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Diversity of *Blastocystis* Subtypes in Humans

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Abstract

Blastocystis is a widespread parasite which is highly prevalent in humans and many animal hosts in developing countries. It is transmitted mainly by a fecal-oral route. Blastocystis infections are more common in low-income communities, crowded conditions, and places with poor hygiene and sanitation. Currently, 13 or more subtypes (STs) have been identified in humans and animals based on characterization of the small-subunit ribosomal RNA (SSU rRNA) gene. Nine of these subtypes, ST1-ST9, have been detected in humans, with ST1-ST4 being most common. Several studies have revealed that some subtypes of Blastocystis isolated from humans can be observed in animals as well, suggesting that animal-to human or zoonotic transmission of Blastocystis can occur. Differences in subtype distribution of Blastocystis spp. depends on different reservoir hosts, geographical areas, and sources of infection, however, ST3 is the predominant subtype distributed worldwide. Most people with Blastocystis infection are carriers, and the clinical manifestations of symptomatic blastocystosis include gastrointestinal complaints, anorexia, joint pain, skin rash and diarrhea. The pathogenicity of this organism remains unclear and further studies are required for better understanding.

Keywords: *Blastocystis*, humans, subtypes, fecal-oral route

Introduction

Blastocystis is an enteric protozoan parasite commonly found in humans and a wide range of animals including pigs, cattle, poultry, and insects. It is a unicellular and ubiquitous intestinal parasite and one of the most common protozoa found in stool samples [1-3]. *Blastocystis* was first described in intestinal tracts of humans in 1912 and was named *Blastocystis hominis*, but the name was later changed to *Blastocystis* spp. due to an indistinguishable difference between those found in humans and in other animals. It belongs to the phylum Stramenopila, a group of unicellular and multicellular eukaryotes, such as water mold, brown algae, and slime nets [4]. The genetic diversity of this parasite population is determined by small-subunit ribosomal RNA (*SSU rRNA*) gene analysis, showing 13 subtypes (STs) [5]. *Blastocystis* is globally distributed, especially in developing countries [6,7]. In Thailand, the prevalence of parasitic infections has been continuously studied, including *Blastocystis* which has prevalence as high as 45% [8-11]. The parasite inhabits the gastrointestinal tract of hosts and can trigger clinical manifestations such as gastrointestinal complaints, anorexia, joint pain, skin rash and diarrhea [12-14].

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Morphology

Blastocystis is an organism with various morphological forms including vacuolar, granular, amoeboid, cyst, multivacuolar and avacuolar forms. All forms can be found in stool samples [7,15-20].

• Vacuolar form: has a central vacuole which occupies 70-90% of the whole cell volume. The size varies from 2-200 μm and contains 1-4 nuclei in each cell.

• Granular form: contains granules within the central vacuole. The size varies from 6.5-8 μm, and also contains 1-4 nuclei in each cell.

• Multivacuolar form: is rarely seen in stool and the size varies from 5-8 μm. Vacuoles can be present or absent in this form. Each cell contains 1-2 nuclei.

• Avacuolar form: also rarely seen in stool. It has no vacuole and the size is approximately 5 μm. This form is also found in the intestine.

• Amoeboid form: this form is rarely reported. Its size varies from 3-8 μm. It has a central vacuole and contains 1-2 nuclei. This form is associated with pathogenicity.

• Cyst form: this form is rarely found in culture. Its size varies from 3-10 μm. It has no central vacuole and can contain 1-4 nuclei. This form is infectious and can survive outside the host
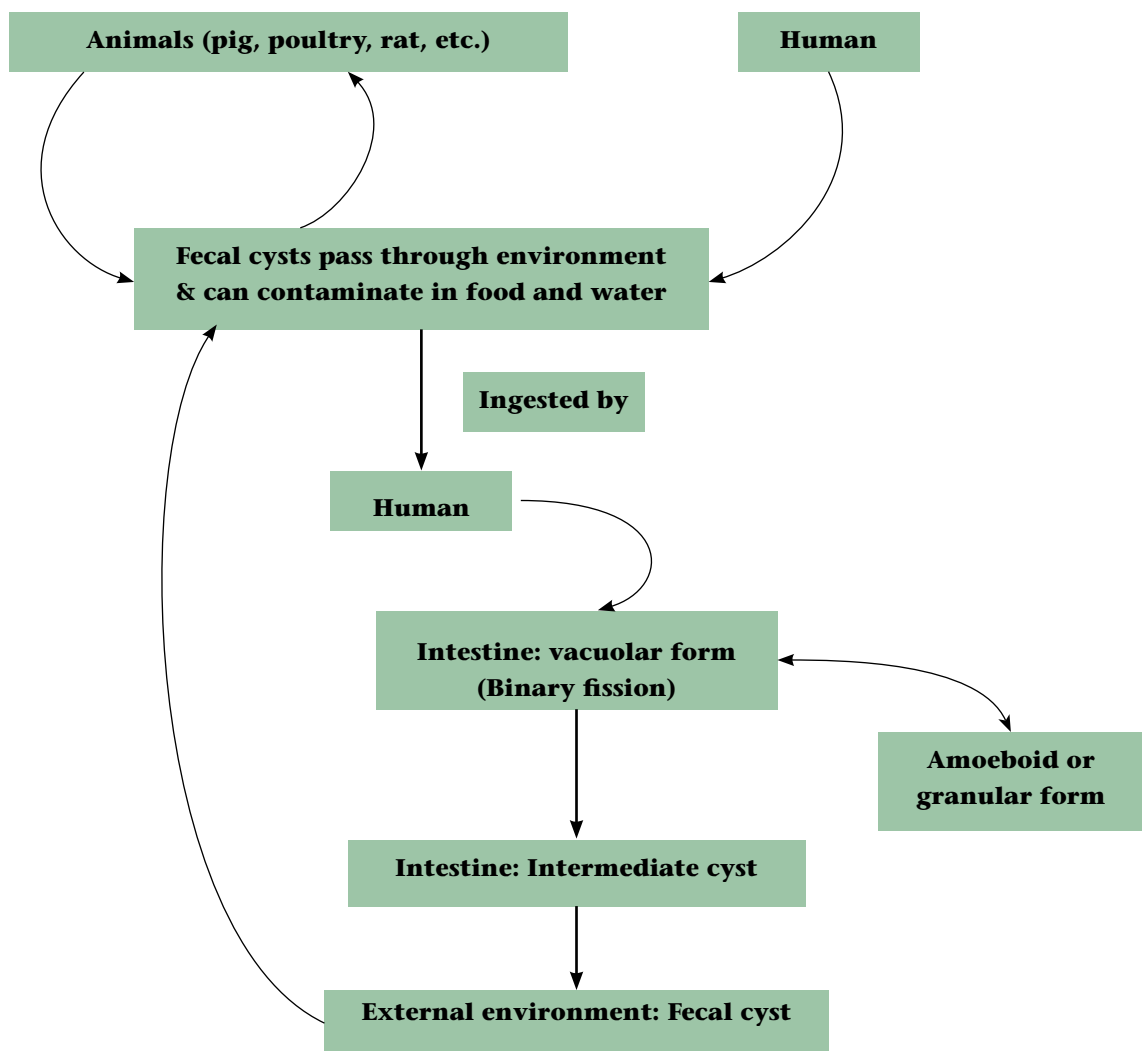

Fig1   The life cycle of *Blastocystis* spp. in humans and many animal hosts

for more than 1 or 2 months in water at 25 °C or 4 °C, respectively.

Life Cycle and Transmission

The life cycle of *Blastocystis* is still not completely understood [2]. Hosts are infected from cysts via a fecal-oral route, such as consumption of contaminated food and water or from practicing poor hygiene. After ingestion, the cyst develops into a vacuolar form, which can asexually reproduce by binary fission, or may change to an amoeboid or granular form, or may form a cyst in the host's intestinal lumen. Information on the transitions from the amoeboid to vacuolar form, and from vacuolar to cyst form is however, not clearly understood [21]. A cyst which is formed in the intestine is surrounded by a thick fibrillar layer, which is subsequently expelled when passing to an external environment, as shown in Figure 1. The excreted cyst is resistant to environmental exposure, and can thus contaminate water or food, causing subsequent *Blastocystis* infection via fecal-oral route [22-24]. Transmission can occur from human to human, human to animal or animal to human.

Epidemiology

Blastocystis is a common parasite in the gastrointestinal tract of humans and its geographic distribution has been reported globally (Table 1). Its prevalence varies from country to country, as well as in different areas within the same country. Prevalence is higher in developing countries (30-50%), such as Brazil and Egypt, than in developed countries (1.5-10%), such as Singapore and Japan [25-27]. This may be due to poor hygiene, exposure to animals, or consumption of contaminated food and water in developing countries. In addition, the wide range of prevalence may be due to the difference in sensitivity of diagnostic techniques, such as molecular methods, culture and microscopy examination.

In Thailand, the prevalence of *Blastocystis* varies from 10-45% in different communities [9-11]. High prevalence has been observed in army bases, orphanages, and among migrant workers from Myanmar. *Blastocystis* infections in orphanages for young children or in homes for girls in Bangkok may be due to direct person-to-person transmission [8]. Sexual activity is also another primary mode of transmission resulting in a high prevalence of enteric parasitic infections in male homosexuals. This includes the high prevalence of *Blastocystis* infection in Australian men who have sex with other men (MSM). There is a significant difference in detection of *Blastocystis* between MSM and non-MSM [28], and infection is continually increasing despite public health campaigns.

Subtypes

It is believed that different subtypes have different reservoir hosts, geographical distribution, and routes of transmission. Therefore, subtyping *Blastocystis* is important for epidemiological studies because it helps to identify potential sources and routes of transmission of a specific subtype in a particular area [43]. However, there is no information of *Blastocystis* subtypes in certain geographical areas, thereby limiting our knowledge of the distribution of *Blastocystis* subtypes. Table 2 shows the distribution of *Blastocystis* subtypes in different geographic regions. Based on gene analysis of small-subunit ribosomal RNA (*SSU rRNA*), at least 13 STs have been identified in mammalian and avian hosts. ST1-ST9 were found in human stool samples [44,45], with ST1-ST4 being the most common subtypes in humans. *Blastocystis* isolated from humans belong to the same subtypes seen in animals, suggesting that animals may act as reservoirs for *Blastocystis* and may be linked to zoonotic transmission [46-48].

ST1 is found in humans and several kinds of animals such as pigs, monkeys, cattle, birds, and rodents [46,49,50]. Some studies report that ST1 is related to zoonotic transmission from farm animals [2,51]. In Libya, ST1 was the predominant subtype and it was suggested that the Libyan population may be exposed to animal feces, which could be the source of *Blastocystis* infection. In Thailand, Leelayoova et al. (2008) found that ST1 was the most prevalent subtype, followed by ST2,

Table 1   The prevalence of *Blastocystis* infection in various geographical distributions.

Countries/ communities	Prevalence (%)	Methods
Brazil	41	Microscopy [29]
China, Shanghai district	1.9	STS primers[a] [30]
China, Eryuan county	18.4	STS primers [30]
China, Menghai county	32.6	STS primers [30]
Egypt	33	Culture [31]
France	3	Microscopy [32]
India, children in rural /urban areas	14.7/18	Microscopy [33]
Indonesia	60	Microscopy [34]
Nepal	26.1	STS primers [35]
Thailand, army base (Chonburi Province)	36.9	Culture [36]
Thailand, immunocompromised host	10.9	Microscopy [37]
Thailand, Myanmar migrant workers	41.5	Culture [38]
Thailand, orphanage (age less than 5 years)	17.3	Culture [8]
Thailand, orphanage (age 5-15 years)	10	Microscopy [39]
Thailand, orphanage (age 10-82 months)	45.5	Culture [11]
Thailand, primary school (age 7-13 years)	13.5	Culture [40]
UK (United Kingdom)	3.9	Culture [41]
USA (United State of American)	23	Microscopy [42]

[a] polymerase chain reaction using sequence-tagged site primers

in schoolchildren due to water-born transmission [50]. Furthermore, Thathaisong et al. (2013) reported that ST1 was the most common subtype, followed by ST6 and ST2, due to person-to-person transmission in a home for Thai girls [43].

ST2 is rare and has a detection rate of about 7% in Africa, Australia, and East Asia [52]. In Ireland, ST2 was the most frequently detected subtype in humans, followed by ST3 [53]. ST2 was also found in children and local rhesus monkeys in Kathmandu (Nepal), suggesting that the rhesus monkey was a possible source of *Blastocystis* spp. infection in that area [54]. This subtype can be isolated from primates and pigs [2].

ST3 is the most common subtype found in humans, and appears to have a cosmopolitan distribution as shown in Japan, Singapore, Egypt, ,Germany, Oregon USA and Turkey [55]. This subtype is also isolated from non-human primates (NHPs) and other mammals such as pigs, dogs, cattle, and rodents [56]. Jantermtor et al. (2013) reported that ST3 was predominant in asymptomatic and symptomatic patients admitted

to major hospitals in the northeastern part of Thailand [55]. However, the route of transmission could not be explained because patients came from different geographical areas.

ST4 is the second most common subtype in the UK and may be restricted to Europe and North America [52]. It is rarely detected in the Far East, South America and North Africa. It was noted that ST4 has been found in rodents as well as NHPs [56].

ST5 and ST9 are rarely found in human stool [57]. Surprisingly, ST6 and ST7 which are avian subtypes were isolated from Thais for the first time by Jantermtor et al. [55]. However, these subtypes are rarely reported in Asia. At present, there have not been any reports about ST10-ST13 isolated from humans.

Pathogenicity

Most people that are infected with *Blastocystis* show neither signs nor symptoms of infection, and it was previously believed that it was a non-pathogenic intestinal protozoa. However, several

Table 2 Distribution of *Blastocystis* subtypes in different geographic regions.

Country/region (no. of samples)	Predominant subtyping (%)	Methods
Bangladesh (26)	3 (92.3)	STS primers[a] [58]
China, Eryuan county (407)	2 (50.7)	STS primers [30]
China, Shanghai district (1,505)	3 (58.6)	STS primers [30]
Denmark (116)	4 (37.5)	Sequencing [3]
Egypt (20)	3 (61.9)	STS primers [45]
France (40)	3 (33.9)	Sequencing [59]
Germany (12)	3 (41.7)	STS primers [58]
Greece (45)	3 (60)	SSCP[c] [60]
Ireland (14)	2 (42.9)	Sequencing [61]
Italy (30)	3 (53.3)	Sequencing [62]
Japan (50)	3 (52)	STS primers [58]
Nepal (82)	3 (60)	STS primers [54]
Nepal (241)	4 (84.1)	STS primers [35]
Pakistan (157)	3 (53)	STS primers [58]
Singapore (276)	3 (77.8)	PCR-RFLP [63]
Spain (51)	4 (94.1)	PCR-RFLP [64]
Sweden (63)	3 (47.6)	Sequencing [65]
Thailand (675)	1 (77.9)	Sequencing [50]
Thailand (370)	1 (94.8)	Sequencing [43]
Thailand (562)	3 (57.1)	STS primers [55]
Turkey (66)	3 (68.2)	STS primers [66]

[a] polymerase chain reaction using sequence-tagged site primers
[b] polymerase chain reaction with restriction fragment length polymorphism
[c] single strand conformational polymorphism

reports have revealed that its pathogenicity [67-69] depends on subtypes, intensity of infection, and host immunity. This parasite is believed to cause irritable bowel syndrome (IBS) in humans, as well as a wide variety of intestinal disorders including nausea, vomiting, abdominal pain, anorexia, flatulence, and acute or chronic diarrhea. *Blastocystis* is difficult to differentiate from other intestinal parasites because there are no specific signs and symptoms. Extraintestinal symptoms, including joint pain and skin rash have also been reported [12,13]. Non-specificity and the variety of symptoms of *Blastocystis* have led to a lack of understanding of its potential pathogenicity [14]. In addition, immunocompromised hosts are more susceptible to this organism than immunocompetent hosts [28,70]; *Blastocystis* infection is frequent in HIV/AIDS and cancer patients with gastrointestinal symptoms [71].

Research has been conducted on rats, mice, and chickens in order to study the pathogenesis of different *Blastocystis* subtypes [72], however, these specimens may not be suitable because they are not typical hosts of some *Blastocystis* subtypes which cause disease in humans. Currently, studies of clinical relevance and *Blastocystis* subtypes are used to find relationships among them. The success of these studies depends on several factors such as the method for detection and type of study group (healthy with/without other diseases, symptomatic, etc.). Scanlan suggested that studies about the clinical relevance of different *Blastocystis* subtypes, their virulence, and the zoonotic potential within and between humans and animals can fill the gaps of incomplete knowledge about pathogenicity of *Blastocystis* [73].

Diagnosis

Microscopy

As described earlier in the morphologic section, *Blastocystis* spp. can be present in polymorphic forms and can vary in size from 2 µm to >200 µm. These variations make microscopic diagnosis detection difficult and inconsistent. The vacuolated form is the most common form found in feces, with an average size of 8-10 µm in diameter and round in shape. In normal saline fecal smear, a large central vacuole containing 1-4 nuclei with peripheral cytoplasm can be observed. When iodine solution is inside the central vacuole, the cytoplasm is a yellowish peripheral area with small peripheral nuclei. When *Blastocystis* is stained with trichrome, the central vacuole may be red and peripheral nuclei stained purple. However, this method has poor sensitivity and can present false-negative results, as shown by the underestimation of the real prevalence of *Blastocystis* in Turkey [74,75].

Culture

Direct cultivation of *Blastocystis* spp. from fecal samples was performed using Jones medium supplemented with 10% horse serum, incubated at 37 °C for 48 h [76]. The sedimentation was examined by light microscopy. Several reports have shown that culture is more sensitive than microscopic methods for the detection of *Blastocystis* parasites in stool samples [76-78]. The culture method is the gold standard for detection of this parasite, but is time consuming (2-3 days) and is not routinely done in many laboratories. In addition, results from the culture can be biased because the growth of one subtype of *Blastocystis* can overcome another subtype.

Polymerase chain reaction (PCR)

PCR detection of small-subunit ribosomal RNA (*SSU rRNA*) gene is a powerful tool for analyzing subtypes of *Blastocystis* from stool specimens [58], and is becoming more widely used for the detection of enteric parasites in both humans and animals [79]. The PCR protocol provides not only high sensitivity when compared with microscopic examinations and the culture method, but also high specificity for the detection of the organism's DNA. The majority of the primer target, the *SSU rRNA* gene, contains highly variable regions that allow phylogenetic analysis of *Blastocystis*. In many countries, PCR with subtype-specific sequence-tagged-site primers (STS) method has been developed and used in several studies to detect genetic variations of *Blastocystis* [80,81].

Treatment and prevention

The pathogenic role of *Blastocystis* is still unclear as the parasite produces non-specific symptoms (asymptomatic to symptomatic), thereby making it difficult to use clinical manifestations for indicating their true cause. In symptomatic patients, metronidazole is the drug of choice for treatment; however, this infection may be self-limiting [82]. The majority of *Blastocystis* infection is transmitted by fecal-oral route; therefore, prevention should involve improved personal and community hygiene and sanitation conditions. In addition, health education and health promotion are important tools in parasitic prevention.

Conclusion

Blastocystis spp. is a common worldwide intestinal parasite. Its pathogenicity is still debatable and its pathogenesis remains unclear. Further investigations and research regarding the pathogenicity of this protozoan are essential to better understand blastocystosis. In addition, proper health education and increased sanitary conditions are recommended to reduce the prevalence of *Blastocystis* spp.

Acknowledgements

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