**Blastocystis** in Health and Disease: Are We Moving from a Clinical to a Public Health Perspective?

Lee O’Brien Andersen, Christen Rune Stensvold
Department of Microbiology and Infection Control, Statens Serum Institut, Copenhagen, Denmark

*Blastocystis* is a genus of common single-celled intestinal parasitic protists with an unsettled role in human health and disease. Being a stable component of intestinal microbiota, once established, the *Blastocystis* parasite appears more common in healthy individuals than in patients with infectious, functional, or inflammatory bowel disease. Recent data suggest that the parasite is associated with certain gut microbiota profiles and health indices. Convincing data and tools differentiating asymptomatic colonization from infection are yet to be demonstrated. Although the parasite may elicit disease under certain circumstances, the focus on *Blastocystis* may be shifting from a clinical to a public health perspective.

**Blastocystis** is a genus of common single-celled intestinal parasitic protists that are among the most common parasites found in human stool samples in clinical microbiology laboratories. "*Blastocystis hominis*" is still the name for human isolates of *Blastocystis* in clinical, diagnostic specimens; in the current review, however, we prefer not to adhere to the traditional binomial nomenclature for reasons previously described (1).

In contrast to the understanding of "acknowledged" pathogens such as *Giardia, Cryptosporidium, and Entamoeba* spp., the clinical significance of *Blastocystis* has been uncertain since its discovery more than 100 years ago (1). Hence, for many decades, *Blastocystis* parasites have suffered mainly epidemiological research that has sought to identify links between colonization and development of symptoms. Randomized controlled treatment studies serving to identify any role for *Blastocystis* in disease, including alleviation of symptoms upon parasite eradication, are practically nonexistent, which is one of the reasons why no known effective strategy exists with regard to eliminating *Blastocystis* from the intestine (2, 3). The pathogenic potential of *Blastocystis* remains controversial primarily due to the following reasons: (i) asymptomatic colonization is very common; (ii) evidence suggesting *Blastocystis*-induced pathogenicity has been inferred mainly from in vitro studies (some of which were reviewed in recent publications [4, 5]), while pathogenicity remains to be robustly demonstrated in vivo; (iii) in contrast to the above-mentioned protozoa, no striking phenotypic virulence properties, such as the presence of flagella, lectins, or rhoptries, have been identified, and phagocytosis has been described only once (6); (iv) no *Blastocystis*-associated outbreaks have been verified; (v) only anecdotal evidence exists regarding clinical improvement upon *Blastocystis* eradication in patients with gastrointestinal symptoms; and (vi) when epidemiological studies are used for inferring hypotheses on the basis of the pathogenic status of enteric microorganisms, distinctions between endemically and intermittently exposed populations are rarely—if ever—made; such distinctions may be critical to understanding differences in symptom development in the event that host immune response plays a significant role in *Blastocystis*-associated disease.

**GENETIC DIVERSITY AND HOST SPECIFICITY**

The genus of *Blastocystis* is known to comprise at least 17 different ribosomal lineages (7), the so-called subtypes, which are arguably separate species, and of which nine (ST1 to ST9) have been found in humans. In Europe, ST1 to ST4 are found with more or less equal frequencies, whereas ST4 is rarely found outside Europe (8). ST5 to ST9 are reported in humans only rarely, although ST6 and ST7, for instance, may not be uncommon in some regions, e.g., Egypt (9).

Although the subtypes commonly found in humans (i.e., ST1 to ST4) are also found in animals, analysis of subtype alleles (see below) has led to the recognition that ST3 strains from humans and nonhuman primates are genetically different (10), and so cryptic host specificity within subtypes exists, further complicating efforts to approach a relevant binominal nomenclature for *Blastocystis*. On the other hand, it was recently suggested that close contact between pigs and their handlers may increase the risk of zoonotic transmission of *Blastocystis*, based on evidence of ST5—a subtype commonly found in pigs and very rarely in humans—in piggery staff in Queensland, Australia (11). Studies comparing subtypes, and, especially, subtype alleles isolated from different hosts in the same location, would shed more light on *Blastocystis* transmission, including the potential for zoonotic transmission.

**DETECTION AND MOLECULAR CHARACTERIZATION**

Traditional detection of *Blastocystis* in stool samples typically involves microscopy of fecal concentrates or permanently stained smears of stool preserved in, for instance, sodium acetate acetic acid formalin and polyvinyl alcohol prior to processing. However, the sensitivities of such methods are inferior to those of nucleic acid-based methods, including conventional and real-time PCR (12–14). Hence, since prevalence data typically stem from general surveys of intestinal parasites employing the formol acetate concentration technique, such figures should be interpreted with utmost caution.

PCR and sequencing represent the current state of the art of *Blastocystis* subtyping. Several methods involving restriction fragment length polymorphism (RFLP) and diagnostic subtype PCRs have been used; however, the limitations of these methods were
recently described (15), and “barcoding,” either in its original form (16) or in a recently modified form (17), still appears to be the method of choice. Blastocystis barcoding involves subtype identification based on analysis of consensus DNA sequences reflecting the ~600 5′-most bases of the small-subunit (SSU) rRNA gene obtained by PCR and Sanger sequencing. Advantages, apart from high applicability and reproducibility, include the fact that this method has been used in numerous studies, and this example of standardization has been essential for interstudy comparisons. Moreover, the online facility “Blastocystis subtype (185) and Sequence Typing (MLST) Databases” (http://pubmlst.org/blastocystis/) enables effective, effortless, and rapid analysis of individual or batches of fasta files generated by sequencing of PCR products obtained using the barcoding primers or other primers spanning this particular region (16, 17). Use of this facility secures standardization of results and thereby reduces the risk of making erroneous subtype calls, an issue that has been blurring global research into the molecular epidemiology of Blastocystis. A further advantage of the online query facility is the fact that fasta files are automatically queried not only against subtypes but also against subtype alleles, which may be considered “genotypes,” reflecting intrasubtype genetic variation across the barcoding region. Hence, the use of subtype alleles enables discrimination between strains within subtypes, which is critical to molecular epidemiological studies.

Blastocystis parasites are among the easiest to cultivate in vitro. Whereas xenic growth (Blastocystis culture in the presence of metabolically active bacteria) is easy to obtain and maintain for weeks and even months using weekly subcultivation, no robust protocol appears to exist for axenic growth of Blastocystis (Blastocystis culture in the absence of metabolically active bacteria). A variety of growth media can be used for xenic culture, including Jones’ or Robinson’s medium. DNA from xenic strains works well for molecular characterization of strains and as control DNA in DNA-based diagnostic assays; however, for biochemical studies, production of monon- and polyclonal antibodies, and generation of molecular data for studies of comparative genomics and proteomics, including identification of virulence factors and other effector proteins, axenic strains are preferred.

INFECTION OR COLONIZATION?

On the basis of published surveys on parasitic infections and the insensitivity of the methods typically used in such studies, the number of individuals colonized by Blastocystis must be expected to exceed 1 billion worldwide. Recent studies using DNA-based methods to assess the positive rate in different cohorts have seen prevalence rates ranging from about 50% in healthy adults in highly industrialized countries to 100% in healthy Senegalese children (18, 19). There are also data suggesting that long-term carriage of Blastocystis may exist, with some individuals testing positive for the same strain over a span of 10 years (18). It would be fair to expect that many Blastocystis carriers are asymptomatic, and so it would appear relevant to differentiate between asymptomatic colonization and “infection,” with the term “infection” referring to Blastocystis-induced pathology resulting in symptom development. The term “blastocystosis” was coined to denote intestinal symptoms caused by Blastocystis. However, no data or tools are yet available to differentiate between infections; nor do any symptoms appear to be specific to the presence of Blastocystis.

Blastocystis parasitizes among the easiest to cultivate in vitro. Whereas xenic growth (Blastocystis culture in the presence of metabolically active bacteria) is easy to obtain and maintain for weeks and even months using weekly subcultivation, no robust protocol appears to exist for axenic growth of Blastocystis (Blastocystis culture in the absence of metabolically active bacteria). A variety of growth media can be used for xenic culture, including Jones’ or Robinson’s medium. DNA from xenic strains works well for molecular characterization of strains and as control DNA in DNA-based diagnostic assays; however, for biochemical studies, production of monon- and polyclonal antibodies, and generation of molecular data for studies of comparative genomics and proteomics, including identification of virulence factors and other effector proteins, axenic strains are preferred.

INFECTION OR COLONIZATION?

On the basis of published surveys on parasitic infections and the insensitivity of the methods typically used in such studies, the number of individuals colonized by Blastocystis must be expected to exceed 1 billion worldwide. Recent studies using DNA-based methods to assess the positive rate in different cohorts have seen prevalence rates ranging from about 50% in healthy adults in highly industrialized countries to 100% in healthy Senegalese children (18, 19). There are also data suggesting that long-term carriage of Blastocystis may exist, with some individuals testing positive for the same strain over a span of 10 years (18). It would be fair to expect that many Blastocystis carriers are asymptomatic, and so it would appear relevant to differentiate between asymptomatic colonization and “infection,” with the term “infection” referring to Blastocystis-induced pathology resulting in symptom development. The term “blastocystosis” was coined to denote intestinal symptoms caused by Blastocystis. However, no data or tools are yet available to differentiate between infections; nor do any symptoms appear to be specific to the presence of Blastocystis.

Blastocystis harbors mitochondrion-like organelles (MLO), and both nuclear and MLO genomes are currently being surveyed with a view to shedding light on parasite evolution, metabolism, and potential virulence. So far, nuclear genomes of ST4 and ST7 are available, but more nuclear genome data are in the pipeline (Andrew Roger, unpublished data). Phylogenetic analysis of MLO genomes of ST1, -2, -3, -4, -6, -7, -8, and -9 confirms the evolutionary relationships between subtypes as inferred from analysis of ribosomal genes (Alison Jacob, unpublished data).

The surge in studies aiming to investigate the role of the intestinal microbiota in human health and disease has more or less ignored the presence of the intestinal microbial fauna (20). Meanwhile, it is clear that eukaryotic organisms such as Blastocystis are common and stable components of the human intestinal microbiome (18, 21). The first study taking a metagenomics approach to studying Blastocystis appeared in 2015 (22); that study was one of only two studies published to date to study associations between Blastocystis carriage and intestinal microbial communities (22, 23). By retrospective analysis of fecal DNA metagenomics data, we recently showed that Blastocystis is highly associated with certain bacterial communities (22). The study took advantage of the entertype classification already applied to the data (24), and it was demonstrated that Blastocystis parasites were significantly less common in individuals with the Bacteroides entertype than in those with the Ruminococcus or Prevotella enterootypes (Fig. 1).

The study included data on 316 individuals, including 110 classified as obese by body mass index (BMI), 62 classified as overweight, and 143 classified as lean (information was missing for the 1 remaining individual). Overall, there was a tendency of Blastocystis being found more commonly in lean individuals, and when only Danish individuals were included in the analysis (n = 177), this tendency became significant (P = 0.008). There were no associations between BMI and bacterial richness (diversity) or between BMI and entertype; however, significant associations were
noted between low BMI and Blastocystis, high bacterial richness and Blastocystis, Blastocystis and enterotype (Bacteroides), and enterotype (Bacteroides) and high bacterial richness (Fig. 1). From an “omics” point of view, this indicates either that something in the bacterial flora of lean individuals might favor the presence of Blastocystis or that the presence of Blastocystis can favor bacterial microbiota specific to lean healthy individuals. The negative correlation between high bacterial richness and the Bacteroides enterotype suggests that certain other enterotypes promote high bacterial diversity, and high bacterial diversity has been shown in numerous studies to be a marker of health. With the positive correlation between low BMI and Blastocystis and between Blastocystis and high bacterial richness, a correlation between low BMI and high bacterial diversity would appear plausible; however, such a correlation could not be confirmed in the study by Andersen et al. (22). For this reason, it is possible that the correlation between leanness and high bacterial diversity requires the presence of Blastocystis to be significant.

Analyzing fecal DNAs from 23 individuals in agrarian communities in Malawi, 13 U.S. residents, and 22 other mammals using 454 sequencing of amplicons generated by broad-specificity eukaryotic primers, Parfrey et al. (25) recently compared the diversities of intestinal eukaryotic microbes as “symbionts” rather than “parasites” in order to make way for a term that covers mutualists, commensals, and parasites, thereby conveying the diverse interactions they have with hosts other than and beyond pathogenicity. Reporting on asymptomatic giardiasis in children, Ish-Horowicz et al. concluded that healthy day care children with asymptomatic Giardia infection show no disadvantage and perhaps even an advantage in nutritional status and freedom from other illnesses (32).

Studies of the interaction between Blastocystis and gut bacteria appear intriguing: for instance, it would be interesting to learn more about the microbial and physiological factors critical to Blastocystis colonization and, in contrast, about the impact of Blastocystis colonization on surrounding microbiota.

Given the apparent ubiquity of Blastocystis in sub-Saharan populations and the apparent scarcity of these organisms in U.S. communities, it would be worth exploring whether the large difference in prevalence is due to differences in exposure only or whether gut ecological factors may account for this situation. To this end, given the popularity of the hygiene hypothesis, the role of Blastocystis and other parasites in host immunomodulation is, of course, also worth studying (33). High diversity across all components of the gut ecosystem, including the eukaryote component of the microbiota, is associated with health and a lower incidence of autoimmune and inflammatory disease (31), and studies of the relationship between Blastocystis and intestinal microbial diversity and host immunity therefore appear highly relevant. The impact of Blastocystis on the intestinal flora should be studied by in vitro and in vivo experiments with a view to identifying any role for Blastocystis as a probiotic and/or immunomodulatory agent and as a potential inducer of a lean phenotype.

CONCLUSION

Most research into Blastocystis to date has aimed at identifying a role for the parasite in the development of disease. Still, ambiguous and conflicting data have resulted from decades of research, and so it might be appropriate to try and look into any beneficial roles of the organism. We believe that taking an “omics” approach to studying the public health impact of Blastocystis is potentially useful; in particular, the interactions between Blastocystis and host-associated intestinal bacterial communities appear worth exploring.

FUNDING INFORMATION

The Lundbeck Foundation provided funding to Christen Rune Stensvold under grant number R108-A10123. Marie Curie Actions provided funding to Christen Rune Stensvold under grant number 321614.

Lee O’Brien Andersen’s work is partly funded by the Lundbeck Foundation (grant R108-A10123). Christen Rune Stensvold’s work is partly funded by the Marie Curie Actions (call FP7-PEOPLE-2012-CIG; grant 321614).

REFERENCES


C. Rune Stensvold, B.Med.Sc., M.Sc., Ph.D., is a parasitologist and senior scientist at the Department of Microbiology and Infection Control, Statens Serum Institut, Copenhagen, Denmark. He did his postdoc training at the Faculty of Infectious and Tropical Diseases, London School of Hygiene and Tropical Medicine. His chief academic areas include the development and optimization of molecular diagnostics and typing methods for parasitic infections. Dr. Stensvold, moreover, takes a vast research interest in exploring the genetic diversity, host specificity, and public health significance of intestinal parasites, with special emphasis on Blastocystis, Entamoeba, and Dientamoeba. Together with Lee O’Brien Andersen, he is currently developing a DNA-based exhaustive method for detection of bacteria, protists, helminths, and fungi in stool samples based on next-generation sequencing, bespoke software, and a locally curated database. In 2013, Dr. Stensvold was awarded the Fritz Kauffmann Prize for his contributions to clinical microbiology in Denmark.