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Pathogenic mechanism for *Blastocystis* sp.: A review

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Highlights

1. *Blastocystis* sp. is an important pathogenic microorganism that causes a variety of intestinal diseases.

2. *Blastocystis* sp. causes symptomatic or asymptomatic infection, resulting in mucosal shedding, increased goblet cell mucin, and decreased intestinal permeability.

3. The host immune response to *Blastocystis* sp. is regulated by inducing apoptosis of host cells, modulating the inflammatory pathway, and altering the microbial composition.

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Abstract

Blastocystis sp. is a unicellular, anaerobic protozoan that resides in the intestinal tract of diverse vertebrates and is linked to cutaneous lesions and urticaria with a worldwide distribution and variable prevalence. *Blastocystis* sp. causes symptomatic or asymptomatic infection via fecal-oral transmission. *Blastocystis* sp. is primarily located in the lumen or mucosal edge of the cecum and colon, causing mucosal shedding, increased goblet cell mucin, and intestinal permeability. The parasite lysate exhibits high cysteine protease activity, leading to the apoptosis and degradation of tight junction proteins. *Blastocystis* sp. also regulates the host immune response by regulating the inflammatory pathway and altering the microbial composition to stimulate the up-regulation of pro-inflammatory cytokines and tumor necrosis factors. In this review, we summarize the findings of in vitro and in vivo studies, with the aim to summarize the existing research results on the pathogenicity of *Blastocystis* sp. and the specificity of host subtypes, providing a valuable reference for future research.

Keywords

Blastocystis sp.; Protozoan; Transmission; Localization; Pathogenic mechanism

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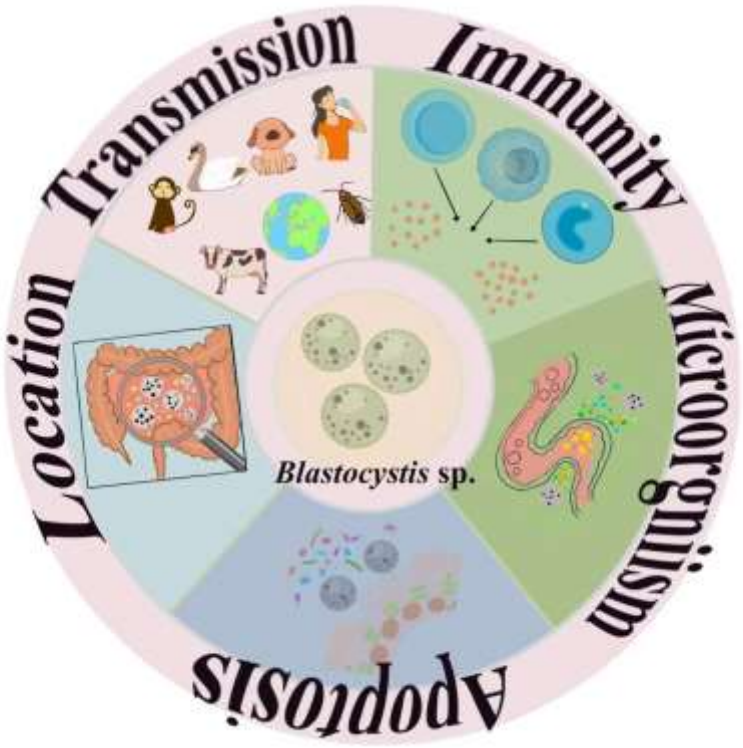


Fig 1. This circular diagram illustrates five key aspects of *Blastocystis* sp. pathogenicity: Transmission**:** *Blastocystis* sp. has a global distribution, which is primarily transmitted via the fecal–oral route. It can spread through direct contact among humans and animals (e.g., monkeys, swans, dogs, cows), or indirectly through contaminated water, food, and insects. Location: The parasite colonizes the host's intestinal tract, particularly the cecum and colon. It is often observed in the luminal space, interacting with the gut epithelium. Apoptosis**:** *Blastocystis* sp. secretes cysteine proteases that disrupt tight junction proteins in intestinal epithelial cells, leading to increased permeability and apoptosis Microorganism Interaction: Colonization by *Blastocystis* sp. alters gut microbial composition, including a shift in the balance of bacterial communities (e.g., Firmicutes, Bacteroides), and may influence microbial diversity and gut homeostasis. Immunity**:** The parasite stimulates the release of various pro- and anti-inflammatory cytokines (e.g., IL-6, IL-10) and interacts with immune cells. The central region of the diagram shows the morphological features of *Blastocystis* sp., including its vacuolar form. This comprehensive model emphasizes the complex interactions between the parasite, host cells, and the gut environment.

1. Introduction

Blastocystis sp. is a prevalent single-celled anaerobic eukaryotic organism inhabiting the intestine. It demonstrates excellent adaptation to the hypoxic or microaerobic conditions of the intestinal environment, including encoding oxygen stress genes (Tsaousis et al. 2018) and mitochondrial vectors capable of transporting glycolysis intermediates (Pyrihova et al. 2024). Blastocystis sp. can be isolated from fresh fecal samples of mammals, birds, invertebrates and

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bivalves (Cian et al. 2017; Ning et al. 2020; Rauff-Adedotun et al. 2020) and grow in large quantities in heterogeneous media (Clark and Stensvold 2016; Naguib et al. 2023; Naguib et al. 2024). Indeed, these animals may serve as the cause of numerous human infections. Moreover, various hosts exhibit distinct mesh morphology and bacterial adhesion characteristics of *Blastocystis* sp., which correlates with its pathogenic potential (Sanggari et al. 2023). The parasite displays multiple morphological forms, including vacuolar, granular, amoeboid, and cystic stages (Kumarasamy et al. 2018) .

Regarding the epidemiology and clinical associations of *Blastocystis* sp. infection, *Blastocystis* sp. is one of the most common intestinal protozoa, with over 1 billion people infected worldwide. The prevalence rate varies from 1% to 100%, depending on geographic location and socioeconomic conditions (Andersen and Stensvold 2016) . Its clinical symptoms include fever (Thergarajan et al. 2019) , nausea, vomiting, diarrhea and so on (Shariati et al. 2019) . The parasite has been linked to diverse conditions, including inflammatory bowel disease (Shirvani et al. 2020) , urticaria (Bahrami et al. 2020) , irritable bowel syndrome (Abedi et al. 2022) , and colorectal cancer (Taghipour et al. 2022) . Emerging evidence also suggests potential associations with thyroid dysfunction (Ghassan Saeid et al. 2023) , HIV (Fontanelli Sulekova et al. 2019) , and neurological diseases (Franklin et al. 2022) . People with compromised immune function are more likely to become infected (Asghari et al. 2021; Khorshidvand et al. 2021) . This includes individuals with diabetes (Mohd Shaari et al. 2024; Taghipour et al. 2024) , COVID-19 patients (Marangi et al. 2024) , pregnant women (Malatyalı et al. 2020; Taghipour et al. 2021) , children with Crohn's disease or iron deficiency anemia (Hamdy et al. 2024; Hurych et al. 2024) .

Blastocystis sp. displays significant genetic diversity. Using the small subunit of the ribosomal RNA gene (SSU rRNA), 42 effective *Blastocystis* sp. subtypes (ST1-ST17, ST21, ST23-ST46) have been identified as meeting the criteria and the most common subtypes are ST1-ST4 in humans and animals (Aykur et al. 2024; Baek et al. 2022; Hernández-Castro et al. 2023; Higuera et al. 2021;

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Martín-Escolano et al. 2023; M. Yu et al. 2023) . However, according to reports, the consequences of *Blastocystis* sp. have ranged from pathogens that cause inflammation and disease to organisms that are beneficial to gut microbes. Most individuals carrying subtypes ST1, ST3, or ST4 are asymptomatic, and the presence of *Blastocystis* is often associated with a more diverse and resilient intestinal microbial community. In particular, ST4 may exert protective effects and has been linked to improved metabolic health. Conversely, ST7—and possibly ST3 under certain host conditions—has been associated with gut dysbiosis and gastrointestinal symptoms such as irritable bowel syndrome (IBS) (E. Piperni et al. 2024) , so the assessment of *Blastocystis* sp. as a pathogen or beneficial symbiont in the intestine should consider environmental factors and host background.

Our primary focus is on the pathogenic mechanisms of *Blastocystis* sp. and the associated controversies. Multiple factors have been implicated in *Blastocystis* sp. pathogenicity, including disruption of the mucin barrier, alterations in the gut microbiota, degradation of tight junctions (TJs) , and induction of pro-inflammatory cytokines in the intestinal wall (SS Ajjampur et al. 2016; Ajjampur and Tan 2016; Lepczyńska and Dzika 2019) . Most of the symptoms linked to cysticercosis infection arise from the innate immune response following barrier destruction, including the infiltration and damage of the intestinal epithelium, which leads to the activation of membrane receptors, such as Toll-like Receptors (TLRs), immunoglobulin A and so on (Vitetta et al. 2016) . Recent studies highlight the role of cysteine proteases, localized in the parasite's central vacuole, as virulence factors that degrade host epithelial junctions and induce apoptosis (Adao and Rivera 2022; Argüello-García et al. 2023; Rajamanikam et al. 2019) . Moreover, changes in intestinal microbial composition or diversity caused by *Blastocystis* sp. may contribute to the pathogenicity of the parasite. Its colonization may be related to the anti-inflammatory response, leading to alterations in the bacterial composition of the gut microbiota, such as an increase in *Firmicutes* levels and the promotion of greater bacterial diversity (Even et al. 2021) . Given that Blastocystis sp. is frequently

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detected in individuals, and the probability of asymptomatic infection and symptomatic infection is equally high (E. Piperni et al. 2024) . However, its pathogenicity and clinical relevance remain a subject of ongoing debate and controversy (Andersen and Stensvold 2016) . Controversies over the pathogenicity of gut microbiota from variations in host regions, isolations from symptomatic or asymptomatic hosts, differences in *Blastocystis* sp. subtypes, and in vitro or in vivo studies, therefore, this review presents a summary of the advancements in the research of transmission, localization, and pathogenesis of *Blastocystis* sp.

2. Transmission

Blastocystis sp. does not seem to have high host specificity, which provides suitable conditions for the spread of zoonosis (AbuOdeh et al. 2019) . *Blastocystis* sp. is transmitted through the fecal-oral route, and infection can be obtained via several direct (human–human, animal–animal, animal–human, and human–animal) or indirect (water-borne food-borne and soil transmission (Oliveira et al. 2020)) transmission routes (Hublin et al. 2020) . In general, the prevalence of the parasite in developing countries (22.1-100%) is higher than in developed countries (0.5–23.1%) (Sanprasert et al. 2016) . Factors such as immune system function, host behavior, age, geographical location, level of contact with livestock and pets, climatic conditions, and other variables can influence the occurrence and transmission of *Blastocystis* sp. (Fig1) (Cociancic et al. 2021; Lhotská et al. 2020) . For human immunodeficiency virus (HIV) infected patients, their immune systems are destroyed by consuming CD4+ T lymphocyte counts, thereby increasing the risk of infection with *Blastocystis* sp. (Popruk et al. 2021; Shehata et al. 2019; Zhang et al. 2023) . It is well known that many parasite infections come from the daily living environment, mostly depending on the behavior of the host. In the subjects selected from urban and rural areas, there exist differences in the types of parasites (Greigert et al. 2018) . Scholars have demonstrated that the possibility of *Blastocystis* sp. infection is significantly elevated in regions with limited economic conditions, poor sanitation, and

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sufficient humidity (Javanmard et al. 2018) . In that conditions, it is easy to breed some arthropods, such as flies and cockroaches, which can act as vectors for *Blastocystis* sp. (Dokmaikaw and Suntaravitun 2020) . Eleven species of intestinal parasites, including *Blastocystis* sp., were also identified from dust samples collected in the household bedroom and living room, indicating that the important of the environment for the parasite transmission (Mejia et al. 2020) . In addition, some researches indicate the infection of the parasite is also related to the education, sleep, and diet. A review of the literature reveals that individuals employed in the food industry with the lower educational background are 20% more likely to contract infections than their more educated counterparts (Teimouri et al. 2021) . At the same time, a positive correlation was observed between the presence of *Blastocystis* sp. and an increase in sleep time per night (E. Piperni et al. 2024) . With regard to dietary habits, vegetables and fruits, though rich in essential nutrients and minerals, are also common vehicles for food-borne human pathogens (Li et al. 2020) .

Blastocystis sp. has been observed in various animals, including livestock and wildlife. In recent years, ecotourism has encouraged closer contact, especially with wildlife inhabiting in ecotourism areas, which may contribute to the spread of zoonosis (Mengshi Yu et al. 2023) . The significance of wild animals in the transmission of the parasite should not be underestimated. Furthermore, mobile animals play a positive role in the direct and indirect transmission of the parasite between animals and between animals and humans through the pollution of the environment with feces. It is postulated that water pollution may be a key factor in the transmission of *Blastocystis* sp. In oceans and lagoons, the *Blastocystis* sp. ST1-5 subtypes have been detected and surprisingly, these subtypes are widely distributed in humans (Attah et al. 2022) . In addition, the presence of *Blastocystis* sp. in farmland irrigation (Javanmard et al. 2019) , surface water (Karaman et al. 2017) , fountains (Hublin et al. 2020) , and sewage (Padukone et al. 2016) has been documented. The worry is that *Blastocystis* sp. has strong resistance to water treated with conventional chlorine and hydrogen peroxide

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(Martín-Escolano et al. 2022) . Consequently, the potential of transmission of *Blastocystis* sp. via water and the necessity for preventive measures cannot be ignored.

3. Colonization

If its pathogenicity is discussed, its location in the human body is also worth describing. At present, the location of *Blastocystis* sp. includes the cecum, ascending colon, transverse colon, the rectum, descending colon, and sigmoid colon (Pavanelli et al. 2016; Sulżyc-Bielicka et al. 2021) . Histological studies in mouse models have shown that *Blastocystis* sp. are localized to the lumens or mucosal margins of the cecum and colon (SSR Ajjampur et al. 2016) . An in vitro mouse model was established to characterize the tissue structure of adhesion and the presence of mucin layers. Two isolates of *Blastocystis* sp. ST7, ST7-B and ST7-H, were incubated with intestinal epithelial cells (IEC) respectively, and the two strains had significant differences in adhesion, with ST7-H demonstrating stronger adhesion to colon tissue compared with the cecum and terminal ileum. Both isolates also possessed mucin-solubilizing effects. Compared with the cecum and terminal ileum, ST7-H demonstrated a preference for binding to the proximal and distal colons, whereas ST7-B did not exhibit similar tissue tropism and instead exhibited more even binding across all intestinal segments (SS Ajjampur et al. 2016) . *Blastocystis* sp. DNA was primarily found in the large intestine, all colons, and approximately 90% of the cecum and rectum (Elisa Piperni et al. 2024) . However, it was also detected in small intestine of some immunosuppressed pigs (Wang et al. 2014) . To sum up, the colonization of *Blastocystis* sp. is in the large intestine.

4. Pathogenic mechanism

4.1 Altering the host intestinal permeability

Intestinal permeability is a normal function of the intestine to exhibit some permeability, to allow nutrients to pass through the gut, while also maintaining a

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barrier function to keep potentially harmful substances (such as antigens) from leaving the intestine and migrating to the body more widely (Jang and Jeong 2023) . *Blastocystis* sp. inhabits the gastrointestinal tract (SS Ajjampur et al. 2016) and increases intestinal permeability. But the damage that *Blastocystis* sp. induces to epithelial barrier function may be different, at least in vitro, based on ST virulence and may be related to host specificity (Argüello-García et al. 2023) Firstly, tight junction proteins (TJs) are recognized for preserving the integrity of the intestinal barrier, and disturbance of these proteins leads to increased paracellular and transcellular permeability (Schoultz and Keita 2020) . *Blastocystis* sp. disrupts the TJs between the cells through some ways. Speca et al. revealed a noticeable increase in the serine protease activity and a significant decrease in the expression of occludin in the supernatant of feces of rats infected with *Blastocystis* sp. (Speca and Dubuquoy 2017) . This intestinal permeability is related to changes in the expression of TJs, including decreased expression of octreotide and Zonula Occludens-138 (Defaye et al. 2020) , which in turn disrupts the TJs between the cells. Additionally, microRNAs (miRNAs) are a class of endogenous small non-coding RNA molecules that are widely present in eukaryotic organisms (Bartel 2009) . The integrity and permeability of intestinal TJs are also considered to be regulated by miRNAs (Kozieł et al. 2021; Su et al. 2016; Zhou et al. 2009) . ST3 significantly changes the expression of miRNAs related to intestinal barrier integrity, significantly upregulates mir-223 and mir-874, and down-regulates mir-29a and claudin-7 (Mohammad Rahimi et al. 2022) . This research offers insights into the role of miRNAs in the development of *Blastocystis* sp., but further in vitro and in vivo studies are required to clarify the changes in the expression of miRNAs in both *Blastocystis* sp. and its hosts. Secondly, proteases are a class of enzymes capable of hydrolyzing peptide bonds in proteins, cleaving intercellular junction proteins, or activating protease-activated receptors (PARs) leading to the opening of junction proteins, thereby increasing intestinal permeability (Rao and Grover 2023) . Cysteine protease, which is secreted by *Blastocystis* sp. and known as legume-activated

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cathepsin B, increases the paracellular permeability of the intestinal Caco-2 cell monolayer, where its positive charge enables to bind to a negatively charged surface (Yason et al. 2016) . Especially for lipid-accumulating *Blastocystis* sp., cysteine protease is up-regulated and has higher activity, which increases the damage to Caco-2 cells (Liao et al. 2023) . The previous vitro studies demonstrated that cysteine proteases produced by ST7 induce the expression of zonula occludens-1 (ZO-1) and F-actin in the intestinal epithelium, which is in a Rho-associated protein kinase (ROCK)-dependent manner (Deng et al. 2021) . Similarly, ST4 has demonstrated the ability to increase epithelial permeability in IEC-6 cell monolayers (Argüello-García et al. 2023) . Significant intra-subtype differences were also observed. Compared with isolates ST7-B, ST7-C, ST7-E, and ST7-G, isolate ST7-H exhibited the highest level of adhesion (Wu et al. 2014) . Variation in this bacterium also has a rank correlation with virulence, as more adhesive strains are associated with greater degradation of tight junction protein ZO-1 and occluding, leading to an increase in intestinal permeability (Fig2). Moreover, *Blastocystis* sp. damages to the mucus barrier. Joel et al. initially demonstrated that α-L-fucosidase (ALFuc) is a secretory product of *Blastocystis* sp., specifically identified in ST1–3 (Martínez-Ocaña et al. 2022) . Its catalytic hydrolysis removes L-fucose residues at the non-reducing end of the binding glycan chain, such as mucin, and may cause damage to the mucus barrier, leading to increased intestinal permeability. However, further investigation is necessary to gain a better understanding of this phenomenon.

4.2 Inducing apoptosis of host cells

Excessive apoptosis of intestinal epithelial cells may lead to the impairment of intestinal barrier function and exacerbate inflammation (Blander 2016) . *Blastocystis* sp. infection causes an significantly increase of the Bax/Bcl-2 ratio, a marker of apoptosis (Basuony et al. 2021) meaning the parasite can induce apoptosis of host cells. This phenomenon has been confirmed by in both in vitro and in vivo settings (Chandramathi et al. 2013; Kapczuk et al. 2020; Mirza et al.

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2012) . In addition, Kumarasamy et al. (Kumarasamy et al. 2017) showed that in vivo infection of male Wistar rats with *Blastocystis* sp. damaged intestinal epithelial cells, intensified oxidative injury, and markedly promoted azoxymethane-induced carcinogenesis; these effects were accompanied by a significant increase in the pro-apoptotic protein Bax and elevated Caspase-3 activity. Recent studies further demonstrated that in Tumor Necrosis Factor (TNF)-dependent intestinal inflammation, combined in vivo oral infection of Wistar rats and in vitro infection of Caco-2 colon cancer cells resulted in a pronounced rise in TUNEL-positive late-apoptotic/DNA-fragmented cells and activation of all three apoptotic pathways (Caspase-3, -8, and -9), leading to impaired renewal of damaged villous epithelial cells, irreversible shortening of villi, and an increased rate of apoptosis (Kapczuk et al. 2020) (Fig2) . Similarly, there is also a study reporting that in murine macrophages, serine proteases from *Blastocystis* sp.ST7 induce the upregulation of IL-1β, IL-6, and TNF-α through the MAP kinase-dependent signaling cascade (ERK/JNK) However, Cysteine Proteases (CP) exert its proteolytic activity to directly cleave adhesion proteins such as E-cadherin, disrupting intercellular junctions. This triggers the mitochondrial apoptosis pathway (an increase in Bax, a decrease in Bcl-2, and an elevation of Caspase-3 activity), ultimately inducing apoptosis. This entire process occurs independently of ERK/JNK/p38 phosphorylation and elicits a comparable inflammatory response via the non-MAPK pathway (Lim et al. 2014) . These observations indicate that CP plays a proactive role in disrupting the intercellular connections of epithelial cells and ultimately inducing apoptosis in host cells. Induction of apoptosis in host cells may one of ways for *Blastocystis* sp. to interact with their host, and therefore, it is a matter of concern as to how *Blastocystis* sp. can impact apoptosis in host cells.

4.3 Immunomodulatory effects

The immune system can recognize and eliminate foreign pathogens, such as bacteria and viruses, but when viruses or microbes invade the human body, they

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may trigger an immune response that leads to tissue damage (Zmora et al. 2017) . Statistics show that people with low immune function have a higher prevalence of *Blastocystis* sp. and the parasite can aggravate the symptoms experienced by people with low immune function, leading to death (Karamati et al. 2021) . Therefore, the interaction between *Blastocystis* sp. and the host immune system has attracted the attention of many researchers.

Masar Hadi Ismail et al. study has shown that serum levels of Interleukin-6 (IL-6), Interleukin-10 (IL-10), Interleukin-18 (IL-18), IFN-γ, and TNF-α significantly increase in IBS patients infected with *Blastocystis* sp.(Ismail et al. 2022) . In another, Zhang et al. study has also shown that Blastocystis sp. exhibits immunomodulatory effects by degrading IgA, inhibiting INOS, and increasing levels of cytokines, including IL-8, GM-CSF, IL-1β, IL-6, and TNF-α.(Lu et al. 2021) . Some studies support the above ideas. Abdel-Hafeez et al. compared the effects of the parasite on normal and immunodeficient mice. Their findings indicated that infected immunosuppressed mice exhibited severe goblet cell hyperplasia and inflammatory cell infiltration, affecting all layers of the colon. Furthermore, the expression of interleukin-12 (IL-12) and TNF-α significantly increased in these mice compared to infected immunocompetent mice, while the expression of anti-inflammatory cytokines such as interleukin-4 (IL-4) and interleukin-10 (IL-10) notably decreased (Abdel-Hafeez et al. 2016) . Ekhlas H et al. verified *Blastocystis* sp. can also induce HT-29 and T-84 human colonic epithelial cells to produce GM-CSF and IL-8 (Lim et al. 2014) . A large number of cytokines may protect the host or produce a strong immune response to the host. These data are consistent with the degradation of proteases secreted by invasive *Blastocystis* sp.(Hussein et al. 2022) .

However, different *Blastocystis* sp. subtypes cause different immune responses in the host. *Blastocystis* sp. ST7 can amplify the expression of proinflammatory cytokines, including IL-6, IL-1β, and TNF-α in mouse macrophages (Dogruman-Al et al. 2010) . Studies have also shown that *Blastocystis* sp. ST7 downregulates INOS (Mirza et al. 2011) , which is an enzyme that plays a key role

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in immune activation and inflammation (Cinelli et al. 2019) . Previously infected ST7 aggravates colitis by inducing proinflammatory Th1 and Th17 immune responses. *Blastocystis* sp. ST4 and ST1 colonization triggered effective Th2 and Treg immune responses (Deng et al. 2023) . Colonization of *Blastocystis* sp. ST4 and ST1 protects against DSS-induced colitis by increasing the abundance of beneficial bacteria. In addition, ST4 colonization promotes the production of short-chain fatty acids (SCFAs), which have been associated with anti-inflammatory effects. Moreover, it increases the proportion of CD4+ T cells expressing Forkhead box P3 (Foxp3) and IL-10, both of which are involved in regulating immune responses (Fig 2) (Lei et al. 2023) . SCFA also exerts anti-inflammatory effects by inhibiting histone deacetylase (HDAC) activity or regulating immune cells, especially Transforming Growth Factor, through G Protein-Coupled (GPRC) Receptors (Park et al.) . In addition, ST1 colonization can induce intestinal epithelial cells to secrete antimicrobial peptide (IL-37). ST1 upregulates IL-6 and TNF-α concomitant to IL-4 and IL-10 downregulation (Argüello-García et al. 2023) . This subtype may have a strain-dependent differential effect on host health (Ferrer-Picón et al. 2020) . ST2/ST3 downregulated IL-10 and ST3 upregulated IL-6 (Norouzi et al. 2022) . In Dinitrobenzene sulfonic acid (DNBS)-induced colitis mice, long-term colonization of *Blastocystis* sp. ST3 can promote faster recovery of colitis and reduce the expression of TNF-α and IL-1β (Vincent Billy et al. 2021) . There are also studies on the isolation of *Blastocystis* sp. from symptomatic human isolates to explore the relationship between mucosal immune surveillance in infected rat precancerous polyps. Immune monitoring revealed that ST3 caused an imbalance in IFN-γ, and showed that the level of sIgA in rats infected with the ST1 and ST3 subtypes was lower than that in rats without polyps.

Most studies have not determined whether the impact of *Blastocystis* sp. on host health is a direct result of immune regulation or an indirect consequence of microbiome regulation. For instance, *Blastocystis* sp. ST3 appears to indirectly enhance the host immune response and promote the proliferation of beneficial

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bacterial populations (Lei et al. 2023) . ST7, which appears to be a pathogenic bacterium, has been found to diminish the production of *Bifidobacteria* and *Lactobacilli*, both of which are considered beneficial bacteria. Some of the functions of *Bifidobacteria* are to maintain the epithelial barrier and exert anti-inflammatory properties, which can reduce the production of pro-inflammatory cytokines such as IL-6 and TNF-α (Ling et al. 2016; Liliana Rojas-Velázquez et al. 2022) . Moreover, indole-3-acetaldehyde (I3AA) alters the sensitivity of Treg cells to Transforming Growth Factor β (TGFβ) signaling, thereby affecting their function and capacity to travel to the intestine. (Fig2.) (Nieves-Ramírez et al. 2018) . A study reported that indole propionic acid (IPA) and indole 3-acetaldehyde (I3AA) are derivatives of indole produced by *Blastocystis* sp. subtype ST7 (DengLee and Tan 2022) . And CD4+ T cells also depend on the tryptophan metabolite indole-3-acetaldehyde (I3AA) produced by single-celled eukaryotes (Wojciech et al. 2023) . The host immune response acts as a way to fight *Blastocystis* sp., but the parasite produces immune escape in a clever way. The function of IL-8 induced by *Blastocystis* sp. in HT-84 cells was dependent on NF-κB and involved cysteine protease. The surprise is that cysteine protease cleaves sIgA (Kumarasamy et al. 2018) resulting in the secretion of anti-lysozyme and anti-lactoferrin factors, which facilitate *Blastocystis* sp. immune evasion (Fig2). In addition, the epithelial cells of the intestinal wall secrete antimicrobial peptides, including beta-defensins and cathelicidin, which against parasitic infections. LL-37, a fragment of cathelicidin, can induce membrane disruption and result in alterations in the shape of *Blastocystis* sp., but subtype ST7 isolate B (ST7-B) releases proteases that degrade peptides and make the environment acidic, resulting in decreased LL-37 activity. Simultaneously, it was also observed that the isolate ST7-B had a thick surface coating, which could protect the parasite from being directly killed by LL-37 (Yason et al. 2016) .

Although reports of this parasite have become more common in human intestinal studies, direct *Blastocystis* sp. mucosal immune responses in mouse models have not yet been explored. Therefore, the impacts of *Blastocystis* sp. on host immune

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regulation require further exploration. Therefore, how this parasite affects the human immune regulation through changes in immune modulatory factors or metabolites is a question that warrants further investigation.

4.4 Microorganism effects

The interaction between intestinal microorganisms is beneficial to the normal progress of intestinal activity and also helps to enhance the immune defense against pathogen invasion (Basolo et al. 2020; Kho and Lal 2018; Rowland et al. 2017) . As an intestinal microorganism, the role of *Blastocystis* sp. on host intestinal microbes is divergent, one is that the parasite causes imbalance of intestinal flora (Stensvold et al. 2022) , the other is that it is symbiotic with other intestinal microorganisms (Vincent Billy et al. 2021) , and the different roles seem to be related to the subtype of the parasite. The results by Raul Y et al. showed that different *Blastocystis* sp. subtypes lead to different intestinal microbiota composition in patients (Tito et al. 2018) , and its pathogenic characteristics may not be completely exerted by the parasite itself, but may also be affected by the ecological balance of intestinal flora (Rajamanikam et al. 2023) . Indeed, in vitro studies have attempted to co-culture *Blastocystis* sp. and *Clostridium* difficile and found that in the case of a decrease in beneficial bacteria in the microbiota, the colonization of *Blastocystis* sp. is prevented (Vega et al. 2021) . When ST3 isolated from symptomatic individuals was co-cultured with a bacterial suspension of *Blastocystis* sp. from asymptomatic individuals, the potential pathogenicity of the parasites was reduced. Therefore, by altering the bacterial composition of cultured *Blastocystis* sp., it is possible that a harmful protozoan parasite can become harmless or even die through changes in the microbiota associated with the gut (Rajamanikam et al. 2023).

Increasing evidence suggests that intestinal colonization by *Blastocystis* sp. induces the activity of intestinal flora and the relationship between *Blastocystis* sp. and intestinal bacteria has consistently been a hot research topic in recent years (Beghini et al. 2017; Siegwald et al. 2017) . A previous study also revealed

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that, compared with the control group, the level of intestinal protective bacteria decreased in 117 men with *Blastocystis* sp. colonization, whereas the level of *Bifidobacterium* decreased in men suffering from IBS. Significant changes in two dominant phyla were observed in patients with IBS, regardless of the state of *Blastocystis* sp. infection, which involved a significant increase in the relative abundance of *Firmicutes* and a correspondingly significant decrease in the relative abundance of *Bacteroidetes* (three-fold increase in the ratio of *Firmicutes* to *Bacteroidetes*) (Fig2) (Beghini et al. 2017) . At the same time, *Blastocystis* sp. in both symptomatic and asymptomatic infections seem to be associated with different bacterial taxa. Studies have revealed that *Faecalibacterium prausnitzii* is abundant in symptomatic subjects, whereas the asymptomatic group shows a high abundance of bacteria classified under *Firmicutes*, *Bacteroidetes*, *Verrucous Microbiota*, and *Desulfurizing Microbiota*. Therefore, asymptomatic *Blastocystis* sp. infection may alter the composition of intestinal bacteria in healthy individuals (Behboud et al. 2022) , but the consequences are not well understood, and further studies are needed to determine whether the parasite alters the gut bacterial composition to induce an anti-inflammatory environment that supports asymptomatic colonization. Studies have shown that *Bifidobacteria* can enhance the expression of TJ proteins such as occludin, claudin-3 and ZO-1, while simultaneously decreasing the production of proinflammatory cytokines, such as IL-6 and TNF-α, but *Blastocystis* sp. ST7 can decrease the population of beneficial bacteria, such as *Bifidobacteria* and *Lactobacilli* (Ling et al. 2016) . These findings indicate that *Bifidobacteria* can eliminate the cytopathic effect of *Blastocystis* sp. on the host (Yason et al. 2019) . Moreover, tryptophanase is a prokaryotic enzyme that plays a crucial role in converting tryptophan into indole, which, as a compound, can influence gene expression and induce behavioral changes in bacteria (Li and Young 2013) . The purified ST7-B tryptophanase enzyme showed a preference for converting indole to tryptophan, suggesting that *Blastocystis* sp. may have the capability to establish a localized “indole-free” environment. This unique ability to consume indole could enhance the toxicity of

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certain neighboring bacterial species (Leonardi et al. 2021) . ST7 was also found to be associated with lower bacterial diversity. Indeed, in patients with ST7-infected colitis, the microbial structure changed and the bacterial diversity decreased, with a significant increase in the proportion of Enterobacteriaceae and Shigella. At the same time, in patients with oral ulcers, the diversity of bacteria infected with *Blastocystis* sp. was found to be lower than that of non-infected patients (DengLee and Tan 2022) . An alternative view is that *Blastocystis* sp. is a gut beneficial organism. the colonization of ST1 can alter the composition of intestinal microbiota and is positively correlated with beneficial bacteria, such as *Alloprevotella* and *Akkermansia* (Deng et al. 2023) . Moreover, ST3 creates a symbiotic condition characterized by favorable *Blastocystis* sp. and *Blastocystis* sp. subtypes (Simona Gabrielli et al. 2020; Nieves-Ramírez et al. 2018) . Positive associations with beneficial bacterial species (e.g., *Prevotella*, *Methanobrevibacter*, and *Ruminococcus*) can lead to changes in microbial composition but not to higher bacterial diversity (V. Billy et al. 2021; S. Gabrielli et al. 2020) . It has been reported that this may indirectly benefit the host immune response (Andersen et al. 2015) . *Blastocystis* sp. ST4 exerts a similar probiotic effect in vitro (Speca and Dubuquoy 2017) , inhibiting the ability of *Bacteroides vulgatus* to disrupt the intestinal epithelial barrier. In vitro experiments have shown that *Bacteroides*. *vulgatus* is associated with high levels of Reactive Oxygen Species (ROS) and upregulation of oxidative stress-related genes, which can invade colon epithelial cells (SW-480 and HT-29) and activate the expression of pro-inflammatory cytokines (Fig2) (Paiva and Bozza 2013) . Meanwhile, *Blastocystis* sp. ST4 promotes the abundance of intestinal beneficial bacteria of *Akkermansia*, *Lachnospiraceae* and *Clostridium*, increasing the diversity of the intestinal microbiome (DengWojciech et al. 2022; Feranmi 2022) and negatively regulating *Akkermansia* (Forsell et al. 2017; Tito et al. 2018) . Interestingly, Deng et al. reported that ST4 altered the microbiota by promoting the growth of beneficial microbes such as *Akkermansia* in Rag 1−/− mice and subsequently enhanced mucus production and reduced inflammation (L. Rojas-Velázquez et al.

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2022) . However, In a study by Defaye et al., inoculation of rats with ST4 resulted in higher relative abundance of *Oscillospira*, reduced levels of *Clostridium*, and decreased levels of SCFAs (Defaye et al. 2020) . Therefore, whether ST4 has a positive or negative effect on the intestinal microbial composition of the host, or even its pathogenic ability to the host, is different due to environmental factors, the parasite itself and other factors, needs to be further investigated.

5. Practical implications

In terms of public health, *Blastocystis* sp. is transmitted via the fecal-oral route, and outbreaks are often associated with untreated surface water, contaminated vegetables, and poor hand hygiene. It is necessary to combine household water treatment using "filtration + chlorine/ultraviolet", hygiene training for food handlers, and annual PCR screening for high-risk groups, such as HIV patients, as well as carry out joint monitoring at the wildlife-livestock-human interface. For diagnosis, PCR or on-site rapid LAMP testing (completed within 45 minutes) should be promoted.

In terms of treatment, metronidazole, once the main therapeutic drug, cannot completely eradicate the parasite. It is prone to causing drug resistance, promoting parasite growth, may convert asymptomatic infections to symptomatic ones, and has side effects when used in excessive doses, so it should not be the first choice (Rajamanikam et al. 2019) . Certain probiotics and their metabolites can disrupt the cell cycle of the parasite; for example, proton pump inhibitors (PPIs) such as pantoprazole can indirectly inhibit the growth of the parasite by regulating intestinal bacteria and can be used as a preventive measure (Lepczyńska et al. 2021) . Silver nanoparticles (AgNPs) can destroy the structure and function of the parasite (Younis et al. 2020) . The combined use of silver nanoparticles, iron oxide nanoparticles (Fe_3O_4 NPs) and light-emitting diodes (LEDs) can significantly reduce the number of parasites, showing potential for the development of new drugs (Alexeree et al. 2024) . Given its widespread

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infectivity, whole-genome sequencing of isolates should be performed every 3–5 years to provide data for vaccine updates and public health early warning.

6. Conclusions and future perspectives

Blastocystis sp. is transmitted through fecal-oral routes, which can be acquired via both direct (human–human, animal–animal, animal–human, and human–animal) and indirect (water-borne and food-borne) modes of transmission (Hublin et al. 2020) . The parasite is frequently found in asymptomatic individuals. The parasite's intestinal colonization has been investigated through in vivo and in vitro experiments, focusing mainly on the cecum and colon (Sulżyc-Bielicka et al. 2021) . This colonization leads to apoptosis and increased intestinal permeability through proteolysis, specifically the degradation of tight junction proteins (Ajjampur and Tan 2016).

Proteases play an essential role in host-pathogen interactions and are widely implicated in the pathogenesis of parasitic infections (Nourrisson et al. 2016) . Thus, it is imperative to investigate the specific pathogenic mechanism of cysteine enzymes in each subtype of *Blastocystis* sp. In addition, the disruption of intestinal barrier function may allow lumen antigens to spread to the submucosa, thereby activating the host immune system. *Blastocystis* sp. demonstrates both immunomodulatory functions and host specificity. However, the underlying mechanisms remain unclear. It is still debated whether its immunomodulatory capacity is derived solely from the parasite, in conjunction with changes in the gut microbiota, or due to differences between pathogenic and non-pathogenic subtypes. Furthermore, the specific mechanisms enabling immune evasion by Blastocystis sp. require further investigation.

In addition, the intestinal flora not only increases the susceptibility to parasitic infections but also leads to parasitic infections. The transformation of *Firmicutes* and *Bacteroides* into the intestinal cavity inhibits the oxidation of butyric acid and increases oxygen in the intestinal cavity. The imbalance between the two bacteria is considered a primary factor for microbial–host homeostasis (Stensvold

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and van der Giezen 2018) . These studies suggest that *Blastocystis* sp. infection plays a role in modulating the intestinal microbiota to some extent; however, whether *Blastocystis* sp. can stimulate the host immune response by altering the intestinal flora remains unclear.

Regarding to the pathogenicity debate and research directions, the information currently available on *Blastocystis* sp. does not explain in detail whether the parasite is a pathogen, a nonpathogen, a symbiont, a mutually beneficial symbiont, or a modifier of the host intestinal bacteria. Differences in factors including the source of the isolates (symptomatic or asymptomatic patients), regional variations in patient population, differences among subtypes and genetic variations within subtypes, variations in vitro models, and colonization times in vivo and in vitro studies have led to variable findings on *Blastocystis* sp. It is likely to be an opportunistic pathogen that can acquire pathogenic characteristics under certain circumstances. Future research should focus on the use of animal models to understand the mechanistic links between *Blastocystis* sp. colonization, immune signal transduction, and the role of bacterial-derived SCFAs. These models can also help clarify the functional differences among various *Blastocystis* sp. subtypes in their interactions with the host. Ultimately, such investigations may uncover the ecological niche of the parasite and its potential role in regulating intestinal health.

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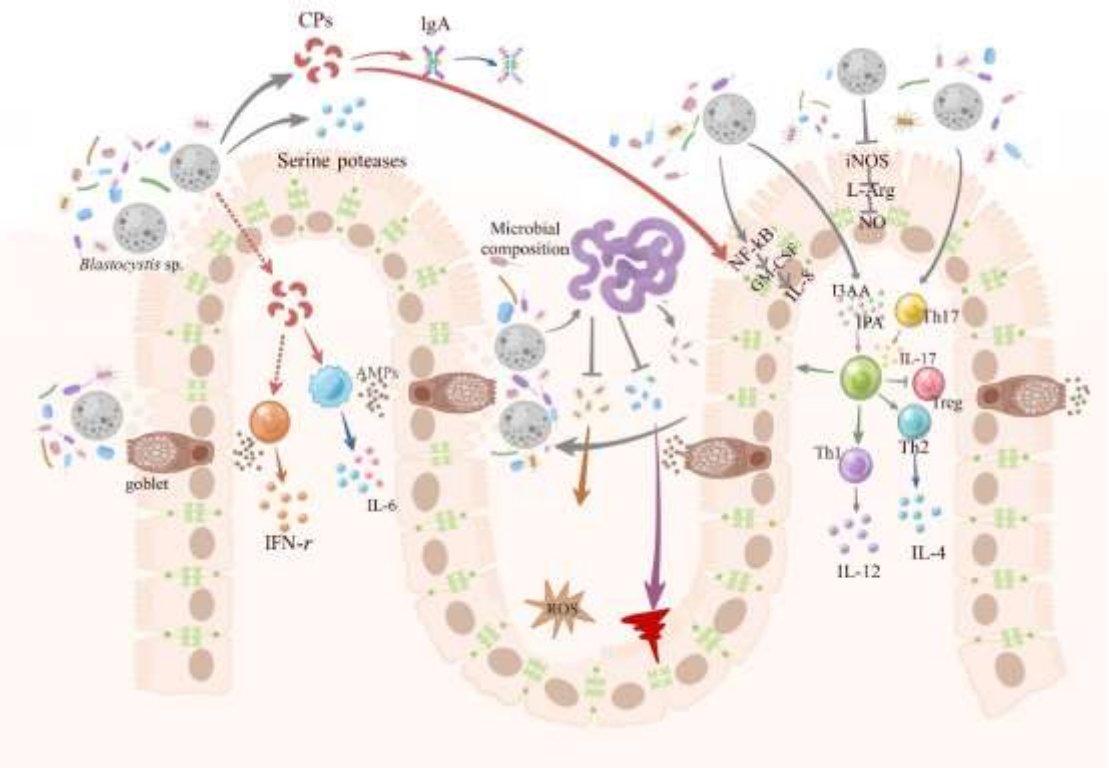


Fig 2. The pathogenic mechanism of *Blastocystis* sp. in the intestine. *Blastocystis* sp. can cause cell fragmentation and degradation of tight junction protein ZO-1 after contact with intestinal epithelium, as well as stimulate goblet cells to secrete AMPs, leading to enhanced intestinal epithelial permeability. At the same time, *Blastocystis* sp. that adheres to cells also releases cysteine enzymes (CPs) and serine proteases. The cysteine enzymes degrade antibodies (IgA) in vitro, stimulate an increase in pro-inflammatory substances in macrophages and T lymphocytes in the epithelium, and affect the expression of pro-inflammatory factors by regulating the NF-kB pathway. In terms of immunity, *Blastocystis* sp. can also evade the host immune response by inhibiting iNOS, which prevents the conversion of arginine into NO. In addition, it can also promote the secretion of CD4 + cells by promoting the secretion of I3AA and IPA. .In turn, CD4 + cells promote the up-regulation of inflammatory factors by regulating various immune cells such as Th1, Th2 and Treg. When *Blastocystis* sp., acting as a pathogenic bacterium, alters the microbiota composition, it can result in an increase of harmful bacteria and a decrease in beneficial bacteria in the intestinal tract. This imbalance may lead to oxidative stress and disruption of cell connections. Left y-axis: “Pro-inflammatory response”; right y-axis: “Barrier integrity / Anti-inflammatory response.” Horizontal axis depicts the spatial gradient from the lumen (left) to the epithelial layer (right). Key symbols and color codes: Solid arrows (→): promote secretion or up-regulation. Blunt-ended arrows (⊤): inhibit secretion or down-regulation.

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Conflict of interest statement

The authors declare that they have no financial interests.

CRediT authorship contribution statement

XinYang: Conceptualization, Writing—original draft preparation, Writing—review and editing. **Huizhu Nan:** Supervision, Project administration. **Jing Zhang:** Literature search and data collection, Conceptualization. **Lei Ma:** Resources, Funding acquisition, Writing-review & editing.

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