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MINIREVIEW

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

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***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 been subject to 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

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recently described (15), and "barcoding," either in it 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 (18S) 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 mono- 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*.

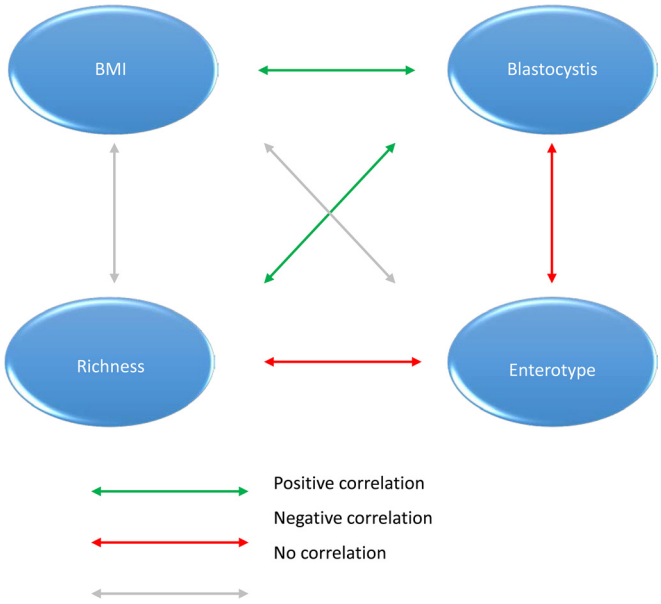

FIG 1 The figure depicts observed significant relations between BMI, bacterial diversity, enterotypes, and *Blastocystis* colonization in healthy Danish individuals, as recently described by Andersen et al. (22).

BLASTOCYSTIS IN THE "OMICS" ERA

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 enterotype classification already applied to the data (24), and it was demonstrated that *Blastocystis* parasites were significantly less common in individuals with the *Bacteroides* enterotype than in those with the *Ruminococcus* or *Prevotella* enterotypes (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 enterotype; 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 microbial eukaryotic gut communities in different populations and showed what could be interpreted as a "defaunation" of individuals adapted to a Western life style. Interestingly, the level of intestinal eukaryotic diversity observed in Malawian individuals was comparable to that seen in the nonhuman hosts investigated; meanwhile, eukaryotic microbiota of the U.S. cohort appeared depauperate. In particular, of the 13 children and adults in Boulder, CO, and Philadelphia, PA, included in the study, none were positive for *Blastocystis*, whereas 16/23 (70%) of the Malawian adults and children were positive, with all individuals with negative test results being infants or toddlers. Indeed, evidence is accumulating that the presence of *Blastocystis* is positively associated with age, with colonization being more common in older children and adults (26, 27).

MOVING FROM A CLINICAL TO A PUBLIC HEALTH PERSPECTIVE

Recent data suggest that *Blastocystis* infections are less common in patients with infectious diarrhea and in those with functional (irritable bowel syndrome [IBS]) and inflammatory bowel diseases (28–30). For instance, a recent Danish study revealed that the proportions of *Dientamoeba*- and *Blastocystis*-positive IBS patients versus healthy controls were 23% and 15% versus 35% and 22%, respectively (28). In patients with inflammatory bowel disease, *Blastocystis* appears to be relatively rare and, possibly, present only in patients in disease remission (30).

When all of the above-mentioned studies are taken into account, it might be speculated that the presence of *Blastocystis* parasites may be an indicator of intestinal and maybe even general health. Indeed, a general shift in the paradigm appears to be emerging and to be appropriate with regard to views on stable eukaryotic intestinal colonizers (31). While, to the knowledge of the authors, no such descriptions are available from human studies, studies of flagellates and ciliates in nonhuman hosts have led to the recognition that such "parasites" may in fact be assisting the host with breakdown of cellulose, hence maintaining vital metabolic processes within the host. It was therefore recently proposed to refer to 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.

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REFERENCES

1. **Clark CG, van der Giezen M, Alfellani MA, Stensvold CR.** 2013. Recent developments in blastocystis research. Adv Parasitol **82**:1–32. http://dx.doi.org/10.1016/B978-0-12-407706-5.00001-0.
2. **Stensvold CR, Smith HV, Nagel R, Olsen KE, Traub RJ.** 2010. Eradication of Blastocystis carriage with antimicrobials: reality or delusion? J Clin Gastroenterol **44**:85–90. http://dx.doi.org/10.1097/MCG.0b013e3181bb86ba.
3. **Nagel R, Bielefeldt-Ohmann H, Traub R.** 2014. Clinical pilot study: efficacy of triple antibiotic therapy in Blastocystis positive irritable bowel syndrome patients. Gut Pathog **6**:34. http://dx.doi.org/10.1186/s13099-014-0034-0.

4. **Wawrzyniak I, Poirier P, Viscogliosi E, Dionigia M, Texier C, Delbac F, Alaoui HE.** 2013. Blastocystis, an unrecognized parasite: an overview of pathogenesis and diagnosis. Ther Adv Infect Dis **1:**167–178.
5. **Roberts T, Stark D, Harkness J, Ellis J.** 2014. Update on the pathogenic potential and treatment options for Blastocystis sp. Gut Pathog **6:**17. http://dx.doi.org/10.1186/1757-4749-6-17.
6. **Dunn LA, Boreham PF, Stenzel DJ.** 1989. Ultrastructural variation of Blastocystis hominis stocks in culture. Int J Parasitol **19:**43–56. http://dx.doi.org/10.1016/0020-7519(89)90020-9.
7. **Alfellani MA, Taner-Mulla D, Jacob AS, Imeede CA, Yoshikawa H, Stensvold CR, Clark CG.** 2013. Genetic diversity of blastocystis in livestock and zoo animals. Protist **164:**497–509. http://dx.doi.org/10.1016/j.protis.2013.05.003.
8. **Alfellani MA, Stensvold CR, Vidal-Lapiedra A, Onuoha ES, Fagbenro-Beyioku AF, Clark CG.** 2013. Variable geographic distribution of Blastocystis subtypes and its potential implications. Acta Trop **126:**11–18. http://dx.doi.org/10.1016/j.actatropica.2012.12.011.
9. **Fouad SA, Basyoni MM, Fahmy RA, Kobaisi MH.** 2011. The pathogenic role of different Blastocystis hominis genotypes isolated from patients with irritable bowel syndrome. Arab J Gastroenterol **12:**194–200. http://dx.doi.org/10.1016/j.ajg.2011.11.005.
10. **Alfellani MA, Jacob AS, Perea NO, Krecek RC, Taner-Mulla D, Verweij JJ, Levecke B, Tannich E, Clark CG, Stensvold CR.** 8 April 2013. Diversity and distribution of Blastocystis sp subtypes in non-human primates. Parasitology http://dx.doi.org/10.1017/S0031182013000255.
11. **Wang W, Owen H, Traub RJ, Cuttell L, Inpankaew T, Bielefeldt-Ohmann H.** 2014. Molecular epidemiology of Blastocystis in pigs and their in-contact humans in Southeast Queensland, Australia, and Cambodia. Vet Parasitol **203:**264–269. http://dx.doi.org/10.1016/j.vetpar.2014.04.006.
12. **Stensvold CR, Arendrup MC, Jespersgaard C, Mølbak K, Nielsen HV.** 2007. Detecting Blastocystis using parasitologic and DNA-based methods: a comparative study. Diagn Microbiol Infect Dis **59:**303–307. http://dx.doi.org/10.1016/j.diagmicrobio.2007.06.003.
13. **Poirier P, Wawrzyniak I, Albert A, El Alaoui H, Delbac F, 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. http://dx.doi.org/10.1128/JCM.01392-10.
14. **Stensvold CR, Ahmed UN, Andersen LO, Nielsen HV.** 14 March 2012. Development and evaluation of a genus-specific, probe-based, internal process controlled real-time PCR assay for sensitive and specific detection of Blastocystis. J Clin Microbiol http://dx.doi.org/10.1128/JCM.00007-12.
15. **Stensvold CR.** 2013. Comparison of sequencing (barcode region) and sequence-tagged-site PCR for blastocystis subtyping. J Clin Microbiol **51:**190–194. http://dx.doi.org/10.1128/JCM.02541-12.
16. **Scicluna SM, Tawari B, Clark CG.** 2006. DNA barcoding of blastocystis. Protist **157:**77–85. http://dx.doi.org/10.1016/j.protis.2005.12.001.
17. **Scanlan PD, Stensvold CR, Cotter PD.** 2015. Development and application of a Blastocystis subtype-specific PCR assay reveals that mixed-subtype infections are common in a healthy human population. Appl Environ Microbiol **81:**4071–4076. http://dx.doi.org/10.1128/AEM.00520-15.
18. **Scanlan PD, Stensvold CR, Rajilić-Stojanović M, Heilig HG, De Vos WM, O'Toole PW, Cotter PD.** 15 September 2014. The microbial eukaryote Blastocystis is a prevalent and diverse member of the healthy human gut microbiota. FEMS Microbiol Ecol http://dx.doi.org/10.1111/1574-6941.12396.
19. **El Safadi D, Gaayeb L, Meloni D, Cian A, Poirier P, Wawrzyniak I, Delbac F, Dabboussi F, Delhaes L, Seck M, Hamze M, Riveau G, Viscogliosi E.** 2014. Children of Senegal River Basin show the highest prevalence of Blastocystis sp. ever observed worldwide. BMC Infect Dis **14:**164. http://dx.doi.org/10.1186/1471-2334-14-164.
20. **Andersen LO, Nielsen HV, Stensvold CR.** 2013. Waiting for the human intestinal Eukaryotome. ISME J **7:**1253–1255. http://dx.doi.org/10.1038/ismej.2013.21.
21. **Scanlan PD, Marchesi JR.** 2008. Micro-eukaryotic diversity of the human distal gut microbiota: qualitative assessment using culture-dependent and -independent analysis of faeces. ISME J **2:**1183–1193. http://dx.doi.org/10.1038/ismej.2008.76.
22. **Andersen LO, Bonde I, Nielsen HB, Stensvold CR.** 29 June 2015. A retrospective metagenomics approach to studying Blastocystis. FEMS Microbiol Ecol http://dx.doi.org/10.1093/femsec/fiv072.
23. **Nourrisson C, Scanzi J, Pereira B, NkoudMongo C, Wawrzyniak I, Cian A, Viscogliosi E, Livrelli V, Delbac F, Dapoigny M, Poirier P.** 2014. Blastocystis is associated with decrease of fecal microbiota protective bacteria: comparative analysis between patients with irritable bowel syndrome and control subjects. PLoS One **9:**e111868. http://dx.doi.org/10.1371/journal.pone.0111868.
24. **Arumugam M, Raes J, Pelletier E, Le Paslier D, Yamada T, Mende DR, Fernandes GR, Tap J, Bruls T, Batto JM, Bertalan M, Borruel N, Casellas F, Fernandez L, Gautier L, Hansen T, Hattori M, Hayashi T, Kleerebezem M, Kurokawa K, Leclerc M, Levenez F, Manichanh C, Nielsen HB, Nielsen T, Pons N, Poulain J, Qin J, Sicheritz-Ponten T, Tims S, Torrents D, Ugarte E, Zoetendal EG, Wang J, Guarner F, Pedersen O, de Vos WM, Brunak S, Doré J; MetaHIT Consortium, Antolín M, Artiguenave F, Blottiere HM, Almeida M, Brechot C, Cara C, Chervaux C, Cultrone A, Delorme C, Denariaz G, et al.** 2011. Enterotypes of the human gut microbiome. Nature **473:**174–180. http://dx.doi.org/10.1038/nature09944.
25. **Parfrey LW, Walters WA, Lauber CL, Clemente JC, Berg-Lyons D, Teiling C, Kodira C, Mohiuddin M, Brunelle J, Driscoll M, Fierer N, Gilbert JA, Knight R.** 2014. Communities of microbial eukaryotes in the mammalian gut within the context of environmental eukaryotic diversity. Front Microbiol **5:**298.
26. **Abdulsalam AM, Ithoi I, Al-Mekhlafi HM, Khan AH, Ahmed A, Surin J, Mak JW.** 2013. Prevalence, predictors and clinical significance of Blastocystis sp. in Sebha, Libya. Parasit Vectors **6:**86. http://dx.doi.org/10.1186/1756-3305-6-86.
27. **Cabrine-Santos M, do Nascimento Cintra E, do Carmo RA, Gabriel Antônio Nogueira Nascentes GA, Pedrosa AL, Correia D, de Oliveira-Silva MB.** 2015. Occurrence of Blastocystis spp. in Uberaba, Minas Gerais, Brazil. Rev Inst Med Trop Sao Paulo **57:**211–214. http://dx.doi.org/10.1590/S0036-46652015000300005.
28. **Krogsgaard LR, Engsbro AL, Stensvold CR, Nielsen HV, Bytzer P.** 2015. The prevalence of intestinal parasites is not greater among individuals with irritable bowel syndrome: a population-based case-control study. Clin Gastroenterol Hepatol **13:**507–513.e502. http://dx.doi.org/10.1016/j.cgh.2014.07.065.
29. **Stensvold CR, Christiansen DB, Olsen KE, Nielsen HV.** 2011. Blastocystis sp. subtype 4 is common in Danish Blastocystis-positive patients presenting with acute diarrhea. Am J Trop Med Hyg **84:**883–885. http://dx.doi.org/10.4269/ajtmh.2011.11-0005.
30. **Petersen AM, Stensvold CR, Mirsepasi H, Engberg J, Friis-Møller A, Porsbo LJ, Hammerum AM, Nordgaard-Lassen I, Nielsen HV, Krogfelt KA.** 25 March 2013. Active ulcerative colitis associated with low prevalence of Blastocystis and Dientamoeba fragilis infection. Scand J Gastroenterol http://dx.doi.org/10.3109/00365521.2013.780094.
31. **Lukeš J, Stensvold CR, Jirkù-Pomajbíková K, Wegener Parfrey L.** 2015. Are human intestinal eukaryotes beneficial or commensals? PLoS Pathog **11:**e1005039. http://dx.doi.org/10.1371/journal.ppat.1005039.
32. **Ish-Horowicz M, Korman SH, Shapiro M, Har-Even U, Tamir I, Strauss N, Deckelbaum RJ.** 1989. Asymptomatic giardiasis in children. Pediatr Infect Dis J **8:**773–779. http://dx.doi.org/10.1097/00006454-198911000-00009.
33. **Scanlan PD, Stensvold CR.** 2013. Blastocystis: getting to grips with our guileful guest. Trends Parasitol **29:**523–529. http://dx.doi.org/10.1016/j.pt.2013.08.006.

Continued next page

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