Epizootiology of *Blastocystis* spp.: A Parasite with Zoonotic Concern

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ABSTRACT

Blastocystosis is an enteric infection caused by globally distributed unicellular Protista Blastocystis. Various animals' species as well as human can be parasitized by Blastocystis species. Due to the existence of genetic diversity between Blastocystis isolates in different hosts recently the organisms were defined as Blastocystis spp. or subtypes, although they are morphologically alike. About 34 valid subtypes have been reported in different hosts. Dissimilarity in Blastocystis genotypes have significant effect on their pathogenicity. The organism is poly morphic, and several distinct morphological forms have been observed including vacuolar, granular, cyst, and amebic forms.

Blastocystis spp. are transmit through feco- oral rout via contaminated food and water. The risk for human infection might be higher in the existence of infected animals with Blastocystis, as well as in poor hygienic conditions. Although most of reported cases are asymptomatic, Blastocystis infection can associated with gastrointestinal disorders and appearance of nonspecific symptoms of nausea, abdominal pain, bloating, and diarrhea which might be self-limiting or severe. Blastocystosis also accompany with extra-intestinal urticaria signs such as palmoplantar pruritus. The organism also reported from cases of irritable bowel syndrome. Diagnosis of Blastocystis infection can be done conventionally based on the parasitological methods including microscopic examination of fecal smear. In vitro cultivation of fecal samples in supplemented medium was another detection method. The development of molecular techniques provides a sensitive, and rapid detection procedures for Blastocystis spp., also aid in genotypes differentiation. Metronidazole is the treatment of choice for Blastocystosis. Control measures include improvement in hygiene, and sanitation conditions, increased health awareness also essential in preventing enteric parasites.

Keywords: Blastocystis, Zoonotic, Polymorphic, Vacuolar form, Enteric protozoa.

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1. INTRODUCTION

Blastocystis is a globally distributed unicellular, anaerobic, eukaryotic protist (Tan 2008) belonging to the phylum Stramenopila inhabiting various hosts' gastrointestinal tracts, including humans and animals (Villalobos et al. 2014). Infection with *Blastocystis* is known as blastocystosis.

Blastocystis has been detected in various domestic and wild animals, including cattle, dogs, cats, rats (Clark et al. 2013; Ramírez et al. 2014), birds, reptiles, and even insects (Rauff-Adedotun et al. 2020). Similarly, it has been reported in pigs and monkeys (Parkar et al. 2007), also recovered from fish (Gantois et al. 2020), indicating a possible threat of zoonotic transmission to humans.

Due to the morphological similarity between *Blastocystis* isolated from humans and animals, it is challenging to differentiate isolates based on morphology. However, broad genetic diversity exists among isolates of human and animal origins. The organism is no longer mentioned as *B. hominis* based on such diversity. Instead, it is called *Blastocystis* spp. or *Blastocystis* subtype (Tan 2008).

The description of *Blastocystis* subtypes depends upon assessing small ribosomal RNA subunit genes (Stensvold et al. 2007a). The genetic diversity among human and animal isolates of *Blastocystis* was interpreted using gene sequences (Baek et al. 2022). There are 38 subtypes (STs), numbered ST1 to ST38 (Maloney et al. 2022). Thirty-four of these subtypes are validated, but ST18–ST20 and ST22 are potential PCR amplification artifacts (Stensvold and Clark 2020).

Variability in *Blastocystis* genotypes plays a significant role in pathogenicity (Souppart et al. 2009). Studies showed that ST3 is the most common isolate in patients suffering from gastrointestinal disorders (Rajamanikam and Govind 2013). Approximately 90% of the human isolates belong to ST1–ST4 (Alfellani et al. 2013a). Some STs are shared between animals and humans, studies demonstrating that zoonotic STs occur frequently in livestock and pet animals (Higuera et al. 2021).

Human infection with *Blastocystis* spp. is around 50% in developing countries compared to 23.1% in developed countries (Alfellani et al. 2013b; Osman et al. 2016). An estimated one billion people are infected with *Blastocystis* worldwide (Alfellani et al. 2013b), most of whom are asymptomatic carriers (Andersen and Stensvold 2016).

The pathogenicity of *Blastocystis* remains debatable, while its association with different gastrointestinal disorders like inflammatory bowel disease (IBD), and irritable bowel syndrome (IBS) (Jimenez-Gonzalez et al. 2012) is established. Infection severity relies on the number of parasites discovered in stool samples (Roberts et al. 2011), and the parasitic subtype (Yan et al. 2006).

The prevalence estimates of *Blastocystis* rely on the applied diagnostic methods. Molecular methods based on PCR have better sensitivity and specificity (Stensvold et al. 2007b). SSU rRNA gene fragments analysis can identify the presence of *Blastocystis* and genetically characterize the organism (Gong et al. 2019). Metronidazole is commonly the first-line therapy, if it is ineffective, trimethoprim-sulfamethoxazole or nitazoxanide is the second-line treatment (Coyle et al. 2012).

Following hygienic stranded, proper waste clearance, contact with infected animals, and consuming contaminated food and water can alter the prevalence rates (Alfellani et al. 2013b). Poor hygiene increases the opportunity for *Blastocystis* transmission (Gong et al. 2019). However, in recent years awareness has been developed about the extensive prevalence of the *Blastocystis* species as a common emerging condition in humans and animals (Li et al. 2018).

2. CLASSIFICATION

The nomenclature of *Blastocystis* has remained indefinable for a long time, classified as flagellate, yeast or fungus, and protist based on its morphological structure (Zierdt 1991). Subsequently, it was suggested to classify *Blastocystis* in the subkingdom of protozoa, subphylum sporozoan, in a separate suborder Blastocystina based on the morphological and physiological criteria (Boreham and Stenzel 1993).

The species name was granted according to the host; the isolated organism from human isolates is called *B. hominis,* and the rat isolate is called *B. ratti*. *Blastocystis* was subsequently isolated from various hosts (Noël et al. 2005), and two other non-human species were described, *B. galli* from chicken and *B. lapemi* from sea snakes (Teow et al. 1991).
Meanwhile, due to its massive diversity, the parasite is no longer mentioned as *B. hominis;* instead, it is called *Blastocystis* spp. or *Blastocystis* spp. subtype n. The n is the number of subtypes based on the classification by Stensvold (Tan et al. 2008).

3. MORPHOLOGY

Previous studies indicated that *Blastocystis* was a polymorphic organism with several distinct morphological forms (Kukoschke et al. 1990). Four primary morphological forms exist: vacuolar, granular, cyst, and amebic. Other morphological forms were also reported by electron microscopy, including vacuolar and multi-vacuolar forms, which are small in size and rarely present. Moreover, the organism can also undertake strange forms like medusa head form on exposure to oxygen and chestnut burr cell in aging culture (Zierdt 1991). The organism can reproduce by five distinct modes: binary fission, budding, endodyogeny, plasmotomy, and schizogony (Zhang et al. 2007).

3.1. VACUOLAR FORM

This spherical form contains a large central vacuole, occupying approximately 90% of the cell space and limiting the cytoplasm and intercellular components in a thin peripheral layer (Lee and Stenzel 1999).
Following morphological identification of vacuolar form by wet mount microscopy, a central space has been observed and labeled as a vacuolar form. Later, the space was found to be a membrane-bound body containing fine granular materials (Yoshikawa et al. 1995).
The membrane-enclosed vacuole contains carbohydrates, fats, and proteins, accumulating due to the Golgi apparatus's action via clathrin-mediated endocytosis (Mehlhorn et al. 2012). The central body is a storage organelle called the central body form (Stenzel et al. 1989).
The nuclei are distributed at the periphery throughout the cytoplasm. More than one nuclease is present; typically, two nuclei are situated at the opposite end of the cell. More than two nuclei were reported rarely (Do Bomfim and Do Couto 2013). There is variation in the size of vacuolar form ranging from 3µm to 120µm in diameter, with an average of 5µm to 15µm, commonly observed in asymptomatic carrier individuals with *Blastocystis* (Sekar and Shanthi 2015).
Mitochondria and other organelles surround the nucleus as a rosette, making thickened pods in the cytoplasmic rim. Occasionally, a surface coat or capsule of various thicknesses surrounds the vacuolar form, especially in fresh clinical isolates, which protects the organism from osmotic shock. It was also assumed to take part in bacterial tapping for nutrition (Zaman et al. 1997).

3.2. GRANULAR FORM

This form is like the vacuolar form but contains centrally situated granules within the central body and cytoplasm (Cassidy et al. 1994). The granular form has two nuclei; at maximum, four are present. Based on the size, it is smaller than the vacuolar form (Do Bomfim and Do Couto 2013), and exhibits a lower degree of pleomorphism than the vacuolar form. The average diameter is 15–25µm, while the largest might reach 80µm (Zierdt and Williams 1974).
Based on electron microscopy studies, three kinds of granules have been identified: metabolic, reproductive, and lipid granules. The first type is mainly found in the cytoplasm and is involved in various

metabolic pathways in the organism. Reproductive granules are only observed in the central body and were proposed to have a role in schizogony (Tan and Stenzel 2003). Lipid granules are a storage space in the central body and cytoplasm (Tan and Zierdt 1973).

3.3. AMEBOID FORM

This form is highly uncommon, and it is irregular in shape, provided with one to two pseudopodia, and has strong adhesive properties that permit attachment to the bowel mucosa. The cytoplasm contains a large vacuole or multiple smaller vacuoles (Tan and Suresh 2006). It is non-motile and measures about 10µm in size (Tan and Zierdt 1973).

The ameboid form converts into a cystic form, and because of its small size and morphology, it looks like neutrophils and macrophages. In standard stool examination, it can easily escape recognition. Zierdt proposed staining an unfixed smear with Gram's stain for identification since the ameboid form of *Blastocystis* lyses when exposed to air, but leukocytes remain intact (Zierdt 1991). The ameboid form is progressively recognized in patients with diarrhea and has been reported to be pathogenic (Zhang et al. 2012).

3.4. CYSTIC FORM

This form is round to oval in shape and smaller in size, measuring 3–6µm, and it is surrounded by a thin, multilayer wall with or without a surface envelope (Do Bomfim and Do Couto 2013). The number of nuclei is from one to four, and the cytoplasm is dense and comprises several mitochondria and storage vacuoles produced from lipids or glycogen. The cystic form can remain viable for about a month even when exposed to air and at 25°C, enabling further infection spread (Tan 2008). The cysts are infective in a proper host and develop into vacuolar forms (Yoshikawa et al. 2004b).

Other forms, like the a vacuolar and multi-vacuolar, gained importance lately. These forms are about 5–8µm in diameter, with no size variations. A vacuolar form lacks the central body, and the multi-vacuolar has several different-sized small vacuoles interlinked or lying separated in the cytoplasm. Both forms are commonly uninucleate, but two nuclei are present sometimes (Parija and Jeremiah 2013).

4. TRANSMISSION

The cystic form of *Blastocystis* is the only transmittable form transmitted by the fecal-oral route (Yoshikawa et al. 2004b). Appropriate hosts may get infected infection by ingesting cysts from drinking contaminated water or eating raw aquatic plants (Lee et al. 2012). Unclean hands can serve as a fomite for transmitting cysts from suspected individuals on direct contact or from contaminated soil (Anuar et al. 2013). Close contact with animals is another source of *Blastocystis* infection (Javanmard et al. 2018).

Human-to-human and inter-species transmission between humans and animals might occur due to poor host specificity (Parkar et al. 2007). However, only 14 subtypes (ST1–ST10, ST12, ST14, ST16, and ST23) were found in humans, with different frequencies (Osorio-Pulgarin et al. 2021), and ST9 has only been described in humans (Andersen and Stensvold 2016). The globally available epidemiological data in several countries represented that > 90% of human subtypes were ST1–ST4 (Alfellani et al. 2013a). Consequently, the four subtypes are transmitted among humans, and the remaining subtypes found infrequently in humans are of animal origin as they predominate in particular mammal or bird groups (Hublin et al. 2021). The existence of these STs in humans explains zoonotic transmission, and isolates exhibit the same gene sequence of SSU rRNA in both animals and humans (Wang et al. 2014; Greige et al. 2018).

The ST1 occurs in several species, like cattle, pigs, primates, rodents, and birds (Yoshikawa et al. 2004b; Thathaisong et al. 2013). ST2 was isolated from pigs and monkeys (Tan 2008), while ST3 is cosmopolitan in humans, non-human primates, and other mammals, like cattle, pigs, dogs, rodents, and horses (Stensvold et al. 2009; Thathaisong et al. 2013). ST4 has been found in rodents (Stensvold et al. 2009), and ST5 occurred in dogs, cats, and pigs (Rauff-Adedotun et al. 2020). ST6 and ST7 are dominant avian subtypes. ST5 and ST9 seldom occur in human (Stensvold et al. 2009), and ST10 is reported in cattle, goats, and deer (Rauff-Adedotun et al. 2020).

5. EPIDEMIOLOGY

Blastocystis spp. have been observed worldwide with various prevalence rates between areas and populations (Yan et al. 2006). Variation in the prevalence rate might be due to poor hygienic conditions, animal exposure, and intake of contaminated water or food (Tan 2008). People in developing countries have a higher incidence of blastocystosis due to a lack of sanitary conditions and contaminated water and food. A seasonal impact on *Blastocystis* prevalence is also reported, with a higher incidence in summer (Yan et al. 2006).

Most of the isolated human subtypes have also been reported in animals, bringing up the issue of the role of animal reservoirs in the parasite's epidemiology (Hublin et al. 2021).

The opportunity for human infection might increase when there are infected animals with *Blastocystis*. Animal feces might contaminate streams and rivers via surface overflow after heavy rain, causing water contamination and spreading in a wide geographical area (Gong et al. 2019). Non-human primates, mammals, and birds are the primary reservoirs for most subtypes (Stensvold et al. 2007a). Regardless of the country of study, the prevalence of different subtypes shows the absence of geographic limitation of these subtypes' distribution (Rauff-Adedotun et al. 2020).

Both zoonotic and enzootic STs have been reported in farm animals (Hublin et al. 2021). Zoonotic STs (ST1, ST3–ST5, ST7) and enzootic STs (ST10, ST12, ST14) usually occur in ruminants (Alfellani et al. 2013b; Song et al. 2017). In cattle zoonotic STs (ST1–7 and ST12) and enzootic STs (ST10, ST14, ST17, ST21, ST23–ST26) are documented (Suwanti et al. 2020; Hublin et al. 2021).

Horses become host for different ST such as ST1, ST3–6, ST10, ST14, ST24–26, ST33 and ST34 (Baek et al. 2022). Eight STs were reported in pigs, which are ST1–ST5, ST7, ST10, and ST15. All these STs, except ST10 and ST15, are considered zoonotic and reported in humans (Hublin et al. 2021).

Ten *Blastocystis* STs (ST1–ST5, ST7, ST8, ST10, ST13, ST17) were described in rodents (Hublin et al. 2021). In monkeys, ST1–ST5 and ST8 have also been identified (Rauff-Adedotun et al. 2020).

Dogs and cats, could be reservoirs for enteric parasites in humans, including *Blastocystis*. wandering stray dogs and cats may contaminate soil, water, and food with zoonotic STs and contribute to transmitting *Blastocystis* (Hublin et al. 2021).

Eight subtypes, including ST1–ST6, ST8, and ST10, were identified in in dogs (Nagel et al. 2012). All subtypes except ST10 are described in humans, implying that dogs might play a role in transmitting *Blastocystis* to humans, or humans could be a source of infection for dogs. Also, ST1, ST3, and ST4 have been reported from cats, which are often found in humans and regarded as zoonotic STs (Hublin et al. 2021).

Birds can harbor enzootic and zoonotic subtypes, and have a critical role in transmitting the protozoa between wildlife, livestock, and humans (Chandrasekaran et al. 2014). *Blastocystis* was reported globally in wild and domestic birds, with prevalence rates of 2.1%–100% (Hublin et al. 2021). ST6 and ST7 are common subtypes, but other STs are also reported, like ST1, ST2, ST4–ST5, and ST8, and enzootic STs (ST10, ST13, ST14, ST20, ST24, ST27–ST29) (Ramírez et al. 2014; Greige et al. 2018; Maloney et al. 2021).

Subtypes 1–4, the primary subtypes infecting humans, have been identified in cockroaches, which implies that cockroaches could be a possible source of infection in humans (Rauff-Adedotun et al. 2020).
Infection with *Blastocystis* is higher among immunocompromised people, immigrants, and travelers who visit developing tropical countries (Tan 2008). Also, a higher prevalence was observed among patients taking immunosuppressive drugs (Rao et al. 2003). Furthermore, infections are widespread in occupations requiring animal exposure, like food handlers, animal handlers, and abattoir workers (Parkar et al. 2010).
Blastocystis was detected in various water sources like rivers and sewage, implying that human and animals infection can occur from contaminated water. The protozoa can occur as high as 50% in tap water and 100% in household water storage tanks and containers, further confirming the possible transmission of this parasite through contaminated water. *Blastocystis* identification in water supplies implies that water can be a source of parasite transmission to humans and animals (Hublin et al. 2021). Studies also identified *Blastocystis* in raw vegetables, and eating unwashed fruit was related to an eight fold increase in an infection rate (Canete et al. 2012).

6. LIFE CYCLE

The life cycle for *Blastocystis* was first proposed by Alexeieff in 1911 (Boreham and Stenzel 1993). Infection occurs upon an intake of cysts (Lee et al. 2012). In a suitable host, the cyst becomes vacuolar inside the large bowel lumen via cyst excitation. Further life cycle continuation relies on subtype-host compatibility (Sekar and Shanthi 2015). After a period of infection, other forms can also develop. The vacuolar forms can convert into other forms. The ameboid, a vacuolar, and multi-vacuolar forms are often observed in diarrhea, suggesting that these forms may have a role in the pathogenesis (Zhang et al. 2012). The encystation of the vacuolar form happens throughout the lumen of the large intestine, and the cysts are shed with feces (Fig. 1) (Vdovenko 2000).
Blastocystis can pass through a complex cycle to form primary cysts, comprising various reproduction modes, binary fission of a binucleate stage plasmotomy, and autogamy, a sexual phenomenon. These cysts produce spores by multiple budding, and the spores, or secondary cysts, are the resistant form. Studies demonstrated that *Blastocystis* can also reproduce through multiple fission, endodyogeny, and schizogony, but the vacuolar form's binary fission is the most common mode (Zhang et al. 2007).

7. PATHOGENESIS

Although the potential of *Blastocystis* spp. subtypes to be pathogenic is yet arguable, some authors showed its connection with gastrointestinal disorders, whereas others have rejected such involvement (Abedi et al. 2022). The pathogenicity of *Blastocystis* is suggested to be dependent on the subtypes and parasite burden. However, individuals may show symptoms even with small numbers of cysts (Coyle et al. 2012).
The proteases are the most virulent enzymes excreted by the ameboid form (Abdel-Hameed and Hassanin 2011; Scanlan 2012). Demonstrating numerous ameboid forms in a patient with severe diarrhea supports the idea that this form is virulent (Zierdt and Tan 1976).
Vassalos et al. (2010) reported an intriguing observation in favor of the pathogenicity of ameboid forms while they studied ST3 intra-subtype variations. In that study, a patient was a carrier of vacuolar and granular forms of ST3 without symptoms but became symptomatic quickly, shedding ameboid forms in his stool.
Proteases can result in secretory IgA splitting and supporting the parasite's survival through immune evasion (Parija and Jeremiah 2013), and provoke inflammatory cytokines (Puthia et al. 2008). Additionally, other hydrolytic enzymes have been detected through electrophoresis. For example, Lysates lead to cytoskeletal changes and stimulate apoptosis in epithelial cells, increasing bowel permeability. Cysteine proteases induce interleukin-8 production by mucosal cells, causing fluid loss and bowel inflammation in infected individuals (Parija and Jeremiah 2013).

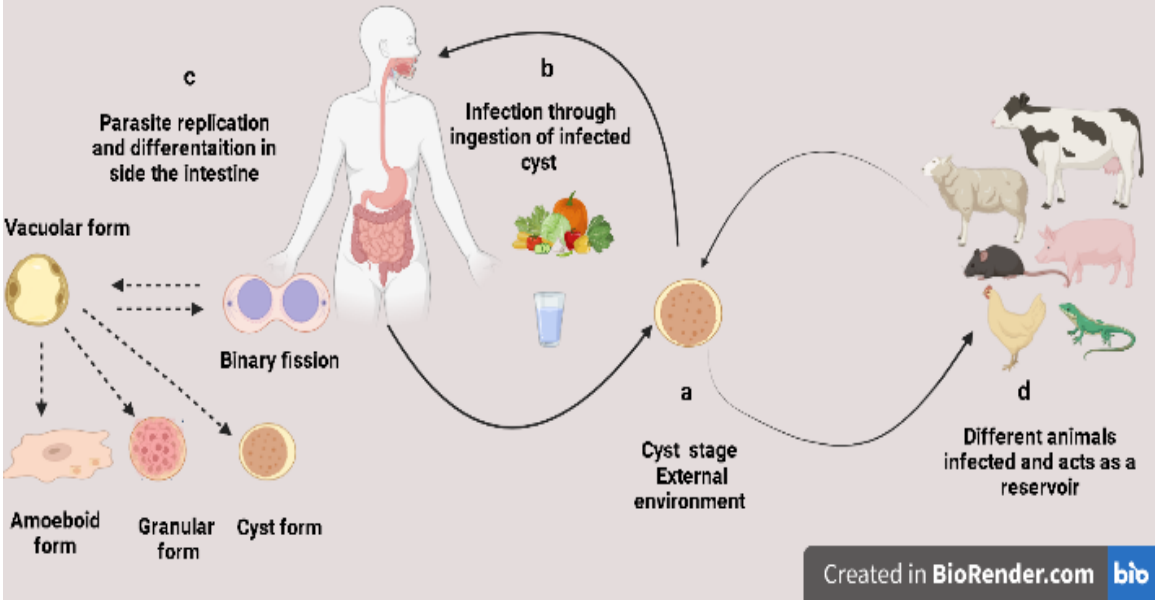

Fig. 1: Life cycle of *Blastocystis* spp.

Preliminary studies for defining the subtype pathogenicity stated a strong association of ST3 with symptomatic disease (Tan 2008). An investigation of ST3 from symptomatic isolates discovered that it typically possessed protease activity, particularly at 32 kDa, assumed to be a virulent element (Abdel-Hameed and Hassanin, 2011). Later, subtypes ST1, ST2, ST4, and ST6 were isolated from symptomatic patients (Nagel et al. 2012).

Intra-subtype pathogenicity variations have also been notable, meaning that some subtype strains can be nonpathogenic (Scanlan 2012), and subtyping alone cannot predict pathogenicity (Nagel et al. 2012).

Preceding studies reported *Blastocystis* association with IBS (Nourrisson et al. 2014), a complex functional bowel disorder (Longstreth et al. 2006). It has been proposed that IBS may be influenced by low-grade inflammation brought on by current immunological activation with *Blastocystis* infection, which provides prolonged antigenic exposure (Stark et al. 2007).

Blastocystis infection might also be associated with skin disorders, the ameboid form of ST3 was detected in acute urticaria cases, and it was believed that a disturbance in the immune homeostasis could trigger cutaneous symptoms (Katsarou-Katsari et al. 2008).

Furthermore, it has been proposed that luminal protozoa can cause allergy-like cutaneous lesions through activating specific cytokines by parasite molecules, such as interleukin 3 (IL-3)-, IL-4-, IL-5-, or IL-13 (Pasqui et al. 2004). According to previous theories, *Blastocystis* antigens induce T-helper 2 cells resulting in an IgE-mediated allergic reaction (Valsecchi et al. 2004; Yavasoglu et al. 2008). Eosinophils were assumed to have a direct role in urticaria pathology (Staumont-Sallé et al. 2006). Another theory supposed that *Blastocystis* might also trigger the complement pathway and production of anaphylatoxins C3a and C5a., These molecules interact with mast cells and basophils to stimulate histamine release and subsequently bring together skin-related disorders (Valsecchi et al. 2004).

8. CLINICAL SIGNS

Blastocystis-associated symptoms continue for 1–14 days and are usually self-limiting, but some untreated infections may last for months. The symptoms may be mild, moderate, severe, acute, or chronic (Roberts et al. 2011).

The host's immune status appears to be the primary risk factor for transmission of *Blastocystis* (Wawrzyniak et al. 2013). Immunodeficient/immunocompromised individuals are more vulnerable to *Blastocystis*, demonstrating opportunistic pathogenesis. Also, the occurrence and severity of clinical symptoms are related to the pathogen's density in the gut and the subtype's virulence (Matiut and Hritcu 2015).

Studies revealed that some individuals are more susceptible to infection. The parasite has been detected in HIV-infected individuals, cancer patients, immunodeficient patients, and frequent travelers. A higher prevalence of infection occurs in children in underdeveloped countries (Sekar and Shanthi 2015).

Intestinal colonization by *Blastocystis* is sometimes connected to asymptomatic conditions (Vassalos et al. 2008). Otherwise, nonspecific symptoms have been observed, such as nausea, anorexia, abdominal pain, bloating, and flatulence. Acute or chronic diarrhea, which may be mild and self-limited, and acute gastroenteritis, have been reported (Tan et al. 2010). There is also ulcerative colitis associated with inflammatory bowel conditions (Cekin et al. 2012). In addition to constipation and fatigue (Öner et al. 2022), vomiting and weight loss are other observed symptoms (Skotarczak 2018).

Reports imply the correlation of *Blastocystis* infection with IBS. When the intestine undergoes modifications, it favors the progression of the parasite (Sekar and Shanthi 2015). Also, its association with other conditions, such as nonspecific colitis and chronic IBD (including Crohn's disease and ulcerous colitis), has been documented (Özyurt et al. 2008; Basak et al. 2014).

There are extra-intestinal symptoms associated with *Blastocystis* infection, including acute or chronic urticaria (Gupta and Parsi 2006), palmoplantar pruritus (Kick et al. 2002), chronic angioedema (Micheloud et al. 2007), and joint pain (Cassano et al. 2005). However, *Blastocystis* infection is usually unrelated to animal clinical manifestation (Hublin et al. 2021).

9. DIAGNOSIS

Several diagnostic methods have been applied to detect *Blastocystis* infection, like direct examination of fecal smear, examination of iodine or trichrome-stained smear, concentration techniques using formalin-ether, *in vitro* cultivation, PCR, and sequencing (Wang et al. 2014).

9.1. MICROSCOPIC EXAMINATION

Conventionally *Blastocystis* detection was based on the clinical parasitological methods used to detect enteric parasites, including direct smear microscopic examination (Kukoschke et al. 1990).

Since the polymorphic forms of *Blastocystis* spp. can be present and range from 2µm to > 200µm in size (Celik et al. 2006). Variations in size make microscopic diagnosis in fecal samples challenging and require significant expertise. Furthermore, only the cyst and vacuolar forms can be detected in feces, while other forms are frequently lost during the processing of fecal samples (Navarro et al. 2008).

Ethyl acetate (formol-ether) concentration techniques have also been defined for diagnosing of *Blastocystis* infection (Suresh and Smith 2004). Nevertheless, incorrect identification can occur due to *Blastocystis* polymorphic structure and misinterpretation as fungi, *Cyclospora* spp., and fat drops (Sekar and Shanthi 2015).

Several staining procedures, including Giemsa, Gram, Wright, and iron hematoxylin, can be applied to detect *Blastocystis* forms (Stenzel and Boreham 1996). Staining of direct fecal smears with trichrome is a commonly used staining procedure. It is sensitive and provides permanent records for the sample (Tan 2008).

9.2. CULTURE METHOD

Culturing *in vitro* is a helpful diagnostic method for low parasite numbers (Suresh et al. 2005). This method was previously considered the gold standard for detecting *Blastocystis* (Popruk et al. 2013). Fecal samples are cultivated in supplemented Jones medium with 10% horse serum and incubated at 37 °C for 48h (Termmathurapoj et al. 2004). Following cultivation, the suspected colonies are examined through light microscopy (Basak et al. 2014). The culture method is more sensitive than direct microscopic examination for diagnosing *Blastocystis* from stool samples (Yakoob et al. 2010).

Blastocystis cells can be cultured on a solid medium, and the parasite clones resemble bacterial colonies microscopically. They can survive up to two weeks and longer in liquid or solid media (Tan et al. 2000). The cell densities reach maximal level around four days post-inoculation, move into the death phase at day five, and are challenging to subculture later on (Tan 2008).

Blastocystis isolates' pure cultures are vital for molecular and biochemical research. Antibiotic combinations can be added to the culture to obtain an axenic culture by eliminating bacteria and fungi. However, some isolates require bacterial existence for survival, and removing all bacteria can lead to the parasite's death (Nourrisson et al. 2014).

Although culture is a sensitive method, it is time overwhelming (2–3 days), and usually not performed in all laboratories. Additionally, culture methods may favor the growth of one subtype, leading to biased results (Navarro et al. 2008; Popruk et al. 2013).

9.3. SERODIAGNOSIS

Blastocystis infection can stimulate IgA, and IgG immune responses. Indirect immunofluorescent (IFA), and enzyme-linked immunosorbent assay (ELISA) can be used to detect antibodies (Mahmoud and Saleh 2003).

Secretory IgA, and serum IgA, and IgG levels in *Blastocystis*-positive people with and without symptoms were looked into by ELISA, and the data revealed that patients with symptoms had significantly higher IgA, and antibodies reactive to *Blastocystis* (Mahmoud and Saleh 2003). Furthermore, (Tasić et al. 2017) reported that symptomatic cases have elevated IgG titers and IgA response is weak or absent in asymptomatic infections.

9.4. MOLECULAR TECHNIQUES

The detection of *Blastocystis* has become more accessible with the advance of molecular methods (Sanggari et al. 2022). PCR targeting the SSU rRNA detects *Blastocystis* spp. effectively from stool (Yoshikawa et al. 2004a).

It has become prevalent owing to its capability to identify *Blastocystis* and its STs after sequencing (Wang et al. 2014). It provides high sensitivity and specificity for detecting the organism's DNA, and the SSU rRNA gene holds highly variable regions allowing phylogenetic analys*is* for *Blastocystis* (Rivera and Tan 2005; Dogruman-Al et al. 2008).

The advancement of real-time PCR for the sensitive and rapid detection of *Blastocystis* spp. and effective differentiation between the genotypes helps with screening and epidemiological studies (Tan 2008).

PCR-RFLP analysis is another technique for detecting of *Blastocystis* SS rRNA gene, which is professional for prevalence studies, and different primers have been described for parasite recognition from unpreserved stool samples (Stensvold et al. 2007a).

10. TREATMENT

In cases where diarrhea is persistent and *Blastocystis* is the only pathogen identified in stool specimens, treatment should be considered. Although infection might be self-limiting, several antimicrobial agents are available to treat *Blastocystis* infections (Sekar and Shanthi 2015).

Metronidazole is the first-line drug of choice for treatment. Nevertheless, *Blastocystis* elimination with this drug is between 0% to 100% (Sekar and Shanthi 2015). Trimethoprim-sulfamethoxazole is a second-line treatment in patients who may not tolerate or respond to metronidazole (Ok et al. 1999).

Treatment is not inevitably necessary in asymptomatic individuals, and detection of *Blastocystis* in symptomatic individuals demands a thorough investigation of other gastrointestinal disorders' causes. Initiating an antimicrobial therapy trial is justifiable in patients with persistent diarrhea, gastrointestinal symptoms, and many cysts in the stool, which might be a candidate for therapy. Furthermore, for those with *Blastocystis* in the stool and with an associated skin eruption, treatment should be considered after eliminating other causes (Coyle et al. 2012).

Paromomycin is a broad-spectrum antibiotic indicated for acute and chronic intestinal amebiasis. It is a successful therapy for *Blastocystis* cases linked to skin lesions, predominantly urticaria (Valsecchi et al. 2004). Other drugs like cotrimoxazole and nitazoxanide may also be applied (Tan 2008).

11. PREVENTION

The fecal-oral route is a major responsible route for the transmission of most *Blastocystis* infections. Hence, improved hygiene and sanitation conditions help prevent infections. Also, increased health awareness is essential in preventing parasitic diseases (Popruk et al. 2013).

12. CONCLUSION

Molecular epidemiological studies identified *Blastocystis* subtypes in different hosts globally. Contact with infected animals is a risk factor for the spread of zoonotic enteric protozoan parasites, including *Blastocystis*. Owing to the genetic diversity of the organisms, developing molecular techniques for genomic studies is essential for identifying genes responsible for the organism's virulence. Studies for establishing transmission outline and the host's specificity are also critical. Due to its zoonotic potential, investigating different subtypes in various hosts, including domestic and wild animals in different areas, is vital for recognizing possible animal reservoirs and finding a novel subtype. Health education, including good personal hygiene and improved sanitation, is highly recommended to prevent *Blastocystis* infection.

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