman *et al.* 1996; Arisue *et al.* 2002; Riisberg *et al.* 2009]. Interestingly, *Blastocystis* sp. is the only stramenopiles known to cause infection in humans. Four major morphological forms of *Blastocystis* sp. were described in stools or *in vitro* cultures: vacuolar (Figure 1), granular, amoeboid and cyst forms [Stenzel and Boreham, 1996; Tan, 2008; Suresh *et al.* 2009]. The two former forms are the most easily recognizable and frequently observed in laboratory culture and stool samples. Although rarely reported, the irregular amoeboid form was postulated to play a role in pathogenesis [Tan *et al.* 2006, Katsarou-Katsari *et al.* 2008 but this hypothesis was contradicted [Souppart *et al.* 2009]. Experimental infectivity studies in animals with the cyst form demonstrated that the water-resistant and environmentally resistant infective cysts represented the transmissible stage of the parasite [Suresh *et al.* 1993, 2005; Moe *et al.* 1996; Yoshikawa *et al.* 2004]. Taking into account these observations and those of *in vitro* encystations studies [Suresh *et al.* 1993; Villar *et al.* 1998; Chen *et al.* 1999], a life cycle for *Blastocystis* sp. was proposed with the cyst as the infectious stage [Tan, 2008]. Upon ingestion of cysts, the parasite undergoes excystation in the large intestine and develops into vacuolar forms. These vacuolar forms divide by binary fission and may develop into amoeboid or granular forms. Then, encystation may occur while crossing along the colon before cyst excretion in the faeces [Tan, 2008]. Therefore, *Blastocystis* sp. lives in oxygen-poor environments and is

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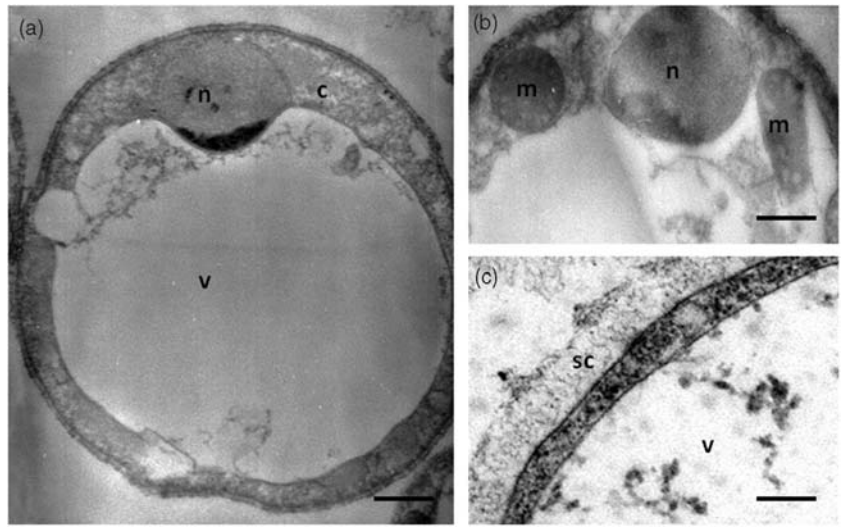

Figure 1. Vacuolar form of *in vitro* cultivated *Blastocystis* sp. viewed under transmission electron microscopy (a). This form is spherical with a large central vacuole (v) and a thin peripheral band of cytoplasm (c) around the vacuole. (b) The cytoplasm contains the nucleus (n) and mitochondrion-like organelles (m). (c) *Blastocystis* sp. cell is surrounded by a surface coat (sc). Bars, 2 μm for (a) and 500 nm for (b) and (c).

characterized by the presence of some double-membrane surrounded organelles called mitochondria-like organelles (MLOs) (Figure 1) [Nasirudeen and Tan, 2004]. Sequencing of complete circular DNA in the MLO of these parasites by different authors [Perez-Brocal and Clark, 2008; Stechmann *et al.* 2008; Wawrzyniak *et al.* 2008] showed that the cellular compartments have the metabolic properties of both aerobic and anaerobic mitochondria. The nuclear genome of *Blastocystis* sp. was recently sequenced and revealed a compact nature and an intriguing architecture [Denoeud *et al.* 2011]. This genome has also provided clues to decipher the genetic diversity and pathogenesis of this parasite.

Summary of diagnosis tools

The most common approaches for the detection of *Blastocystis* sp. [Stenzel and Boreham, 1996; Stensvold *et al.* 2007a; Tan, 2008] consist of direct smear examination by light microscopic or xenic *in vitro* culture. However, given the occurrence of different forms of *Blastocystis* sp. (especially the hardly recognizable cystic form), deterioration caused by environmental conditions or drug treatment and the fact that *Blastocystis* sp. can be confused with other microorganisms, this method seems to have largely underestimated this parasite in the context of enteric parasite diagnosis. Moreover, culturing this parasite is time consuming and can bias subsequent genotyping due to the different ability of isolates to grow in selective medium [Roberts *et al.* 2011]. Therefore, to overcome these limitations, several molecular polymerase chain reaction (PCR)-based diagnostic approaches using faeces directly or after culture of faecal specimens have been described [Santin *et al.* 2011; Abe *et al.* 2003a; 2003b; Yoshikawa *et al.* 2004; Scicluna *et al.* 2006; Stensvold *et al.* 2006; Roberts *et al.* 2011]. Studies comparing the relative performances of these various diagnostic methods [Suresh and Smith, 2004; Parkar *et al.* 2007; Stensvold *et al.* 2007a] showed that the PCR approach was as sensitive as the culture approach. More recently, Poirier and colleagues reported a highly sensitive real-time quantitative PCR (qPCR) assay developed to detect *Blastocystis* sp. in stool samples [Poirier *et al.* 2011]. This assay targets a region of the small subunit rRNA gene (SSU-rDNA) and allowed subtyping of isolates by direct sequencing of qPCR products. Moreover, Stensvold and colleagues developed a qPCR on stool samples using the SSU-rDNA marker, including an internal process control enabling the evaluation of potential PCR inhibitors [Stensvold *et al.* 2012]. This approach had the advantage of increasing the specificity and avoiding the amplification of false positives. Therefore, currently, SSU-rDNA genotyping is the method of choice for diagnosis [Poirier *et al.* 2011; Stensvold *et al.* 2012].

Blastocystis sp., a highly prevalent and divergent parasite

Numerous epidemiological surveys carried out in different countries identify *Blastocystis* sp. as the most common eukaryotic parasite reported in human faecal samples [Tan, 2008]. Overall the prevalence of *Blastocystis* sp. is higher than those of other intestinal protozoan parasites such as *Giardia*, *Entamoeba* and *Cryptosporidium*, as observed in France [The ANOFEL Cryptosporidium National Network, 2010] and the United States [Boorom *et al.* 2008]. An increasing trend in identification of *Blastocystis* sp. suggests that it is an emerging parasite with a worldwide distribution [WHO, 2008]. Prevalence varies widely from country to country and within communities of the same country [Tan, 2008; Souppart *et al.* 2009; Alfellani *et al.* 2013]. Developing countries have a higher prevalence of the parasite than industrialized countries. This difference can be explained by poor hygiene practices, close animal contact and consumption of contaminated food or water [Li *et al.* 2007; Leelayoova *et al.* 2008; Eroglu and Koltas, 2010; Baldursson and Karanis, 2011; Ithoi *et al.* 2011; Lee *et al.* 2012; Nagel *et al.* 2012] since the faecal–oral route is considered to be the main mode of transmission of this parasite [Yoshikawa *et al.* 2004]. Prevalence is low in developed countries such as Japan (0.5–1%) [Hirata *et al.* 2007] and Singapore (3.3%) [Wong *et al.* 2008] and high in developing nations including Brazil (40.9%) [Aguiar *et al.* 2007], Egypt (33.3%) [Rayan *et al.* 2007] and Indonesia (60%) [Pegelow *et al.* 1997]. In some countries, the prevalence can be rather variable and ranges from 1.9% to 32.6% in China [Li *et al.* 2007] and from 0.9% to 45.2% in Thailand [Saksirisampant *et al.* 2003, 2006], depending on the subpopulation studied. Such variations within the same country could reflect true differences between communities or the use of different diagnostic approaches [Stensvold, 2013].

Blastocystis sp. isolates from humans and other animals have been reported to be morphologically indistinguishable. However, extensive genetic variation among numerous *Blastocystis* sp. isolates from both humans and animals was mainly observed by PCR restriction fragment length polymorphism [Hoevers *et al.* 2000; Kaneda *et al.* 2001; Abe *et al.* 2003b; Rivera and Tan, 2005] and PCR using sequenced-tagged site primers [Yoshikawa *et al.* 2004; Yan *et al.* 2006; Li *et al.* 2007; Yoshikawa *et al.* 2009]. This considerable genetic divergence among isolates was subsequently confirmed by molecular phylogenies, mainly inferred from SSU-rDNA sequences [Arisue *et al.* 2003; Abe, 2004; Noël *et al.* 2005; Scicluna *et al.* 2006; Jones *et al.* 2008; Ozyurt *et al.* 2008; Souppart *et al.* 2009, 2010; Stensvold *et al.* 2009; Parkar *et al.* 2010; Whipps *et al.* 2010]. From these molecular analyses, a consensus on *Blastocystis* sp. terminology was proposed in an international collaborative project [Stensvold *et al.* 2007b]. In this new classification, all isolates should be designated *Blastocystis* sp. and assigned to one of the described subtypes (STs). Each of the STs exhibiting sufficient genetic diversity should be classified as separate species. Thereafter, a new ST was identified from primates and artiodactyls and designated as *Blastocystis* sp. ST10 [Stensvold *et al.* 2009]. More recently, seven additional STs (ST11–17) were identified from zoo animals [Parkar *et al.* 2010; Fayer *et al.* 2012; Alfellani *et al.* 2013; Roberts *et al.* 2013].

Most of the samples included in published epidemiological surveys represented simple infections. Mixed infections (i.e. infections by at least two different STs) probably result from multiple sources of infection. The prevalence of these infections is similar in different countries and roughly comprised between 2.6% and 14.3% [Yan *et al.* 2006; Li *et al.* 2007; Dogruman-Al *et al.* 2008; Souppart *et al.* 2009; Meloni *et al.* 2012]. However, the true distribution of mixed infections remains difficult to ascertain in a particular individual and is likely underestimated as this depends on the method employed for subtyping [Meloni *et al.* 2012]. In almost all the studies reported so far, including those in Europe [Souppart *et al.* 2009; Meloni *et al.* 2011; Forsell *et al.* 2012; Alfellani *et al.* 2013], Africa [Souppart *et al.* 2010; Alfellani *et al.* 2013], Oceania [Roberts *et al.* 2013], Asia [Jantermtor *et al.* 2013] and the Middle East [Moosavi *et al.* 2012], a large majority of human infections with *Blastocystis* sp. were attributable to ST3 isolates. Only a few exceptions showed a higher prevalence of ST4 in Spain [Dominguez-Marquez *et al.* 2009], Denmark [Stensvold *et al.* 2011] and in a region of France [Poirier *et al.* 2011], and of ST1 in Thailand [Thathaisong *et al.* 2013]. Collectively, these studies suggest that the dominant ST3 was the only ST of human origin as was first proposed by Noël and colleagues [Noël *et al.* 2005], even if it can also be found in some

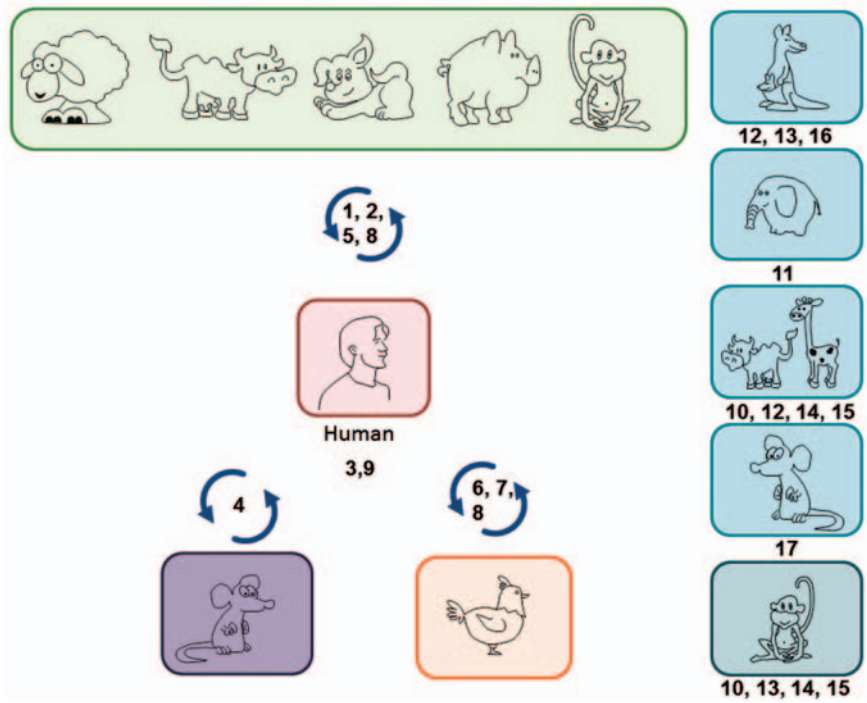

Figure 2. *Blastocystis* sp. subtypes (STs 1–9) with various host specificities. Humans can be infected by nine STs, some being mainly found in humans (ST3 and ST9). ST1, 2, 5 and 8 are found both in human and mammalian isolates (primate, pig, human, cattle and pig), while ST4 is also present among rodent isolates, and ST6, 7 and 8 among avian isolates. Some STs are exclusively found in animals (ST10–17). ST10 and 15 are present among *Artiodactyla* and nonhuman primates, ST11 among *Proboscidea*, ST12 among *Artiodactyla* and marsupials, ST13 among nonhuman primates and marsupials, ST14 among *Artiodactyla*, ST16 among marsupials and ST17 among rodents.

animals. Consequently, the predominance of this ST might be mainly explained by large-scale human to human transmission [Yoshikawa *et al.* 2000]. Proportions of ST1 to ST4 differ between locations. ST6 and ST7 are common in Asia but rarely observed in European countries. ST5, ST8 and ST9 are found episodically in humans [Yan *et al.* 2007; Tan, 2008; Stensvold *et al.* 2009; Tan *et al.* 2010; Moosavi *et al.* 2012].

Comparison of SSU-rDNA gene sequences, cross-transmission experiments and respective prevalence of different STs in the human population indicate that almost all of the known STs of supposed animal origin are likely zoonotic and able to infect human (Figure 2) [Noël *et al.* 2005; Parkar *et al.* 2007, 2010; Yan *et al.* 2007; Yoshikawa *et al.* 2009]. Therefore, a higher risk of *Blastocystis* sp. infection was found in people with close animal contact, including zoo keepers [Parkar *et al.* 2010] and abattoir workers [Rajah Salim *et al.* 1999; Parkar *et al.* 2010], indicating that animals may represent a significant zoonotic source of this parasite for humans.

Insights into *Blastocystis* sp. pathogenesis

The pathogenic status of *Blastocystis* sp. was widely debated in the literature to determine whether this microorganism was a truly pathogenic or commensal organism [Stenzel and Boreham, 1996; Boorom *et al.* 2008; Tan, 2008; Tan *et al.* 2010], although an increasingly number of recent studies cited *Blastocystis* sp. as an emerging pathogen [Tan, 2008, Tan *et al.* 2010; Poirier *et al.* 2012; Scanlan, 2012]. This is mainly due to the fact that *Blastocystis* sp. can be found in both symptomatic and asymptomatic patients [Dogruman-Al *et al.* 2008; Eroglu *et al.* 2009; Souppart *et al.* 2009]. However, recent *in vitro* and *in vivo* studies show that *Blastocystis* sp. infection is associated with a variety of gastrointestinal disorders (called blastocystosis), especially in irritable bowel syndrome (IBS) and cutaneous lesions.

Blastocystis sp. is associated with various clinical symptoms in humans

In humans, blastocystosis is mainly characterized by nonspecific gastrointestinal symptoms, like

diarrhoea, abdominal pain, flatulence, nausea, vomiting, constipation, weight loss or fatigue [Stenzel and Boreham, 1996; Boorom *et al.* 2008; Tan, 2008; Tan *et al.* 2010]. The severity of these diseases is variable and ranges from acute to chronic infections [Tan, 2008]. A hypothesis to explain differences in the disease caused by *Blastocystis* sp. is its genetic diversity [Hussein *et al.* 2008; Tan, 2008; Tan *et al.* 2010; Scanlan, 2012], although no association was detected between symptoms and *Blastocystis* subtypes in several studies [Ozyurt *et al.* 2008; Dogruman-Al *et al.* 2009; Souppart *et al.* 2009; Jantermtor *et al.* 2013]. However, ST4 isolates are more common in symptomatic patients in Sweden, Denmark and Spain [Dominguez-Marquez *et al.* 2009; Stensvold *et al.* 2011; Forsell *et al.* 2012], arguing for an important role of this subtype that needs to be investigated further.

In addition to aspecific gastrointestinal symptoms, studies associated the parasite with cutaneous disorders and chronic or acute urticaria [Vogelberg *et al.* 2010; Hameed *et al.* 2011; Zuel-Fakkar *et al.* 2011; Verma and Delfanian, 2013]. These diseases were correlated with the presence of *Blastocystis* sp. belonging to the ST2 [Vogelberg *et al.* 2010] or ST3 [Zuel-Fakkar *et al.* 2011] in the patient stools. An association was also found between urticaria and amoeboid forms of a ST3 isolate [Katsarou-Katsari *et al.* 2008]. It was suggested that the amoeboid form adheres efficiently to the intestinal epithelium, affecting gut immune homeostasis and causing an inflammatory response against the parasite that led to urticaria [Valsecchi *et al.* 2004].

Blastocystis sp. is also suspected to be involved in IBS [Tan *et al.* 2010; Poirier *et al.* 2012; Scanlan, 2012]. Indeed several studies reported a higher incidence of the parasite in patients with IBS compared with healthy populations [Poirier *et al.* 2012]. IBS is a common gastrointestinal disorder characterized by abdominal pain and discomfort associated with changes in bowel habits [Longstreth *et al.* 2006]. Studies on the IBS population showed a higher prevalence of ST1 and ST3 isolates of *Blastocystis* sp. [Yakoob *et al.* 2010; Fouad *et al.* 2011; Jimenez-Gonzalez *et al.* 2012]. However, these studies did not conclude that *Blastocystis* sp. was the sole etiologic agent. Moreover, a recent study demonstrated that even though *Blastocystis* sp. was more frequent in symptomatic patients with IBS, the differences compared with controls were not significant [Cekin *et al.* 2012]. The presence of *Blastocystis* sp. in symptomatic patients can also indicate that this parasite could be involved with other factors in this disease pathophysiology [Poirier *et al.* 2012]. It is possible that the alteration of the intestinal environment, provoked by pathogens (bacteria), genetic or environmental factors promotes its development. Furthermore, studies showing the concomitant eradication of *Blastocystis* sp. with the disappearance of symptoms in patients with IBS are needed to clarify the role of this parasite.

High-risk populations

As in many parasitic infections, some populations are more susceptible to *Blastocystis* sp. infection. Therefore, this parasite is frequently found in immunocompromised individuals such as those with human immunodeficiency virus/acquired immunodeficiency syndrome or cancer [Kurniawan *et al.* 2009; Tan *et al.* 2010]. Children from developing countries and those who are immunocompromised are also more susceptible [Calik *et al.* 2011; Canete *et al.* 2012; Daryani *et al.* 2012]. The socioeconomic status, the quality of drinking water, the consumption of contaminated food and the personal hygiene habits are the major risks explaining contamination in children [Abdulsalam *et al.* 2012; Canete *et al.* 2012]. A higher risk of *Blastocystis* sp. infection was also found in people who are in close contact with animals, which increases exposure to the parasite [Yoshikawa *et al.* 2009; Parkar *et al.* 2010], reinforcing the zoonotic nature of *Blastocystis* sp.

Pathophysiology of blastocystosis

One of the major obstacles to the study of the pathogenesis of *Blastocystis* sp. is the lack of animal models to test Koch's postulate. However, a variety of experimental infections involving different animals have been described, including rats, mice, guinea pigs or chickens [Tan, 2008; Tan *et al.* 2010]. From these, it was deduced that laboratory mice are not suitable as animal models. Infections were generally self limiting, although some mice showed weight loss and lethargy. However, histological examination of the cecum and colon revealed intense inflammatory cell infiltration, oedematous lamina propria and mucosal sloughing [Moe *et al.* 1997]. These authors also showed that there was an age-related susceptibility to *Blastocystis* sp. in mice. Indeed, juvenile BALB/c mice were more susceptible

than adult mice and 8-week-old adult BALB/c mice were totally resistant to *Blastocystis* sp.

Studies on rat models suggest that this species is more suitable for developing an animal model [Tan, 2008]. Ten to a hundred cysts were able to establish an infection *via* intracecal or oral inoculations in 3-week-old Wistar rats and all contents from the cecum and large intestine were positive for *Blastocystis* sp. infection [Yoshikawa *et al.* 2004]. Hussein and colleagues tested the infectivity of human ST1–4 isolates obtained from both asymptomatic and symptomatic patients in 4-week-old male Wister rats orally inoculated with 4-day-old cultures of *Blastocystis* sp. negative for *Cryptosoridium*, *Cyclospora*, *Isospora*, *Microsporidium* and common bacterial pathogens [Hussein *et al.* 2008]. Interestingly, the moderate and severe degrees of pathological changes observed 6 weeks post infection were found only with symptomatic isolates while mild changes were found only with asymptomatic isolates. Interestingly, ST1 symptomatic isolates induced 25% mortality in rats, indicating its pathogenesis. In parallel, the authors described intense inflammatory reaction and sloughing mucosa, oedema and precancerous polyps in cecum and proximal colon tissues in symptomatic infected rats. It was suggested that the inflammation induced by *Blastocystis* sp. had the ability to alter tight junctions between the intestinal epithelial cells and the intestinal content, which led to disturbances of the barrier function and permeability [Hussein *et al.* 2008]. However, the possibility that these pathologies could be of bacterial or viral origin was not excluded.

Some *in vitro* studies were conducted to investigate the mechanisms of physiopathology by studying the cytopathic effects of *Blastocystis* sp. on mammalian cell cultures. A first study [Long *et al.* 2001] showed that 24 h incubation with *Blastocystis* sp. ST1 cells or culture filtrates induced the production of proinflammatory cytokines interleukin (IL)-8 and granulocyte–macrophage colony-stimulating factor, suggesting that the parasite was able to modulate the host immune response (Figure 3). The induction of IL-8 production from human colonic epithelial cells (HT84) was demonstrated to be activated

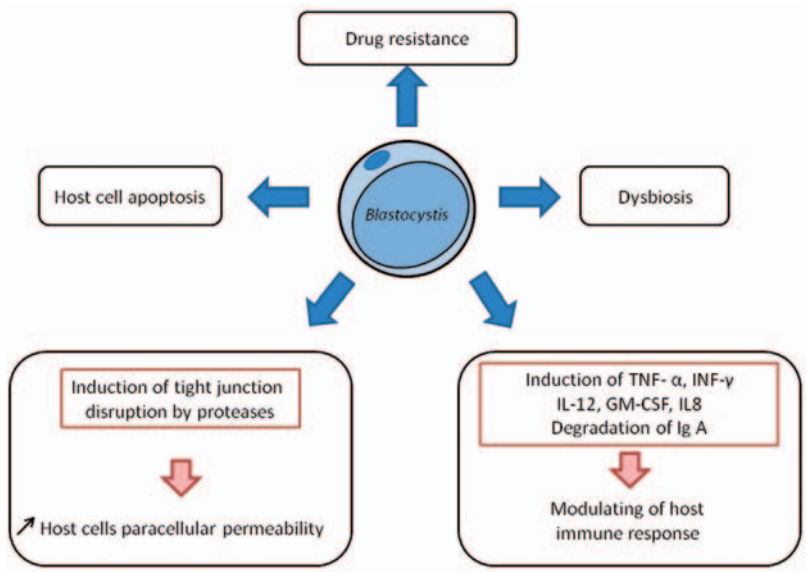

Figure 3. Mechanisms of blastocystosis physiopathology. *Blastocystis* sp. may release cysteine protease that may participate in the attack of intestinal epithelium with other hydrolases and may cause the increase in paracellular permeability that is observed in several digestive pathologies such as irritable bowel syndrome (IBS). *Blastocystis* sp. is able to induce physiological disturbances linked to IBS: host cell apoptosis, the modulation of host immune response and a microinflammation. Some as yet uncharacterized secondary metabolites produced by the polyketide synthase or nonribosomal peptide synthases could participate in host intestinal symptoms by inducing changes in the host microbiota, another feature of IBS. Finally, drug-resistant isolates of the parasite could be explained by the presence of multidrug resistance proteins that could eject active drugs. GM-CSF, granulocyte–macrophage colony-stimulating factor; Ig, immunoglobulin; IL, interleukin; INF, interferon; TNF, tumour necrosis factor.

by cysteine proteases from *Blastocystis* sp. ST4 in a nuclear factor κB dependent manner [Puthia *et al.* 2008]. The same authors also showed that coincubation of intestinal epithelial cells of rat interstitial IEC6 with either *Blastocystis* sp. ST4 isolates or parasite lysates induced the apoptosis of host cells [Puthia *et al.* 2006] in a contact-independent manner. A decrease in transepithelial resistance and an increase in epithelial permeability were also observed and could be explained by the rearrangement of actin filaments [Tan *et al.* 2010; Mirza *et al.* 2012]. These data suggest that *Blastocystis* sp. is able to disturb host gut homeostasis (Figure 3). As observed in other parasitic protozoa [Sajid and McKerrow, 2002], cysteine proteases of *Blastocystis* sp. should be involved in parasite survival *in vivo* and represent virulence factors [Tan *et al.* 2010; Scanlan, 2012]. Variations in cysteine protease activity were observed between ST4 and ST7 isolates, which may be attributable to differences in virulence [Mirza and Tan, 2009]. Protease activities were identified in *Blastocystis* sp. secretory products and were able to cleave human secretory immunoglobulin A, the prevalent immunoglobulin defence at the mucosal surface [Puthia *et al.* 2005]. Another study revealed that cysteine proteases increased permeability of human epithelium by reorganization of the tight junction complex and modulation of the rho associated kinase/phosphorylation of myosin light chain pathway [Mirza *et al.* 2012]. The sequence of the *Blastocystis* sp. ST7 complete genome provides molecular candidates that could be involved in pathogenesis [Denoeud *et al.* 2011]. Twenty-two proteases were predicted to be secreted [Denoeud *et al.* 2011] and could therefore play a role at the host–parasite interface. Among them, 20 cysteine proteases, 1 serine protease and 1 aspartic protease were characterized and 2 cysteine proteases were experimentally identified and characterized in the secretory products by mass spectrometry analysis [Wawrzyniak *et al.* 2012]. These secreted proteases are serious candidates to explain gut function disruption observed in intestinal pathologies [Poirier *et al.* 2012]. Apart from proteases, hydrolases and protease inhibitors were predicted to be secreted and could participate in the blastocystosis physiopathology. Moreover, genes coding a polyketide synthase and nonribosomal peptide synthases were identified [Denoeud *et al.* 2011]. These enzymes may produce molecules with interesting pharmacological activities like antibiotics and toxins and may be implicated in dysbiosis (Figure 3). The next challenge is to understand the role of *Blastocystis* sp. in gut dysfunctions [Poirier *et al.* 2012].

Treatment of blastocystosis

This treatment is usually considered if diarrhoea is persistent and no other pathogen apart from *Blastocystis* sp. is identified in faecal specimens [Coyle *et al.* 2012]. In this case, metronidazole is considered as the first-line therapy for *Blastocystis* sp. infection [Nigro *et al.* 2003; Cassano *et al.* 2005; Moghaddam *et al.* 2005; Stensvold *et al.* 2010]. In a first evaluation of this drug efficacy, Nigro and colleagues showed that immunocompetent individuals with *Blastocystis* sp. infection as the only evident cause of diarrhoea responded to metronidazole treatment and consequently they suggested that the parasite induced intestinal disease [Nigro *et al.* 2003]. However, there were accumulating reports of treatment failure, particularly in patients with severe *Blastocystis* sp. infections [Haresh *et al.* 1999; Moghaddam *et al.* 2005; Stensvold *et al.* 2008, 2010], suggesting the existence of extensive variations in drug susceptibility [Mirza *et al.* 2011]. Accordingly, some genes coding for multidrug resistance pump proteins (ATP-binding cassette transporters) were identified in the *Blastocystis* sp. ST7 genome (Figure 3) [Denoeud *et al.* 2011]. Several standard antimicrobials (cotrimoxazole, ornidazole, nitazoxanide, paromomycin, chloroquine, trimethoprim-sulfamethoxazole, iodoquinol, tinidazole, emetine, pentamidine, iodochlorhydroxyquin and furazolidone) may be considered as second-choice drugs [Ok *et al.* 1999; Cimerman *et al.* 2003; Diaz *et al.* 2003; Rossignol *et al.* 2005; Stensvold *et al.* 2008; Mirza *et al.* 2011; Coyle *et al.* 2012]. Even if some of these drugs were shown to be globally effective against *Blastocystis* sp., treatment failures were also largely reported [Haresh *et al.* 1999; Nigro *et al.* 2003; Mirza *et al.* 2011].

Conclusion

Blastocystis sp. was included in the Water Sanitation and Health programmes of the World Health Organization [WHO, 2008]. Increasing interest of the scientific and medical community in *Blastocystis* sp. was coupled with new data about epidemiology, pathogeny and more recently the first whole genome of *Blastocystis* ST7. Accumulating *in vivo*, *in vitro* and *in silico* data assessed the importance of *Blastocystis* sp. in human health, with probably the association with major host and environmental factors to be determined. To answer most of

the crucial questions regarding *Blastocystis* sp., we need to develop and standardize axenization of new STs and diagnosis tools, transfection and animal models, microarrays and genotyping markers. Sequencing of genomes from different STs and comparative genomic projects are in progress and will be useful to identify specific virulence factors. Multicentre studies are also required to further our comprehension of the clinical implications of *Blastocystis* sp. in IBS and other pathologies. To conclude, the interaction of *Blastocystis* sp. with gut microbiota needs to be studied because of the increasing interest in microbiota disturbances in the genesis of various gastrointestinal dysfunctions.

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Conflict of interest statement

The authors declare no conflicts of interest in preparing this article.

References

Abdulsalam, A., Ithoi, I., Al-Mekhlafi, H., Ahmed, A., Surin, J. and Mak, J. (2012) Drinking water is a significant predictor of *Blastocystis* infection among rural Malaysian primary schoolchildren. *Parasitology* 139: 1014–1020.

Abe, N. (2004) Molecular and phylogenetic analysis of *Blastocystis* isolates from various hosts. *Vet Parasitol* 120: 235–242.

Abe, N., Wu, Z. and Yoshikawa, H. (2003a) Molecular characterization of *Blastocystis* isolates from birds by PCR with diagnostic primers and restriction fragment length polymorphism analysis of the small subunit ribosomal RNA gene. *Parasitol Res* 89: 393–396.

Abe, N., Wu, Z. and Yoshikawa, H. (2003b) Zoonotic genotypes of *Blastocystis* hominis detected in cattle and pigs by PCR with diagnostic primers and restriction fragment length polymorphism analysis of the small subunit ribosomal RNA gene. *Parasitol Res* 90: 124–128.

Aguiar, J., Goncalves, A., Sodre, F., Pereira Sdos, R., Boia, M., De Lemos, E. *et al.* (2007) Intestinal protozoa and helminths among Terena Indians in the State of Mato Grosso do Sul: high prevalence of *Blastocystis* hominis. *Rev Soc Bras Med Trop* 40: 631–634.

Alfellani, M., Stensvold, C., Vidal-Lapiedra, A., Onuoha, E., Fagbenro-Beyioku, A. and Clark, C. (2013) Variable geographic distribution of *Blastocystis* subtypes and its potential implications. *Acta Trop* 126: 11–18.

Alfellani, M., Taner-Mulla, D., Jacob, A., Imeede, C., Yoshikawa, H., Stensvold, C. *et al.* (2013) Genetic diversity of *Blastocystis* in livestock and zoo animals. *Acta Trop* 126: 11–18.

Arisue, N., Hashimoto, T. and Yoshikawa, H. (2003) Sequence heterogeneity of the small subunit ribosomal RNA genes among *Blastocystis* isolates. *Protist* 164: 497–509.

Arisue, N., Hashimoto, T., Yoshikawa, H., Nakamura, Y., Nakamura, G., Nakamura, F. *et al.* (2002) Phylogenetic position of *Blastocystis* hominis and of stramenopiles inferred from multiple molecular sequence data. *J Eukaryot Microbiol* 49: 42–53.

Baldursson, S. and Karanis, P. (2011) Waterborne transmission of protozoan parasites: review of worldwide outbreaks – an update 2004–2010. *Water Res* 45: 6603–6614.

Boorom, K., Smith, H., Nimri, L., Viscogliosi, E., Spanakos, G., Parkar, U. *et al.* (2008) Oh my aching gut: irritable bowel syndrome, *Blastocystis*, and asymptomatic infection. *Parasit Vectors* 1: 40.

Calik, S., Karaman, U. and Colak, C. (2011) Prevalence of microsporidium and other intestinal parasites in children from Malatya, Turkey. *Indian J Microbiol* 51: 345–349.

Canete, R., Diaz, M., Avalos Garcia, R., Laud Martinez, P. and Manuel Ponce, F. (2012) Intestinal parasites in children from a day care centre in Matanzas City, Cuba. *PLoS One* 7: e51394.

Cassano, N., Scoppio, B., Loviglio, M. and Vena, G. (2005) Remission of delayed pressure urticaria after eradication of *Blastocystis* hominis. *Acta Derm Venereol* 85: 357–358.

Cekin, A., Cekin, Y., Adakan, Y., Tasdemir, E., Koclar, F. and Yolcular, B. (2012) Blastocystosis in patients with gastrointestinal symptoms: a case-control study. *BMC Gastroenterol* 12: 122.

Chen, X., Singh, M., Howe, J., Ho, L., Tan, S. and Yap, E. (1999) In vitro encystation and excystation of *Blastocystis* ratti. *Parasitology* 118: 151–160.

Cimerman, S., Ladeira, M. and Iuliano, W. (2003) Blastocystosis: nitazoxanide as a new therapeutic option. *Rev Soc Bras Med Trop* 36: 415–417.

Coyle, C., Varughese, J., Weiss, L. and Tanowitz, H. (2012) Blastocystis: to treat or not to treat. *Clin Infect Dis* 54: 105–110.

Daryani, A., Sharif, M., Nasrolahei, M., Khalilian, A., Mohammadi, A. and Barzegar, G. (2012) Epidemiological survey of the prevalence of intestinal parasites among schoolchildren in Sari, Northern Iran. *Trans R Soc Trop Med Hyg* 106: 455–459.

Denoeud, F., Roussel, M., Noel, B., Wawrzyniak, I., Da Silva, C., Diogon, M. *et al.* (2011) Genome sequence of the stramenopile *Blastocystis*, a human anaerobic parasite. *Genome Biol* 12: R29.

Diaz, E., Mondragon, J., Ramirez, E. and Bernal, R. (2003) Epidemiology and control of intestinal parasites with nitazoxanide in children in Mexico. *Am J Trop Med Hyg* 68: 384–385.

Dogruman-Al, F., Dagci, H., Yoshikawa, H., Kurt, O. and Demirel, M. (2008) A possible link between subtype 2 and asymptomatic infections of *Blastocystis* hominis. *Parasitol Res* 103: 685–689.

Dogruman-Al, F., Yoshikawa, H., Kustimur, S. and Balaban, N. (2009) PCR-based subtyping of *Blastocystis* isolates from symptomatic and asymptomatic individuals in a major hospital in Ankara, Turkey. *Parasitol Res* 106: 263–268.

Dominguez-Marquez, M., Guna, R., Munoz, C., Gomez-Munoz, M. and Borras, R. (2009) High prevalence of subtype 4 among isolates of *Blastocystis* hominis from symptomatic patients of a health district of Valencia (Spain). *Parasitol Res* 105: 949–955.

Eroglu, F., Genc, A., Elgun, G. and Koltas, I. (2009) Identification of *Blastocystis* hominis isolates from asymptomatic and symptomatic patients by PCR. *Parasitol Res* 105: 1589–1592.

Eroglu, F. and Koltas, I. (2010) Evaluation of the transmission mode of B. hominis by using PCR method. *Parasitol Res* 107: 841–845.

Fayer, R., Santin, M. and Macarisin, D. (2012) Detection of concurrent infection of dairy cattle with *Blastocystis*, *Cryptosporidium*, *Giardia*, and *Enterocytozoon* by molecular and microscopic methods. *Parasitol Res* 111: 1349–1355.

Forsell, J., Granlund, M., Stensvold, C., Clark, C. and Evengard, B. (2012) Subtype analysis of *Blastocystis* isolates in Swedish patients. *Eur J Clin Microbiol Infect Dis* 31: 1689–1696.

Fouad, S., Basyoni, M., Fahmy, R. and Kobaisi, M. (2011) The pathogenic role of different *Blastocystis* hominis genotypes isolated from patients with irritable bowel syndrome. *Arab J Gastroenterol* 12: 194–200.

Hameed, D., Hassanin, O. and Zuel-Fakkar, N. (2011) Association of *Blastocystis* hominis genetic subtypes with urticaria. *Parasitol Res* 108: 553–560.

Haresh, K., Suresh, K., Khairul., Anus, A. and Saminathan, S. (1999) Isolate resistance of *Blastocystis* hominis to metronidazole. *Trop Med Int Health* 4: 274–277.

Hirata, T., Nakamura, H., Kinjo, N., Hokama, A., Kinjo, F., Yamane, N. *et al.* (2007) Prevalence of *Blastocystis* hominis and Strongyloides stercoralis infection in Okinawa, Japan. *Parasitol Res* 101: 1717–1719.

Hoevers, J., Holman, P., Logan, K., Hommel, M., Ashford, R. and Snowden, K. (2000) Restriction-fragment-length polymorphism analysis of small-subunit rRNA genes of *Blastocystis* hominis isolates from geographically diverse human hosts. *Parasitol Res* 86: 57–61.

Hussein, E., Hussein, A., Eida, M. and Atwa, M. (2008) Pathophysiological variability of different genotypes of human *Blastocystis* hominis Egyptian isolates in experimentally infected rats. *Parasitol Res* 102: 853–860.

Ithoi, I., Jali, A., Mak, J., Wan., Sulaiman, W. and Mahmud, R. (2011) Occurrence of *Blastocystis* in water of two rivers from recreational areas in Malaysia. *J Parasitol Res* 2011: 123916.

Jantermtor, S., Pinlaor, P., Sawadpanich, K., Pinlaor, S., Sangka, A., Wilailuckana, C. *et al.* (2013) Subtype identification of *Blastocystis* spp. isolated from patients in a major hospital in Northeastern Thailand. *Parasitol Res* 112: 1781–1786.

Jimenez-Gonzalez, D., Martinez-Flores, W., Reyes-Gordillo, J., Ramirez-Miranda, M., Arroyo-Escalante, S., Romero-Valdovinos, M. *et al.* (2012) Blastocystis infection is associated with irritable bowel syndrome in a Mexican patient population. *Parasitol Res* 110: 1269–1275.

Jones, M., 2nd., Ganac, R., Hiser, G., Hudson, N., Le, A. and Whipps, C. (2008) Detection of *Blastocystis* from stool samples using real-time PCR. *Parasitol Res* 103: 551–557.

Kaneda, Y., Horiki, N., Cheng, X., Fujita, Y., Maruyama, M. and Tachibana, H. (2001) Ribodemes of *Blastocystis* hominis isolated in Japan. *Am J Trop Med Hyg* 65: 393–396.

Katsarou-Katsari, A., Vassalos, C., Tzanetou, K., Spanakos, G., Papadopoulou, C. and Vakalis, N. (2008) Acute urticaria associated with amoeboid forms of *Blastocystis* sp. subtype 3. *Acta Derm Venereol* 88: 80–81.

Kurniawan, A., Karyadi, T., Dwintasari, S., Sari, I., Yunihastuti, E., Djauzi, S. *et al.* (2009) Intestinal parasitic infections in HIV/AIDS patients presenting with diarrhoea in Jakarta, Indonesia. *Trans R Soc Trop Med Hyg* 103: 892–898.

Lee, L., Chye, T., Karmacharya, B. and Govind, S. (2012) Blastocystis sp: waterborne zoonotic organism, a possibility?. *Parasit Vectors* 5: 130.

Leelayoova, S., Siripattanapipong, S., Thathaisong, U., Naaglor, T., Taamasri, P., Piyaraj, P. *et al.* (2008) Drinking water: a possible source of *Blastocystis* spp. subtype 1 infection in schoolchildren of a rural community in central Thailand. *Am J Trop Med Hyg* 79: 401–406.

Li, L., Zhang, X., Lv, S., Zhang, L., Yoshikawa, H., Wu, Z. *et al.* (2007) Cross-sectional surveys and subtype classification of human *Blastocystis* isolates from four epidemiological settings in China. *Parasitol Res* 102: 83–90.

Li, L., Zhou, X., Du, Z., Wang, X., Wang, L., Jiang, J. *et al.* (2007) Molecular epidemiology of human *Blastocystis* in a village in Yunnan Province, China. *Parasitol Int* 56: 281–286.

Long, H., Handschack, A., Konig, W. and Ambrosch, A. (2001) Blastocystis hominis modulates immune responses and cytokine release in colonic epithelial cells. *Parasitol Res* 87: 1029–1030.

Longstreth, G., Thompson, W., Chey, W., Houghton, L., Mearin, F. and Spiller, R. (2006) Functional bowel disorders. *Gastroenterology* 130: 1480–1491.

Meloni, D., Poirier, P., Mantini, C., Noel, C., Gantois, N., Wawrzyniak, I. *et al.* (2012) Mixed human intra- and inter-subtype infections with the parasite *Blastocystis* sp. *Parasitol Int* 61: 719–722.

Meloni, D., Sanciu, G., Poirier, P., El., Alaoui, H., Chabe, M., Delhaes, L. *et al.* (2011) Molecular subtyping of *Blastocystis* sp. isolates from symptomatic patients in Italy. *Parasitol Res* 109: 613–619.

Mirza, H. and Tan, K. (2009) Blastocystis exhibits inter- and intra-subtype variation in cysteine protease activity. *Parasitol Res* 104: 355–361.

Mirza, H., Teo, J., Upcroft, J. and Tan, K. (2011) A rapid, high-throughput viability assay for *Blastocystis* spp. reveals metronidazole resistance and extensive subtype-dependent variations in drug susceptibilities. *Antimicrob Agents Chemother* 55: 637–648.

Mirza, H., Wu, Z., Teo, J. and Tan, K. (2012) Statin pleiotropy prevents rho kinase-mediated intestinal epithelial barrier compromise induced by *Blastocystis* cysteine proteases. *Cell Microbiol* 14: 1474–1484.

Moe, K., Singh, M., Howe, J., Ho, L., Tan, S., Chen, X. *et al.* (1997) Experimental *Blastocystis* hominis infection in laboratory mice. *Parasitol Res* 83: 319–325.

Moe, K., Singh, M., Howe, J., Ho, L., Tan, S., Ng, G. *et al.* (1996) Observations on the ultrastructure and viability of the cystic stage of *Blastocystis* hominis from human feces. *Parasitol Res* 82: 439–444.

Moghaddam, D., Ghadirian, E. and Azami, M. (2005) Blastocystis hominis and the evaluation of efficacy of metronidazole and trimethoprim/sulfamethoxazole. *Parasitol Res* 96: 273–275.

Moosavi, A., Haghighi, A., Mojarad, E., Zayeri, F., Alebouyeh, M., Khazan, H. *et al.* (2012) Genetic variability of *Blastocystis* sp. isolated from symptomatic and asymptomatic individuals in Iran. *Parasitol Res* 111: 2311–2315.

Nagel, R., Cuttell, L., Stensvold, C., Mills, P., Bielefeldt-Ohmann, H. and Traub, R. (2012) Blastocystis subtypes in symptomatic and asymptomatic family members and pets and response to therapy. *Intern Med J* 42: 1187–1195.

Nasirudeen, A. and Tan, K. (2004) Isolation and characterization of the mitochondrion-like organelle from *Blastocystis* hominis. *J Microbiol Methods* 58: 101–109.

Nigro, L., Larocca, L., Massarelli, L., Patamia, I., Minniti, S., Palermo, F. *et al.* (2003) A placebo-controlled treatment trial of *Blastocystis* hominis infection with metronidazole. *J Travel Med* 10: 128–130.

Noël, C., Dufernez, F., Gerbod, D., Edgcomb, V., Delgado-Viscogliosi, P., Ho, L. *et al.* (2005) Molecular phylogenies of *Blastocystis* isolates from different hosts: implications for genetic diversity, identification of species, and zoonosis. *J Clin Microbiol* 43: 348–355.

Ok, U., Girginkardesler, N., Balcioglu, C., Ertan, P., Pirildar, T. and Kilimcioglu, A. (1999) Effect of trimethoprim-sulfamethaxazole in *Blastocystis* hominis infection. *Am J Gastroenterol* 94: 3245–3247.

Ozyurt, M., Kurt, O., Molbak, K., Nielsen, H., Haznedaroglu, T. and Stensvold, C. (2008) Molecular epidemiology of *Blastocystis* infections in Turkey. *Parasitol Int* 57: 300–306.

Parkar, U., Traub, R., Kumar, S., Mungthin, M., Vitali, S., Leelayoova, S. *et al.* (2007) Direct characterization of *Blastocystis* from faeces by PCR and evidence of zoonotic potential. *Parasitology* 134: 359–367.

Parkar, U., Traub, R., Vitali, S., Elliot, A., Levecke, B., Robertson, I. *et al.* (2010) Molecular characterization of *Blastocystis* isolates from zoo animals and their animal-keepers. *Vet Parasitol* 169: 8–17.

Pegelow, K., Gross, R., Pietrzik, K., Lukito, W., Richards, A. and Fryauff, D. (1997) Parasitological and nutritional situation of school children in the Sukaraja district, West Java, Indonesia. *Southeast Asian J Trop Med Public Health* 28: 173–190.

Perez-Brocal, V. and Clark, C. (2008) Analysis of two genomes from the mitochondrion-like organelle of the intestinal parasite *Blastocystis*: complete sequences, gene content, and genome organization. *Mol Biol Evol* 25: 2475–2482.

Poirier, P., Wawrzyniak, I., Albert, A., El., Alaoui, H., Delbac, F. and Livrelli, V. (2011) Development and evaluation of a real-time PCR assay for detection and quantification of *Blastocystis* parasites in human stool samples: prospective study of patients with hematological malignancies. *J Clin Microbiol* 49: 975–983.

Poirier, P., Wawrzyniak, I., Vivares, C., Delbac, F. and El Alaoui, H. (2012) New insights into *Blastocystis* spp.: a potential link with irritable bowel syndrome. *PLoS Pathog* 8: e1002545.

Puthia, M., Lu, J. and Tan, K. (2008) Blastocystis ratti contains cysteine proteases that mediate interleukin-8 response from human intestinal epithelial cells in an NF-kappaB-dependent manner. *Eukaryot Cell* 7: 435–443.

Puthia, M., Sio, S., Lu, J. and Tan, K. (2006) Blastocystis ratti induces contact-independent apoptosis, F-actin rearrangement, and barrier function disruption in IEC-6 cells. *Infect Immun* 74: 4114–4123.

Puthia, M., Vaithilingam, A., Lu, J. and Tan, K. (2005) Degradation of human secretory immunoglobulin a by *Blastocystis*. *Parasitol Res* 97: 386–389.

Rajah., Salim, H., Suresh., Kumar, G., Vellayan, S., Mak, J., Khairul., Anuar, A., Init, I. *et al.* (1999)

Blastocystis in animal handlers. *Parasitol Res* 85: 1032–1033.

Rayan, H., Ismail, O., El. and Gayar, E. (2007) Prevalence and clinical features of Dientamoeba fragilis infections in patients suspected to have intestinal parasitic infection. *J Egypt Soc Parasitol* 37: 599–608.

Riisberg, I., Orr, R., Kluge, R., Shalchian-Tabrizi, K., Bowers, H., Patil, V. *et al.* (2009) Seven gene phylogeny of heterokonts. *Protist* 160: 191–204.

Rivera, W. and Tan, M. (2005) Molecular characterization of *Blastocystis* isolates in the Philippines by riboprinting. *Parasitol Res* 96: 253–257.

Roberts, T., Barratt, J., Harkness, J., Ellis, J. and Stark, D. (2011) Comparison of microscopy, culture, and conventional polymerase chain reaction for detection of *Blastocystis* sp. in clinical stool samples. *Am J Trop Med Hyg* 84: 308–312.

Roberts, T., Stark, D., Harkness, J. and Ellis, J. (2013) Subtype distribution of *Blastocystis* isolates from a variety of animals from New South Wales, Australia. *Vet Parasitol* 196: 85–89.

Rossignol, J., Kabil, S., Said, M., Samir, H. and Younis, A. (2005) Effect of nitazoxanide in persistent diarrhea and enteritis associated with *Blastocystis* hominis. *Clin Gastroenterol Hepatol* 3: 987–991.

Sajid, M. and McKerrow, J. (2002) Cysteine proteases of parasitic organisms. *Mol Biochem Parasitol* 120: 1–21.

Saksirisampant, W., Nuchprayoon, S., Wiwanitkit, V., Yenthakam, S. and Ampavasiri, A. (2003) Intestinal parasitic infestations among children in an orphanage in Pathum Thani Province. *J Med Assoc Thai* 86(Suppl. 2): S263–S270.

Saksirisampant, W., Prownebon, J., Kulkumthorn, M., Yenthakam, S., Janpla, S. and Nuchprayoon, S. (2006) Prevalence of intestinal parasitic infections among school children in the central region of Thailand. *J Med Assoc Thai* 89: 1928–1933.

Santin, M., Gomez-Munoz, M., Solano-Aguilar, G. and Fayer, R. (2011) Development of a new PCR protocol to detect and subtype *Blastocystis* spp. from humans and animals. *Parasitol Res* 109: 205–212.

Scanlan, P.D. (2012) Blastocystis: past pitfalls and future perspectives. *Trends Parasitol* 28: 327–334.

Scicluna, S., Tawari, B. and Clark, C. (2006) DNA barcoding of *Blastocystis*. *Protist* 157: 77–85.

Silberman, J., Sogin, M., Leipe, D. and Clark, C. (1996) Human parasite finds taxonomic home. *Nature* 380: 398.

Souppart, L., Moussa, H., Cian, A., Sanciu, G., Poirier, P., El., Alaoui, H. *et al.* (2010) Subtype analysis of *Blastocystis* isolates from symptomatic patients in Egypt. *Parasitol Res* 106: 505–511.

Souppart, L., Sanciu, G., Cian, A., Wawrzyniak, I., Delbac, F., Capron, M. *et al.* (2009) Molecular epidemiology of human *Blastocystis* isolates in France. *Parasitol Res* 105: 413–421.

Stechmann, A., Hamblin, K., Perez-Brocal, V., Gaston, D., Richmond, G., Van Der., Giezen, M. *et al.* (2008) Organelles in *Blastocystis* that blur the distinction between mitochondria and hydrogenosomes. *Curr Biol* 18: 580–585.

Stensvold, C. (2013) Comparison of sequencing (barcode region) and sequence-tagged-site PCR for *Blastocystis* subtyping. *J Clin Microbiol* 51: 190–194.

Stensvold, C., Ahmed, U., Andersen, L. and Nielsen, H. (2012) Development and evaluation of a genus-specific, probe-based, internal-process-controlled real-time PCR assay for sensitive and specific detection of *Blastocystis* spp. *J Clin Microbiol* 50: 1847–1851.

Stensvold, C., Alfellani, M., Norskov-Lauritsen, S., Prip, K., Victory, E., Maddox, C. *et al.* (2009) Subtype distribution of *Blastocystis* isolates from synanthropic and zoo animals and identification of a new subtype. *Int J Parasitol* 39: 473–479.

Stensvold, C., Arendrup, M., Jespersgaard, C., Molbak, K. and Nielsen, H. (2007a) Detecting *Blastocystis* using parasitologic and DNA-based methods: a comparative study. *Diagn Microbiol Infect Dis* 59: 303–307.

Stensvold, C., Arendrup, M., Nielsen, H., Bada, A. and Thorsen, S. (2008) Symptomatic infection with *Blastocystis* sp. subtype 8 successfully treated with trimethoprim-sulfamethoxazole. *Ann Trop Med Parasitol* 102: 271–274.

Stensvold, C., Christiansen, D., Olsen, K. and Nielsen, H. (2011) Blastocystis sp. subtype 4 is common in Danish *Blastocystis*-positive patients presenting with acute diarrhea. *Am J Trop Med Hyg* 84: 883–885.

Stensvold, C., Smith, H., Nagel, R., Olsen, K. and Traub, R. (2010) Eradication of *Blastocystis* carriage with antimicrobials: reality or delusion? *J Clin Gastroenterol* 44: 85–90.

Stensvold, C., Suresh, G., Tan, K., Thompson, R., Traub, R., Viscogliosi, E. *et al.* (2007b) Terminology for *Blastocystis* subtypes – a consensus. *Trends Parasitol* 23: 93–96.

Stensvold, R., Brillowska-Dabrowska, A., Nielsen, H. and Arendrup, M. (2006) Detection of *Blastocystis* hominis in unpreserved stool specimens by using polymerase chain reaction. *J Parasitol* 92: 1081–1087.

Stenzel, D. and Boreham, P. (1996) Blastocystis hominis revisited. *Clin Microbiol Rev* 9: 563–584.

Suresh, K., Ng, G., Ramachandran, N., Ho, L., Yap, E. and Singh, M. (1993) In vitro encystment and experimental infections of *Blastocystis* hominis. *Parasitol Res* 79: 456–460.

Suresh, K. and Smith, H. (2004) Comparison of methods for detecting *Blastocystis* hominis. *Eur J Clin Microbiol Infect Dis* 23: 509–511.

Suresh, K., Smith, H. and Tan, T. (2005) Viable *Blastocystis* cysts in Scottish and Malaysian sewage samples. *Appl Environ Microbiol* 71: 5619–5620.

Suresh, K., Venilla, G., Tan, T. and Rohela, M. (2009) In vivo encystation of *Blastocystis* hominis. *Parasitol Res* 104: 1373–1380.

Tan, K. (2004) Blastocystis in humans and animals: new insights using modern methodologies. *Vet Parasitol* 126: 121–144.

Tan, K. (2008) New insights on classification, identification, and clinical relevance of *Blastocystis* spp. *Clin Microbiol Rev* 21: 639–665.

Tan, K., Mirza, H., Teo, J., Wu, B. and Macary, P. (2010) Current views on the clinical relevance of *Blastocystis* spp. *Curr Infect Dis Rep* 12: 28–35.

Tan, T. and Suresh, K. (2006) Predominance of amoeboid forms of *Blastocystis* hominis in isolates from symptomatic patients. *Parasitol Res* 98: 189–193.

Thathaisong, U., Siripattanapipong, S., Mungthin, M., Pipatsatitpong, D., Tan-Ariya, P., Naaglor, T. *et al.* (2013) Identification of *Blastocystis* subtype 1 variants in the home for girls, Bangkok, Thailand. *Am J Trop Med Hyg* 88: 352–358.

The ANOFEL Cryptosporidium National Network. (2010) Laboratory-based surveillance for Cryptosporidium in France, 2006–2009. *EuroSurveill* 15: pii=19642.

Valsecchi, R., Leghissa, P. and Greco, V. (2004) Cutaneous lesions in *Blastocystis* hominis infection. *Acta Derm Venereol* 84: 322–323.

Verma, R. and Delfanian, K. (2013) Blastocystis hominis associated acute urticaria. *Am J Med Sci* 346: 80–81.

Villar, J., Carbajal, J., Lanuza, M., Munoz, C. and Borras, R. (1998) In vitro encystation of *Blastocystis* hominis: a kinetics and cytochemistry study. *Parasitol Res* 84: 54–58.

Vogelberg, C., Stensvold, C., Monecke, S., Ditzen, A., Stopsack, K., Heinrich-Grafe, U. *et al.* (2010) Blastocystis sp. subtype 2 detection during recurrence of gastrointestinal and urticarial symptoms. *Parasitol Int* 59: 469–471.

Wawrzyniak, I., Roussel, M., Diogon, M., Couloux, A., Texier, C., Tan, K. *et al.* (2008) Complete circular DNA in the mitochondria-like organelles of *Blastocystis* hominis. *Int J Parasitol* 38: 1377–1382.

Wawrzyniak, I., Texier, C., Poirier, P., Viscogliosi, E., Tan, K., Delbac, F. *et al.* (2012) Characterization of two cysteine proteases secreted by *Blastocystis* St7, a human intestinal parasite. *Parasitol Int* 61: 437–442.

Whipps, C., Boorom, K., Bermudez, L. and Kent, M. (2010) Molecular characterization of *Blastocystis* species in Oregon identifies multiple subtypes. *Parasitol Res* 106: 827–832.

WHO. (2008) *Guidelines for Drinking-Water Quality* (3rd edition, incorporating first and second addenda), World Health Organization: Geneva.

Wong, K., Ng, G., Lin, R., Yoshikawa, H., Taylor, M. and Tan, K. (2008) Predominance of subtype 3 among *Blastocystis* isolates from a major hospital in Singapore. *Parasitol Res* 102: 663–670.

Yakoob, J., Jafri, W., Beg, M., Abbas, Z., Naz, S., Islam, M. *et al.* (2010) Blastocystis hominis and Dientamoeba fragilis in patients fulfilling irritable bowel syndrome criteria. *Parasitol Res* 107: 679–684.

Yan, Y., Su, S., Lai, R., Liao, H., Ye, J., Li, X. *et al.* (2006) Genetic variability of *Blastocystis* hominis isolates in China. *Parasitol Res* 99: 597–601.

Yan, Y., Su, S., Ye, J., Lai, X., Lai, R., Liao, H. *et al.* (2007) Blastocystis sp. subtype 5: a possibly zoonotic genotype. *Parasitol Res* 101: 1527–1532.

Yoshikawa, H., Abe, N., Iwasawa, M., Kitano, S., Nagano, I., Wu, Z. *et al.* (2000) Genomic analysis of *Blastocystis* hominis strains isolated from two long-term health care facilities. *J Clin Microbiol* 38: 1324–1330.

Yoshikawa, H., Morimoto, K., Wu, Z., Singh, M. and Hashimoto, T. (2004) Problems in speciation in the genus *Blastocystis*. *Trends Parasitol* 20: 251–255.

Yoshikawa, H., Wu, Z., Kimata, I., Iseki, M., Ali, I., Hossain, M. *et al.* (2004) Polymerase chain reaction-based genotype classification among human *Blastocystis* hominis populations isolated from different countries. *Parasitol Res* 92: 22–29.

Yoshikawa, H., Wu, Z., Pandey, K., Pandey, B., Sherchand, J., Yanagi, T. *et al.* (2009) Molecular characterization of *Blastocystis* isolates from children and rhesus monkeys in Kathmandu, Nepal. *Vet Parasitol* 160: 295–300.

Yoshikawa, H., Yoshida, K., Nakajima, A., Yamanari, K., Iwatani, S. and Kimata, I. (2004) Fecal–oral transmission of the cyst form of *Blastocystis* Hominis in rats. *Parasitol Res* 94: 391–396.

Zuel-Fakkar, N., Abdel., Hameed, D. and Hassanin, O. (2011) Study of *Blastocystis* hominis isolates in urticaria: a case-control study. *Clin Exp Dermatol* 36: 908–910.