Review

Blastocystis: past pitfalls and future perspectives

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Blastocystis **is a genetically heterogeneous protist found in the intestinal tract (IT) of many vertebrates, and although it is implicated in a variety of human intestinal disorders, data regarding the clinical relevance of *Blastocystis* is at best speculative. Several research issues, including a lack of standardization across studies, the potential for intrasubtype variation in pathogenicity, and difficulties associated with diagnostics for many idiopathic disorders of the human IT have led to conflicting reports in support of a role for *Blastocystis* pathogenicity. Here, several research areas and methodologies are reviewed that if integrated appropriately into a prospective study may prove useful and facilitate a better understanding of the role of *Blastocystis* in human health and disease.**

Blastocystis: a controversial protist

Blastocystis is a single-celled, genetically heterogeneous protist, phylogenetically placed within the Stramenopiles [1,2]. Not only is *Blastocystis* a common inhabitant of the human intestinal tract (IT), it is also found in a diverse array of other vertebrates including pigs, cows, chickens, and reptiles and has a worldwide distribution, highlighting both its low host specificity and zoonotic potential [3–5]. *Blastocystis* is also cited as an emerging human pathogen and many epidemiological, as well as *in vitro* and *in vivo*, studies have strongly implicated this microorganism in a variety of disorders [3,6–9].

The human IT is a complex open-ended ecosystem that is host to a diversity of microorganisms including bacteria, archaea, viruses, fungi, and protozoa [10–12]. Crucially, the human intestinal microbiota and specific components of the intestinal microbiota (including *Blastocystis*) have been identified as possible risk factors in a range of both intestinal and extraintestinal diseases [6,13,14]. However, despite reported differences in the composition and stability of intestinal microbial communities of various disease groups compared with healthy control groups [15,16], definitive causal links between disease states and particular microbes are difficult to establish [17]. These difficulties are largely due to the complexity of interactions between the host, host microbiota, and a wide range of other environmental factors that may contribute to the etiology of intestinal illness and functional bowel disorders. Although *Blastocystis* is highlighted as a possible causative agent in intestinal illness and idiopathic disorders of the human IT [6,18–20], and despite increasing research interest in this area, the pathogenic status of this organism remains controversial and there remain many outstanding questions [18,21–23]. To quote the Centre for Disease Control (CDC), "A full understanding of the biology of *Blastocystis* and its relationship to other organisms is not clear, but is

Glossary

Amoeboid: nonspherical, irregular polymorphic form of *Blastocystis* varying in size from 2 to 8 μm. This form is strongly adherent and it is been suggested that this morphotype may be associated with pathogenicity as it has been isolated from symptomatic carriers.

CD dysbiosis: an altered intestinal microbiota and environment in individuals with Crohn's disease (CD) that play an important role in disease onset and maintenance.

Cyst: although very little is known about the lifecycle and zoonotic potential of different *Blastocystis* genotypes and morphological forms, *Blastocystis* transmission is believed to be via cysts through the fecal–oral route. Cysts range in size from 3 to 10 μm and lack a central vacuole. The characteristically thick multilayered cyst wall provides *Blastocystis* with resistance to adverse conditions and water thus facilitating survival outside the host.

Granuolar: this morphotype is very similar to the vacuolar form with the exception of distinct granules present in vacuoles and/or within the cytoplasm. Variation in granules has also been observed, with distinct forms possibly involved in metabolic and reproductive functions. Typical size ranges from 6 to 80 μm.

Health: for this review, the term health or healthy individuals is used with reference to individuals or groups of individuals with normal intestinal functioning and no history of any intestinal complications, infections, or diseases including IBS and IBD. Individuals that host *Blastocystis* but do not show any clinical symptoms of carriage (i.e., asymptomatic carriers) are also considered as healthy. Conversely, as several intestinal diseases and also symptomatic carriage of *Blastocystis* are referred to throughout this review, the term disease is used throughout the text in general reference to individuals or groups with gastrointestinal symptoms (such as abdominal pain, diarrhea, etc.), symptomatic carriers, and/or diseases such as IBS or IBD.

Koch's postulates: were originally devised in 1884 and are a set of four criteria that serve as important guidelines in establishing the etiology of an organism of interest. These four criteria are outlined below; however, it is important to note that these now serve as general guidelines in research studies and many known pathogens do not fulfill all criteria.

1. The microorganism must be present in all organisms suffering with the disease and should be absent in healthy organisms.
2. The microorganism must be isolated from a diseased organism and grown in pure culture.
3. The isolated microorganism must cause symptoms and disease when inoculated into a healthy organism.
4. The microorganism must be re-isolated and verified as the pathogenic agent causing disease from the inoculated, diseased experimental host.

Vacuolar form: this is the most commonly observed *Blastocystis* form and is characterized by a large central body, or 'vacuole' with a thin band or rim of cytoplasm around the periphery. The cell typically varies between 8 and 10 μm, with a range of 5–30 μm reported. Vacuoles are typically associated with food storage and/or osmoregulation in protists, but the exact role of this form in *Blastocystis* remains unclear. Avacuolar forms and multivacuolated forms have been observed for *Blastocystis* and these are morphological variants where no vacuoles and multiple small vacuoles within a single cell are observed, respectively. Unfortunately the relevance of these forms is also unknown.

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Keywords: Blastocystis; intestinal health and disease; pathogen; IBD; IBS; Crohn's disease.

1471-4922/$ – see front matter http://dx.doi.org/10.1016/j.pt.2012.05.001

an active area of research" (http://www.cdc.gov/parasites/*Blastocystis*/).

Blastocystis prevalence in disease

In addition to symptomatic carriage (blastocystosis), *Blastocystis* is hypothesized to play a functional role in bowel disorders such as irritable bowel syndrome (IBS) and possibly inflammatory bowel disease (IBD) [which is an umbrella term for Crohn's disease (CD) and ulcerative colitis (UC)] [18,20,24]. A key issue complicating research into this topic is that together with an absence of clear markers for both IBD and IBS, these intestinal disorders share several nonspecific clinical symptoms that are also correlated with parasitic infections (including diarrhea, abdominal pain, and inflammation), which make it difficult to interpret a pathogenic role for *Blastocystis* in these diseases [6,18]. A variety of different of animal models (primarily chickens, rats, and mice) have been used in experimental infection studies to investigate the pathogenicity of different *Blastocystis* subtypes [23]. However, a key issue with existing models is that mice do not appear to be natural hosts of *Blastocystis* and the range of *Blastocystis* subtypes reported for both chickens and rats are not typically hosted by humans [23,25]. Although studies using these animal models are useful and have provided evidence in support of pathogenicity [26], a key research priority is the development of a more suitable animal model to facilitate further research [23,27].

Consequently, in the absence of a suitable animal model to address Koch's postulates (see Glossary), researchers have relied on the relative presence or absence of *Blastocystis* and/or specific *Blastocystis* subtypes in different cohorts of individuals to assess its putative role in disease. Prevalence data is only as good as and dependent on the sensitivity and accuracy of the detection method used and unfortunately numerous methodological issues associated with the variety of morphological, biochemical, and molecular techniques (see [4] and [21] for an overview of different methodologies) used to detect *Blastocystis* have led to widely conflicting prevalence reports across broad geographic groups of individuals from both health and disease groups, thus exacerbating the controversy (Table 1).

A key issue with standardization in epidemiological surveys of *Blastocystis* is a continued reliance on cultivation and microscopy based methods which have low sensitivities compared with molecular based methods such as PCR [3,28–30]. The accurate detection of *Blastocystis* using microscopy is a considerable challenge due to its morphological variation with over four major forms recognized including amoeboid, cyst, granular, and vacuolar and several other forms such as avacuolar and multivacuolar also reported [21,31–33]. Similarities between *Blastocystis* and

Table 1. Variation in *Blastocystis* prevalence across studies

Case	Controls	Prevalence		*P*	Country	Detection method	Refs
		Case	Control				
IBS	Controls were individuals who had attended the gastroenterology clinic but were not diagnosed with IBS	11/66 (16.7%)	6/60 (10%)	0.203	Thailand	Microscopy and *in vitro* cultivation	[87]
IBS	Patients that attended a gastroenterology outpatient clinic and those with upper abdominal discomfort not suggestive of IBS are cited as healthy controls	95/158 (60%)	38/157 (20%)	<0.001	Egypt	Microscopy and culture	[7]
IBS	Control group consisted of individuals with a diversity of bowel alterations including polyps, diverticulitis, and hemorrhoids	14/45 (31%)	6/45 (13%)	0.043	Mexico	Fecal DNA extraction and PCR	[8]
Hospital patients – no patient data provided	None	9/276 (3.3%)	NA	NA	Singapore	Cultivation followed by PCR for subtyping only	[36]
HM	Nonimmunocompromised patients (non-HM)	15/94 (16%)	12/92 (13%)	0.678[a]	France	Comparison of microscopy, culture, and Q-PCR	[30]
None	Randomly sampled healthy individuals	NA	14/17 (82%)	NA	Ireland	Fecal DNA extraction and PCR	[10]
Symptomatic – individuals were assessed for gastrointestinal symptoms based on questionnaire	Asymptomatic – a sample was taken from a family member that was asymptomatic	38/670 (5.7%)	23/670 (3.4%)	0.07	Iran	Cultivation and microscopy	[88]
Acute diarrhea	None	25/444 (5.6%)	NA	NA	Denmark	Culture PCR for subtyping	[89]
Clinical stool samples – no patient data provided	NA	98/513[b] (19%)	NA	NA	Australia	Cultivation, microscopy, and fecal DNA extraction and PCR	[29]

[a]Own calculation using Fisher's exact test.

[b]A total of 513 samples taken from 462 patients.

Abbreviations: IBS, irritable bowel syndrome; HM, hematological malignancies; NA, not applicable.