GLOBAL WATER PATHOGEN PROJECT

PART THREE. SPECIFIC EXCRETED PATHOGENS: ENVIRONMENTAL AND EPIDEMIOLOGY ASPECTS

BLASTOCYSTIS

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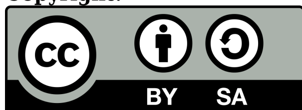

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Citation:

de la Cruz, C. and Stensvold, R. 2017. Blastocystis. In: J.B. Rose and B. Jiménez-Cisneros, (eds) Global Water Pathogen Project. http://www.waterpathogens.org (R. Fayer and W. Jakubowski, (eds) Part 3 *Protists*) http://www.waterpathogens.org/book/blastocystis-hominis Michigan State University, E. Lansing, MI, UNESCO.

https://doi.org/10.14321/waterpathogens.31

Acknowledgements: K.R.L. Young, Project Design editor; Website Design: Agroknow (http://www.agroknow.com)

Last published: November 9, 2017

Summary

Blastocystis is one of the most common single-celled intestinal parasites found in human stool samples and in a wide variety of domestic animals and wildlife. To date, 17 different subtypes (STs)—arguably separate species—have been identified using molecular techniques. At least eight subtypes (ST1–ST8) are shared by human and non-human hosts, suggesting potential zoonotic and anthroponotic transmission. Prevalence in humans may amount to 100% in some areas, with higher records reported in areas where sanitation standards and personal hygiene are relatively poor.

Following its discovery more than 100 years ago, *Blastocystis* remains enigmatic in several respects, including taxonomy, life cycle, pathogenicity, and transmission. Numerous studies incriminate it as being pathogenic, although the parasite is frequently seen in healthy individuals, and no tools are available to distinguish disease from colonization. Symptoms attributed to *Blastocystis* are often non-specific and overlap with infections involving bacteria, viruses, or other parasitic protists.

Blastocystis is highly polymorphic, existing in multiple forms, including a cyst stage, which measures 3–6 µm. *Blastocystis* may be easily missed on microscopy due to its small size and irregular shape, sometimes being misidentified as yeast, lipid, or even an artefact. Reagents are available commercially that facilitate identification of *Blastocystis* by immunofluorescence microscopy. Meanwhile, PCR-based detection is currently state-of-the-art, and analysis of *Blastocystis* nuclear ribosomal DNA enables subtype identification.

Susceptible hosts acquire colonisation/infection via the fecal-oral route, i.e., through accidental ingestion of cyst-contaminated food or water. *Blastocystis* has been recovered from drinking water, sewage/wastewater, and river systems suggesting that water can serve as a source of human infection. Information on the removal and/or inactivation of *Blastocystis* by water and wastewater treatment processes is limited, and little is known regarding sensitivity to disinfection; however, as for (oo)cysts of protozoa, the cyst form may to some extent retain infectivity following disinfection processes. Prevention of source water contamination by human or animal waste may prove critical to reducing transmission of this parasite.

1.0 EPIDEMIOLOGY OF THE DISEASE AND PATHOGEN

1.1 Global Burden of Disease

1.1.1 Global Distribution

Blastocystis is possibly the most prevalent micro-eukaryotic parasite colonizing and/or infecting the large intestine of at least one billion people worldwide (Scanlan et al., 2015; Stensvold, 2015). Several reviews indicate a generally higher prevalence in developing countries where the standards of sanitation and personal hygiene are poor, and where exposure to domesticated animals and consumption of fecally contaminated food and water is common (Tan, 2008; Suresh et al., 2010; Kumar and Tan, 2013). Prevalence also appears to vary among countries and within different communities of the same country. However, these studies have used different diagnostic methods with different levels of sensitivity; hence, the data presented in these reports may not entirely represent the true prevalence of *Blastocystis* (Tan, 2008; Kumar and Tan, 2013).

It should be emphasized that molecular tools now offer a remarkably better screening efficiency for *Blastocystis* when compared to direct microscopy (Abu-Madi et al., 2015). A recent molecular survey performed in rural villages in Senegal, Africa showed a surprising 100% prevalence among children living in close contact with animals and with little or no access to clean water and sanitation (El-Safadi et al., 2014). Updates on prevalence were also recently presented in other parts of Africa, UK, Qatar, United Arab Emirates, and Australia employing molecular approaches for screening (Alfellani et al., 2013a; Wang et al., 2014; Abu-Madi et al., 2015; Pandey et al., 2015; Abuodeh et al., 2016). Subtypes 1 to 4 appear to account for more than 95% of *Blastocystis* in humans, and subtypes such as 1, 2, and 3 appear to have a global distribution (Alfellani et al., 2013a; Roberts et al., 2014a). ST1 and ST3 were the only subtypes found in humans in India (Pandey et al., 2015; Das et al., 2016). Similarly, ST3 has been found to predominate among Malaysian subjects (Nithyamathi et al., 2015). The prevalence of ST4 is subject to remarkable variation, being virtually absent in most countries outside of Europe. ST4 comprises at least two genotypes (Stensvold et al., 2012), one of which is most commonly found in animals, with the other one accounting for most human cases. The genotype found in humans is virtually clonal, representing the subtype allele 42 (Stensvold et al., 2012). Subtypes such as ST5, ST6, ST7, and ST8 are rarely seen in humans, possibly reflecting cases of zoonotic transmission. ST9 has been found only in humans, and only very rarely so.

1.1.2 Symptomatology

This is a parasite that primarily colonizes the coecum and large intestine (Fayer et al., 2014; Wang et al., 2014). *Blastocystis* is most probably confined to the lumen of the gut, although close proximity to the mucosal lining has not been ruled out and colonisation of the small intestine has been noted in pigs (Fayer et al., 2014; Wang et al., 2014). It is shed with stool, and day-to-day variation in shedding has been described (Vennila et al., 1999).

The clinical and public health significance of *Blastocystis* in human health and disease is still an unresolved issue, despite the availability of large amounts of data provided by multi-disciplinary studies. Without a relevant animal model to test Koch's postulates, our

knowledge on the pathogenicity of *Blastocystis* will remain limited (Elwakil and Hewedi, 2010; Coyle et al., 2012). Insights from molecular studies have initially indicated pathogenesis to be subtype-related (Yakoob et al., 2010; Coyle et al., 2012; Roberts et al., 2014b). However, it is clear from subsequent studies that the same subtypes occur in patients with gastrointestinal complaints and asymptomatic carriers (Dogruman-Al et al., 2009), making the issue even more confusing and difficult to resolve. Symptoms that have been linked to symptomatic carriage are usually non-specific and may include diarrhea, constipation, abdominal pain, nausea, flatulence, fatigue, weight loss, and sometimes, skin manifestations, and intestinal inflammation (Tan et al., 2010; Stensvold et al., 2009b). The severity of symptoms may be influenced by factors innate to the host such as age and immune status (Dogruman-Al et al., 2009).

Blastocystis has been proposed as a natural component of the human intestinal microbiota (Andersen and Stensvold, 2016). Recent independent studies suggest that *Blastocystis* is linked to a certain gut microbiota structure. Andersen et al. (2015) managed to identify *Blastocystis*-specific signatures in metagenomics data originally developed for investigation of bacterial organisms only. They confirmed that *Blastocystis* is rare in patients with organic bowel disease but common in healthy individuals. Being the first ever to investigate the association between *Blastocystis* and gut bacterial communities, they found that *Blastocystis* was positively associated with enterotypes (Arumugam et al., 2011) driven by Ruminococcus and Prevotella and negatively associated with the enterotype driven by Bacteroides. This association was later confirmed using a different approach, namely qPCR for detection of major groups of bacteria in samples positive and negative for *Blastocystis* (Andersen et al., 2016). On a similar note, Audebert et al. (2016) using Ion Torrent technology-based 16S rDNA sequencing to decipher *Blastocystis*-associated gut microbiota found higher gut microbiota diversity in patients colonized with *Blastocystis* than in those who were not. Taken together, these studies appear to suggest that *Blastocystis* is associated with gut microbiota generally perceived as "healthy" rather than with gut that is impaired in terms of its function with a microbial imbalance.

Although *Blastocystis* appear to be a predictor of a healthy gut microbiota, any direct role for the parasite in the development of gastrointestinal disease has not yet been ruled out. For instance, it remains unclear to which extent development of disease is dependent on infection intensity; but the presence of five or more cells per high-power/oil immersion objective microscopic field has been suggested to reflect symptomatic carriage (Tan, 2008). Mono-infection with a particular subtype is commonly encountered among individuals, but some cases of mixed-subtype infections have been reported as well (Yoshikawa et al., 2004; Stensvold et al., 2009b; Malheiros et al., 2011; Abdulsalam et al., 2013). Using subtype-specific PCR primers also revealed that mixed-subtype infections were common in a healthy human population (Scanlan et al., 2015).

Blastocystis has been suspected of being associated with irritable bowel syndrome (IBS) (Yakoob et al., 2004; Ustün and Turgay, 2006; Vargas-Sanchez et al., 2015). Regional data on disease burden of *Blastocystis* infection associated with IBS have been reported from 48 states in the US, Argentina, Switzerland, and Chile (FAO/WHO, 2014). Increased prevalence of *Blastocystis* in IBS patients compared with controls has been demonstrated (Yakoob et al., 2004; Dogruman-Al et al., 2010; Yakoob et al., 2010). One study from Pakistan showed a high number of IBS patients harboring *Blastocystis* accompanied by high levels of antibodies against *Blastocystis* (Hussain et al., 1997). ST4 has been potentially linked to IBS and other symptoms or diseases (Stensvold et al., 2011; Alfellani et al., 2013b; Mattiucci et al., 2016); whereas, a study conducted in Denmark showed a greater proportion in the control group than IBS cases carried *Blastocystis* (Krogsgaard et al., 2015). On the other hand, the incidence of *Blastocystis* infection in European and American cohorts did not significantly differ from IBS patients (Giacommeti et al., 1999). Similar findings were reported in a survey that involved Thai subjects (Tungtrongchitr et al., 2004).

It is possible that *Blastocystis* becomes an opportunistic pathogen when the host's immune system is negatively impacted (Gassama et al., 2001; Kurniawan et al., 2009; Alemu et al., 2011; Hotez et al., 2015). In a study involving rats, the induction of physical stress exacerbated infectivity and pathogenicity of *Blastocystis* through dysregulation of the host's oxidant-antioxidant regulatory system and suppression or alteration of the normal development and physiological functions of immune cells and antibody production (Chandramathi et al., 2014). Recently, a 29-kDA parasite protein (Abou-Gamra et al., 2011) and a parasite-associated protease (Abdel-Hameed and Hassanin, 2011) have received attention as potential markers of pathogenicity. Elevated protease activity in cultures having a higher percentage of amoebic forms seen in symptomatic isolates may imply that amoebic forms play a role in exacerbation of intestinal symptoms during *Blastocystis* infection (Rajamanikam and Govind, 2013). The elucidation of the entire genome sequence of ST7 revealed some important processes identifying genes coding for proteins that are responsible for host protease inhibition (Denoeud et al., 2011). These proteins can modulate the host protease activity, thereby disturbing intestinal homeostasis (Parija and Jeremiah, 2013).

1.2 Taxonomic Classification of the Agent

The earliest accounts of *Blastocystis* can be traced back to 1849 with reports of what appeared as cystic forms – known as cholera bodies – observed in feces of cholera patients by Brittan, 1849 and by Swayne and Budd, 1849 according to Kumar and Tan, 2013; Parija and Jeremiah, 2013). With limited tools for isolation and identification available at that time, it was not until the early 20th century when Alexieff and Brumpt studied isolates from humans that the name *Blastocystis* hominis was applied to yeast-like structures (Zierdt, 1991). Nearly forty years after, *Blastocystis* was reclassified as an alga (Cifferi and Redaelli, 1938) and later became known as a protist based on cellular morphology and ultrastructure (Zierdt et al., 1967; Zierdt and Tan, 1976) and following an experiment showing: i) the inability of *Blastocystis* to grow in fungal

media and ii) a positive response to anti-protozoal drugs (Zierdt et al., 1983). However, the taxonomic position of *Blastocystis* remained elusive until molecular tools became available and provided reliable data for a better classification (Silberman et al., 1996; Ho et al., 2000; Arisue et al., 2002). The use of molecular markers has led to the positioning of *Blastocystis* in the Phylum Heterokontophyta; a molecular phylogeny-based heterogeneous group known as “Stramenopiles”, which includes “plant-like protists” such as algae, diatoms, and slime molds (Keeling et al., 2005; Tan, 2008; Riisberg et al., 2009).

1.2.1 *Blastocystis* subtypes

The earliest attempt to designate species epithets for any member of the genus *Blastocystis* was based on the host from which it was isolated. For instance, B. hominis and B. ratti were names applied to all *Blastocystis* detected in humans and rodents, respectively (Clark et al., 2013). Cellular morphology and ultrastructural features were elucidated through electron microscopy and soon considered as diagnostic tools for identifying and classifying species of *Blastocystis* (Cassidy et al., 1994; Stenzel et al., 1997). However, the above-mentioned approaches were proven unreliable soon after analysis of molecular data revealed the remarkable genetic diversity among *Blastocystis* isolates (Clark, 1997). The use of host identity and cell features as criteria for species identification was therefore discontinued, and the terminology has been limited to *Blastocystis* sp. (mostly followed by a subtype number) when referring to any isolates of animal and human origin (Tan et al., 2010; Stensvold et al., 2007; Stensvold, 2013). Currently, 17 different subtypes –arguably separate species– have been identified (inferred from phylogenetic analysis of nuclear small subunit rRNA genes) in humans, non-human primates, other mammals, and birds (Stensvold et al., 2007; Stensvold, 2013; Wang et al., 2014). Molecular studies have shown important aspects of host specificity and cross-infectivity of the different subtypes among mammalian hosts (Noel et al., 2005; Stensvold et al., 2009c; Alfellani et al., 2013a; Alfellani et al., 2013b).

1.2.2 Physical description of the agent

Aside from being one of the two known stramenopiles parasitic to the human body (Stensvold, 2015), *Blastocystis* is peculiar in the sense that it lacks the typical flagellum possessed by all other stramenopiles at some point in their life cycle (Silberman et al., 1996). By microscopy, the parasite can be observed typically as a spherical cell, variable in size, containing numerous mitochondria-like organelles (MLO), Golgi complex, and other features common to eukaryotes (Clark et al., 2013). *Blastocystis* is polymorphic, displaying a great variety in morphology, and a number of forms/life cycle stages have been mentioned, including the vacuolar form, the avacuolar from, the multivacuolar form, the amoeboid form, the granular form, and the cyst form, some of which will be described in further detail below (Tan et al., 2002; Tan, 2008; Stenzel and Boreham, 1991).

The vacuolar form exhibits extensive size variation and is also the form that is usually encountered during direct stool examination and by in vitro culturing. The average cell diameter is approximately 4–15 µm, with some cells reaching the size of about 200 µm in diameter when cultured in vitro (Stenzel and Boreham, 1996; Yamada and Yoshikawa, 2012). It has been suggested that the size varies depending on the geographic source of the isolates (Kumar and Tan, 2013), which, however, requires confirmation; there is also the possibility that cell size varies with fitness, since larger (swollen) cells have been observed typically in cultures to which fresh growth medium has not been provided on a regular basis (Stensvold, unpublished). The vacuolar form is typically round to ovoid in shape, exhibiting a large central vacuole, which occupies almost the entire cytoplasmic space. Histochemical methods have demonstrated the metabolic and storage functions of the central vacuole for carbohydrates, lipids, and basic proteins (Yamada and Yoshikawa, 2012), but this requires further confirmation. The thin rim of cytoplasm found in the periphery contains the nuclei, mitochondria-like organelle, and Golgi complexes (Boreham and Stenzel, 1993; Yamada and Yoshikawa, 2012).

The granular forms were observed to predominantly co-exist with the vacuolar forms in fresh fecal samples or in vitro cultures (Yamada and Yoshikawa, 2012). Moussa (2009) noted the transformation of the vacuolar forms into the granular types after 48 hours of culture. Numerous granules found within the large central vacuole have been suggested to have roles in reproduction (Kumar and Tan, 2013). Using electron microscopy, Suresh et al. (1994) observed small “grape-like” clusters of what was believed to be progeny of *Blastocystis*. A similar observation was made by Singh et al. (1995) when large cells were stained with acridine-orange. Such fluorochrome dye highlights nucleic acids and can be used to localize DNA structures (Grecu et al., 2013). Acridine-orange stain has been used to differentiate the various morphological stages of *Blastocystis* sp. (Suresh et al., 1994; Grecu et al., 2013). It was also used to elucidate the biochemical difference between vacuolar and granular forms of *Blastocystis* sp. cultured at varying temperatures (Gaythri et al., 2014).

The amoeboid form is rarely seen in fresh fecal samples but frequently observed in old or antibiotic-treated cultures (Tan et al., 1996; Tan et al., 2002; Kumar and Tan, 2013). Using light and transmission electron microscopy, cells exhibiting the amoebic form have been described as irregular in shape and with distinct pseudopod-like extensions (Tan et al., 1996; Tan et al., 2001), which have no known function in terms of locomotion (Kumar and Tan, 2013). The role of the amoeboid form in the life cycle of *Blastocystis* remains rather elusive (Tan et al., 2002), and several authors have proposed conflicting views on the transformation into the amoeboid stage. An in vitro encystation study showed that the amoebic form could act as an intermediate stage between the vacuolar stage and the cyst (Suresh et al., 1994). During this stage, the cells may consume bacteria and other cellular debris, possibly to meet nutritional requirements for encystment (Singh et al., 1995; Tan et al., 2001). Indeed, the formation of cyst-like

stages was observed when solubilized bacteria were added under *in vitro* conditions (Suresh et al., 1993). Zhang et al. (2012) suggested that the amoeboid form develops from the vacuolar form, in response to stress during *in vitro* cultivation. Tan and Suresh (2006) proposed a pathogenic role of the amoeboid form as this form was mostly seen in samples from symptomatic carriers. Rajamanikam and Govind (2013) also demonstrated a positive correlation between protease activity and abundance of amoeboid forms in cultures of isolates from patients with gastrointestinal symptoms. Still others have regarded the amoebic form as an artifact resulting from poor handling of the samples (Boreham and Stenzel, 1993).

The cyst is considered the transmissible stage (Suresh et al., 1993). It is very difficult to detect by direct examination of stool samples due to its very small size (Stenzel and Boreham, 1991), which may vary between human and animal isolates (Stenzel et al., 1997). Generally, however, it can be seen in fresh specimens by light microscopy as a round to ovoid refractile object, ranging in size from 2 to 5 µm in diameter (Moussa, 2009; Stenzel and Boreham, 1996; Moe et al., 1996). Despite being smaller in size relative to other forms, the cysts contains one to four nuclei (Stenzel and Boreham, 1996; Yoshikawa et al., 2003), and the cell is encapsulated within a thick wall structure, which provides protection against environmental pressure outside of the host's body (Gajadhar and Allen, 2004; Suresh et al., 2005). Within the cysts, granular bodies have been observed and suggested to contain "hibernating" progeny of *Blastocystis*, which are released during excystation (Suresh et al., 2009). Glycogen was also observed clumping in the cytoplasm (Zaman et al., 1995). Cysts were usually observed in very old cultures (Moussa, 2009) and frequently encountered in stool samples from symptomatic patients (Moe et al., 1999). Cysts were viable for up to 19 days at room temperature (Suresh et al., 1993; Moe et al., 1996).

1.3 Transmission

1.3.1 Routes of transmission

The life cycle proposed by Tan (2008) provides a plausible mechanism for *Blastocystis* transmission, highlighting the remarkable genetic diversity and distribution of the parasite among humans and non-human hosts. Molecular data indicates the apparent host-preference of certain subtypes (Noel et al., 2005; Stensvold, 2013) and the potential of some subtypes involved in zoonotic transmission (Tan, 2008). What remains unclear up to this point are the factors influencing the transition between the intestinal forms of the parasite (Tan, 2008), and whether differences in intestinal forms reflect differences in clinical significance.

Similar to most protozoal agents implicated in diarrheal disease, *Blastocystis* is transmitted via the fecal-oral route through human-to-human or animal-to-human transfer. This has been demonstrated in a transmission model using rats with an infective $dose_{20}$ of at least 10 cysts (data showed this dose corresponded to infection in 20% to 100% of those subjects exposed) (Yoshikawa et al., 2004). In humans, infection is likely initiated upon accidental ingestion of feces-derived cysts via contaminated food and water (Roberts et al., 2014a).

Excystation of viable cysts in the large intestine was demonstrated in laboratory mice (Moe et al., 1997). Vacuolated forms are released and develop into other vegetative forms (Wawrzyniak et al., 2013). Moe et al. (1999) observed large numbers of the vacuolar forms within 24 hours following in vitro inoculation of cysts isolated from human feces. Vacuolar forms multiply asexually through binary fission, the primary mode of reproduction in *Blastocystis* – giving rise to both granular and amoebic forms (Moe et al., 1999). Binary fission commenced in some cysts (*B. ratti*), even before completion of excystation (Chen et al., 1999). Other reproductive means such as budding and plasmotomy have been confirmed recently in *Blastocystis* (Zhang et al., 2012). Others (Kumar and Tan, 2013) suggest a more rapid mode of reproduction in *Blastocystis*– citing the sudden rise in cell population of certain human isolates seen within just a few hours of cultivation (Tan, 2008). Progeny of *Blastocystis* that resembled grape-like structures when observed by electron microscopy appeared to reproduce by a multiple fission-like mode (Suresh et al., 1994, Suresh et al., 1997). Of course, further experimental evidence is warranted to confirm this mode of reproduction.

Vegetative cells can revert into the cystic forms, with the pre-cyst being the transitional structure. Encystation involves extensive dehydration of the cytoplasm coupled with active synthesis of proteinaceous materials as precursors of the wall found in mature cysts (Suresh et al., 2009). Multivacuolar and amoebic forms are postulated to produce two types of cysts: the thin- and thick-walled cyst, respectively (Singh et al., 1995). The thick-walled cyst is the type being released into the external environment during defecation. Excretion of thick-walled cysts was inversely correlated with the abundance of vacuolar forms seen in feces and may depend on host factors such as diet, lifestyle, drugs, and immune status (Suresh et al., 2009). Maintenance of disease through autoinfection is attributed to the thin-walled cyst (Singh et al., 1995) and is most likely since both the process of encystation and excystation could occur in the large intestine (Moe et al., 1997).

1.3.2 Excretion

The vacuolar and cyst forms of *Blastocystis* are the usual stages that are encountered during stool examinations. Venilla et al. (1999) investigated the shedding pattern of the cysts in both human and rodent infection. The number of cysts encountered in human stool appears to vary greatly from day to day, with cysts sometimes being absent in the stool. When present, the number of cysts may reach up to an estimate of 7.4×10^5 cysts per gram of stool. However, no discernable pattern has been observed over the course of infection. A similar irregular pattern was observed in rats over a 20-day monitoring period. Moreover, the abundance of cysts appears to vary from host to host. These findings

underscore the need for repeated stool examination in patients suspected to have *Blastocystis* infection (Venilla et al., 1999). A follow-up study to determine some factors influencing excretion was performed involving a human volunteer with a ST3 infection. Stool samples were submitted and examined within a 30-day monitoring period. High numbers of cysts in stools were associated with frequent visits to the toilet, defecation during the morning, and semi-solid stool form. Emotional fluctuations also appeared to be associated with high variations in the excretion pattern of the cyst (Ragavan et al., 2015).

1.3.3 Population susceptibility

In places where sanitation and/or hygiene practices are poor, the infective cysts may be acquired through direct encounter with an infected person or contact with objects contaminated with faeces (Waikagul et al., 2002; Pipatsatitpong et al., 2012; Anuar et al., 2013). Studies suggest that susceptibility to infection among human hosts appears to be associated with certain subtypes (Souppart et al., 2009; Stensvold et al., 2009a; Coyle et al., 2012; El-Safadi et al., 2014) and immune/ health status of the person during establishment of infection (Horiki et al., 1999; Kurniawan et al., 2009). Other risk factors have been mentioned, including consumption of unboiled water and raw vegetables, and absence of or under-maintained sanitary systems (Kumar and Tan, 2013). A study from Aragua State, Venezuela suggests that persons belonging to relatively lower social class are more likely predisposed to *Blastocystis* infection (Serna et al., 2005). The same findings were obtained in a recently concluded extensive survey involving 1,760 respondents from 26 schools throughout Malaysia (Nithyamathi et al., 2015). Age has been associated with *Blastocystis* infection, with adults being more susceptible than children (Gonzalez-Moreno et al., 2011). However, a remarkably high prevalence of 58% has been recorded among day-care children aged 5 and below from Calarcá, Colombia (Londoño-Franco et al., 2014).

1.3.4 Animal reservoirs

Blastocystis has been categorized as rank 3 zoonotic pathogen associated with waterborne diseases (Suresh et al., 2012). The zoonotic potential of *Blastocystis* is highlighted in several epidemiological surveys that analyzed specimens from humans and animals. *Blastocystis* sp. has been found in farm animals, circus animals, pets as well as in non-human primates (Pakandl, 1991; Boreham and Stenzel, 1993; Stenzel et al., 1994; Abe et al., 2002; de Bomfim and do Couto, 2013; de La Cruz et al., 2016), just to mention a few major groups of host types. Evidence of frequent exposure to animals as a potential risk factor for human infection exists. In Malaysia, a higher prevalence of up to 41% was observed in feces from animal handlers compared with feces from individuals in the city with minimal animal exposure (Salim et al., 1999). Similarly, dog ownership was associated with a high prevalence of *Blastocystis* infection according to one study done in an urban area in Manila, Philippines (Belleza et al., 2015).

Molecular studies performed in certain parts of the world have recorded the occurrence and subtype distribution of *Blastocystis* in a number of animal species. Cats and dogs from the Pacific Northwest region of the US were found to harbour several STs of *Blastocystis*, with ST10 reported to be the predominant subtype (Rauax and Stang, 2015). One study from Australia implicated 18 species of farm and zoo animals as hosts of nine subtypes of *Blastocystis* (ST1–ST5, ST11–ST13, and ST17). Among these animal hosts, moderate to high rates of prevalence was observed in pigs, gorillas, and elephants (Roberts et al., 2013a). Surveys done in pig farms in Southern Queensland, Australia and rural villages in Cambodia, for example, showed a predominance of *Blastocystis* ST5 in pigs with a prevalence ranging from 45%–77%. In the same study, faecal samples from Australian pig handlers were shown to be positive for ST5 (Wang et al., 2014). In another molecular survey carried out in de Colombia, Ramirez et al. (2014) revealed the predominance of ST1 (34%) and ST2 (23%) in more than 350 fecal samples from humans and different species of mammals. These subtypes (ST1 and ST2) together with ST3 were found to co-infect humans and domesticated animals alike. Subtypes 3, 4, 6 and 8 were also observed in a few samples, with the latter two found exclusively in birds and marsupials (Ramirez et al., 2014). In the Philippines, isolates from pig farmers shared 100% sequence similarities to a pig subtype (Rivera, 2008). Subtype analysis of animal isolates coming from pigs (ST5) from China (Yan et al., 2007); and samples also from a pig and horse in Thailand (Thathaisong et al., 2003) showed sequence similarity with human isolates.

Indeed, the use of molecular tools has further provided insights into the cross-transmissibility of certain *Blastocystis* subtypes between man and his domesticated animals. Recently, an online tool (www.pubmlst.org/blastocystis) was developed for allele identification of *Blastocystis* subtypes, which enables investigation into host specificity on a more discriminative level than that representing mere 18S analysis. Hence, each subtype comprises up to several different alleles, some of which appear to exhibit strict host specificity and some of which appear to be linked to geography.

1.4 Population and Individual Control Measures

1.4.1 Drug therapy and hygiene measures

The disputable pathogenic nature of *Blastocystis* has limited our approach to treatment of *Blastocystis* infection. Antimicrobial agents have been investigated individually or in combination for their efficacy to eradicate *Blastocystis* infection in certain cases. To date, metronidazole is still considered the primary drug of choice in treating blastocystosis, especially in symptomatic patients (Kurt et al., 2016). The drug is usually administered to adults for 10 days, usually 3x a day with a dosage ranging from 250 to 750 mg or 1.5 g per day (Sekar and Shanthi, 2013). However, metronidazole does not always result in total eradication, with some cases being clearly resistant even in higher dosage (Engsbro and Stensvold, 2012). Initial treatment with metronidazole has been found ineffective in

eliminating ST1, ST3, ST4, and ST5 from feces of patients with intestinal symptoms (Roberts et al., 2013b). Other treatment drugs including nitazoxanide, ornidazole, paromomycin, and trimethoprim were also tested and found to have varying efficacy (Roberts et al., 2014b). ST1, ST3, ST4, and ST8 have been shown to have little sensitivity against single-treatment with metronidazole or paromomycin and to triple-treatment with zolidone, nitazoxanide and secnidazole (Roberts et al., 2015) Drug susceptibility has been suggested to be subtype-related which can only be elucidated through the use of high-resolution molecular markers (Stensvold et al., 2010; Roberts et al., 2015). A recent study investigated the effects of several herbal extracts on *Blastocystis* and found garlic and ginger to significantly reduce the number of cells after 48 hours (Abdel-Hafeez et al., 2015). Another study have also demonstrated the anti-*Blastocystis* effects of some ethanolic extracts from 21 medicinal plants collected from Ghana (Bremer et al. (2015).

As with other parasites transmitted via the oral-fecal route, *Blastocystis* infection can be minimized through proper hygiene at the individual and community level. These include consumption of treated water and thoroughly-cooked food and maintenance of waste disposal systems.

2.0 ENVIRONMENTAL OCCURRENCE AND PERSISTENCE

2.1 Detection Methods

State-of-the-art detection of *Blastocystis* now relies on real-time PCR using genomic DNA extracted from faeces. DNAs can be obtained by one of numerous commercial kits, and at least three real-time PCRs have been published, two of which (Poirier et al., 2011; Stensvold et al., 2012) enable the detection of all the subtypes so far identified in humans (ST1–ST9). DNA-based detection may prompt a wish for subtyping *Blastocystis* in positive cases, and so the DNA can also be used for this purpose; here, one of the most commonly used methods is “barcoding”, which relies on amplification and sequencing of small subunit ribosomal genes (Scicluna et al., 2006; Stensvold et al., 2013). Sequences obtained by the barcoding method can be analysed individually or in bulk, using the above mentioned online database (www.pubmlst.org/blastocystis) (Stensvold et al., 2012a), where both subtype and subtype allele information will be provided upon sequence query. This tool recently enabled detailed epidemiological analysis of *Blastocystis* from human and non-human primates (Alfellani et al., 2013b); although shared subtypes were identified, potentially suggesting overlapping host specificity, the strains found in human and non-human primates differed, as evidenced by subtype allele analysis.

For diagnostic purposes, culture of fresh stool using one or more of a variety of suitable and quite simple media (Clark and Diamond, 2002) can be used in those settings where real-time PCR or PCR and sequencing is not available. Although not as sensitive as PCR, culture is a better alternative than traditional microscopy of faecal concentrates (Stensvold et al., 2007b; Rene et al., 2009). The diagnostic PCRs published so far remain to be evaluated with regard to their applicability on water samples.

Commercial assays are available, including one for detection of *Blastocystis*-specific antigens in stool (CoproELISA BlastocystisTM, Savyon Diagnostics); the assay was recently evaluated by Dogruman-Al et al. (2015). Detection by immunofluorescence (IFA) microscopy using ParaFlor B™ antibody (Boulder Diagnostics, Boulder, CO) has been described by Fayer et al. (2012 and 2014).

2.2 Data on Occurrence in the Environment

2.2.1 Raw sewage and sludge

Few studies have reported direct evidence for the presence of *Blastocystis* cysts in wastewater samples. It has been found that influent and effluent samples from Pakistan were positive with *Blastocystis* (Zaman and Khan, 1994). Suresh et al. (2005) also detected viable cysts in sewage treatment plants from Scotland and Malaysia. In vitro cultivation revealed that 50% the total influent samples and 28% of effluents contained viable cysts. The study of Banaticla and Rivera (2011) using in vitro cultivation coupled with SSU rRNA gene sequencing revealed the occurrence of ST1 and ST2 from 9 of 62 (14.5%) wastewater samples from residential, commercial, recreational areas and hospitals. Of these, 7 (23%) were detected in 31 influent samples; whereas two (6%) were recovered from 31 effluent samples. The sequence identities of the isolates were also found to be highly similar to those found in humans and non-human primates. The viability of cysts recovered in these studies as revealed by in vitro cultivation presents evidence of the probability of waterborne *Blastocystis* infection.

2.2.2 Surface water

Water samples collected from the Indrawati River and Sindukhola River in Nepal showed the presence of *Blastocystis* ST1 and ST4; both subtypes were known to occur in humans and their farm animals within the area. Results of this study strongly imply the possibility of waterborne zoonotic transmission of *Blastocystis* (Lee et al., 2012). *Blastocystis* was also found in two recreational rivers in Malaysia. A positive correlation was found between coliforms and *Blastocystis* in river samples (Ithoi et al., 2011).

2.2.3 Ground waters and drinking waters

Waterborne outbreaks of *Blastocystis* have been reported only in China (Wu et al., 2000) and Italy (Guglielmetti et al., 1989). Nonetheless, the presence of *Blastocystis* in drinking water highlights the possibility of waterborne transmission of this parasite and the presence of cysts has been demonstrated in a number of epidemiological surveys. *Blastocystis* cysts were observed in 1% of the total 840 potable water samples analysed in Egypt (El Shazy et al., 2007). Similarly, 10% (one of 10) water samples collected in a rural community in Argentina

was positive for *Blastocystis* (Basualdo et al., 2007). Screening of drinking water in La Planta urban center, Argentina resulted in an estimate of finding four cells per 100 liter of water that corresponded to 4.2% probability of occurrence (Basualdo et al., 2000). However, the use of direct microscopy with low sensitivity, even with the aid of staining techniques, as the method of detection in these studies might have underestimated the actual prevalence of the cysts from these areas.

The development of molecular approaches has increased the detection sensitivity for *Blastocystis*, especially in water samples. A study that used sequenced-tag primers demonstrated ST1 in tap water and fecal samples from human subjects, suggesting the former as the source of human infection (Eroglu and Koltas, 2010). Fecal and water samples coming from a primary school in central Thailand were also analyzed through direct PCR and sequencing of the SSU rRNA gene. The study revealed 18.9% prevalence with subtypes 1 and 2 detected in human samples. ST1 was also detected in water collected from sources near the school (Leelayoova et al., 2008). Exposure to well water was also linked to *Blastocystis* infection among patients examined in a Children's hospital of Pittsburg, Germany (O'Gorman et al., 1993). The occurrence of some protozoa in household tanks was investigated and *Blastocystis* was found in 12% of the water samples (Khalifa et al., 2001).

Several studies have also linked human blastocystosis with environmental contamination and poor sanitary or personal hygiene practices – factors that could elevate the environmental occurrence of the resistant cysts. Investigation of gastroenteritis outbreak in Izmer, Turkey has been linked to a recent heavy rainfall that resulted in cross-contamination of drinking water pipes with intestinal parasites, including *Blastocystis* (Tuncay et al., 2008). Another survey conducted in Nakhon Ratchasima Province, Thailand has linked several intestinal parasitic infections, including blastocystosis with the consumption of unboiled drinking water (Kitvatanachai et al., 2008). Investigations of *Blastocystis* infection in certain children populations in different areas suggested environmental contamination as one major factor. Faeces from primary school children from Chile showed a high prevalence of *Blastocystis*, reaching up to 64.3% and this was attributed to fecal contamination of the river (Navarrete and Torres, 1994). *Blastocystis* infection was also frequently encountered among children aged 10 and below and consume stored water in lid-covered containers (Seran et al., 2005 as cited by Suresh et al., 2010). Similarly, Abdulsalam et al. (2012) observed an estimated 25.7% prevalence among 300 primary school children in rural communities in Pahang state, Malaysia. The source of the water supply was a significant predictor of infection in children, alongside the low educational level of their mothers. A high prevalence of *Blastocystis* (61.8%) along with other intestinal parasites was found in persons residing in houses in Chile with no sanitary feces disposal system (Torres et al., 1992). In Yunnan Province, China, unboiled/ raw drinking water and exposure to pigs were risk factors. Consumption of raw water was positively correlated with ST1 infections; while ST3 with unboiled drinking water (Li et al., 2007). A remarkable 100% prevalence of *Blastocystis* was found among children residing along the Senegal River (El-Safadi et al., 2014). This community used water from the river and wells for drinking, cooking, and other household purposes. Water collected from such sources may have been contaminated with human and animal wastes that contain the environmentally-resistant cysts.

At an army base in Thailand, 334 (36.9%) of 904 stool samples from army personnel were positive for *Blastocystis* (Leelayoova et al., 2004). Consumption of unboiled drinking water was found to be significantly associated with *Blastocystis* infection in this cohort. Similar to their previous study from another army base (Taasmari et al., 2000), drinking unboiled water presented a higher risk of acquiring *Blastocystis* infection. Both studies have provided strong evidence for the role of waterborne transmission for this parasite. In addition, preference for unboiled drinking water also predisposed Orang Asli tribal communities in Malaysia towards infection with *Blastocystis* (Anuar et al., 2013). *Blastocystis* was also found the most prevalent intestinal parasite (27.2%) among 504 people surveyed in a rural community in Argentina. Among the environmental variables investigated in this study, the presence of communal pump and public faucet as possible sources of drinking water as well as body waste disposal by latrine were all associated with human blastocystosis (Basualdo et al., 2007). Two parasitological surveys among preschool children in Northern Jordan showed *Blastocystis* as one of the most frequent parasites found in stool samples. In these studies, consumption of contaminated water was suspected to be the source of infection. For instance, the potential of *Blastocystis* for water transmission was suggested due to association with giardiasis, the infective agent known for being a waterborne pathogen (Nimri, 1993). Also, children from rural places that drank untreated rainwater stored in wells had higher *Blastocystis* infection rates than the children coming from the city (Nimri and Batchoun, 1994).

2.2.4 Seawater and shellfish

Blastocystis cysts were recovered in samples of mussels (Anodonta anatine) collected from Lake Malta, a municipal reservoir in Poland. This finding demonstrates the waterborne nature of *Blastocystis* and the potential role of shellfish as a source of *Blastocystis* (Słodkowicz-Kowalska et al., 2015).

2.3 Persistence

No data available.

3.0 REDUCTIONS BY SANITATION MANAGEMENT

3.1 Wastewater Treatment

According to a recent meta-analysis utilizing data from 54 papers, access to and use of sanitation facilities and water treatment were found to lower the odds of infection with intestinal protozoa (Speich et al., 2016). This highlights the importance of improving water safety,

sanitation, and hygiene behavior to minimize spread of infectious parasitic agents, including *Blastocystis*.

3.1.1 Composting of fecal wastes

No data available.

3.1.2 Wastewater treatment facilities

No data available.

3.1.3 Tertiary treatment

No data available.

3.2 Disinfection

3.2.1 Chlorine and ozone

Effects of chemical and physical intervention on cyst viability have been subject to investigation. The morphological integrity of the cysts remained intact when they were placed in distilled water (Zaman, 1994; Zaman et al., 1995), with cysts able to survive for up to 10 days (Wu et al., 2000). The effect of ozonation on the viability/infectivity of some parasitic protists, including *Blastocystis*, was studied by Khalifa et al. (2001). While the viability of Giardia cysts, Cryptosporidia oocysts, and microsporidia were completely inactivated following exposure to ozonated water at a concentration of 1 ppm for nine minutes, some *Blastocystis* survived and remained infective to laboratory animals (Khalifa et al., 2001). When exposed to varying concentrations of chlorine, the cysts of *Blastocystis* remained viable even at concentration of 2.2 ppm for up to three hours (Zaki et al., 1997). As such, routine chlorination set at the standard concentration of 0.5 to 1.0 ppm to disinfect drinking water against vegetative bacteria and inactivation of viruses was proven ineffective with regard to metabolically inactivating *Blastocystis* cysts. These studies indicate a highly resistant nature of *Blastocystis* cysts relative to those of other intestinal protozoa.

3.2.2 Irradiation and UV disinfection

No data available.

References

Abdel-Hafeez, E.H., Ahmad, A.K., Abdelgelil, N.H., Abdellatif, M.Z.M., Kamal, A.M. and Mohamed, R.M. (2015). In vitro effect of some Egyptian herbal extracts against *Blastocystis hominis*. Journal of the Egyptian Society of Parasitology. 45, pp. 93–100.

Abdel-Hameed, D.M. and Hassanin, O.M. (2011). Proteaese activity of *Blastocystis hominis* subtype3 in symptomatic and asymptomatic patients. Parasitology Research. 109, pp. 321–327. doi: 10.1007/s00436-011-2259-x.

Abdulsalam, A.M., Ithoi, I., Al-Mekhlafi, H.M., Ahmed, A., Surin, J. and Mak, J. (2012). Drinking water is a significant predictor of *Blastocystis* infection among rural Malaysian primary school. Parasitology. 139, pp. 1014–1020. doi: 10.1017/S0031182012000340.

Abdulsalam, A.M., Ithoi, I., Al-Mekhlafi, H.M., Al-Mekhlafi, A.M., Ahmed, A. and Surin, J. (2013). No Title. PLoS ONE. 8, pp. e84372. doi: 10.1371/journal.pone.0084372.

Abe, N., Nagoshi, M., Takami, K., Sawano, Y. and Yoshikawa, H. (2002). A survey of *Blastocystis* sp. in livestock, pets, and zoo animals in Japan. Veterinary Research. 106, pp. 203–212.

Abou-Gamra, M.M., Elwakil, H.S., Deeb, H.K., Khalifa, K.E. and Abd-Elhafiz, H.E. (2011). The potential use of 29 kDa protein as a marker of pathogenicity and diagnosis of symptomatic infections with *Blastocystis hominis*. Parasitology Research. 108, pp. 1139–1146. doi: 10.1007/s00436-010-2156-8.

Abu-Madi, M., Aly, M., Behnke, J.M., Clark, C.G. and Balkhy, H. (2015). The distribution of *Blastocystis* subtypes in isolates from Qatar. Parasit Vectors. 8, pp. 465. doi: 10.1186/s13071-015-1071-3.

AbuOdeh, R., Ezzedine, S., Samie, A., Stensvold, C.R. and El-Bakri, A. (2016). Prevalence and subtype distribution of *Blastocystis* in healthy individuals in Sharjah, United Arab Emirates. Infection, Genetics, and Evolution. Genetics and Evolution. 37, pp. 158–162. doi: 10.1016/j.meegid.2015.11.021.

Alemu, A., Shiferaw, Y., Getnet, G., Yalew, A. and Addis, Z. (2011). Opportunistic and other intestinal parasites among HIV/AIDS patients attending Gambi higher in Bahir Dar City, North West Ethiopia. Asian Pacific Journal of Tropical Medicine. 4, pp. 661–665. doi: 10.1016/S1995-7645(11)80168-5.

Alfellani, M.A., Jacob, A.S., Perea, N.A., Krecek, R.S., Taner-Muella, D., Verweij, J.J. *et al*. (2013). Diversity and distribution of *Blastocystis* sp. subtypes in non-human primates. Parasitology. 140, pp. 966–971. doi: 10.1017/S0031182013000255.

Alfellani, M.A., Stensvold, C.R., Vidal-Lapiedra, A., Onuoha, E.S., Fagbenro-Beyioku, A.F. and Clark, C.G. (2013). Variable geographic distribution of *Blastocystis* subtypes and its potential implications. Acta Tropica. 126, pp. 11–18. doi: 10.1016/j.actatropica.2012.12.011.

Andersen, L., Karim, A.B., Roager, H.M., Vigsnæs, L.K., Krogfelt, K.A., Licht, T.R. *et al*. (2016). European Journal of Clinical Microbiology and Infectious Diseases. doi: 10.1007/s10096-016-2680-2.

Anuar, T.S., Ghani, M.K.A., Azreen, S.N., Salleh, F.M. and Moktar, N. (2013). *Blastocystis* infection in Malaysia: evidence of waterborne and human-to-human transmissions among Proto-Malay, Negrito, and Senoi tribes of Orang Sali. Parasites and Vectors. 6, pp. 40. doi: 10.1017/S0031182012000340.

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. Journal of Eukaryotic Microbiology. 49, pp. 42–53.

Arumugam, M., Raes, J., Pelletier, E., Le Paslier, D., Yamada, T. and Mende, D.R. (2011). Enterotypes of the human gut microbiome. Nature. 473, pp. 174–180. doi: 10.1068/nature09944.

Audebert, C., Even, G., Cian, A., Group, B.Investigat, Loywick, A., Merlin, S. *et al*. (2016). No Title. Scientific Reports. 6, pp. 25255. doi: 10.1038/srep25255.

Banaticla, J.E. and Rivera, W.L. (2011). Detection and subtype identification of *Blastocystis* isolates from wastewater samples in the Philippines. Journal of Water and Health. 9, pp. 128–137. doi: 10.2166/wh.2010.127.

Basualdo, J., Pezzani, B., De Luca, M., Córdoba, A. and Apezteguía, M. (2000). Screening of the municipal water system of La Plata, Argentina, for human intestinal parasites. International Journal of Hygiene and Environmental Health. 203, pp. 177–182.

Basualdo, J.A., Cordoba, M.A., De Luca, M.M., Ciarmela, M.L., Pezzani, B.C., Grenovero, M.A. *et al*. (2007). Intestinal parasitoses and environmental factors in a rural population of Argentina, 2002-2003. Revista do Instituto de Medicina Tropical de São Paulo. 49, pp. 251–255.

Belleza, M.L.B., Cadacio, L.C., Borja, M.P., Solon, J.A.A., Padilla, M.A., Tongol-Rivera, P. *et al*. (2015). Epidemiologic study of *Blastocystis* infection in an urban community in the Philippines. Journal of environmental and public health. doi: 10.1155/2015/894297.

Bomfim, T.C.B. and Couto, M.C.M. (2013). Morphological diagnosis and occurrence of *Blastocystis* spp. obtained from the stool samples of domestic bird species commercialized in municipal markets. Journal of Parasitology and Vector Biology. 5, pp. 20–26.

Boreham, P.F.L. and Stenzel, D.J. (1993). *Blastocystis* in humans and animals: morphology, biology, and epizootiology. Advances in Parasitology. 32, pp. 1–70.

Bremer, C.C., Soelberg, J., Stensvold, C.R. and Jäger, A.K. (2015). Activity of medicinal plants from Ghana against the parasitic gut protist *Blastocystis*. Journal of Ethnopharmacology. 174, pp. 569–575. doi: 10.1016/j.jep.2015.03.006.

Cassidy, M.F., Stenzel, D.J. and Boreham, P.F.L. (1994). Electron microscopy of surface structures of *Blastocystis* sp. from different hosts. Parasitology Research. 80, pp. 505–511.

Chandramathi, S., Kumar, S., Sivanandam, S. and Kuppusamy, U.R. (2014). Stress exacerbates infectivity and pathogenicity of *Blastocystis hominis*: *in vitro* and in vivo evidences. PLOS One. 9, pp. 294567. doi: 10.1371/journal.pone.0094567.

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

Ciferri, R. and Redaelli, P. (1938). A new hypothesis on the nature of *Blastocystis*. Mycopathologia. 1, pp. 3-6.

Clark, C.G. (1997). Extensive genetic diversity in *Blastocystis hominis*. Molecular and Biochemical Parasitology. 87, pp. 79–83.

Clark, C.G. and Diamond, L.S. (2002). Methods for cultivation of luminal parasitic protists of clinical importance. Clinical Microbiology Reviews. 15, pp. 329–341.

Clark, C.G., Van Der Giezen, M., Alfellani, M.A. and Stensvold, C.R. (2013). Recent developments in *Blastocystis* research. Advances in Parasitology. 82, pp. 1–32. doi: 10.1016/B978-0-12-407706-5.00001-0.

Coyle, C.M., Varughese, J., Weiss, L.M. and Tanowitz, H.B. (2012). *Blastocystis*: to treat or not to treat. Clinical Infectious Diseases. 54, pp. 105–110. doi: 10.1093/cid/cir810.

Das, R., Khalil, S., Mirdha, B.R., Makharia, G.V., Dattagupta, S. and Chaudhry, R. (2016). Molecular characterization and Subtyping of *Blastocystis* species in irritable bowel syndrome patients from North India. PLoS One. 11, pp. e0147055. doi: 10.1371/journal.pone.0147055.

De La Cruz, C.P.P., Gorospe, M. and Paller, V.G.V. (2016). *Blastocystis* infection among backyard-raised pigs in Bay, Province of Laguna, The Philippines. Asian Journal of Microbiology, Biotechnology, and Environmental Science,.

Denoeud, F., Roussel, M., Noel, B., Wawrzyniak, I. and Da Silva, C. (2011). Genome sequence of the stramenopile *Blastocystis*, a human anaerobic parasite. Genome Biology. 12(R29), doi: 10.1186/gb-2011-12-3-r29.

Dogruman-Al, F., Kustimur, S., Tuncer, C., Simsek, Z., Tanyuksel, M., Araz, E. *et al*. (2009). *Blastocystis* subtypes in irritable bowel syndrome and inflammatory bowel disease in Ankara, Turkey. Memórias do Instituto Oswaldo Cruz. 104, pp. 724–727. doi: 10.1590/S0074-02762009000500011.

Dogruman-Al, F., Simsek, Z., Boorom, K., Ekici, E., Sahin, M., Tuncer, C. *et al*. (2010). Comparison of methods for detection of *Blastocystis* infection in routinely submitted stool samples, and also in IBS/IBD patients in Ankara, Turkey. PLoS ONE. 5, pp. e15484. doi: 10.1371/journal.pone.0015484.

Dogruman-Al, F., Turk, S., Adiyaman-Korkmaz, G., Hanenel, A., Levi, L., Kopelowitz, J. *et al*. (2015). Dogruman-Al, F., Turk, S., Adiyaman-Korkmaz, G., Hanenel, A., Levi, L., Kopelowitz, J., Babai, O., Gross, S., Greenberg, Z., Herschkovitz, Y., And Mumcuoglu, I. Parasitology Research. 114, pp. 495–500.

El-Safadi, D., Gaayeb, L., Meloni, D., Cian, A. and Poirier, P. (2014). Children of Senegal River Basin show the highest prevalence of *Blastocystis* sp. ever observed worldwide. BMC Infectious Diseases. 14, pp. 164. doi: 10.1186/1471-2334.

El-Shazly, A.M., Elsheikha, H.M., Soltan, D.M., Mohammad, K.A. and Morsy, T.A. (2007). Protozoal pollution of surface water sources in Dakahlia Governorate, Egypt. Journal of the Egyptian Society of Parasitology. 37, pp. 51–64.

Elwakil, H.S. and Hewedi, I.H. (2010). Pathogenic potential of *Blastocystis hominis* in laboratory mice. Parasitology Research. 107, pp. 685–689.

Engsbro, A.L. and Stensvold, C.R. (2012). *Blastocystis*: to treat or not to treatłdots but how?. Clinical Infectious Diseases. 55, pp. 1431–1432. doi: 10.1093/cid/cis699.

Eroglu, F. and Koltas, I.S. (2010). Evaluation of the transmission of B. hominis by using PCR method. Parasitology Research. 107, pp. 841–845. doi: 10.1007/s00436-010-1937-4.

FAO, WHO (2014). Multicriteria-based ranking for risk management of food-borne parasites. Microbiological Risk Assessment Series No. 23. pp. 302.

Fayer, R., Elasser, T., Gould, R., Solano, G., Urban, Jr, J. and Santin, M. (2014). *Blastocystis* tropism in the pig intestine. Parasitology Research. 113, pp. 1465–1472.

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. Parasitology Research. 111, pp. 1349–1355.

Gajadhar, A.A. and,. (2004). Factors contributing to the public health and economic importance of waterborne zoonotic parasites. Veterinary Parasitology. 126, pp. 3–14.

Gassama, A., Sow, P.S., Fall, F., Camara, P., Guèye-N'diaye, A., Seng, R. *et al*. (2001). Ordinary and opportunistic enteropathogens associated with diarrhea in Senegalese adults in relation to human immunodeficiency virus serostatus. International Journal of Infectious Diseases. 5, pp. 192–198.

Gaythri, T., Suresh, K., Subha, B. and Kalyani, R. (2014). Identification and characterization of heat shock protein 70 in thermal stressed *Blastocystis* sp. PLoS ONE. 9, pp. e95608. doi: 10.1371/journal.pone.0095608.

Giacommeti, A., Cirioni, O., Fiorentini, A., Fortuna, M. and Scalise, G. (1999). No Title. Journal of clinical microbiology and infectious diseases. 18, pp. 436–439.

Gonzalez-Moreno, O., Domingo, L., Teixidor, J. and Gracenea, M. (2011). No Title. Parasitology Research. 108, pp. 87–93.

Grecu, D., Neagu, A., Harmanescu, E. and Moglan, I. (2013). In vitro division modalities developed by *Blastocystis hominis* examined with the acridine orange stain. Analele Științifice ale Universității „Alexandru Ioan Cuza” din Iași, s. Biologie animal.

Guglielmetti, P., Cellesi, C., Figura, N. and Rossolini, A. (1989). Family outbreak of *Blastocystis hominis* associated gastroenteritis. Lancet. 2, pp. 1394.

Ho, L.C., Jeyeseelan, K. and Singh, M. (2000). Use of the elongation factor-1 alpha gene in a polymerase chain reaction-based restriction-fragment-length polymorphism analysis of genetic heterogeneity among *Blastocystis* species. Molecular and Biochemical Parasitology. 112, pp. 287–291.

Horiki, N., Kaneda, Y., Maruyama, M., Fujita, Y. and Tachibana, H. (1999). Intestinal blockage by carcinoma and *Blastocystis hominis* infection. American Journal of Tropical Medicine and Hygiene. 60, pp. 400.402.

Hussain, R., Jaferi, W., Zuberi, S., Bagai, R., Abrar, N., Ahmed, A. *et al*. (1997). Significantly increased IgG2 subclass antibody levels to *Blastocystis hominis* in patients with irritable bowel syndrome. American Journal of Tropical Medicine and Hygiene. 5, pp. 301–306.

Ithoi, I., Jali, A., Mak, J.W., Sulaiman, W. and Mahmud, R. (2011). Occurrence of *Blastocystis* in Water of Two Rivers from Recreational Areas in Malaysia. Journal of Parasitology Research. 6, pp. 123916. doi: 10.1155/2011/123916.

Keeling, P.J., Burger, G., Durnford, D.G., Lang, B.F., Lee, R.W., Pearlman, R.E. *et al*. (2005). The tree of eukaryotes. Trends in Ecology and Evolution. 20, pp. 670–676.

Khalifa, A.M., El-Temsahy, M.M. and Naga, IF.Abou-El (2001). Effect of ozone on the viability of some protozoa in drinking water. Journal of the Egyptian Society of Parasitology. 31, pp. 603–616.

Kitvatanachai, S., Boonslip, S. and Watanasatitarpa, S. (2008). Intestinal parasitic infections in Srimum suburban area of Nakhon Ratchasima Province, Thailand. Tropical Biomedicine. 25, pp. 237–242.

Krogsgaard, L.R., Engsbro, A.L., Stensvold, C.R., Nielsen, H.V. and Bytzer, P. (2015). The prevalence of intestinal parasites is not greater among individuals with irritable bowel syndrome: a population-based case-control study. Clinical Gastroenterology and Hepatology. 13, pp. 507–513. doi: 10.1016/j.cgh.2014.07. 065.

Kumar, S. and Tan, T. (2013). Romancing *Blastocystis*: a 20-year affair. Parasites and their Vectors: A Special Focus on Southeast Asia. Springer. pp. 131–154.

Kurniawan, A., Karyadi, T., Dwintasari, S.W., Sari, I.P., Yunihastuti, E., Djauzi, S. *et al*. (2009). Intestinal parasitic infections in HIV/AIDS patients presenting with diarrhoea in Jakarta, Indonesia. Transactions of the Royal Society of Tropical Medicine and Hygiene. 103, pp. 892–898. doi: 10.1016/j.trstmh.2009.02.017.

Kurt, O., Dogruman, A. and Mehmet, T. (2016). Eradication of *Blastocystis* in humans: really necessary for all?. Parasitology International. 10.1016/j.parint.2016.01.010,.

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

Leelayoova, S., Rangsin, R., Taamasri, P., Naaglor, T., Thathaisong, U. and Mungthin, M. (2004). Evidence of waterborne transmission of *Blastocystis hominis*. American Journal of Tropical Medicine and Hygiene. 70, pp. 658–662.

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. American Journal of Tropical Medicine and Hygiene. 79, pp. 401–406.

Li, L., Zhou, X., Du, Z., Wang, X. and Wang, L. (2007). . Molecular epidemiology of human *Blastocystis* in a village in Yunnan Province, China. Parasitology International. 56, pp. 281-286.

Londono-Franco, A.L., Loaiza-Herrera, J., Lora-Suárez, F.M. and Gómez-Marín, J.E. (1999). *Blastocystis* sp. frequency and sources among children from 0 to 5 years of age attending public day care centers in Calarca, Colombia. Biomedica. 34, pp. 2218–227. doi: 10.1590/S0120-41572014000200008.

Malheiros, A.F., Stensvold, C.R., Clark, C.G., Braga, G.B. and Shaw, J.J. (2011). Short report: Molecular characterization of *Blastocystis* obtained from members of the indigenous Tapirapé ethnic group from the Brazilian Amazon region, Brazil. Am J Trop Med Hyg. 85, pp. 1050–1053. doi: 10.4269/ajtmh.2011.11-0481.

Mattiucci, S., Crisafi, B., Gabrielli, S., Paoletti, M. and Cancrini, G. (2016). Molecular epidemiology and genetic diversity of *Blastocystis* infection in humans in Italy. Epidemiology and infection. 144, pp. 635–646.

Moe, K.T., Singh, M., Howe, J., Ho, L.C., Tan, S.W., Chen, X.Q. *et al*. (1997). Experimental *Blastocystis hominis* infection in laboratory mice. Parasitology Research. 83, pp. 319–325.

Moe, K.T., Singh, M., Howe, J., Ho, L.C., Tan, S.W., Chen, X.Q. *et al*. (1999). Development of *Blastocystis hominis* cysts into vacuolar forms *in vitro*. Parasitology Research. 85, pp. 103–108.

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

Moussa, H.M.E. (2009). In vitro cultivation for detection and phenotypic study of *Blastocystis* spp. in stool samples. Parasitologists United Journal. 2, pp. 33–46.

Murray, C.J.L., Barber, R.M., Foreman, K.J., Ozgoren, A.A., Abd-Allah, F., Abera, S.F. *et al*. (2015). Global, regional, and national disability-adjusted life years (DALYs) for 306 diseases and injuries and healthy life expectancy (HALE) for 188 countries, 1990-2013: quantifying the epidemiological transition. Lancet (London, England). 386, pp. 2145–2191. doi: 10.1016/S0140-6736(15)61340-X.

Navarette, N. and Torres, P. (1994). Prevalence of infection by intestinal helminths and protozoa in school children from a coastal locality in the province of Valdivia, Chile. Boletín Chileno de Parasitología. 49, pp. 79–80.

Nimri, L. (1993). Evidence of an epidemic of *Blastocystis hominis* infection in preschool children in Northern Jordan. Journal of Clinical Microbiology. 31, pp. 2706–2708.

Nimri, L. and Batchoun, R. (1994). Intestinal colonization of symptomatic and asymptomatic schoolchildren with *Blastocystis hominis*. Journal of Clinical Microbiology. 32, pp. 2865–2866.

Nithyamathi, K., Chandramathi, S. and Kumar, S. (2015). Predominance of *Blastocystis* sp. infection among school children in Peninsular Malaysia. PLoS One. 11, pp. e0136709. doi: 10.1371/journal.pone.0136709.

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

O' brien Andersen, L., Bonde, I., Nielsen, H.B. and Stensvold, C.R. (2015). A retrospective metagenomics approach to studying *Blastocystis*. FEMS Microbiology and Ecology. 91, doi: 10.1093/femsec/fov072.

O' Brien Andersen, L. and Stensvold, C.R. (2016). *Blastocystis* in health and disease: are we moving from a clinical to a public health perspective. Journal of clinical microbiology. 54, pp. 524–528. doi: 10.1128/JCM.02520-15.

O'Gorman, M.A., Orenstein, S.R., Proujansky, R., Wadowsky, R.M., Putnam, P.E. and Kocoshis, S.A. (1993). Prevalence and characteristics of *Blastocystis hominis* infection in children. Clin Pediatr (phila). 32, pp. 91-96.

Pakandl, M. (1991). Occurrence of *Blastocystis* sp. in pigs. Folia Parasitologica. 38, pp. 297–301.

Pandey, P.K., Verma, P., Marathe, N., Shetty, S., Bavdekar, A., Patole, M.S. *et al*. (2015). No Title. Infection, Genetics and Evolution. 31, pp. 296–299. doi: http://dx.doi.org/10.1016/j.meegid.2015.02.012.

Parija, S.C. and Jeremiah, S.S. (2013). *Blastocystis*: taxonomy, biology, and virulence. Tropical Parasitology. 3, pp. 17–25. doi: 10.4103/2229-5070.113894.

Pipatsatitpong, D., Rangsin, R., Leelayoova, S., Naaglor, T. and Mungthin, M. (2012). Incidence and risk factors of *Blastocystis* infection in an orphanage in Bangkok, Thailand. Parasites and Vectors. 5, pp. 37.

Poirier, P., Wawrzyniak, I., Albert, A., H Alaoui, E., 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. Journal of Clinical Microbiology. 49, pp. 975–983.

Quiroz-Santiago, C., Vázquez-Salinas, C., Natividad-Bonifacio, I., Barrón-Romero, B.L. and Quiñones-Ramírez, E.I. (2014). *Rotavirus* G2P4 detection in fresh vegetables and oysters in Mexico City. Journal of Food Protection. 77, pp. 1953–1959. doi: 10.4315/0362-028X.JFP-13-426.

Ragavan, N.D., Govind, S.K., Chye, T.T. and Mahadeva, S. (2015). Factors that influence the shedding of *Blastocystis* cysts in an irritable bowel syndrome (IBS) patient–an evidence-based case study. Parasitology Research. 114, pp. 2999–3005. doi: 10.1007/s00436-015-4502-3.

Rajamanikam, A. and Govind, S.K. (2013). No Title. Parasites and Vectors. 6, pp. 295. doi: 10.1186/1756-3305-6-295.

Rene, B.A., Stensvold, C.R., Badsberg, J.H. and Nielsen, H.V. (2009). Subtype analysis of *Blastocystis* isolates from *Blastocystis* cyst excreting patients. American Journal of Tropical Medicine and Hygiene. 80, pp. 588–592.

Republica de Colombia (2014). Decreto nº 1287. Criterios para el uso de biosólidos generados en plantas de tratamiento de aguas residuales municipales. Gobierno de Colombia. Ministerio de Vivienda Ciudad y Territorio. Bogota, Colombia.

Riisberg, I., Orr, R.J., Kluge, R., Shalchian-Tabrizi, K., Bowers, H.A., Patil, V. *et al*. (2009). Seven gene phylogeny of heterokonts. Protist. 160, pp. 191–204. doi: 10.1016/j.protis.2008.11.004.

Rivera, W.L. (2008). Phylogenetic analysis of *Blastocystis* isolates from animal and human hosts in the Philippines. Veterinary Parasitology. 156, pp. 178–182. doi: 10.1016/j.vetpar.2008.06.001.

Robertson, W.D., Van Stempvoort, D.R., Solomon, D.K., Homewood, J., Brown, S.J., Spoelstra, J. *et al*. (2013). Persistence of artificial sweeteners in a 15-year-old septic system plume. Journal of Hydrology. 477, pp. 43-54.

Roberts, T., Bush, S., Ellis, J., Harkness, J. and Stark, D. (2015). No Title. Antimicrob Agents Chemother. 59, pp. 4417–4423. doi: 10.1128/AAC.04832-14.

Roberts, T., Stark, D., Harkness, J. and Ellis, J. (2014). Update on the molecular epidemiology and diagnostic tools for *Blastocystis* sp. Journal of Medical Microbiology and Diagnosis. 3, pp. 131. doi: 10.4172/2161-0703.1000131.

Roberts, T., Stark, D., Harkness, J. and Ellis, J. (2014). Update on the pathogenic potential and treatment options for *Blastocystis* sp. Gut Pathogens. 6, pp. 17. doi: 10.1186/1757-4749-6-17.

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. Veterinary Parasitology. 196, pp. 85–89.

Ruaux, C.G. and Stang, B.V. (2015). Prevalence of *Blastocystis* in shelter-resident and client-owned companion animals in the US Pacific Northwest. PLoS ONE. 9, pp. e107496. doi: 10.1371/journal.pone.0107496.

Salim, H., Kumar, G., Vellayan, S., Mak, J.W., Anuar, A., Init, I. *et al*. (1999). *Blastocystis* in animal handlers. Parasitology Research. 86, pp. 1032–1033.

Scanlan, P.D., Stensvold, C.R. and Cotter, P.D. (2015). Development and application of a *Blastocystis* subtype-specific PCR assay reveals that mixed-subtype infections are common in a healthy human population. Applied and environmental microbiology. 81, pp. 4071–4076. doi: 10.1128/AEM.00520-15.

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

Sekar, U. and Shanti, M. (2013). *Blastocystis*: consensus of treatment and controversies. Tropical Parasitology. 3, pp. 35–39. doi: 10.4103/2229-5070.113901.

Serna, R., Veliz, I. and Guevara, M. (2005). Social and cultural factors influencing *Blastocystis hominis* infection in two parishes of the Girardot Municipality in Aragua State. (August–December 2001 to January-February 2002.). Salus. 9, pp. 31–47.

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

Singh, M., Suresh, K., Ho, L.C., Ng, G.C. and Yap, E.H. (1995). Elucidation of the life cycle of the intestinal protozoan *Blastocystis hominis*. Parasitology Research. 81, pp. 446–450.

Słodkowicz-Kowalska, A., Majewskaa, A.C., Piotr, R., Łukasz, S. and Wernera, A. (2015). Human waterborne protozoan parasites in freshwater bivalves(Anodonta anatina and Unio tumidus) as potential indicators of fecal pollution in urban reservoir. Limnologica. 51, pp. 32–36.

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

Speich, B., Croll, D., Fürst, T., Utzinger, J. and Keiser, J. (2016). Effect of sanitation and water treatment on intestinal protozoa infection: a systematic review and meta-analysis. The Lancet Infectious Diseases. 16, pp. 87–99. doi: 10.1016/S1473-3099(15)00349-7.

Stensvold, C.R. (2015). Laboratory diagnosis of *Blastocystis* spp. Tropical Parasitology. 5, pp. 3–5. doi: 10.4103/2229-5070.149885.

Stensvold, C.R. (2013). Comparison of sequencing (barcode region) and sequence-tagged-site PCR for *Blastocystis* subtyping. J Clin Microbiol. 51, pp. 190–194. doi: 10.1128/JCM.02541-12.

Stensvold, C.R., Alfellani, M. and Clark, C.G. (2012). Levels of genetic diversity vary dramatically between *Blastocystis* subtypes. Infection, Genetics and Evolution. 12, pp. 263–273. doi: 10.1016/j.meegid.2011.11.002.

Stensvold, C.R., Alfellani, M.A., Nørskov-Lauritsen, S., Prip, K., Victory, E.L., Maddox, C. *et al*. (2009). Subtype distribution of *Blastocystis* isolates from synanthropic and zoo animals and identification of a new subtype. International Journal for Parasitology. 39, pp. 473–479. doi: 10.1016/j.ijpara.2008.07.006.

Stensvold, C.R., Arendrup, M.C., Jespersgaard, C., Mølbak, K. and Nielsen, H.V. (2007). Detecting *Blastocystis* using parasitologic and DNA-based methods: a comparative study. Diagnostic Microbiology and Infectious Disease. 59, pp. 303–307.

Stensvold, C.R., Arendrup, M.C., Nielsen, H.V. and Mølbak, K. (2009). *Blastocystis*–an enigmatic parasite. Ugeskr Laeger. 171, pp. 2388–2390.

Stensvold, C.R., Christiansen, D.B., Olsen, K.E.P. and Nielsen, H.V. (2011). No Title. American Journal of Tropical Medicine and Hygiene. 84, pp. 883–885. doi: 10.4269/ajtmh.2011.11-0005.

Stensvold, C.R., Smith, H.V., Nagel, R., Olsen, K.E.P. and Traub, R.J. (2010). Eradication of *Blastocystis* carriage with antimicrobials: reality of delusion?. Journal of Clinical Gastroenterology. 44, pp. 85–90. doi: 10.1097/MCG.0b013e3181bb86ba.

Stensvold, C.R., Suresh, G.K., Tan, K.S., Thompson, R.C., Traub, R.J., Viscogliosi, E. *et al*. (2007). Terminology for *Blastocystis* subtypes–a consensus. Trends in Parasitology. 23, pp. 93–96.

Stenzel, D.J. and Boreham, P.F. (1991). A cyst-like stage of *Blastocystis hominis*. International Journal for Parasitology. 21, pp. 613–615.

Stenzel, D.J. and Boreham, P.F.L. (1996). *Blastocystis hominis* revisited. Clinical Microbiology Reviews. 9, pp. 563–584.

Stenzel, D.J., Cassidy, M.F. and Boreham, P.F. (1994). Morphology of *Blastocystis* sp. from domestic birds. Parasitology Research. 80, pp. 131–137.

Stenzel, D.J., Lee, M.G. and Boreham, P.F. (1997). Morphological differences in *Blastocystis* cysts-an indication of different species?. Parasitology Research. 83, pp. 452–457.

Suresh, K., Howe, J., Ng, G.C., Ho, L.C., Ramachandran, N.P., Loh, A.K. *et al*. (1994). A multiple fission-like mode of asexual reproduction in *Blastocystis hominis*. Parasitology Research. 80, pp. 523–527.

Suresh, K., Mak, J.W., Chuong, L.S., Ragunathan, T. and Init, I. (1997). Sac-like pouches in *Blastocystis* from the house lizard Cosymbotus platyurus. Parasitology Research. 83, pp. 523–525.

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

Suresh, K., Smith, H. and Tan, T.C. (2005). Viable *Blastocystis* cysts in Scottish and Malaysian sewage samples. Applied and Environmental Microbiology. 71, pp. 5619–5620.

Suresh, K., Tan, T.C. and Illi, F. (2010). *Blastocystis* in water – needs screening?. Water Practice and Technology. 4, pp. 72. doi: 10.2166/wpt.2009.072.

Suresh, K., Toranzos, G.A., Fayer, R., Nissaparton, V., Olveda, R., Ashbolt, N. *et al*. (2012). Assessing the importance of zoonotic waterborne pathogens. IWA Publishing. London, UK.

Suresh, K., Venilla, G.D., Tan, T.C. and Rohela, M. (2009). In vitro encystation of *Blastocystis hominis*. Parasitology Research. 104, pp. 1373–1380.

Taamasri, P., Mungthin, M., Rangsin, R., Tongupprakarn, B., Areekul, W. and Leelayoova, S. (2000). Transmission of intestinal blastocystosis related to the quality of drinking water. The Southeast Asian Journal of Tropical Medicine and Public Health. 31, pp. 112-117.

Tan, K.S.W., Howe, J., Yap, E.H. and Singh, M. (2001). Do *Blastocystis hominis* colony forms undergo programmed cell death?. Parasitology Research. 87, pp. 362–367.

Tan, K.S.W., Mizra, H., Teo, J.D., Wu, Z. and Macary, P.A. (2010). Current views on the clinical relevance of *Blastocystis* spp. Current infectious disease reports. 12, pp. 28–35. doi: 10.1007/s11908-009-0073-8.

Tan, K.S.W., Singh, M., Thong, K.T., Ho, L.C., Moe, K.T., Chen, X.Q. *et al*. (1996). Clonal growth of *Blastocystis hominis* in soft agar with sodium thioglycollate. Parasitology Research. 82, pp. 737–739.

Tan, K.S.W., Singh, M. and Yap, E.H. (2002). Recent advances in *Blastocystis hominis* research: hot spots in terra incognita. International journal for parasitology. 32, pp. 789–804.

Tan, T.C. and Suresh, K.C. (2006). Predominance of amoeboid forms of *Blastocystis hominis* in isolates from symptomatic patients. Parasitology Research. 98, pp. 189–193.

Tan, T.S.W. (2008). New insights on classification, identification, and clinical relevance of *Blastocystis* spp. Clinical Microbiology Reviews. 21, pp. 639–665.

Thathaisong, U., Worapong, J., Mungthin, M., Tan-Ariya, P., Viputtigul, K., Sudatis, A. *et al*. (2003). *Blastocystis* isolates from a pig and a horse are closely related to *Blastocystis hominis*. Journal of Clinical Microbiology. 41, pp. 967–975.

Torres, P., Miranda, J.C., Flores, L., Riquelme, J., Franjola, R., Perez, J. *et al*. (1992). Blastocystosis and other intestinal protozoan infections in human riverside communities of the Valdivia River Basin, Chile. Revista do Instituto de Medicina Tropical de São Paulo. 34, pp. 557–564.

Tuncay, S., Delibaş, S., Inceboz, T., Over, L., Oral, A.M., Akisü, C. *et al*. (2008). An outbreak of gastroenteritis associated with intestinal parasites. Türkiye Parazitoloji Dergisi. 32, pp. 249–252.

Tungtrongchitr, A., Manatsathit, S., Kositchaiwat, C. and Chaicumpa, W. (2004). *Blastocystis hominis* infection in irritable bowel syndrome patients. The Southeast Asian Journal of Tropical Medicine and Public Health. 35, pp. 705–710.

Ustün, S. and Turgay, N. (2006). *Blastocystis hominis* and bowel diseases. Türkiye Parazitoloji Dergisi. 30, pp. 73–77.

Vargas-Sanchez, G.B., Romero-Valdovinos, M., Ramirez-Guerrero, C., Vargas-Hernandez, I., Ramirez-Miranda, M.E., Martinez-Ocaña, J. *et al*. (2015). *Blastocystis* isolates from patients with irritable bowel syndrome and from asymptomatic carriers exhibit similar parasitological loads, but significantly different generation times and genetic variability across multiple subtypes. PLoS ONE. 10, pp. e0124006.

Vennila, G.D., Suresh, K.G., Anuar, A., Rajah, S., Saminathan, R., Sivanandan, S. *et al*. (1999). Irregular shedding of *Blastocystis hominis*. Parasitology Research. 85, pp. 162–164.

Waikagul, J., Krudsood, S., Radomyos, P., Radomyos, B., Chalemrut, K., Jonsuksuntigul, P. *et al*. (2002). A cross-sectional study of intestinal parasitic infections among schoolchildren in Nan Province, Northern Thailand. The Southeast Asian Journal of Tropical Medicine and Public Health. 33, pp. 218–223.

Wang, W., Owen, H., Traub, R.J., Cuttell, L., Inpankaew, T. and Bielefeldt-Ohman, H. (2014). Molecular epidemiology of *Blastocystis* in pigs and their in-contact humans in Southeast Queensland, Australia, and Cambodia. Veterinary Parasitology. 203, pp. 264–269. doi: 10.1016/j.vetpar.2014.04.006.

Wawrzyniak, I., Poirier, P., Viscogliosi, E., Dionigia, M., Texier, C., Delbac, F. *et al*. (2013). *Blastocystis*, an unrecognized parasite: an overview of pathogenesis and diagnosis. Therapeutic Advances in Infectious Disease. 1, pp. 167–178. doi: 10.1177/2049936113504754.

Wu, G., Xiong, Y., Cao, G., Li, G., Liu, M. and Zhu, J. (2000). Investigation of an epidemic outbreak of blastocystiasis. Chinese Journal of Parasite Diesease. 13, pp. 25–27.

Yakoob, J., Jafri, W., Asim-Beg, M., Abbas, Z., Naz, S., Islam, M. *et al*. (2010). Irritable bowel syndrome: is it associated with genotypes of *Blastocystis hominis*. Parasitology Research. 106, pp. 1033–1038. doi: 10.1007/s00436-010-1761-x.

Yakoob, J.J.W., Jafri, N., Khan, R., Islam, M., Asim-Beg, M. and Zaman, V. (2004). Irritable bowel syndrome: in search of an etiology: role of *Blastocystis hominis*. American Journal of Tropical Medicine and Hygiene. 70, pp. 383–385.

Yamada, M. and Yoshikawa, H. (2012). Morphology of human and animal *Blastocystis* isolates with special references to reproductive modes. Parasitology Research Monographs. Springer. doi: 10.1007/978-3-642-32738-4_2.

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

Yoshikawa, H., Abe, N. and Wu, Z. (2004). PCR-based identification of zoonotic isolates of *Blastocystis* from mammals and birds. Microbiology. 150, pp. 1147–1151. doi: 10.1099/mic.0.26899-0.

Yoshikawa, H., Nagashima, M., Morimoto, K., Yamanouti, Y., Yap, E.H. and Singh, M. (2003). Freeze fracture and cytochemical studies on the *in vitro* cyst form of reptilian *Blastocystis* pythoni. Journal of Eukaryotic Microbiology. 50, pp. 70–75.

Zaki, M., Zaman, V. and Sheikh, N. (1997). Resistance of *Blastocystis hominis* cysts to chorine. Journal Of Pakistan Medical Association. 46, pp. 178–179.

Zaman, V. (1994). Morphology of the cyst of *Blastocystis hominis*. The Southeast Asian Journal of Tropical Medicine and Public Health. 25, pp. 790–791.

Zaman, V., Howe, J. and Ng, J. (1995). Ultrastructure of *Blastocystis hominis* cysts. Parasitology Research. 81, pp. 465–469.

Zhang, X., Zhang, S., Qiao, J., Wu, X., Zhao, L., Lui, Y. *et al*. (2012). Ultrastructure insights into morphology and reproductive mode of *Blastocystis hominis*. Parasitology Research. 110, pp. 1527-1532.

Zhang, X., ZHANG, S., QIAO, J., WU, X., ZHAO, L., Liu, Y. *et al*. (2011). Ultrastructural insights into morphology and reproductive mode of *Blastocystis hominis*. Parasitology Research. 110, pp. 1165-1172.

Zierdt, C.H. (1991). *Blastocystis hominis* – past and future. Clinical Microbiology Reviews. 4, pp. 61-79.

Zierdt, C.H., Swan, J.C. and Hosseini, J. (1983). In vitro response of *Blastocystis hominis* to antiprotozoal drugs. Journal of Protozoology. 30, pp. 332-334.

Zierdt, C.H., Rude, W.S. and Bull, B.S. (1967). Protozoan characteristics of *Blastocystis hominis*. American Journal of Clinical Pathology. 48, pp. 495–501.

Zierdt, C.H. and Tan, H.K. (1976). Ultrastructure and light microscope appearance of *Blastocystis hominis* in a patient with enteric disease. Zeitschrift für Parasitenkunde. 50, pp. 277–283.