

***Blastocystis* spp.: Current Status and Research Issues**

Carolina Valença Barbosa¹, Renata Costa Biot², Heloisa Werneck de Macedo³ and Helena Lúcia Carneiro Santos^{1*}

¹Laboratório de Estudos Integrados em Protozoologia, Instituto Oswaldo Cruz/Fundação Oswaldo Cruz/Fiocruz, Brazil

²Laboratório de Parasitologia do Departamento de Patologia do Hospital Universitário Antônio Pedro/Universidade Federal Fluminense, Brazil

***Corresponding Author:** Helena Lúcia Carneiro Santos, Laboratório de Estudos Integrados em Protozoologia, Instituto Oswaldo Cruz/Fiocruz, Av. Brasil, Pavilhão Arthur Neiva, Mangueiras, Rio de Janeiro, Brazil.

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Abstract

Despite *Blastocystis* being one of the most common parasite in the gastrointestinal tract of human, its role in disease remains inconclusive and its pathogenic potential is still a topic of discussions. However, it has been suggested that its pathogenicity could be directly related to its extensive genetic diversity. There are reports on its ability to cause disease, but none of them has shown a clear correlation between clinical symptoms and subtype. The scenario emerging from this enigmatic parasite, show us a large bottleneck in our knowledge, there is a deficit of basic information about the biology of the parasite, life cycle and the poor performance of animal models used, besides questions involving its zoonotic potential, taxonomic aspects and diagnostic. This review summarizes the current the state of the art on *Blastocystis* spp.

Keywords: *Blastocystis*; Subtypes; Intestinal Parasite; Blastocystosis; Genetic Diversity

Introduction

Blastocystis sp. is one of the most common parasite in the gastrointestinal tract of humans, often more common than other intestinal parasites such as *Entamoeba histolytica*-*Entamoeba dispar* complex, *Cryptosporidium*, and *Giardia lamblia* [1-3]. This parasite is cosmopolitan and its prevalence has been estimated at 1.5% to 10% in industrialized countries [4] and 30% to 76% in developing countries [5,6]. Nonetheless, much is still unknown regarding its biology and its role in disease. This is largely due to difficulties in identification of *Blastocystis*, related to the high polymorphism of this parasite, and to the lack of standardized and sufficiently sensitive detection or diagnoses methods.

Blastocystis prevalence is higher in areas lacking in sanitation infrastructure, especially drinking water and sewage, which led the World Health Organization (WHO) to include *Blastocystis* in its Water Sanitation Health program for control of tropical diseases in 2008. *Blastocystis* have been isolated not only in wastewater, but also in drinking water distribution system [7,8]. The source of infection of *Blastocystis* and mode of transmission have not been definitively established. Although it is currently proposed the mode of transmission is fecal-oral ingestion of food and water contaminated with cystic forms [9], the lack of personal and collective hygiene and care in the preparation of food, and direct contact with animals promote its transmission [7,10]. In Brazil, epidemiological studies aimed at other enteroparasites have demonstrated a high prevalence of *Blastocystis* sp. in all geographic regions. However, there are no studies on the occurrence of *Blastocystis* in different hosts or on genotypes circulating in the Brazilian territory. The molecular epidemiology of *Blastocystis* infections is still unknown in many parts of the world.

Overview of *Blastocystis* spp Hosts, Diversity and Taxonomic Classification

Blastocystis spp. occur in a variety of animals, including pigs, cattle, horses, non-human primates, birds, amphibians, reptiles, fish, arthropods, and annelids [11-16], indicating high host plasticity. One current classification places the genus *Blastocystis* in the kingdom

Chromista, subkingdom Chromobiota, infrakingdom Heterokonta, subphylum Opalimata, class Blastocystea [17,18], but the taxonomic position of *Blastocystis* remains controversial [14]. For many years, the parasite found in human feces was named *Blastocystis hominis*, but recent research suggests referring to *Blastocystis* from humans as *Blastocystis* sp., and isolates from other hosts as *Blastocystis* spp. Molecular studies based mainly on analysis of partial sequences of the small subunit ribosomal DNA (SSU-rDNA), as well as other partial sequences (SSU-rDNA of a similar like mitochondria like organelle, internal transcribed spacers (1 and 2) plus the 5.8S region and elongation factor-1 alpha) have shown that *Blastocystis* sp. has large genetic variability [19,20]. However, discrepant results were observed when different molecular markers were used for subtyping [21]. Currently, sequence analysis identified 17 subtypes, nine of which (ST1–ST9) found both in humans and animals [14,22], while the hosts of the other eight subtypes (ST10–ST17) are exclusively non-human. ST1 and ST3 have a high prevalence in humans [12], with ST3 the most prevalent [23], ST2 and ST4 regularly detected [24], and ST5–ST9 sporadically detected in humans. Subtypes circulating among animals and humans (ST1-ST9) show low host specificity, enabling transmission from animals to humans and vice versa [4]. Thus, transmission may occur through human-to-human, animal-to-human, and possibly, human-to-animal transmission [25-27]. *Blastocystis* spp. were found in captive animals and their caregivers, and infection rates among these professionals are significantly higher than in the general population [14,15]. Further study of the genetic variability of *Blastocystis* spp. may contribute to a better understanding of their biology, taxonomic position, and connection with other potential pathogenic and zoonotic organisms, and to the identification of the subtypes circulating in an area.

Morphology and Reproduction Forms

The parasite is generally spherical in shape and contains multiple nuclei, mitochondria like organelles, Golgi apparatus, lysosomes, and endoplasmic reticulum into a thin rim of cytoplasm surrounding the large central vacuole that often contains several granules. Although *Blastocystis* spp. are highly polymorphic organisms, isolates from humans and animals are morphologically similar [22,28]. To date, the following forms have been described: vacuolar, granular, amoeboid, multivacuolar, and cystic avacuolar [15,29]. Variation in size, shape and type of form of the parasite depends on the particular subtype, and the age and culture conditions [26] and there is no precise definition of each of these forms. Moreover, little is known about the transition from one morphological form to another along the life cycle [30,31] or in fact, about the life cycle and mode of transmission, which, so far, has only been demonstrated in the cystic form of the parasite [26,28]. Different life cycles were proposed for the species [26,32,33], probably due to the different types of reproduction of the parasite: binary division, endodyogeny, budding, plasmotomy and schizogony. In binary division, the mother cell splits into two identical daughter cells, while, in endodyogeny, the central vacuole of mother cell divides into two smaller cells forming a new membrane between the vacuoles of the two new cells. In budding, an outgrowth at a particular site of the cell develops into to a new cell, whereas, in plasmotomy, a prolongation of the plasma membrane separates into another cell. Lastly, schizogony occurs when a large cell with several core-like structures gives rise to other small cells. Binary division, endodyogeny and budding occur in feces, and plasmotomy and schizogony in culture.

Pathogenic Potential of *Blastocystis* spp

The pathogenic potential of *Blastocystis* spp. is still a topic of debate, as some individuals are asymptomatic [3]. Gut microbiota represent a relevant factor that may strongly interfere with the pathophysiology of the infections. The *Blastocystis*-intestinal microbiota relationship is poorly understood and investigated. For example, analyzes of meta-genomic data have revealed *Blastocystis* is highly associated with certain bacterial communities [34-36]. However, another metagenomic study support the hypothesis *Blastocystis* is associated with decrease of fecal microbiota protective bacteria and might be linked to the pathophysiology of Irritable Bowel Syndrome with constipation and intestinal flora imbalance [37]. Metagenomic analyzes, thus far point towards the need to discover whether bacterial community structure and function contributes *Blastocystis* colonization or vice versa. Moreover, it does raise a question 'can *Blastocystis* induce disease or contribute to dysbiosis or could *Blastocystis* merely serve as a biomarker of microbiota homeostasis'.

A positive correlation between protease activity and virulence has been demonstrated for a range of parasites including isolates of *Blastocystis*. Experimental studies suggest a relationship between subtypes and pathogenicity. For instance, the interaction of *Blastocystis* ST4 with rat intestinal epithelial cells (IEC6) caused apoptosis [38]. Conversely, interaction with a *Blastocystis* ST4 mouse isolate caused

no changes to cells from human epithelial cell line Caco-2, whereas an ST7 human isolate caused epithelial barrier dysfunction [39]. An *in vitro* experimental model developed to investigate the interaction of *Blastocystis* with epithelial cells of different lineages showed that the parasite can have immunomodulatory effects, including degradation of IgA, inhibition of iNOS, and upregulation of proinflammatory cytokines IL8 and GM-CSF in intestinal epithelial cells, and of IL1 β , IL6, and TNF α . Interactions with epithelial cell lines HT-29 and T-84 induced a significant increase in the release of cytokine IL-8 [40]. Additionally, the interaction of the parasite with epithelial cell line CHO correlated with a cytopathic effect [41]. However, Souppart and colleagues argue the infection is not associated with specific subtypes, but has multifactorial causes involving transmission routes, other potential pathogenic and zoonotic parasites, and the host immune profile [42]. Studies in rodent models and naturally infected pigs have shown that the parasite localizes to the lumen and mucosal surface of the large intestine mostly in the caecum and colon [43,44].

The lack of animal models to study experimental infection is a major bottleneck to study the pathogenicity of the parasite. It should be pointed out, however, that most studies were performed using different methodologies, making a comparative analysis of results across studies challenging. Thus, the validity of the data used to determine the pathogenic potential of *Blastocystis* should be questioned, as the different results across studies may have been due to differences in the diagnostic methods used.

Diagnosis and Their Limitations

Laboratory diagnosis depends on parasitological examination, using morphological criteria for the main *Blastocystis* forms. While direct examination of stool is a rapid and low-cost method, it has low sensitivity, as the presence of fecal debris makes it difficult to view the internal structures of the parasite. Thus, diagnosis usually employs techniques for concentration of the parasites by spontaneous sedimentation, such as the spontaneous sedimentation method of Hoffman, Pons & Janer (HPJ), or by centrifugation, such as the Ritchie technique. The highest diagnostic sensitivity is achieved by stool culture, but this is a laborious and time-consuming technique [19,45-46]. In short, there is currently no consensus on the best diagnostic method to be used. This, together with the polymorphism of the parasite and inexperience in morphological identification by many in the field is responsible for the conflicting results between different studies [47]. Understanding the dynamics of morphological variations of the parasite is essential for the development of accurate diagnostic methods, and to obtain reliable epidemiological data for the evaluation of the pathogenicity profile of and therapeutic efficacy against the parasite. So far, few studies have compared the sensitivity of different diagnostic methods for the identification of *Blastocystis* in feces.

Clinical Presentation and Treatment

Frequent clinical manifestations of blastocystosis (the infection caused by *Blastocystis* sp.) include abdominal pain, anal itching, flatulence, meteorism, nausea, vomiting, diarrhea, and eosinophilia [11], although infected individuals may be asymptomatic. Studies reporting rates of up to 73% of infection by *Blastocystis* in individuals with irritable bowel syndrome (IBS) [5,47] suggest a role for this parasite in the pathogenesis of IBS, a disease whose prevalence ranges from 35% to 45% in developing countries (36), and 5% to 24% in industrialized countries [49]. IBS may result from infection by *Blastocystis* [3], as *Blastocystis* antigens can cause inflammation in the intestinal mucosa [50] or the changes caused by IBS may create favorable conditions for development of the parasite in the intestinal tract [50]. The role of *Blastocystis* in IBS remains inconclusive, mainly due to our limited knowledge about the pathogenic potential and biology of this parasite.

Although considered an opportunistic pathogen in immunosuppressed individuals [51], a number of reports that have identified *Blastocystis* outside the gastrointestinal tract in immunocompetent patients support the proposal that this pathogen has invasive potential [45]. *Blastocystis* sp. was found in ulcerations of the mucosa without evidence of other causes [52], in appendicitis conditions associated with organ lumen obstruction by *Blastocystis* sp. [53], and in the synovial fluid of a patient with arthritis who experienced full resolution of clinical symptoms after treatment with metronidazole [9]. Indeed, recurrent episodes of arthritis caused by *Blastocystis* have been described in immunocompetent individuals [54]. Additionally, *Blastocystis* subtype 3 was identified in the appendix, recto-uterine pouch, and peritoneal fluid of a nine-year old child [48], and in splenic cysts in a young immunocompetent woman [55]. *Blastocystis* infec-

tion may also associate with urticaria in immunocompetent individuals [56]: several reports describe association of *Blastocystis* with skin rashes [57], albeit without establishing causality. The detection of the parasite in extraintestinal sites indicates that it is able to evade the immune system and settle in sites unfavorable for its development, considering that it preferentially parasitizes the gastrointestinal tract.

Currently, there is no consensus on what the most effective treatment is for *Blastocystosis*. A variety of agents that target anaerobic organisms or other protozoan parasites (Nitazoxanide, tinidazole, ornidazole, secnidazole, ketoconazole, pentamidine, quinine, iodoquinol, emetine, paromomycin and Sulphamethoxazole-trimethoprim) [50] have all been used, but metronidazole is most common treatment [58], with efficiency rates ranging from 33 to 100% depending on the dose administered. Its mode of action has not been fully elucidated [20]. Paromomycin has shown superior efficacy to metronidazole to treat *Blastocystosis* [59].

Concluding Remarks

This review examined the current state of scientific knowledge about *Blastocystis*. Several aspects about this protist, considered an emerging parasite, remain enigmatic, such as questions involving its role as a pathogen, zoonotic potential, taxonomic aspects life-cycle. The standardization of sensitive and specific diagnostic methods and the development of new animal models need to be addressed in order to provide a clearer picture of *Blastocystis* sp. biology and how these infections may impact global health.

Conflicts of Interest

The authors declare no conflicts of interest.

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