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ORIGINAL RESEARCH

Molecular Epidemiology and Risk Factors of *Blastocystis* sp. Infections Among General Populations in Yunnan Province, Southwestern China

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Background: *Blastocystis* is a common enteric parasite of controversial pathogenic roles in human diseases. Although the prevalence of *Blastocystis* infections has been investigated in a diverse range of populations, there is little knowledge on the molecular epidemiology and risk factors of *Blastocystis* infections among general populations in southeastern China.

Materials and Methods: A total of 507 individuals were randomly selected in Yunnan province, China from July 2016 to March 2017. Stool specimens were sampled for detection of *Blastocystis* sp. using PCR assay, and the risk factors of *Blastocystis* infections were identified. *Blastocystis* isolates were subtyped, and the associations of *Blastocystis* infections and subtypes with clinical manifestations were examined.

Results: The overall detection rate of *Blastocystis* sp. was 9.47% (95% *CI*: 7.13–12.44%). Toilet type (*OR* = 3.248, 95% CI: 1.245–8.473), anemia (*OR* = 2.601, 95% *CI*: 1.245–8.473) and type of daily drinking water (*OR* = 3.11, 95% *CI*: 1.557–6.213) were identified as risk factors of *Blastocystis* infections; however, *Blastocystis* infections showed no associations with clinical symptoms. Four subtypes (ST1 to ST4) were characterized in *Blastocystis* isolates, in which ST3 was predominant (4.73%, 95% *CI*: 3.2–6.94%), followed by ST1 (3.16%, 95% *CI*: 1.95–5.07%), ST4 (1.38%, 95% *CI*: 0.07–2.82%) and ST2 (0.2%, 95% *CI*: 0–1.11%). In addition, ST1 subtype infection was found to correlate with anemia (*OR* = 4.66, 95% *CI*: 1.631–14.314).

Conclusions: There is a high prevalence of *Blastocystis* infections among general populations in Yunnan province, southwestern China, and toilet type, anemia and type of daily drinking water are risk factors of *Blastocystis* infections. ST3 is the dominant subtype of *Blastocystis* sp. characterized, and ST1 correlates with anemia. Improving hygiene conditions, developing healthy lifestyles and intensifying health education programs are strongly recommended to reduce the prevalence and transmission potential of *Blastocystis* infections.

Keywords: *Blastocystis* sp., molecular epidemiology, risk factor, subtype, clinical manifestation, southwestern China

Introduction

The single-celled protozoan *Blastocystis*, which was firstly characterized in 1912,[1] is one of the most common enteric parasites that inhabits the gastrointestinal tract of humans and many animals with global distribution.[2] The prevalence of human *Blastocystis* sp. infections varies greatly in countries, ranging from 1.26% to 70%.[3] More importantly, the prevalence of human *Blastocystis* sp. infections appears a tendency towards a rise over years across the world.[4]

Epidemiological studies have shown that *Blastocystis* sp. infections predominantly occur in immunocompromised individuals and those with close contacts with animals.[5] Human infections may manifest diverse clinical syndromes, and severe infections may present abdominal pain, diarrhea, weakness and fever.[6] Although the pathogenic role of *Blastocystis* sp. remains controversial,[5] this intestinal pathogen is linked to gastrointestinal disorders,[7–10] urticaria[11–14] and Steven Johnson's syndrome.[15] In addition, *Blastocystis* sp. infection may cause a growth stunting and a reduction in cognitive and learning abilities and quality of life in children.[16,17] There is increasing evidence proving that *Blastocystis* sp. infection poses a great threat to human health,[18] and some *Blastocystis* subtypes show pathogenic potential.[19] In animal models experimentally infected with *Blastocystis* sp., the *Blastocystis* subtype 1 (ST1) was found to be associated with elevated pathogenicity.[20] In Egyptian urticarial patients, the *Blastocystis* subtype 3 (ST3) was reported to be linked to chronic idiopathic urticaria.[12] Infection with different *Blastocystis* subtypes may present diverse clinical manifestations; however, the underlying mechanisms remain to be investigated.

In China, *Blastocystis* sp. human infections have been detected in more than 12 provinces, and the overall prevalence was 3.37% (range, 0.8% to 100%),[21] and multiple risk factors have been identified for *Blastocystis* sp. human infections, including poor hygiene conditions, not washing hands after using toilets, hepatitis B virus infection, keep pets at home, drinking non-tap water.[22–25] Although a high prevalence of *Blastocystis* sp. human infections has been reported in rural areas of China,[26–28] there is little knowledge on the molecular epidemiology and subtypes of *Blastocystis* sp. in many parts of China, notably in impoverished and underprivileged communities from rural and remote areas, which are of the great need for public health interventions. This cross-sectional study was therefore designed to investigate the molecular epidemiology and risk factors for *Blastocystis* sp. infections among general populations living in remote, impoverished, and rural communities of Yunnan province, southwestern China.

Materials and Methods

Ethics Statement

This study was approved by the Ethical Review Committee of National Institute of Parasitic Diseases, Chinese Center for Disease Control and Prevention. All experimental procedures were performed in accordance with the Declaration of Helsinki, and written informed consent was obtained from all participants following a detailed description of the purpose, potential risks and benefits of the study. Participation was voluntary and individuals were free to withdraw from the study at any time without further obligations.

Study Subjects

A cross-sectional study was performed during the period between July 1, 2016 and March 31, 2017. Participants were randomly selected from Beihai and Qushi towns in suburban areas of Tengchong city (24°38′ N, 98°05′ E), Yunnan province, southwestern China. According to the local government statistics, there were 659,900 residents living in the city, which had a gross domestic product (GDP) of 2.05 billion dollars in 2014, and local residents primarily depend on farming and livestock breeding. As a tropical monsoon climate area, Tengchong city has a mean annual rainfall of 1,135 mm, mean annual temperature of 14.9°C, and mean elevation of 1,596 m above sea level. Both towns are agricultural areas, and more than 60% of local residents live in rural areas. Participants with inadequate fecal samples, unqualified questionnaires, or refusal to participate were excluded from the study.

Questionnaire Survey

A structured questionnaire was employed to capture the demographic (gender, age, height, body weight, ethnicity, residence, marital status, family size, number of children, occupation and education level) and clinical characteristics (blood pressure, heart rate, abdominal pain, abdominal distension, loss of appetite, itchy skin, perianal pruritus and constipation). In addition, the lifestyle and diet habitats were investigated using the questionnaire survey. All surveys were performed by well-trained doctors or nurses.

Identification of *Blastocystis* sp. Infections

Fecal samples (amount >3 g or 3 mL) were collected from each participant under aseptic conditions, transferred to the laboratory of the Tengchong Center for Disease Control and Prevention within 12 hours, and frozen at –70°C. Each frozen stool specimen was delivered to the laboratory of the National Institute of Parasitic Diseases, Chinese Center for Disease Control and Prevention (Shanghai, China), and stored at –70°C for the subsequent experiments.

Blastocystis sp. genomic DNA was extracted from each stool specimen using the QIAamp DNA Stool Mini Kit

(Qiagen; Hilden, Germany) according to the manufacturer's protocol. *Blastocystis* sp. was detected using a polymerase chain reaction (PCR) assay with the primers targeting the 18S small-subunit ribosomal RNA (*SSU rRNA*) coding region gene.[29] PCR assay was performed in a 25 μL of the reaction system containing 12.5 μL of 2 × TaKaRa Taq™ DNA mixture (TaKaRa Bio Inc, Shiga, Japan), 2 μL of DNA template, 1 μL of each primer set (forward: 5′-GGAGGTAGTGACAATAAATC-3′, reverse: 5′-ACTAGGAATTCCTCGTTCATG-3′, each at 10 μM), and 8.5 μL of ddH_2O under the following conditions: at 94°C for 5 min; followed by 40 cycles of at 94°C for 30 s, at 58°C for 1 min, and at 72°C for 1 min; and finally at 72°C for 10 min. PCR products were checked by electrophoresis on 1% agarose gels at 120 V for 40 min and visualized under UV light following ethidium bromide staining, and the observed size of the PCR amplification product was 1100 bp.

Characterization of *Blastocystis* Subtypes

The PCR amplification products of suspected positive cases were purified and sequenced on both strands using the dideoxy-terminal method in Sangon Biotech Company (Shanghai, China).[30] Sequences were edited in the software MEGA version 6.0 (https://www.megasoftware.net/) and aligned with the reference sequences of each *Blastocystis* sp. subtype retrieved from the GenBank database using BLAST queries, including ST1 (GenBank accession number: U51151), ST2 (GenBank accession number: AB070997 and AB070987), ST3 (GenBank accession number: AB070988 and AB070986), ST4 (GenBank accession number: AY244621 and U51152), ST5 (GenBank accession number: AB070999 and AB107966), ST6 (GenBank accession number: AB070990 and AB070994), ST7 (GenBank accession number: AF408427 and AB070991), ST8 (GenBank accession number: AY266472) and ST9 (GenBank accession number: AY266470).

Statistical Analysis

All data were processed using the statistical software SPSS version 25.0 (SPSS, Inc.; Chicago, IL, USA). All measurement data were described as the median and interquartile range (IQR), and comparisons of the measurement data between groups were done using Student *t*-test, Wilcoxon rank sum test or Kruskal–Wallis *H*-test. The risk factors of *Blastocystis* sp. infections were identified using univariate analysis with Fisher exact test and chi-square test, and factors with a *P* value of <0.20 in the univariate analysis were included in the multivariate logistic regression analysis. The associations of *Blastocystis* sp. infections and subtypes with clinical manifestations were examined using chi-square test or Fisher exact test, and odds ratio (*OR*) and 95% confidence intervals (95% *CI*s) were calculated to measure the strength of the association. A *P* value of <0.05 was considered statistically significant.

Results

Subject Characteristics

A total of 507 individuals participated in this study. The subjects included 260 men and 247 women, and had a median age of 49 years (95% *CI*: 48–51 years), a mean weight of 57.75 kg (95% *CI*: 56.77–58.72 kg), a mean height of 160.28 cm (95% *CI*: 158.88–161.69 cm), a median family size of 4 (95% *CI*: 4–5), and a median child number of 1 (95% *CI*: 1–2). Among all participants, 94.87% had a Han ethnicity, 77.71% lived in rural areas, 65.48% had an education level of primary school, 63.12% were farmers and 81.46% were married. The five most common clinical manifestations included loss of appetite (17.95%), abdominal pain (17.95%), abdominal distention (12.43%), anemia (12.23%) and itchy skin (12.23%) (Table 1).

Prevalence of *Blastocystis* sp. Infections

The overall detection rate of *Blastocystis* sp. was 9.47% (95% *CI*: 7.13–12.44%) among the participants. There were no significant differences between individuals with and without *Blastocystis* sp. infections in terms of the median age (*Blastocystis*-positive individuals: 52 years, 95% *CI*: 51–55 years; *Blastocystis*-negative individuals: 51 years, 95% *CI*: 45–61 years; $Z = -0.065$, $P = 0.948$), median height (*Blastocystis*-positive individuals: 163 cm, 95% *CI*: 163–165 cm; *Blastocystis*-negative individuals: 167.5 cm, 95% *CI*: 160–168 cm; $Z = -1.168$, $P = 0.244$) or mean proportion of eosinophilia granulocytes in the peripheral blood (*Blastocystis*-positive individuals: 2%, 95% *CI*: 1.8–2.3%; *Blastocystis*-negative individuals: 1.85%, 95% *CI*: 1.4–2.6%; $Z = -0.212$, $P = 0.832$). However, a higher median weight was measured in individuals with *Blastocystis* infections (60 kg, 95% CI: 60–62) than in those without *Blastocystis* infections (55 kg, 95% *CI*: 55–61 kg) ($Z = -2.431$, $P = 0.013$).

Risk Factors of *Blastocystis* sp. Infections

Univariate analysis showed that the prevalence of *Blastocystis* sp. infections was not associated with age,

Table 1 Univariate Analysis of Factors Affecting *Blastocystis* sp. Infections

Variable		*Blastocysts* sp. Infections (n, %)		Univariate Analysis			
		Positive (n = 48)	Negative (n =459)	χ^2	*P*	*OR*	95% *CI* for *OR*
Age (years)	<40 (n = 134) 40 or greater (n=373)	13 (9.7) 35 (9.4)	121 (90.3) 338 (90.6)	0.012	0.914	0.964	0.493, 1.833
Gender	Male (n=260) Female (n=247)	22 (8.5) 26 (10.5)	238 (91.5) 221 (89.5)	0.63	0.427	1.273	0.701, 2.311
Ethnicity	Minority ethnicity (n=26) Han ethnicity (n=481)	3 (11.5) 45 (9.4)	23 (88.5) 436 (90.6)	–*	0.724	0.791	0.229, 2.739
Residence	Urban area (n=113) Rural area (n=394)	9 (8.0) 39 (9.9)	104 (92.0) 355 (90.1)	0.383	0.536	1.269	0.595, 2.706
Education level	Primary school (n=332) Junior school (n=111) High school (n=43) University or College (n=21)	33 (9.9) 11 (9.9) 2 (4.7) 2 (9.5)	299 (90.1) 100 (90.1) 41 (95.3) 19 (90.5)	1.518	0.678	–*	–*
Marital status	Unmarried (n=69) Married (n=413) Living alone or widowed (n=25)	5 (7.2) 40 (9.7) 3 (12.0)	64 (92.8) 373 (90.3) 22 (88.0)	0.625	0.738	–*	–*
Family member	<5 Individuals (n=376) ≥5 Individuals (n=131)	37 (9.8) 11 (8.4)	339 (90.2) 120 (91.6)	0.236	0.627	0.84	0.415, 1.699
Body mass index	Underweight (n=53) Normal (n=300) Overweight (n=154)	7 (13.2) 34 (11.3) 7 (4.5)	46 (86.8) 266 (88.7) 147 (95.5)	6.436	0.04	–*	–*
Daily drinking water	Boiled water (n=438) Unboiled water (n=69)	33 (7.5) 15 (21.7)	405 (92.5) 54 (78.3)	14.033	<0.001	3.409	1.739, 6.683
Source of drinking water	Well water (n=7) Tap water (n=500)	0 (0.0) 48 (9.6)	7 (100.0) 452 (90.4)	–*	0.999	–*	–*
Toilet types	Water closet (n=149) Waterless toilet (n=358)	5 (3.4) 43 (12.0)	144 (96.6) 315 (88.0)	9.196	0.002	3.931	1.525, 10.133
Washing hands after defecation	No (n=189) Yes (n=318)	24 (12.7) 24 (7.5)	165 (87.3) 294 (92.5)	3.67	0.055	0.561	0.309, 1.02
Raising livestock	No (n=196) Yes (n=311)	17 (8.7) 31 (10.0)	179 (91.3) 280 (90.0)	0.235	0.628	1.166	0.627, 2.168
Keeping pets	No (n=347) Yes (n=160)	31 (8.9) 17 (10.6)	368 (91.1) 143 (89.4)	0.365	0.545	1.212	0.65, 2.261
Contacting animals	No (n=203) Yes (n=304)	19 (9.4) 29 (9.5)	184 (90.6) 275 (90.5)	0.005	0.946	1.021	0.556, 1.876
Chronic diarrhea in family members	No (n=485) Yes (n=22)	46 (9.5) 2 (9.1)	439 (90.5) 20 (90.9)	0.004	0.951	0.954	0.216, 4.213
Anemia	No (n=445) Yes (n=62)	36 (8.1) 12 (19.4)	409 (91.9) 50 (80.6)	8.057	0.005	2.727	1.332, 5.581

(*Continued*)

Table 1 (Continued).

Variable		*Blastocysts* sp. Infections (n, %)		Univariate Analysis			
		Positive (n = 48)	Negative (n =459)	χ^2	*P*	*OR*	95% *CI* for *OR*
Chronic diarrhea	No (n=448) Yes (n=59)	40 (8.9) 8 (13.6)	408 (91.1) 51 (86.4)	1.304	0.253	1.6	0.71, 3.607
Eating raw or semi-raw meat	Never (n=429) Occasionally (n=73) Frequently (n=5)	42 (9.8) 6 (8.2) 0 (0.0)	387 (90.2) 67 (91.8) 5 (100.0)	1.185	0.553	–*	–*
Eating raw or semi-raw eggs	Never (n=500) Occasionally (n=7) Frequently (n=0)	48 (9.6) 0 (0.0) –	452(90.4) 7 (100.0) –	–*	0.999	0.904	0.879, 0.93
Eating raw or semi-raw dairy products	Never (n=503) Occasionally (n=4) Frequently (n=0)	48 (9.5) 0 (0.0) –	455 (90.5) 4 (100.0) –	–*	0.999	0.905	0.879, 0.931
Eating raw vegetables or fruits	Never (n=470) Occasionally (n=26) Frequently (n=11)	45 (9.6) 1 (3.8) 2 (18.2)	425 (90.4) 25 (96.2) 9 (81.8)	2.005	0.367	–*	–*

Note: *Data unavailable or not calculated.
Abbreviations: *OR*, odds ratio; *CI*, confidence interval.

gender, ethnicity, residence, education level, marital status, family size, source of drinking water, raising livestock, keeping pets, contact with animals, chronic diarrhea in family members, chronic diarrhea, eating raw/semi-raw meat, eating raw or semi-raw eggs, eating raw/semi-raw dairy products, eating raw vegetables/fruits or washing hands after defecation ($P > 0.05$), while the prevalence of *Blastocystis* sp. infections significantly correlated with type of daily drinking water, toilet type, anemia and body mass index ($P < 0.05$) (Table 1). Then, five variables were included in the multivariate logistic regression analysis, including washing hands after defecation, daily drinking water, toilet type, anemia and body mass index, and toilet type (*OR* = 3.248, 95% *CI*: 1.245–8.473), anemia (*OR* = 2.601, 95% *CI*: 1.245–-8.473) and type of daily drinking water (*OR* = 3.11, 95% *CI*: 1.557–6.213) were identified as risk factors of *Blastocystis* sp. infections (Table 2).

Association Between *Blastocystissp.* Infection and Clinical Manifestations

There were no associations of *Blastocystis* sp. infections with abdominal distension, loss of appetite, itchy skin, perianal pruritus or constipation ($P > 0.05$) (Table 3).

Prevalence of *Blastocystis* sp. Subtypes

Among the 48 *Blastocystis* isolates, there were four subtypes characterized (Figure 1), including ST3 (n = 24, 50%), ST1 (n = 16, 33.33%), ST4 (n = 7, 14.58%) and ST2 subtypes (n = 1, 2.08%). The prevalence of *Blastocystis* subtypes varied among participants ($\chi^2 = 26.118$, $P < 0.001$), and the highest prevalence was estimated for the ST3 subtype (4.73%, 95% *CI*: 3.20–6.94%), followed by the ST1 (3.16%, 95% *CI*: 1.-95–5.07%) and ST4 subtypes (1.38%, 95% *CI*: 0.07–2.82%) and the lowest for the ST2 subtype (0.2%, 95% *CI*: 0.00–1.11%). We found no age- ($P = 0.316$), gender- ($P = 0.29$),

Table 2 Multivariate Logistic Regression Analysis of Factors Affecting *Blastocystis* sp. Infections

Variables	*Beta*	*SE*	*Wald*	*P*	*OR*	95% *CI* for *OR*
Daily drinking water	1.135	0.353	10.329	0.001	3.11	1.557, 6.213
Toilet types	1.178	0.489	5.799	0.016	3.248	1.245, 8.473
Anemia	0.956	0.378	6.394	0.011	2.601	1.24, 5.457
Constant	–6.871	1.09	39.75	< 0.001	0.001	–*

Note: *Data unavailable or not calculated.
Abbreviations: *SE*, standard error of mean; *CI*, confidence interval; *OR*, odds ratio.

Table 3 Association Between Clinical Manifestations and *Blastocystis* sp. Infection

Variable		*Blastocystis sp.* Infection (n, %)		χ^2	*P*	*OR*	95% *CI* for *OR*
		Positive (n = 48)	Negative (n = 459)				
Abdominal distension	No (n = 444) Yes (n = 63)	46 (10.4) 2 (3.2)	398 (89.6) 61 (96.8)	3.324	0.068	0.284	0.067, 1.199
Loss of appetite	No (n = 416) Yes (n = 91)	41 (9.9) 7 (7.7)	375 (90.1) 84 (92.3)	0.408	0.523	0.762	0.33, 1.758
Itchy skin	No (n = 445) Yes (n = 62)	43 (9.7) 5 (8.1)	402 (90.3) 57 (91.9)	0.162	0.687	0.82	0.312, 2.156
Perianal pruritus	No (n = 479) Yes (n = 28)	48 (10.0) 0 (0.0)	431 (90.0) 28 (100.0)	–*	0.079	–*	–*
Constipation	No (n = 452) Yes (n = 55)	41 (9.1) 7 (12.7)	411 (90.9) 48 (87.3)	0.765	0.382	1.462	0.621, 3.419

Note: *Data unavailable or not calculated.
Abbreviations: *OR*, odds ratio; *CI*, confidence interval.

weight- (P = 0.437), or height-specific prevalence of *Blastocystis* subtypes (P = 0.278). In addition, there was no gender-specific prevalence of *Blastocystis* ST1 (3.1% vs 3.2%, P = 0.917), ST2 (0 vs 0.4%, P = 0.487), ST3 (3.1% vs 6.5%, P = 0.071) or ST4 subtypes (2.3% vs 0.4%, P = 0.123).

Association Between *Blastocystis* Subtypes and Clinical Manifestations

Infection with the *Blastocystis* ST1 subtype was not found to correlate with abdominal pain (P = 0.491), lack of appetite (P = 0.999), itchy skin (P = 0.999), perianal pruritus (P = 0.999) or constipation (P = 0.689), while ST1 subtype infection was associated with anemia (P = 0.008). The ST3 subtype infection had no associations with abdominal pain (P = 0.781), lack of appetite (P = 0.281), itchy skin (P = 0.999), perianal pruritus (P = 0.387), constipation (P = 0.316) or anemia (P = 0.1), and no associations of ST4 subtype infections were seen with abdominal pain (P = 0.327), anemia (P = 0.999), lack of appetite (P = 0.615), itchy skin (P = 0.999), perianal pruritus (P = 0.999) or constipation (P = 0.555).

Discussion

As a unicellular parasite that colonizes the intestines of humans and a wide range of non-human animals, *Blastocystis* sp. is widely distributed in the world, and the prevalence of this parasite infection has shown a remarkable rise during the past decade.[4] Epidemiological data show that *Blastocystis* sp. is highly prevalent in tropical areas, notably in developing countries with poor hygiene conditions.[31] Although the pathogenic potential of *Blastocystis* sp. continues to be debated, this parasite is widely accepted as a pathogenic contributor to diarrhea and abdominal pain in humans.[2,7] Notably, *Blastocystis* sp. infections may induce obvious diarrhea in immunocompromised patients, such as HIV/AIDS and cancer patients.[32] As a genetically diverse parasite, there have been 17 subtypes of *Blastocystis* sp. characterized based on the *SSU rRNA* gene analysis until now.[33] *Blastocystis* ST1 to ST9 and ST12 have been identified in both humans and non-human animals, and there is a risk of zoonotic transmission of ST1–ST8 subtypes to humans.[33]

In China, the prevalence of *Blastocystis* sp. infections has been investigated in diverse populations.[21] In two ethnic minority groups on both sides of the China-Myanmar border, PCR assay detected a 6.29% overall prevalence of *Blastocystis* sp. infections in the study populations, with 4.50% in Yao ethnic participants and 9.3% in Wa ethnic participants.[22] Among the HIV/AIDS patients living in Tengchong city, southwestern China and Fuyang city, central China, the detection rates of *Blastocystis* sp. were 3.86% and 6.78%, respectively.[24,34] In Huainan city, Anhui province, central China, iodine staining and hematoxylin staining detected 1%, 1%, 0 and 5.96% prevalence of *Blastocystis* sp. infections among infants, pupils, middle school students and the patients with diarrhea, respectively.[35] In a rural district of West China, the detection rate of *Blastocystis* sp. was 1.37% using the culture method.[36] Among 850 children with acute diarrhea at ages

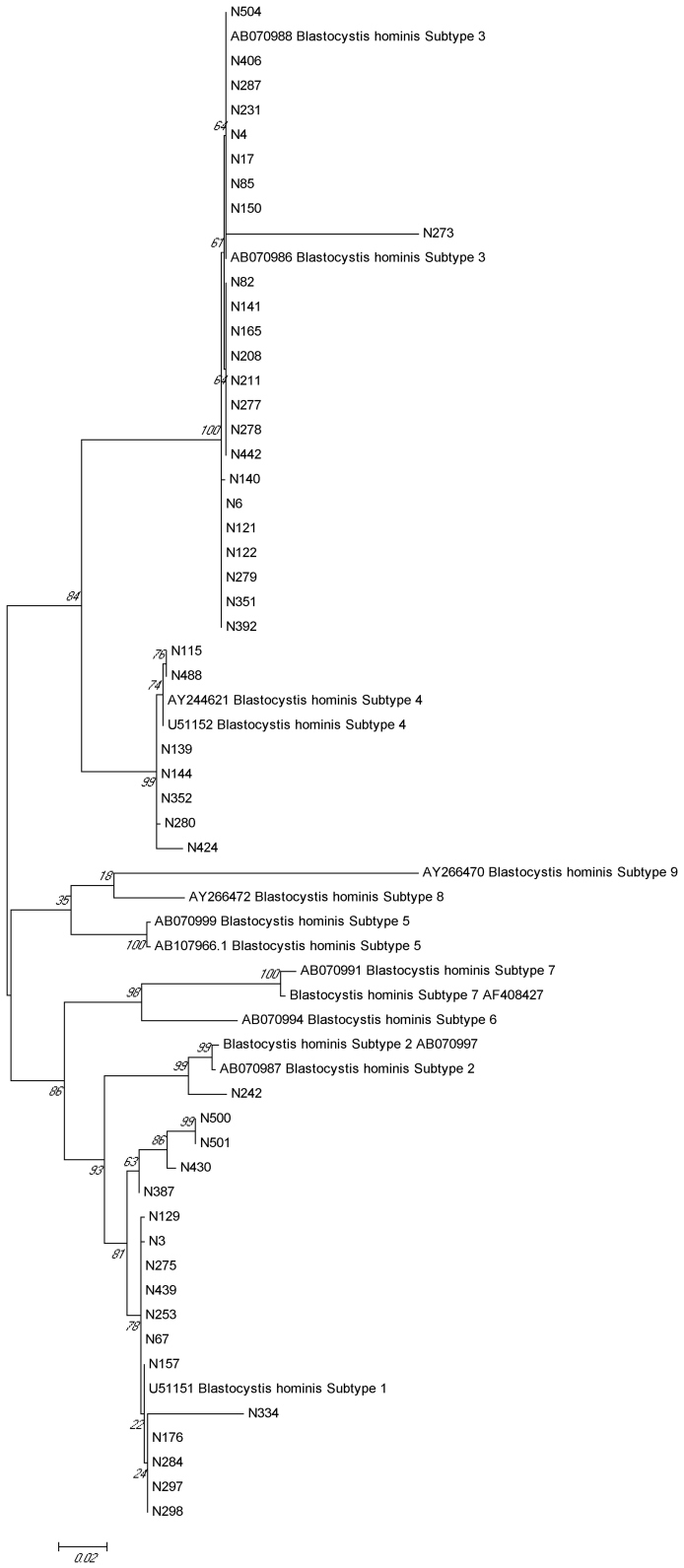

Figure 1 Evolutionary relationships among *Blastocystis* subtypes shown by the maximum likelihood method. The percentage of replicate trees in which the associated *Blastocystis* subtypes are clustered together in the bootstrap test (1,000 iterations) is indicated next to the branches.

of one month to 5 years and 170 healthy controls sampled from four sentinel hospitals in Kunming, Yunnan province, southwestern China, PCR assay detected 3.1% prevalence of *Blastocystis* sp. infections in children with diarrhea and 0.5% in controls (*OR* = 4.7, 95% *CI*: 1.01–112).[37] However, there is little knowledge on the prevalence and risk factors of *Blastocystis* sp. infections, as well as *Blastocystis* sp. subtypes among general populations in southeastern China.

In this study, PCR assay was employed to identify *Blastocystis* sp. infections among general populations sampled from two suburban towns of Tengchong city, Yunnan province, southwestern China, and the detection rate of *Blastocystis* sp. was 9.47% in the study subjects, which is similar to the prevalence in Wa ethnic populations living in the province (9.3%),[22] but lower than in Eryuan county (18.4%),[27] Menghai county (32.6%)[27] and Xishuangbanna prefecture (32.6%),[26] and higher than in HIV/AIDS patients and children with acute diarrhea from the province.[34,37] The variation in the prevalence of *Blastocystis* sp. infections may be attributed to the geographical locations, study subjects and diagnostic techniques.[21]

Previous studies have identified a diverse range of risk factors for *Blastocystis* sp. infections.[22–25] In this study, drinking unboiled water was found to correlate with a high prevalence of *Blastocystis* sp. infections, which was consistent with previous studies.[26] Although Chinese populations have a habit of drinking boiled water, some individuals living in remote areas prefer drinking unboiled raw water directly, which increases the likelihood of intestinal protozoan infections.[26] Improving the disinfection and management of drinking water may reduce the prevalence of *Blastocystis* sp. infections.[38]

In this study, we found that the participants using waterless toilets had a significantly greater prevalence of *Blastocystis* sp. infections than those using water closets ($P = 0.002$). The study sites are agricultural areas located in remote, rural and impoverished regions of Yunnan province, southwestern China, where waterless toilets have not been completely replaced by water closets.[39] As a common zoonotic pathogen, *Blastocystis* sp. is widespread in livestock, and *Blastocystis* sp. cannot be effectively killed in human or livestock feces without high temperature composting or biogas fermentation.[4] If feces containing *Blastocystis* sp. are flushed into the fields through rainfall, this may contaminate vegetables, notably broadleaf vegetables, thereby inducing *Blastocystis* sp. infections via the fecal-oral route.[4] Hence, using sanitary toilets, agricultural production with machines instead of livestock and harmless disposal of livestock fertilizer are likely to reduce *Blastocystis* sp. infections, and improving environmental hygiene is of great importance for the prevention of *Blastocystis* sp. infections.

In the current study, the participants with anemia were found to have a higher prevalence of *Blastocystis* sp. infections than in those without anemia, and multivariate logistic regression analysis identified anemia as a risk factor of *Blastocystis* sp. infections. It is reported that individuals suffering from iron deficiency anemia are more susceptible to have *Blastocystis* sp. infections than non-anemic controls (40% vs 6.3%; $P < 0.000,1$), and *Blastocystis* sp. infection has been identified as a contributing factor to the pathogenesis of iron deficiency anemia in pregnant women.[40] In this study, we found a correlation between ST1 subtype infection and anemia ($P = 0.008$); however, the predominant ST3 subtype, ST2 or ST4 all had no associations with anemia. This is different from previous findings in pregnant women reporting no correlation between *Blastocystis* subtypes and anemia.[40] However, the causal mechanism underlying *Blastocystis* sp. infections and anemia remains to be demonstrated. Previous studies have shown that *Blastocystis* sp. infection is significantly associated with abdominal pain in symptomatic French populationsn;[41] however, the present study examined no associations of *Blastocystis* sp. infection with any clinical manifestations. Further studies to evaluate the correlation between *Blastocystis* sp. infection and clinical syndromes seem justified.

To date, six *Blastocystis* subtypes (ST1 to ST6) have been identified in humans in China, and ST3 is the most dominant subtype.[21] In this study, four subtypes (ST1 to ST4) were characterized in 48 *Blastocystis* isolates, and ST3 was found to be the dominant subtype, which was in agreement with the findings from the same province,[22,26,27] and the isolates from kindergarten children in southern Xinjiang, northwestern China[42] and college students in Guangxi, northern China,[43] as well as from Zhejiang province and Shanghai municipality, eastern China.[27] However, ST1 was also reported as the dominant *Blastocystis* subtype in China.[44] This variation may be attributable to *Blastocystis* isolates from different populations and various geographical locations.

Previous studies have demonstrated the correlations between *Blastocystis* subtypes and clinical manifestations.[45,46] *Blastocystis* subtype ST1, ST2, ST4 and ST6 are reported to correlate with gastrointestinal symptoms,[47,48] and ST2 subtype infection was associated with gastrointestinal and urticarial symptoms,[49] while ST1,

ST3 and ST7 were linked with irritable bowel syndrome.[50,51] In this study, we found that ST1 subtype infection was associated with anemia ($P = 0.008$), while no associations were seen between *Blastocystis* subtypes ST3 or ST4 and any clinical symptoms. Our data demonstrate the pathogenic role of *Blastocystis* subtype ST1 in anemia. Further mechanistic studies are required to investigate the distinct pathogenicity of *Blastocystis* subtypes and the associations between *Blastocystis* subtypes and anemia.

This study has some limitations. First, this is a cross-sectional study, and we are unable to draw causal conclusions. Second, only a fecal specimen was collected from each participant. Third, the number of positive samples of ST1, ST2, ST3 and ST4 was small, so that data sparsity resulted in unstable estimates for the association between *Blastocystis* subtypes and clinical symptoms.

In summary, the results of the present study demonstrate a high prevalence of *Blastocystis* sp. infections among general populations in Yunnan province, southwestern China, and toilet type, anemia and type of daily drinking water are risk factors of *Blastocystis* sp. infections. In addition, there were *Blastocystis* subtypes (ST1, ST2, ST3 and ST4) characterized in the study populations, and ST3, as the dominant subtype, correlates with anemia. Improving hygiene conditions, developing healthy lifestyles and intensifying health education programs are strongly recommended to reduce the prevalence and transmission potential of *Blastocystis* sp. infections.

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Disclosure

The authors declare no competing interests for this work.

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