

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

This article was published in the following Dove Press journal:
Risk Management and Healthcare Policy

Yao Deng,^{1,*} Shunxian Zhang,^{2,*}
Chaoqun Ning,² Yongkang Zhou,³
Xuejiao Teng,² Xiuping Wu,²
Yanhong Chu,² Yingfang Yu,²
Jiaxu Chen,² Liguang Tian,²
Wei Wang¹ 

¹Key Laboratory of National Health Commission on Parasitic Disease Control and Prevention, Jiangsu Provincial Key Laboratory on Parasite and Vector Control Technology, Jiangsu Institute of Parasitic Diseases, Wuxi 214064, Jiangsu Province, People's Republic of China; ²National Institute of Parasitic Diseases, Chinese Center for Disease Control and Prevention, Key Laboratory of National Health Commission on Parasites and Vector Biology, WHO Collaborating Center for Tropical Diseases, Chinese Center for Tropical Diseases Research, Shanghai 200025, People's Republic of China; ³The First Clinical Medical College of Lanzhou University, Lanzhou 730000, Gansu Province, People's Republic of China

*These authors contributed equally to this work

Correspondence: Liguang Tian
National Institute of Parasitic Diseases,
Chinese Center for Disease Control and
Prevention, Key Laboratory of National
Health Commission on Parasites and
Vector Biology, WHO Collaborating
Center for Tropical Diseases, Chinese
Center for Tropical Diseases Research,
Shanghai City 200025, People's Republic
of China
Email jztlg@126.com

Wei Wang
Key Laboratory of National Health
Commission on Parasitic Disease Control
and Prevention, Jiangsu Provincial Key
Laboratory on Parasite and Vector
Control Technology, Jiangsu Institute of
Parasitic Diseases, Wuxi City, Jiangsu
Province 214064, People's Republic of
China
Email wangwei@jipd.com

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 habits 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 μ L of the reaction system containing 12.5 μ L of 2 $\times$ TaKaRa Taq™ DNA mixture (TaKaRa Bio Inc, Shiga, Japan), 2 μ L of DNA template, 1 μ L of each primer set (forward: 5'-GGAGGTAGTGACAATAAATC-3', reverse: 5'-ACTAGGAATTCCTCGTTCATG-3', each at 10 μ M), and 8.5 μ L of ddH₂O 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 *Blastocystis* sp. 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)	46 (10.4)	398 (89.6)	3.324	0.068	0.284	0.067, 1.199
	Yes (n = 63)	2 (3.2)	61 (96.8)				
Loss of appetite	No (n = 416)	41 (9.9)	375 (90.1)	0.408	0.523	0.762	0.33, 1.758
	Yes (n = 91)	7 (7.7)	84 (92.3)				
Itchy skin	No (n = 445)	43 (9.7)	402 (90.3)	0.162	0.687	0.82	0.312, 2.156
	Yes (n = 62)	5 (8.1)	57 (91.9)				
Perianal pruritus	No (n = 479)	48 (10.0)	431 (90.0)	—*	0.079	—*	—*
	Yes (n = 28)	0 (0.0)	28 (100.0)				
Constipation	No (n = 452)	41 (9.1)	411 (90.9)	0.765	0.382	1.462	0.621, 3.419
	Yes (n = 55)	7 (12.7)	48 (87.3)				

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

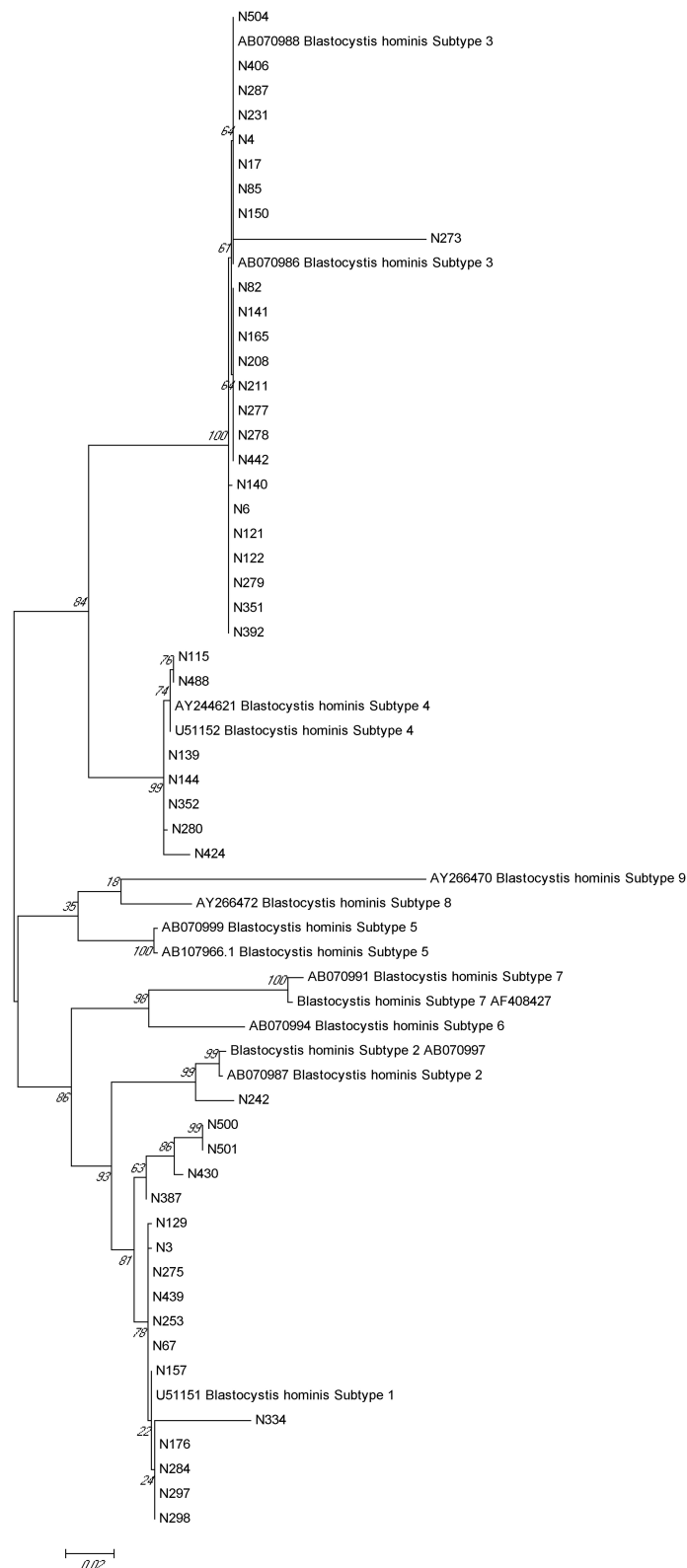


Figure 1 Evolutionary relationships among *Blastocystis* subtypes shown by the maximum likelihood method. The percentage of replicate trees in which the associated *Blastocystis* subtypes are clustered together in the bootstrap test (1,000 iterations) is indicated next to the branches.

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 populations;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 China⁴² 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.

Acknowledgments

We would like to express our sincere thanks to Tengchong Municipal People's Hospital, Tengchong Hospital of Traditional Chinese Medicine and Tengchong Municipal Center for Disease Control and Prevention during the stool specimen collection and epidemiological investigations. This study was supported by the grants from the National Natural Science Foundation of China (grant no. 81473022) and Jiangsu Provincial Young Talents in Medical Sciences (grant no. QNRC2016621).

Disclosure

The authors declare no competing interests for this work.

References

- Plutzer J, Karanis P. Neglected waterborne parasitic protozoa and their detection in water. *Water Res*. 2016;101:318–332. doi:10.1016/j.watres.2016.05.085
- Skotarczak B. Genetic diversity and pathogenicity of *Blastocystis*. *Ann Agric Environ Med*. 2018;25(3):411–416. doi:10.26444/aaem/81315
- Clark CG, van der Giezen M, Alfellani MA, Stensvold CR. Recent developments in *Blastocystis* research. *Adv Parasitol*. 2013;82:1–32.
- Stensvold CR, Clark CG. Current status of *Blastocystis*: a personal view. *Parasitol Int*. 2016;65(6):763–771. doi:10.1016/j.parint.2016.05.015
- Wawrzyniak I, Poirier P, Viscogliosi E, et al. *Blastocystis*, an unrecognized parasite: an overview of pathogenesis and diagnosis. *Ther Adv Infect Dis*. 2013;1(5):167–178.
- Ocaña-Losada C, Cuenca-Gómez JA, Cabezas-Fernández MT, et al. Clinical and epidemiological characteristics of intestinal parasite infection by *Blastocystis hominis*. *Rev Clin Esp*. 2018;218(3):115–120. doi:10.1016/j.rce.2018.01.003
- Kumarasamy V, Anbazhagan D, Subramanian V, Vellamy S. *Blastocystis* sp., parasite associated with gastrointestinal disorders: an overview of its pathogenesis, immune modulation and therapeutic strategies. *Curr Pharm Des*. 2018;24(27):3172–3175. doi:10.2174/1381612824666180807101536
- Kesuma Y, Firmansyah A, Bardosono S, Sari IP, Kurniawan A. *Blastocystis* ST-1 is associated with irritable bowel syndrome-diarrhoea (IBS-D) in Indonesian adolescences. *Parasite Epidemiol Control*. 2019;6:e00112. doi:10.1016/j.parepi.2019.e00112
- Yakoob J, Jafri W, Beg MA, et al. Irritable bowel syndrome: is it associated with genotypes of *Blastocystis hominis*. *Parasitol Res*. 2010;106(5):1033–1038. doi:10.1007/s00436-010-1761-x
- Kök M, Çekin Y, Çekin AH, Uyar S, Harmandar F, Şahintürk Y. The role of *Blastocystis hominis* in the activation of ulcerative colitis. *Türk J Gastroenterol*. 2019;30(1):40–46.
- Verma R, Delfanian K. *Blastocystis hominis* associated acute urticaria. *Am J Med Sci*. 2013;346(1):80–81. doi:10.1097/MAJ.0b013e3182801478
- Hameed DM, Hassanin OM, Zuel-Fakkar NM. Association of *Blastocystis hominis* genetic subtypes with urticaria. *Parasitol Res*. 2011;108(3):553–560. doi:10.1007/s00436-010-2097-2
- Zuel-Fakkar NM, Abdel Hameed DM, Hassanin OM. Study of *Blastocystis hominis* isolates in urticaria: a case-control study. *Clin Exp Dermatol*. 2011;36(8):908–910. doi:10.1111/j.1365-2230.2011.04127.x
- Bahrami F, Babaei E, Badirzadeh A, Riabi TR, Abdoli A. *Blastocystis*, urticaria, and skin disorders: review of the current evidences. *Eur J Clin Microbiol Infect Dis*. 2020;39(6):1027–1042. doi:10.1007/s10096-019-03793-8
- Singh A, Priyadarshi K, Rai T, Banerjee T. A case report of *Blastocystis* infection and Steven Johnson's syndrome. *Trop Biomed*. 2019;36(4):987–992.
- Berkman DS, Lescano AG, Gilman RH, Lopez SL, Black MM. Effects of stunting, diarrhoeal disease, and parasitic infection during infancy on cognition in late childhood: a follow-up study. *Lancet*. 2002;359(9306):564–571. doi:10.1016/S0140-6736(02)07744-9
- Olaya J, Sarmiento D, Jaramillo JF, Ordoñez GA, Cortés F, Sánchez LK. Intestinal parasitic infections and associated factors in children of three rural schools in Colombia. A cross-sectional study. *PLoS One*. 2019;14(7):e0218681. doi:10.1371/journal.pone.0218681
- Andersen LO, Stensvold CR, Kraft CS. *Blastocystis* in health and disease: are we moving from a clinical to a public health perspective? *J Clin Microbiol*. 2016;54(3):524–528. doi:10.1128/JCM.02520-15
- Dogruman-Al F, Dagci H, Yoshikawa H, Kurt O, Demirel M. A possible link between subtype 2 and asymptomatic infections of *Blastocystis hominis*. *Parasitol Res*. 2008;103(3):685–689.
- Hussein EM, Hussein AM, Eida MM, Atwa MM. Pathophysiological variability of different genotypes of human *Blastocystis hominis* Egyptian isolates in experimentally infected rats. *Parasitol Res*. 2008;102(5):853–860. doi:10.1007/s00436-007-0833-z

21. Deng L, Chai Y, Zhou Z, et al. Epidemiology of *Blastocystis* sp. infection in China: a systematic review. *Parasite*. 2019;26:41. doi:10.1051/parasite/2019042
22. Gong B, Liu X, Wu Y, et al. Prevalence and subtype distribution of *Blastocystis* in ethnic minority groups on both sides of the China-Myanmar border, and assessment of risk factors. *Parasite*. 2019;26:46. doi:10.1051/parasite/2019046
23. Chen TL, Chan CC, Chen HP, et al. Clinical characteristics and endoscopic findings associated with *Blastocystis hominis* in healthy adults. *Am J Trop Med Hyg*. 2003;69(2):213–216. doi:10.4269/ajtmh.2003.69.213
24. Zhang SX, Yu YF, Wu XP, et al. Epidemiological characteristics and risk factors of *Blastocystis hominis* infection among patients with HIV/AIDS in Fuyang City Anhui Province. *Zhongguo Xue Xi Chong Bing Fang Zhi Za Zhi*. 2019;31(5):498–503.
25. Kang JM, Li YT, Chen R, et al. Prevalence and risk factors of *Blastocystis hominis* infection in inpatients in Jiangjin District, Chongqing City. *Zhongguo Xue Xi Chong Bing Fang Zhi Za Zhi*. 2019;31(5):479–485.
26. Li LH, Zhou XN, Du ZW, et al. Molecular epidemiology of human *Blastocystis* in a village in Yunnan province, China. *Parasitol Int*. 2007;56(4):281–286. doi:10.1016/j.parint.2007.06.001
27. Li LH, Zhang XP, Lv S, et al. Cross-sectional surveys and subtype classification of human *Blastocystis* isolates from four epidemiological settings in China. *Parasitol Res*. 2007;102(1):83–90. doi:10.1007/s00436-007-0727-0
28. Zhang SX, Yang CL, Gu WP, et al. Case-control study of diarrheal disease etiology in individuals over 5 years in southwest China. *Gut Pathog*. 2016;8:58. doi:10.1186/s13099-016-0141-1
29. Wong KH, Ng GC, Lin RT, Yoshikawa H, Taylor MB, Tan KS. Predominance of subtype 3 among *Blastocystis* isolates from a major hospital in Singapore. *Parasitol Res*. 2008;102(4):663–670. doi:10.1007/s00436-007-0808-0
30. Hattori M, Sakaki Y. Dideoxy sequencing method using denatured plasmid templates. *Anal Biochem*. 1986;152(2):232–238. doi:10.1016/0003-2697(86)90403-3
31. Javanmard E, Niyyati M, Ghasemi E, Mirjalali H, Asadzadeh Aghdaei H, Zali MR. Impacts of human development index and climate conditions on prevalence of *Blastocystis*: a systematic review and meta-analysis. *Acta Trop*. 2018;185:193–203. doi:10.1016/j.actatropica.2018.05.014
32. Marcos LA, Gotuzzo E. Intestinal protozoan infections in the immunocompromised host. *Curr Opin Infect Dis*. 2013;26(4):295–301.
33. Stensvold CR, Suresh GK, Tan KS, et al. Terminology for *Blastocystis* subtypes—a consensus. *Trends Parasitol*. 2007;23(3):93–96. doi:10.1016/j.pt.2007.01.004
34. Zhang SX, Kang FY, Chen JX, Tian LG, Geng LL. Risk factors for *Blastocystis* infection in HIV/AIDS patients with highly active anti-retroviral therapy in Southwest China. *Infect Dis Poverty*. 2019;8(1):89. doi:10.1186/s40249-019-0596-7
35. Wang KX, Li CP, Wang J, Cui YB. Epidemiological survey of *Blastocystis hominis* in Huainan City, Anhui Province, China. *World J Gastroenterol*. 2002;8(5):928–932. doi:10.3748/wjg.v8.i5.928
36. Tang N, Luo NJ. A cross-sectional study of intestinal parasitic infections in a rural district of west China. *Can J Infect Dis*. 2003;14(3):159–162. doi:10.1155/2003/721930
37. Zhang SX, Zhou YM, Xu W, et al. Impact of co-infections with enteric pathogens on children suffering from acute diarrhea in southwest China. *Infect Dis Poverty*. 2016;5(1):64. doi:10.1186/s40249-016-0157-2
38. Sekar U, Shanthi M. *Blastocystis*: consensus of treatment and controversies. *Trop Parasitol*. 2013;3(1):35–39. doi:10.4103/2229-5070.113901
39. Feng L, Yu J, Huang GF. Toilet retrofitting in rural areas of Yunnan province: a cross-sectional survey. *Chin J Public Health*. 2019;35(5):615–617.
40. El Deeb HK, Salah-Eldin H, Khodeer S. *Blastocystis hominis* as a contributing risk factor for development of iron deficiency anemia in pregnant women. *Parasitol Res*. 2012;110(6):2167–2174. doi:10.1007/s00436-011-2743-3
41. El Safadi D, Cian A, Nourrisson C, et al. Prevalence, risk factors for infection and subtype distribution of the intestinal parasite *Blastocystis* sp. from a large-scale multi-center study in France. *BMC Infect Dis*. 2016;16(1):451. doi:10.1186/s12879-016-1776-8
42. Qi M, Wei Z, Zhang Y, et al. Genetic diversity of *Blastocystis* in kindergarten children in southern Xinjiang, China. *Parasit Vectors*. 2020;13(1):15. doi:10.1186/s13071-020-3890-0
43. Zhan TZ, Liu T, Shi HH, He SS, Yan H, Liu DY. PCR-based genotype classification of *Blastocystis hominis* isolates from college students of Guangxi. *Zhongguo Ji Sheng Chong Xue Yu Ji Sheng Chong Bing Za Zhi*. 2014;32(3):209–211.
44. Yan Y, Su S, Lai R, et al. Genetic variability of *Blastocystis hominis* isolates in China. *Parasitol Res*. 2006;99(5):597–601. doi:10.1007/s00436-006-0186-z
45. Tan KS, Mirza H, Teo JD, Wu B, Macary PA. Current views on the clinical relevance of *Blastocystis* spp. *Curr Infect Dis Rep*. 2010;12(1):28–35. doi:10.1007/s11908-009-0073-8
46. Salvador F, Sulleiro E, Sánchez-Montalvá A, et al. Epidemiological and clinical profile of adult patients with *Blastocystis* sp. infection in Barcelona, Spain. *Parasit Vectors*. 2016;9(1):548. doi:10.1186/s13071-016-1827-4
47. Cakir F, Cicek M, Yildirim IH. Determination the subtypes of *Blastocystis* sp. and evaluate the effect of these subtypes on pathogenicity. *Acta Parasitol*. 2019;64(1):7–12.
48. Kaneda Y, Horiki N, Cheng X, Tachibana H, Tsutsumi Y. Serologic response to *Blastocystis hominis* infection in asymptomatic individuals. *Tokai J Exp Clin Med*. 2000;25(2):51–56.
49. Vogelberg C, Stensvold CR, Monecke S, et al. *Blastocystis* sp. subtype 2 detection during recurrence of gastrointestinal and urticarial symptoms. *Parasitol Int*. 2010;59:469–471. doi:10.1016/j.parint.2010.03.009
50. Poirier P, Wawrzyniak I, Vivarès CP, Delbac F, El Alaoui H, Knoll LJ. New insights into *Blastocystis* spp.: a potential link with irritable bowel syndrome. *PLoS Pathog*. 2012;8(3):e1002545. doi:10.1371/journal.ppat.1002545
51. Jimenez-Gonzalez DE, Martinez-Flores WA, Reyes-Gordillo J, et al. *Blastocystis* infection is associated with irritable bowel syndrome in a Mexican patient population. *Parasitol Res*. 2012;110(3):1269–1275. doi:10.1007/s00436-011-2626-7

Risk Management and Healthcare Policy

Dovepress

Publish your work in this journal

Risk Management and Healthcare Policy is an international, peer-reviewed, open access journal focusing on all aspects of public health, policy, and preventative measures to promote good health and improve morbidity and mortality in the population. The journal welcomes submitted papers covering original research, basic science, clinical & epidemiological studies, reviews and evaluations,

guidelines, expert opinion and commentary, case reports and extended reports. The manuscript management system is completely online and includes a very quick and fair peer-review system, which is all easy to use. Visit <http://www.dovepress.com/testimonials.php> to read real quotes from published authors.

Submit your manuscript here: <https://www.dovepress.com/risk-management-and-healthcare-policy-journal>