

Literature Analysis of Blastocystis sp. from 1990 to 2019 in China

Chao-qun Ning

Chinese Center for Disease Control and Prevention

Lin Ai

Chinese Center for Disease Control and Prevention

Li-Guang Tian (✉ jztlg@126.com)

Centers for Disease Control and Prevention <https://orcid.org/0000-0003-0817-6943>

Research Article

Keywords: Blastocystis sp.; literature analysis; China

Posted Date: May 13th, 2020

DOI: <https://doi.org/10.21203/rs.3.rs-26013/v1>

License: © ⓘ This work is licensed under a Creative Commons Attribution 4.0 International License.

[Read Full License](#)

Version of Record: A version of this preprint was published on December 30th, 2020. See the published version at <https://doi.org/10.1186/s40249-020-00779-z>.

Abstract

Background

Blastocystis sp. is ubiquitous presence in animals and humans worldwide and a broad diversity genetically. The aim of this study was to find out the main research progress and research status of *Blastocystis* sp. in China in the past thirty years.

Methods

On the Chinese mainstream database China National Knowledge Infrastructure (CNKI), Wanfang database and PubMed database, the research literatures of Chinese *Blastocystis* sp. were searched and analyzed.

Results

285 articles related to *Blastocystis* sp. were retrieved, including 244 published in Chinese and 41 English on pathogen morphology, epidemiological investigation, laboratory detection and diagnosis, clinical case reports, drug treatment, animal model establishment as well as pathogenicity studies. There were the most epidemiological investigations and researches on pathogenic morphology decreasing recently. Surveys of *Blastocystis* sp. of human has been carried out among a total of 18 provinces, with infection rates ranging from 0.007–48.6%. Infants and young children, school students, hospitalized diarrhea patients, human immunodeficiency virus (HIV) patients, tuberculosis patients, and cancer patients as respondents had been included. ST1-ST7 and ST12 were the main subtypes in Chinese population. Moreover, surveys of *Blastocystis* sp. of animal were also conducted in 12 provinces, with infection rates ranging from 2.54–79.41%. A variety of animals were investigated including pigs, cattle, sheep, yak, giant panda, and *Crested Ibis* et al., with the main subtypes of ST1-ST8, ST10, ST12-ST14. In vitro culture and Polymerase chain reaction (PCR) were commonly used to detect *Blastocystis* sp. in research and less in clinical application. Traditional Chinese medicine and western medicine for clinical drug treatment appeared curative effects. However, there were few reports on the application of traditional Chinese medicine in clinical treatment. The pathogenicity of *Blastocystis* sp. remains controversial and needs further exploration.

Conclusion

various researches on *Blastocystis* sp. had been carried out in China, especially in epidemiology. However, there is no report on the relationship between human *Blastocystis* sp. and animal infection. Furthermore, the relationship between infant diarrhea and its impact on the intestinal microecology have not received

much attention from clinicians. The research on *Blastocystis* sp. still needs to be strengthened to provide effective data for the prevention and control and improve the quality of life of the population.

1 Background

Blastocystis sp. is widely distributed throughout the world. It is an anaerobic intestinal parasite that can infect humans and various animals [1, 2]. It was named by Brumpt in 1912 and had been mistaken for yeast that is harmless to the human body [3]. It had been classified into protozoan, according to its physiological and ultrastructural characteristics in 1967 [4]. *Blastocystis* sp. was proved as pathogenic intestinal parasite until 1988 [5], and gradually attracted the attention of researchers from various countries. The first case of *Blastocystis* sp. infection in China was recorded in 1990 [6]. Since then, Chinese researchers had conducted extensive research in this field, as well as published a large number of research papers. However, most of them were in Chinese which published in domestic journals, foreign counterparts were unable to grasp and understand the published literature materials in Chinese as well as related data and information.

There were three times national human parasite investigations carried out in China since 1988, with twice of which *Blastocystis* sp. investigated as well as distributed among 18 provinces. The investigated population contained infants and young children, students, outpatients with diarrhea, HIV infection, tuberculosis, and cancer patients. Additionally, the investigation of animal infected with *Blastocystis* sp. had been launched in 12 provinces, including pig, cattle, sheep, yak, giant pandas, and crested ibis, etc. Although a large amount of epidemiological data has been accumulated, the research results and research progress of *Blastocystis* sp. in China were rarely understood due to language barriers by foreigners. Therefore, published relevant research reports on *Blastocystis* sp. were searched in two main databases in China, CNKI and Wanfang Database to collect data from 1990. And then, the research results and progress of *Blastocystis* sp. in China over the past three decades were summarized and analyzed, and provided data for domestic and foreign colleagues to understand the research progress of *Blastocystis* sp. in China and carry out further research cooperation and exchanges.

2 Methods

"*Blastocystis* sp.", used as a search term, was searched in two widely used databases CNKI and Wanfang Database in China. The deadline of the search is July 2019. Chinese documents were selected from the search results, and the literatures were obtained from the database. There were 201 and 360 literatures obtained from CNKI as well as Wanfang database, respectively. 136 duplicate articles were deleted and 239 articles were obtained. The earliest literature was published in 1990. Use "*Blastocystis*" + "China" as the search term on PubMed. The deadline was July 2019, there were 46 articles obtained. Finally 285 articles were retrieved from CNKI, Wanfang Database and PubMed. Data were processed using Excel (Microsoft, WA, USA) filters.

3 Results

3.1 Analysis of the age, sources, publications and main contents of *Blastocystis* sp.

A total of 285 articles were retrieved, including 244 in Chinese and 41 in English. The first literature of domestic *Blastocystis* sp. was recorded in 1990, He and others (1990) observed the structure of *Blastocystis* sp. in vitro, published their results in the Journal of Sun Yat-Sen University [6]. Detail information of this parasite literature published from 1990 to 2019 has been shown in Fig. 1. The most published articles were 19 Chinese literatures published in 2012, followed by 18 in 2006, and unpublished in 1996. In 1993, Jiang et al. (1993) published the earliest English literature describing the classification of *Blastocystis* sp. on *Parasitology today* [7]. Moreover, English literatures have been published up to 7 articles in 2019. From 1990 to 2019, there were 71 institutions published Chinese literatures, including 47 (66.20%) institutions published only one article, 11 (15.49%) published two articles, and 13 institutions published three or more articles, 9 published five or more Chinese literatures (Table 1). 19 institutions published English literature, of which 12 published only one article, 2 published two articles, and 5 published more than 3 articles (Table 2). Among the 244 Chinese literatures, 26 were published as master's or doctoral dissertations, 8 were published in conference papers, 210 were published in 74 journals, of which 42 kinds of journals published 1 literature and 18 journals published 2 literatures, 14 magazines published 3 or more literatures. Table 3 shows magazine that published 3 or more literatures. The largest number of literatures on *Blastocystis* sp. has been published in *Chinese Journal of Parasitology and Parasitic Diseases* with 31 articles. Followed by 25 articles have been published *the Chinese Journal of Pathogen Biology (Chinese Journal of Parasitic Disease Control)*. The third was that the *Chinese Journal of Zoonoses* published 12 articles. There were 41 English articles published in 22 English journals, with most (10 articles) published in *Parasitology research*, 4 articles published in *Parasites & vectors* and *Acta tropica*, respectively, as well as 3 articles published in *The American journal of tropical medicine and hygiene*.

The main research fields of 285 articles include: 1. pathogen morphology, 2. epidemiology, 3. laboratory detection and diagnosis, 4. clinical case report and drug treatment, 5. animal model establishment and pathogenicity research, 6. research review and others. Figure 2 shows the proportion of literature with different research directions. The epidemiological survey has a maximum of 116 literatures (116/285, 41%), followed by reviews and other literatures, with 53 (30 reviews) (53/285, 18%). The laboratory research and diagnostic methods occupied the least research literature, all of which were 23 articles (23/285, 8%).

3.2 Classification and pathogenic morphology of *Blastocystis* sp.

3.2.1 Classification

In 1993, Jiang et al. proposed a new categorization standard for *Blastocystis* sp., and believed that *Blastocystis* sp. should be classified as protozoa, proposing a new subphylum Blastocysta, including class Blastocystea, order Blastocystida, family Blastocystidae and genus *Blastocystis*, this classification method has been widely recognized by domestic and foreign counterparts [7].

3.2.2 Morphology of *Blastocystis* sp.

There were 23 research articles on pathogenic morphology, 21 in Chinese and 2 in English. In 1990, He and Jiang et al. [6] published "Light Microscopy and Ultrastructure of *Blastocystis Hominis*" in *Journal of Sun Yat-Sen University*, which was the first record of *Blastocystis* sp. in China and the first observation of the structure of the *Blastocystis* sp. in vitro culture in China, found the original vacuolar, granular, and amoeboid forms and multiple fission form. Moreover, the granular form can reproduce by binary fission. The multiple fission form was also observed under a scanning electron microscope in 1992 [8]. Multiple fission form was also observed in 2001 [9].

In 1998, Qiao et al. [10] observed the morphology of *Blastocystis* sp. from patients with diarrhea and found that there were vacuolar, granular and amoeboid forms, of which the vacuolar form accounts for up to 84% and the granular form accounts for 12.8%, amoeboid form only accounts for 3.1%. In 2006, Qiao et al. [11] observed that the *Blastocystis* sp. have binary fission, endodyogeny, multiple fission and budding.

In 2000, cysts and their photogrammetry were reported in China firstly, which were relatively simple in form, both round or oval [12]. Cyst form was spherical, with two parts, an inner sphere and an outer cyst wall-like structure [13]. Cyst form contained more RNA-like substances under electron microscope [9]. Furthermore, Su et al. [14] observed the morphological characteristics of cysts in different environments and found that the cysts morphology was basically consistent under the light microscopy and the ultrastructure in different culture media, with different sizes and polymorphisms.

In 2007, cyst form in Roswell Park Memorial Institute (RPMI) 1640 medium firstly was observed [15]. In 2012, Xu Zhang et al. [16] observed multivacuolar, vacuolar, and amoeboid form in diarrhea, and found that the vacuolar form can be transformed into a granular form in the RPMI 1640 medium. Trophozoites were observed in different environments and found that the average diameter of vacuolar and granular trophozoites was larger in those with symptoms than in those without symptoms [17]. Moreover, asymptomatic trophozoites own little morphological changes after being cultured. However, after inoculation in the abdominal cavity of mice, the *Blastocystis* sp. enlarged, which was similar to that of symptomatic ones. The trophozoites were varying in shape and size depending on their living environment.

In China, research on the morphology of *Blastocystis* sp. has gradually decreased in recent years. 10 articles were published from 1990 to 1999, 10 articles were published from 2000 to 2009, and only 3 articles were published from 2010 to 2019.

3.3 Epidemiological investigations

There were 116 epidemiological investigations (88 in Chinese and 28 in English), which accounted for 41% of the research on *Blastocystis* sp. in China, including 98 population surveys and 18 animals. China has carried out three national human parasite investigations. In the first national human parasite survey from 1988 to 1992, it found that the nationally weighted average infection rate of *Blastocystis* sp. was 1.473(\pm 0.075)%, with the highest infection rate in Sichuan 8.01% [18]. However, there was no data on *Blastocystis* sp. in the second national survey of important human parasites in 2001–2004. In the third national survey of key human parasites in 2014–2016, the highest weighted average infection rate was 5.69% in Guizhou, followed by Guangxi 4.52% [19].

3.3.1 Infection of *Blastocystis* sp. in humans

According to the current investigation of *Blastocystis* sp., the population infection rate was 0.007–48.6%. In 2000, surveyed the outpatient diarrhea patients in Guangzhou hospitals with the highest infection rate was 48.6% reported by Zhang et al. [20], and the lowest infection rate was 0.007% in the Xinjiang Uygur Autonomous Region, 2015 [21]. The general population, students, children, hospitalized and outpatients, patients with diarrhea, and people with other diseases were contained in the population epidemiological survey.

There were 44 epidemiological surveys of *Blastocystis* sp. infection in the general population, with 36 in Chinese, and 8 in English, of which 6 were genotyped. The percentage of *Blastocystis* sp. infection in the population in different regions of China was ranged from 0.007–43.26%. The infection status and subtype distribution of *Blastocystis* sp. in parts of China has been listed in Table 4. The highest infection rate of human *Blastocystis* sp. was 43.26% in Bama Yao Autonomous County [22]. The lowest infection rate was 0.007% [21]. With the development of molecular detection methods, PCR method was used to identify the subtype of *Blastocystis* sp. in China. Yan and others identified that the subtypes of *Blastocystis* sp. infected by humans was ST1–ST3. ST3 was the main subtype (40.0%), followed by ST1 (37.1%), mixed subtypes of ST1 and ST3 account for 14.3%. This study also detected unknown subtype [23].

The risk factors for *Blastocystis* sp. infection were diverse. A survey of children aged 7–12 years in the border areas of China and Myanmar found that not washing their hands after using the toilet may be a risk factor for *Blastocystis* sp. infection [24]. *Helicobacter pylori* infection and hepatitis B were also risk factors for *Blastocystis* sp. infection [25]. Drinking unboiled tap water, raising livestock and low immune function were the risk factors of *Blastocystis* sp. [26]. Female and body mass index < 19 were risk factors for intestinal parasite infection in patients with tuberculosis. Feeding poultry or livestock was important risk factor for *Blastocystis* sp. infection. Anemia and laboring barefoot in farmland were risk factors for intestinal helminth infection in patients with tuberculosis [27, 28].

There were 8 investigations of *Blastocystis* sp. infections among students, all of which published in Chinese, with prevalence ranging from 1.1–16.61%, 3 of which were tested for subtype. The highest prevalence of students was 16.61% in 2010 grade freshmen admitted to Guilin Medical University [29]. In

1999, the infection rate of a primary school pupil in Nantang Town, Gan County was 1.1% [30], but the infection rate of *Blastocystis* sp. in Grade 2002 of Gannan Medical College reached 10.09% [31]. The prevalence of *Blastocystis* sp. was 14.93% among 2008–2010 grades students in Guangxi Medical University [32]. The comparison between these studies shows that it may be that under the management of parents and teachers, primary school students have developed good hygiene habits such as diligent hand washing. However, there are still regional differences, the prevalence of *Blastocystis* sp. was 1.41% (6/426) in college students in Dali, Yunnan Province, and the infection subtype was ST1 [33]. A study in Guangxi found that infection subtypes include ST1, ST3, ST4, ST6 and ST7, of which ST3 is the main genotype (32.08%) [34].

There were 8 investigations about infection in children of *Blastocystis* sp., including 7 published in Chinese and 1 in English. However, there was no DNA sequence analysis performed. According to these studies, the prevalence was 0.4–35.9%. The highest prevalence of children with diarrhea was 35.9% in Jiangxi Provincial Children's Hospital [35]. Moreover, the prevalence of children with repeated abdominal pain was 35.4% in the First Affiliated Hospital of Gannan Medical University [36]. The prevalence of children with diarrhea was 3.1% and 8.9% in four sentinel hospitals in Kunming, Yunnan Province and the First Affiliated Hospital of Fujian Medical University, respectively [37, 38]. Cao et al performed fecal microscopy on children treated in the outpatient and inpatient departments of Shenzhen Children's Hospital, and found that the infection rate of *Blastocystis* sp. was 0.4% [39]. Children with diarrhea have a higher infection rate, but the infection rate in Jiangxi is the highest.

There were 11 investigations of *Blastocystis* sp. infection in inpatients or outpatients, all of which were published in Chinese with 1 sequence analysis. The prevalence was 3.7–36.6%. The highest prevalence in hospitalized patients was 36.6% [40]. The infection rate of patients in the First Affiliated Hospital of Guangxi Medical University is 22.78%, and the infection rate has a significant difference in gender [41]. The prevalence of some patients who attended the five hospitals were 16.27% and 16.77%, respectively in 2005 and 2013, in Nanning, Guangxi [42]. The infection rate of *Blastocystis* sp. in hospitals in Guangxi region was relatively high, and the infection rate has not changed much over time. It may be that the local population can see the doctor in time after experiencing symptoms such as diarrhea, resulting in a higher rate of infection of *Blastocystis* sp. in local hospitals. The subtypes of patients with *Blastocystis* sp. infection in two hospitals in Guangxi are ST1–ST4, ST6 and ST7, of which ST2 is the main genotype (35.09%) [43]. The prevalence of hospitalized patients was 9.47% in Tengchong City, Yunnan Province [44]. From 2014 to 2017, among the samples sent to the National Institute of Parasitic Diseases for testing, the infection rate of *Blastocystis* sp. was 3.7% [45].

13 (12 published in Chinese and 1 in English) surveys of *Blastocystis* sp. in patients with diarrhea, one of which did sequence analysis, and the prevalence was 0.42–48.6%. The infection rate of patients with diarrhea in outpatient clinics of hospitals in Guangzhou was 48.6% (84/138), which was the highest infection rate of diarrhea patients. Those diagnosed with *Blastocystis* sp. infection mainly complained of abdominal pain or diarrhea, 19% of them were associated with colitis, and some showed symptoms of fatigue and anorexia [20]. The lowest prevalence of *Blastocystis* sp. of patients with diarrhea was 0.42%

in Fuzhou area. *Blastocystis* sp. was one of the main causes of diarrhea in local children. The infection rate of *Blastocystis* sp. in patients with diarrhea is significantly different in seasons, the highest in summer [46]. *Blastocystis* sp. was one of the common pathogens in patients with diarrhea. The prevalence was 4.8% in patients with diarrhea over 5 years old in Kunming, Yunnan, and it was highest in summer [47]. But Zhang et al. found that the infection rate of *Blastocystis* sp. in spring was the highest, followed by summer. In the four sentinel hospitals in Kunming, Yunnan Province the prevalence of diarrhea patients was 4.2%, and the subtypes were ST1 and ST2, of which ST1 occupied the predominate subtype (97.9%) [48]. The infection rate of patients with diarrhea was 18.54% in Guangxi. Most of the patients with *Blastocystis* sp. have a history of drinking raw water or eating outside for a long time, and some of them have or are raising animals. It was found that the mixed infection of *Blastocystis* sp. and other parasites accounted for 31.87%, of which 73.8% were co-infected with liver flukes. And patients with mixed liver fluke infection have eaten sashimi [49].

There were 14 investigations of *Blastocystis* sp. combined with other diseases, 9 published in Chinese and 5 in English. The prevalence was 3.7–20.7%, of which 3 did DNA sequence analysis. These investigations included HIV patients, tuberculosis patients, cancer patients and chronic disease patients co-infection with *Blastocystis* sp.. There were 8, 2, 2 and 2 surveys respectively. The average infection rate was 12.30% [26, 50–56], 6.1% [27, 28], 13.1% [57, 58], 17.8% [59, 60], respectively. Among the co-infections, patients with chronic diseases had the highest rate of *Blastocystis* sp. infection, followed by cancer patients, and tuberculosis patients had the lowest. It has been found that the highest prevalence of *Blastocystis* sp. among HIV patients was 20.7% in Guangxi Province in 2015 [50]. The lowest prevalence was 3.7% among HIV seropositive individuals in Tengchong, Yunnan. The ST1, ST3, ST4, ST7, and ST12 subtypes were found, among which ST12 infection was first discovered in China. CD4 + cell count \leq 500 cells/ μ l, and an HIV-RNA viral load \geq 50 copies/ml were the influencing factors for *Blastocystis* sp. infection among HIV-seropositive individuals [26]. Tian et al suggested that HIV and *Blastocystis* sp. co-infection made IL-2 levels higher than those without HIV infection, altered the Th1/Th2 balance, and accelerated the transformation of HIV infection to AIDS [51]. The prevalence of HIV and *Blastocystis* sp. co-infection was 16.23% (49/302) in rural areas of China [52]. The prevalence of *Blastocystis* sp. among HIV positives was 19.57% in the suburbs of Fuyang City, Anhui Province [54]. The infection rate of *Blastocystis* sp. was 6.2% among tuberculosis patients without HIV infection. However, the infection rate was 7.6% in healthy group, there was no difference between the two groups [28]. The infection rate of *Blastocystis* sp. was 7.1% (27/381) in cancer patients. The subtypes ST1 and ST3 in cancer patients appeared the symptom of diarrhea, of which 66.7% (8/12) was ST1 and 40% (6/15) was ST3. Zhang and others detected *Blastocystis* sp. in cancer patients: lung, stomach, colorectal, liver, esophagus, breast and hematologic. The infection rate of *Blastocystis* sp. in the first three cancer patients was relatively high [57]. The infection rate of *Blastocystis* sp. was 18.29% in chronic disease patients. These patients have abdominal pain, diarrhea, vomiting and other symptoms. Low immune status and poor nutritional status of patients with chronic diseases are the factors that cause *Blastocystis* sp. infection [60].

3.3.2 Distribution of *Blastocysts* sp. in Animals

There were 18 investigations of *Blastocystis* sp. in animals, 5 published in Chinese and 13 in English, of which 13 did gene sequence analysis. Table 5 show the distribution and genetic diversity of *Blastocystis* sp. in different animals in some cities and province. According to references, nonhuman primates, birds, and mammals can be infected with *Blastocystis* sp. [61–63]. The subtypes of *Blastocystis* sp. infection animals were ST1–ST8, ST10, as well as ST12–ST14 in China. However, there was no ST9, ST11, ST15–ST17 subtypes found. ST10 was the main subtype on infection *Blastocystis* sp. in China [64–67], followed by ST5 [61, 68]. Li et al. detected four unknown subtypes and the predominant subtype of sheep infection was ST10. However, only ST1 was observed in goats [66]. Song and others detected that the prevalence of *Blastocystis* sp. have significant difference in different study sites and age groups [61]. The infection rate of *Blastocystis* sp. in cats was relatively low. Among the 6 provinces in eastern China, only *Blastocystis* sp. was detected in Lu'an, Anhui Province [63]. Wang and others detected the infection rate was 9.5%, 8.8% and 5.5% in cattle, pig and sheep, respectively. They found 38.5% of *Blastocystis* sp. isolates were potential zoonotic subtypes [69]. Insects could also be infected. Body surface and digestive tract of Cockroach and Housefly can be infected with *Blastocystis* sp. The infection rate of Cockroach's and housefly's digestive tract was 24.76% and 4.35%, respectively [70].

3.3.3 Investigation on the distribution of *Blastocystis* sp. in different populations and animals

Surveys of *Blastocystis* sp. of human were conducted in 18 provinces, among which the survey in the general population was conducted in 15 provinces (Fig. 3). Calculate the average infection rate of each province, and the infection rate range is 0.82%–21.59%. The highest prevalence was 21.59% in Guangxi Zhuang Autonomous Region, followed by Hunan Provinces (18.4%), Sichuan Province (8.01%), Yunnan Province (7.82%), Fujian Province (5.99%), Shaanxi Province (3.46%), and Zhejiang Province (the lowest prevalence of 0.82%). Investigation of *Blastocystis* sp. infection among students in 3 provinces of Guangxi, Jiangxi and Yunnan, among inpatients or outpatients in 3 provinces of Guangxi, Yunnan, and Shanghai, among children in 4 provinces of Shenzhen, Fujian, Jiangxi, and Yunnan, among patients with diarrhea in 8 provinces including Fujian and Anhui et al., and among patients with other diseases in 5 provinces including Yunnan and Anhui et al.

Investigations of *Blastocystis* sp. among animal have conducted on 12 provinces of China. Based on the number of positive animals and the number of inspections in each province, the rate of *Blastocystis* sp. infection was calculated from 2.54–79.41% (Fig. 4). The highest prevalence of animal was 79.41% in Guangzhou [71], and the lowest animal prevalence was 2.54% in Liaoning Province [62]. The prevalence of *Blastocystis* sp. of primates was 79.41% [71], 75.59% [61], and 19.33% [72] in Guangzhou, Shaanxi, and Guilin investigated, respectively. The infection of *Blastocystis* sp. in birds was investigated in three provinces, Sichuan, Heilongjiang and Shaanxi (Table 5). Surveys of mammal *Blastocystis* sp. have conducted in 10 provinces/cities including Qinghai and Shaanxi (Table 6). The lowest prevalence of the mammal was 2.54% in Liaoning and the highest was 61.74% in Shaanxi Province.

3.4 In vitro culture and clinical detection of *Blastocystis* sp.

3.4.1 In vitro culture of *Blastocystis* sp.

Blastocystis sp. can grow and reproduce in a variety of in vitro media. In 2001, Qiao et al. explored the most suitable culture conditions for the growth of *Blastocystis* sp. in Locke-egg-serum medium (LES), and established the first continuous culture method of *Blastocystis* sp. in China [73]. RPMI1640 medium was found superior to LES medium in the culture of *Blastocystis* sp.. Vacuolar, granular, multiple fission and cyst-like form can be seen in RPMI1640 medium [74]. Iscove's Modified Dulbecco's Medium (IMDM) was more suitable for the growth and reproduction of *Blastocystis* sp. than RPMI1640 and LES media. IMDM can be used for long-term culture in vitro [75]. Moreover, modified Locke-egg serum medium culturing (mLES culturing) has a higher detection rate of *Blastocystis* sp. than the iodine direct smear. Under the same culture conditions, the mLES was simpler, more sensitive than the LES. It was more convenient in the examination of *Blastocystis* sp. [76]. Xu Zhang and others observed the growth of *Blastocystis* sp. in three commercially available liquid media (RPMI1640 medium, 199 single-phase medium and Dulbecco's Modified Eagle Medium (DMEM)), and found that these three liquid media have the characteristics of environmental safety, convenient preparation and storage, high sensitivity, and strong specificity. This result was published in *the International journal of infectious diseases*, in 2012 [77].

3.4.2 Clinical detection of *Blastocystis* sp.

Direct wet-mount, iodine staining, improved centrifugal sedimentation with washing, and improved centrifugal sedimentation with hydrochloric acid and aether were applied to simultaneously stool samples detection. It was found that the detection rate of the improved centrifugal sedimentation with hydrochloric acid and aether was high and the rate of missed diagnosis was low. Although the detection rate of improved centrifugal sedimentation with washing was high, the operation was tedious and time-consuming. Direct wet-mount and iodine staining have lower detection rate and higher missed diagnosis rate [78]. In 2006, PCR was firstly applied to detect *Blastocystis* sp. in China. The molecular detection result was consistent with the results of microscopy [79]. After that, there were applications of PCR detection in research and clinical. In 2015, Yu et al. reported that PCR and in vitro culture can be used in the clinical diagnosis of *Blastocystis* sp. [80].

3.5 Clinical case reports and treatment of *Blastocystis* sp.

There were 19 clinical case reports, 18 published in Chinese and 1 in English. *Blastocystis* sp. patients have symptoms of abdominal pain, diarrhea, fatigue and abdominal distension [81, 82]. 26 children cases for the first time were reported in 1994. Among the 26 cases, the youngest was only 3 months and the oldest was 10 years old [83]. In 1997, was the first to find a feeder infected with *Blastocystis* sp. in chicken farm was reported and believed that the feeder might be infected due to long-term close contact with poultry [84]. In 1999, Wang et al. reported a patient infection *Blastocystis* sp. with jam-like stool, which was treated orally and enema with traditional Chinese medicine *Pulsatilla chinensis* (Bunge) Regel soup. The symptoms of the patient disappeared, but *Blastocystis* sp. was still found in multiple stool tests [85]. In 2003, a case of amoeba complicated with *Blastocystis* sp. was also recorded with colon

pathological manifestations of mucosal congestion, edema, ulcer and extensive erosion [86]. Furthermore, Liu et al. reported that a 4-month-old infant was infected in 2006. After 20 days of treatment with metronidazole, the infant was cured [87]. Wei-Ping Tai et al. found that 6 ulcerative colitis patients with refractory symptoms co-infective with *Blastocystis* sp., and showed the bloody purulent stool and some pathological manifestations including diffused edema, hyperemia and hemorrhage in the rectal and sigmoid flexure. After 3 weeks of metronidazole treatment, edema and hyperemia almost completely disappeared. After multiple stool examinations no *Blastocystis* sp. was found [88]. In 2017, Zhao et al. observed that a patient infection *Blastocystis* sp. but no abdominal pain, abdominal distension, abdominal distension, nausea and other symptoms [89].

There were 17 papers on drug therapy, 16 papers published in Chinese, and 1 paper in English. Among these articles, the drugs effect on killing *Blastocystis* sp. in vitro cultures has been recorded in 6 articles, 4 reports on clinical use of traditional Chinese medicine, 3 reports on western medicine and 4 reports on Chinese and western medicine combination therapy. Drugs that have effects on this protozoa include metronidazole, mefloquine, chloramphenicol and oxytetracycline [90], tinidazole and azithromycin [91], *Coptis chinensis* and *Brucea javanica* [92], *Radix pulsatillae* [93], Oregano oil [94], metronidazole plus cimetidine [95], metronidazole combined with streptomycin [96], erythromycin in combination with *Radix pulsatillae* [97]. Drugs that have a better effect on cultured blastocysts include: oregano oil [94], tinidazole [91]. Drugs that have good therapeutic effects on patients with *Blastocystis* sp. in clinical practice include: Metronidazole and streptomycin have a good combination effect, and the effective rate was 95.8% [96]. Erythromycin combined with *Radix pulsatillae* has better effect on *Blastocystis* sp. patient, the effective rate was 94% [97]. Therefore, it was considered that the combined use of two drugs appeared better effect clinical treatment, including the combination of two western medicines and the combination of Chinese and western medicines.

3.6 Animal model establishment and pathogenicity study

There were 25 studies to explore the pathogenicity of *Blastocystis* sp. by establishing animal models and animal infections, and 9 studies have introduced the relationship between intestinal mucosa and cytokines in patient infection *Blastocystis* sp..

In 1994, the infected female mice of Kunming species with oral *Blastocystis* sp. were firstly used to study its pathogenicity in China. After anatomy it was found that the terminal ileum and cecum had hyperemia and edema [98]. In 2003, Yao selected healthy female mice to infect with *Blastocystis* sp. by orally and rectum. The number of *Blastocystis* sp. in the jejunum was the largest, followed by the stomach, ileum, colon, rectum, and cecum. The small intestine and colon show pathological manifestations of epithelial cell shedding and inflammatory cell infiltration [99]. In 2005, it was found that the higher detection rate was in the ileocecal and cecum in Kunming female mice [100]. However, there were no obvious intestinal pathological changes in infected Sprague-Dawley (SD) rats. However, *Blastocystis* sp. was observed in the cecum [101].

Liu et al. infected SD male rats with different doses of *Blastocystis* sp. trophozoites, and found that the number of mice infected in different dose groups was different after 21 days of infection, of which the highest dose (10^8) group was all infected in 2019. The subtypes of the infected mice were all ST7. It was found the protozoa in the ileum, cecum, and colon, but not in the rest of the intestine [102]. In 2005, Yao et al. infected ICR mice by mouth and rectum, and found that by rectum was more susceptible to infection mice than by mouth, which is consistent with the results of their 2003 study [99]. When the immune function of mice was low, the pathogenicity of *Blastocystis* sp. can be more enhanced, which caused severe intestinal pathological changes [103]. This indicates that *Blastocystis* sp. infection was related to the immune status of the host. In 2011, it was found that cyst was more susceptible to infection ICR mice [104].

In the pathogenicity study, the intestine of ST1 infected SD rats showed pathological the mucus membrane slough and inflammatory cell infiltration [105]. The levels of interleukin-17 (IL-17) and IL-23 were higher in the intestinal mucosa of infected mice than those in the normal control group. The expression levels of IL-17 and IL-23 were higher in the duodenum and jejunum mucosa, and the expression level of IL-17 was also higher in the colon mucosa [106]. In 2003, Cui et al. observed T-cell subsets and membrane interleukin-2 receptor (mIL-2 receptor) in peripheral blood of patient infection *Blastocystis* sp. for the first time. T cell subsets and mIL-2 receptors were found to be closely related to the immune function of *Blastocystis* sp. infection [107]. Moreover, the intestinal epithelial cells of infected patient had a local immune response. Higher levels of IL-8, IL-18 and granulocyte-macrophage colony-stimulating factors were appeared in them. These three cytokines play a certain role in the pathogenesis and increase with the increase of the infection rate [108]. It was consistent with the results by Jin et al. in 2005 [109]. It has also been found that the intestine of patient had varying degrees of hyperemia, edema, erosion, and ulcers. Pathological sections revealed infiltration of eosinophils, and the number of eosinophils infiltration was positively correlated with the degree of *Blastocystis* sp. infection. IL-5 was one of the important cytokines that cause intestinal mucosal damage, and its level gradually increased with the increase of infiltrating eosinophils [110].

4. Discussion

Among the results of literature analysis, it indicated that there were a large number of literatures on the research of *Blastocystis* sp. in 1994, 2006, and 2012. The literatures in 1994 were related to the first national survey of human parasite distribution in 1988–1992. More literature in 2006 was mainly related to the increase of research on the optimization of in vitro culture conditions. More literature in 2012 were the increase of research on the pathogenicity of *Blastocystis* sp.. There was only conducted 1 report or 1 research in most institutions that have published research in China. The institutions that conduct research in the field of *Blastocystis* sp. in China were mainly concentrated in Guangxi Medical University and the First Affiliated Hospital of Guangxi Medical University, Gannan Medical University and First affiliated hospital of Gannan Medical University, Xi'an Jiaotong University and National Institute of Parasitic Diseases, Chinese Center for Disease Control and Prevention.

The research on the morphology of *Blastocystis* sp. was mainly concentrated from 2000 to 2009. With the development of molecular biology technology, scholars have mainly studied the molecular epidemiology, nucleic acid detection technology and genomics. In recent years, with the deepening of cooperation and exchanges between China and all over the world, more and more articles have been published in international journals in the form of English literature. 29 of the 41 English articles have been published in the last ten years, the most of which have been published in the journal *Parasitology research*.

There were 116 articles on the epidemiological investigation in China and 98 articles on the population, of which 14 of them were about molecular epidemiological investigations. According to the survey, the subtypes that can infect humans were ST1–ST7, ST12 at present in China [43, 53, 111], of which ST1–ST3 were the main subtypes [24, 34, 43, 48], including mixed subtypes of ST1 and ST3, ST1 and ST2, and ST2 and ST3 [112]. Foreign studies have found that ST1–ST9 of *Blastocystis* sp. infect humans [113]. However, it has not been found ST8 and ST9 that can infect humans in China. There were 18 articles on animal infection. The subtypes of *Blastocystis* sp. infection animals were ST1–ST8, ST10, as well as ST12–ST14 in China. Some foreign studies have found that ST1–ST17 can infect animals [113]. However, there was no ST9, ST11, ST15–ST17 found. ST10 was the main subtype on infection *Blastocystis* sp. in China [64–67], followed by ST5 [61, 68]. Dogs in the Italian rescue center were infected with ST3 [114]. The subtype infection in the United States was mainly ST8 (20.6%), followed by ST6 (17.3%) and ST5 (15.9%) [115]. Because ST1–ST9 subtypes can infect both humans and animals, there are studies abroad to explore the possibility of the transmission of *Blastocystis* sp. between people and animals. In Lebanon, the infection rate of *Blastocystis* sp. in cow was as high as 63.4% in 2019. Among the seven subtypes ST1, ST2, ST3, ST5, ST7, ST10 and ST14 of cow infection, ST10 and ST14 were the main subtypes. ST1, ST2, and ST3 were found in people who had been touched cows, with ST3 as the main subtype in feeders. ST1, ST2 and ST3 were subtypes that infection feeders and cows meanwhile, but only the ST3 sequence was completely identical between the two. It was believed that *Blastocystis* sp. has a potential risk of transmission from livestock to its contacts [116].

There were fewer investigations of *Blastocystis* sp. infection among students, children, tuberculosis, cancer and patients with chronic diseases. Population and animal infections survey were merely carried out in some provinces. At present, there was no not investigated animals and its feeders at the same time in China, and has not carried out exploratory research on the possibility of zoonoses of *Blastocystis* sp..

Currently, the detection methods of *Blastocystis* sp. in China mainly include direct smear microscopy, iodine-stained smear microscopy, improved acid ether precipitation method, in vitro culture method and PCR detection method. The microscopic diagnosis method is simple, fast and convenient, but its morphological identification is difficult [39]. The in vitro culture methods, such as RMRI1640 medium, 199 single-phase medium and DMEM medium should be considered for clinical diagnosis (except emergency) and field research application [77]. After the first application of the PCR method in China in 2006, the using frequency was increasing. The PCR detection method partially or completely amplifies The Small Subunit Ribosomal RNA (SSU-rRNA) gene, which was very sensitive to the identification and

typing of *Blastocystis* sp. [117], and this method can also be considered for clinical detection and diagnosis. Until now, there are many applications of PCR methods in China, but few applications of other PCR methods. Foreign countries have used restriction fragment length polymorphism PCR (PCR-RFLP) to investigate animal *Blastocystis* sp. infections [118], and real-time fluorescence quantitative PCR (RT-PCR) was used to detect the infection status and subtype distribution of animals and their close contacts [116, 119].

In China, the infection of *Blastocystis* sp. is treated with traditional Chinese medicine, western medicine as well as the combination of two drugs. However, the combination of two western medicines and a combination of Chinese and western medicines is more effective. It has been found that traditional Chinese medicines, such as *Coptis chinensis*, *Brucea javanica*, *Oregano* oil, *Radix pulsatillae*, all of which have effect on *Blastocystis* sp. in vitro culture media. However, there were few reports on the application of traditional Chinese medicine in clinical treatment, so the application of traditional Chinese medicine should be increased in clinical treatment.

The research on *Blastocystis* sp. in China includes pathogenic morphology, laboratory testing, epidemiology, pathogenicity, animal models, immunology, molecular biology, clinical case reports and treatment. Particularly, there were a large amount of data and literature accumulated in the field of epidemiological investigation and research. Although the research scope of *Blastocystis* sp. is extensive, many research filed have been ignored in China.

5 Conclusions

Although the research on *Blastocystis* sp in China covered a wide range of fields, the investigation and research on clinical therapeutic drugs, laboratory testing and diagnostic methods, and animal infections were still rare. Furthermore, there were fewer surveys of patients with colon cancer, tuberculosis, and chronic diseases infection *Blastocystis* sp. and fewer surveys on minority areas. Meanwhile, there were fewer reports on the investigation and research on the possibility and related relationship of the spread of *Blastocystis* sp. between humans and animals. Therefore, further studies are needed to reveal the possibility of *Blastocystis* sp. transmission between humans and animals. With the rapid development of the field of intestinal microecology recently, there have been many reports on related research, but there has been no report on the relationship between *Blastocystis* sp. and intestinal microecology in China. Thence, research in this area should be strengthened.

Abbreviations

CNKI, China National Knowledge Infrastructure; DMEM, Dulbecco's Modified Eagle Medium; HIV, human immunodeficiency virus; IMDM, Iscove's Modified Dulbecco's Medium; IL, interleukin; Locke-egg serum medium; LES, Locke-egg-serum medium; mIL, membrane interleukin; mLES, modified SD, PCR, Polymerase chain reaction; PCR-RFLP, restriction fragment length polymorphism PCR; RPMI, Roswell Park

Memorial Institute; RT-PCR, real-time fluorescence quantitative PCR; Sprague-Dawley; SSU-rRNA, The Small Subunit Ribosomal RNA.

Declarations

Ethics approval and consent to participate

Not applicable.

Consent for publication

Not applicable.

Availability of data and materials

All datasets are presented in the main paper.

Competing interests

The authors declare that they have no competing interests.

Funding

This research was funded by the National Natural Science Foundation of China (81473022).

Authors' contributions

C-QN and LA performed the statistical analysis and drafted the paper. C-QN and L-GT conceived and designed the study. LA and L-GT critically reviewed the paper. All authors read and approved the final version of the paper.

Acknowledgements

We sincerely thank all the experts who have insights into this paper.

Authors' information

National Institute of Parasitic Diseases, Chinese Center for Disease Control and Prevention, Key Laboratory for Parasite and Vector Biology, Ministry of Health of China, WHO Collaborating Center for Tropical Diseases, National Center for International Research on Tropical Diseases.

Chao-qun Ning, Lin Ai, Li-guang Tian.

Corresponding author is Li-guang Tian.

References

1. Tan KS. *Blastocystis* in humans and animals: new insights using modern methodologies. *Vet Parasitol.* 2004;126(1–2):121–44. <https://doi.org/10.1016/j.vetpar.2004.09.017>.
2. Tan KS. New insights on classification, identification, and clinical relevance of *Blastocystis* spp. *Clin Microbiol Rev.* 2008;21(4):639–65. <https://doi.org/10.1128/cmr.00022-08>.
3. Brumpt E. *Blastocystis hominis* n. sp. et formes voisines. *Bull Soc Path Exot.* 1912;5:725–30.
4. Z CH. WS. R, BS. B. Protozoan characteristics of *Blastocystis hominis*. *Am J Clin Pathol.* 1967;48:495–501.
5. Zierdt CH. *Blastocystis hominis*, a long-misunderstood intestinal parasite. *Parasitology today* (Personal ed). 1988;4(1):15–7. [https://doi.org/10.1016/0169-4758\(88\)90049-x](https://doi.org/10.1016/0169-4758(88)90049-x).
6. He Jianguo J, Jingbo Z, Hong L. Xiaomei. Optical and ultrastructural studies of *Blastocystis hominis*. *Journal of Sun Yat-sen University (Natural Science Edition)* 1990, (03):122–128.
7. Jiang J-B, He J-G. Taxonomic Status of *Blastocystis hominis*. *Parasitol Today.* 1993;9:2–3.
8. He Jianguo J. Jingbo. Scanning electron microscope observation of *Blastocystis hominis*. *Journal of Sun Yat-sen University (Natural Science Edition)* 1992, (02):93–97.
9. Ni HE, Yue-qing ZHANG, Ming-li HONG, Min CONG. Morphological and Ultrastructural Observation of *Blastocystis hominis*. *Chinese Journal of Parasitology Parasitic Diseases.* 2001;19(03):169–72.
10. Qiao Jiying X, Hongxie T, Ai. Morphological observation of *Blastocystis hominis*. *Chinese Journal of Parasitology and Parasitic Diseases* 1998(02):46–47.
11. Qiao ji-ying, Xu Z, Zhi-chao W, Yang Jun-hua, Li Ya-Qing, Zhang Rong. Study on the biological characteristic of *Blastocystis hominis*: morphology, mode of reproduction and the relation to bacteria. *Journal of Hygiene Research* 2006, 35(06):739–742.
12. Guohong Wu, Gaolu C, Shuilian Su, Aimin H, Xuebin X, Hu Yaqiong. Observation on morphology of trophozoite and cyst of *Blastocystis hominis*. *Journal of Gannan Medical University* 2000, 20(03):203–206 + 305.
13. Zhang Xu Q, Ji-Ying DXiao-Hui, Li Ya-Qing, Li Xiao-Qi, Li Chen. Study on morphology of *Blastocystis hominis* in culture and from diarrhea patients. *Chinese Journal of Parasitology and Parasitic Diseases* 2003, 21(02):116–118.
14. Shuilian Su, Guifeng C, Zhichun LJuan,L, Hua L. Structural observation of *Blastocystis hominis* cysts in different environment. *Chinese Journal of Parasitic Disease Control.* 2003;16(05):287–9.
15. Zhang X, Qiao JY, Zhou XJ, Yao FR, Wei ZC. Morphology and reproductive mode of *Blastocystis hominis* in diarrhea and in vitro. *Parasitol Res.* 2007;101(1):43–51. <https://doi.org/10.1007/s00436-006-0439-x>.
16. Zhang X, Zhang S, Qiao J, Wu X, Zhao L, Liu Y, et al. Ultrastructural insights into morphology and reproductive mode of *Blastocystis hominis*. *Parasitol Res.* 2011;110(3):1165–72. <https://doi.org/10.1007/s00436-011-2607-x>.
17. Shen Jiqing T, Chunlin Lu, Zuochao W, Xiaoling L, Dengyu L, Xiaoquan, et al. Relationship between morphology and pathogenicity of *Blastocystis hominis* trophozoites. *Chinese Journal of*

- Parasitology Parasitic Diseases. 2013;31(02):137–9 + 142.
18. Xu Longqi. Distribution and harm of human parasites in China. People's Medical Publishing House; 1999.
 19. Zhou Xiaonong. Report on the national survey of important human parasitic diseases in China. (2015). People's Medical Publishing House; 2018.
 20. Zhang Ruilin L, Chi C, Ailian H, Ai. Experimental observation on the case of *Blastocystis hominis*. *Guangdong Parasite Society Annual Report 2000*, 22:59–60.
 21. Umar M, Xiao-Ying C, Osman Y, Simayi A, Yan-Yan H, Maimaitiyiming Y, et al. Epidemiological Survey on Human Intestinal Protozoa in Xinjiang Uygur Autonomous Region in 2015. *Chinese Journal of Parasitology Parasitic Diseases*. 2016;34(4):361–5.
 22. He Shanshan. Investigation on the infection of *Blastocystis hominis* various populations in Bama Yao Autonomous County of Guangxi and RAPD analysis of 105 isolates. *Master*. Guangxi Medical University; 2013.
 23. Yan Y, Su S, Lai R, Liao H, Ye J, Li X, et al. Genetic variability of *Blastocystis hominis* isolates in China. *Parasitol Res*. 2006;99(5):597–601. <https://doi.org/10.1007/s00436-006-0186-z>.
 24. Gong B, Liu X, Wu Y, Xu N, Xu M, Yang F, 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. <https://doi.org/10.1051/parasite/2019046>.
 25. Chen TL, Chan CC, Chen HP, Fung CP, Lin CP, Chan WL, et al. Clinical characteristics and endoscopic findings associated with *Blastocystis hominis* in healthy adults. *Am J Trop Med Hyg*. 2003;69(2):213–6.
 26. Xue-jiao TENG, Yan-hong CHU, Cheng-cheng ZHAI, Ying-fang YU, Yu-chun CAI, Shao-hong CHEN, et al. The epidemiological characteristics and influencing factors for *Blastocystis hominis* infection among human immunodeficiency virus seropositive individuals in Tengchong of Yunnan Province. *Chinese Journal of Parasitology Parasitic Diseases*. 2018;36(02):129–34.
 27. Li XX, Chen JX, Wang LX, Tian LG, Zhang YP, Dong SP, et al. Prevalence and risk factors of intestinal protozoan and helminth infections among pulmonary tuberculosis patients without HIV infection in a rural county in P. R. China. *Acta Trop*. 2015;149:19–26. <https://doi.org/10.1016/j.actatropica.2015.05.001>.
 28. Li XX, Chen JX, Wang LX, Tian LG, Zhang YP, Dong SP, et al. Intestinal parasite co-infection among pulmonary tuberculosis cases without human immunodeficiency virus infection in a rural county in China. *Am J Trop Med Hyg*. 2014;90(1):106–13. <https://doi.org/10.4269/ajtmh.13-0426>.
 29. Mo Gang Y, Dongyu Z, Lingyou N, Zijun L. Yunping. Infection status of *Blastocystis hominis* in the 2010 freshmen of Guilin Medical University. *Chinese Journal of School Health*. 2012;33(04):502.
 30. Cao Haolu Wu, Guohong G, Zengzhu. *Blastocystis hominis* infection among primary school students in Nantang Town, Gan County. *Journal of Gannan Medical University* 1999, 14(04):364.
 31. Shuilian Su, Guifeng C, Minchang T, Ruiqi Z, Ruichong Z. Liao Hua et al. Investigation and analysis on the infection of intestinal parasites in grade 2002. *Journal of Gannan Medical University*.

- 2002;22(05):540–1.
32. Liu T, Xiaoquan L, Huanhuan S, Shanshan H, Ge W, Fu Ruijia, et al. Investigation of college student infection with *Blastocystis hominis* in Guangxi Medical University. *Journal of Guangxi Medical University*. 2011;28(06):961–3.
 33. Liu Qing. Studies on prevalence and genotypes of *G. lamblia*, *B. hominis* and *E. bieneusi* in humans in Dali. *Master*. Dali University; 2018.
 34. Ting-zheng ZHAN, Teng LIU, Huan-huan SHI, Shan-shan HE, Hui Y. LIU Deng-yu. PCR-based genotype classification of *Blastocystis hominis* isolates from college students of Guangxi. *Chinese Journal of Parasitology Parasitic Diseases*. 2014;32(03):209–11.
 35. Li Z., Shuming D. Chen Fanggen. Study on chronic diarrhea and infection of *Blastocystis* in children in Jiangxi. *Medical Information*. 2015;28(46):62–3.
 36. Wang Ling Z, Ruiqi L, Hongqun, Chen Guifeng. Analysis of 209 cases of intestinal parasites in children with repeated abdominal pain. *Chinese Journal of Misdiagnosis*. 2008;8(31):7697–8.
 37. Zhang SX, Zhou YM, Xu W, Tian LG, Chen JX, Chen SH, et al. Impact of co-infections with enteric pathogens on children suffering from acute diarrhea in southwest China. *Infectious diseases of poverty* 2016, 5(1):64. <https://doi.org/10.1186/s40249-016-0157-2>.
 38. Huang Miaohui C, Suqing Wu, Bin Wu, Lise Z, Ziqing. Clinical epidemiological investigation of intestinal pathogenic parasitic infection in children with diarrhea. *China Public Health*. 1999;15(6):481.
 39. Cao Ke L, Xiaojuan W, Danlin Z, Yi T, Dan C, Yunsheng, et al. A preliminary investigation on the infection of human *Blastocystis hominis* in the children's hospital in Shenzhen. *Chinese Journal of Clinical Laboratory Science*. 2019;37(03):222–5.
 40. Wang Beibei Y, Hui S, Huanhuan L, Dengyu L, Dejun, Liu, Xiaoquan, et al. Investigating *Blastocystis hominis* infection of inpatients in two tertiary hospitals of Guangxi. *Journal of Guangxi Medical University*. 2016;33(01):144–6.
 41. Liu Ying Q, Cheng C, Xiangren Z, Dequan H, Aijuan Y, Yuanhua, et al. Investigation of infectious status of *Blastocystis hominis* in Guangxi. *Journal of Applied Preventive Medicine*. 2008;14(05):285–6.
 42. LU Zuo-chao HU, Ying LIU, Xiao-quan LIU, Deng-yu. LI Yan-wen. Analysis intestinal parasitic infection status of 12 313 cases of hospital patients in Nanning city, Guangxi. *Journal of Tropical Medicine*. 2015;15(05):677–80.
 43. Yan Hui. Analyjs of infection status, genotyping and and drugs senitivity of *Blastocystis hominis* in inpatiets from two tripleastatus hospitals of Guanagxi. *Master*. Guangxi Medical University; 2015.
 44. Tao JIANG, Jia-zhi WANG, Ying-fang YU, Xue-Jiao TENG, Yan-hong CHU, Xiu-ping WU, et al. Hospital-based study on the risk factors for *Blastocystis hominis* infection in the Tengchong City, Yunnan Province, China. *Chinese Journal of Zoonoses*. 2019;35(10):964–9 + 972.
 45. Peng SONG, Hao LI, Jian GUO, Lin AI, Yan LU, Yu-chun CAI, et al. Laboratory detection of parasitic infections in clinical samples from hospitals in Shanghai during 2014–2017. *Chinese Journal of*

- Parasitology Parasitic Diseases. 2018;36(05):489–94.
46. Chen Hao L, Cunhui X, Manling W, Xueying Y, Lijun. The relationship between diarrhea and the infection of intestinal protozoa in Fuzhou district. *Strait Journal of Preventive Medicine* 2002(06):14–16.
 47. Zhang SX, Yang CL, Gu WP, Ai L, Serrano E, Yang P, et al. Case-control study of diarrheal disease etiology in individuals over 5 years in southwest China. *Gut Pathog.* 2016;8:58. <https://doi.org/10.1186/s13099-016-0141-1>.
 48. Shun-xian ZHANG, Li-guang TIAN, Yan LU, Lan-hua LI, Jia-xu CHEN. ZHOU Xiao-nong. Epidemiological characteristics of *Blastocystis hominis* in urban region, southwestern China. *Chinese Journal of Zoonoses.* 2016;32(05):424–8.
 49. Jin Qunxin Yu, Kaimin T, Lianfeng T, Chunlin Lu. Zuochoao. Investigation of infectious status of *Blastocystis hominis* in 1354 outpatients. *China Tropical Medicine.* 2005;5(07):1469–71.
 50. Wang Z. Co-infection with HIV and *Cryptosporidium* or *Blastocystis hominis* in Guangxi and the genotype of *Cryptosporidium*. *Master.* Guangxi Medical University; 2015.
 51. Tian LG, Wang TP, Lv S, Wang FF, Guo J, Yin XM, et al. HIV and intestinal parasite co-infections among a Chinese population: an immunological profile. *Infectious diseases of poverty.* 2013;2(1):18. <https://doi.org/10.1186/2049-9957-2-18>.
 52. Tian LG, Chen JX, Wang TP, Cheng GJ, Steinmann P, Wang FF, et al. Co-infection of HIV and intestinal parasites in rural area of China. *Parasit Vectors.* 2012;5:36. <https://doi.org/10.1186/1756-3305-5-36>.
 53. Teng Xuejiao. Molecular epidemiological investigation of HIV / AIDS combined with intestinal protozoan infection in Tengchong, Yunnan. *Master.* Chinese Center for Disease Control and Prevention; 2018.
 54. Tian Liguang. Co-infection of HIV and intestinal parasites in highly endemic areas of HIV/AIDS in China. *PhD.* Chinese Center for Disease Control and Prevention; 2010.
 55. Li-guang TIAN, Jia-xu CHEN, Guo-jin CHENG, Feng-feng WANG, Jian GUO, Xiao-mei YIN, et al. Survey on *Blastocystis hominis* infection in HIV positive individuals in Fuyang City, Anhui Province. *Chinese Journal of Schistosomiasis Control.* 2012;24(03):303–6 + 310.
 56. Tian Liguang C, Guojin C, Jiayu W, Fengfeng C, Yuchun, Guo J, et al. Investigation on the status of intestinal parasite infection in rural areas with high HIV / AIDS in China. In: *The Fifth National Conference on AIDS, Viral Hepatitis C and National Tropical Diseases of the Chinese Medical Association: 2011; Wuhan, Hubei, China; 2011: 2.*
 57. Zhang W, Ren G, Zhao W, Yang Z, Shen Y, Sun Y, et al. Genotyping of *Enterocytozoon bienersi* and subtyping of *Blastocystis* in cancer patients: relationship to diarrhea and assessment of zoonotic transmission. *Front Microbiol.* 2017;8:1835. <https://doi.org/10.3389/fmicb.2017.01835>.
 58. Ying HU, Xiang-qun SONG, Yan-wen LI, SHI Huan-huan. Research on the infection status of *Blastocystis hominis* in patients with malignant tumour. *Chinese Journal of Health Laboratory Technology.* 2015;25(12):1962–4.

59. Ying HU, Xuelian DENG, Jiying SHI, Yanwen LI. Analysis on status quo of intestinal protozoan infections among patients with chronic diseases in Guangxi area. *Laboratory Medicine Clinic*. 2017;14(17):2497–9. <https://doi.org/10.3969/j.issn.1672-9455.2017.17.002>.
60. Ying HU, Yan-wen LI, Xiao-quan LIU, Ji-ying SHI. Investigation of infection in patients with chronic diseases. *Chinese Journal of Health Laboratory Technology*. 2017;27(17):2558–60.
61. Zhao GH, Hu XF, Liu TL, Hu RS, Yu ZQ, Yang WB, et al. Molecular characterization of *Blastocystis* sp. in captive wild animals in Qinling Mountains. *Parasitol Res*. 2017;116(8):2327–33. <https://doi.org/10.1007/s00436-017-5506-y>.
62. Wang J, Gong B, Liu X, Zhao W, Bu T, Zhang W, et al. Distribution and genetic diversity of *Blastocystis* subtypes in various mammal and bird species in northeastern China. *Parasit Vectors*. 2018;11(1):522. <https://doi.org/10.1186/s13071-018-3106-z>.
63. Li W, Liu X, Gu Y, Liu J, Luo J. Prevalence of *Cryptosporidium*, *Giardia*, *Blastocystis*, and trichomonads in domestic cats in East China. *J Vet Med Sci*. 2019;81(6):890–6. <https://doi.org/10.1292/jvms.19-0111>.
64. Song J-K, Yin Y-L, Yuan Y-J, Tang H, Ren G-J, Zhang H-J, et al. First genotyping of *Blastocystis* sp. in dairy, meat, and cashmere goats in northwestern China. *Acta Trop*. 2017;176:277–82. <https://doi.org/10.1016/j.actatropica.2017.08.028>.
65. Zhu W, Tao W, Gong B, Yang H, Li Y, Song M, et al. First report of *Blastocystis* infections in cattle in China. *Vet Parasitol*. 2017;246:38–42. <https://doi.org/10.1016/j.vetpar.2017.09.001>.
66. Li WC, Wang K, Gu Y. Occurrence of *Blastocystis* sp. and *Pentatrichomonas hominis* in sheep and goats in China. *Parasit Vectors*. 2018;11(1):93. <https://doi.org/10.1186/s13071-018-2671-5>.
67. Ren M, Song JK, Yang F, Zou M, Wang PX, Wang D, et al. First genotyping of *Blastocystis* in yaks from Qinghai Province, northwestern China. *Parasit Vectors*. 2019;12(1):171. <https://doi.org/10.1186/s13071-019-3436-5>.
68. Song J-K, Hu R-S, Fan X-C, Wang S-S, Zhang H-J, Zhao G-H. Molecular characterization of *Blastocystis* from pigs in Shaanxi province of China. *Acta Trop*. 2017;173:130–5. <https://doi.org/10.1016/j.actatropica.2017.06.014>.
69. Wang J, Gong B, Yang F, Zhang W, Zheng Y, Liu A. Subtype distribution and genetic characterizations of *Blastocystis* in pigs, cattle, sheep and goats in northeastern China's Heilongjiang Province. *Infection Genetics Evolution*. 2018;57:171–6. <https://doi.org/10.1016/j.meegid.2017.11.026>.
70. Li Jihong C, Qian Wu, Yang L, Mingchang. Investigation of *Blastocystis hominis* infected by omnivorous insect *Chinese Journal of Zoonoses* 2004(09):728.
71. He Jianguo Z, Guojuan Z, Zhongxing. Investigation and research of primate human *Blastocystis hominis* in Guangzhou area. *Chinese Journal of Zoology* 1994(01):1–3.
72. Nong Zijun L, Yunping M, Gang J, Liping L. Xinde. Investigation of rhesus monkey infection with *Blastocystis hominis* in Guilin. *Heilongjiang Animal Science and Veterinary Medicine* 2012(01):105–106.

73. Qiao Jiying Z, Xu D, Xiaohui L, Xiaoqi Y, Junhua Y Shengfang. Studies on the vitro culture of *Blastocystis hominis*. *Journal of Tropical Medicine* 2001, 1(02):144–147.
74. Fanrong Y, Jiying Q, Junhua Y, Xiaoqi L, Xu Z, Rong D. Comparison of *Blastocystis hominis* in RPMI1640 and LES Culture. *Journal of Shaanxi Normal University (Natural Science Edition)* 2003(S2):259–264.
75. Wu Lingyuan T, Lili Fu, Ruijia H, Shanshan Lu, Zuocho L. Dengyu. Observation of the effects of culturing *Blastocystis hominis* in different media and under different conditions. *Journal of Parasitic Biology*. 2013;8(04):336–8 + 349.
76. Xu-ning SUN, De-jun LIAO, Jing LIU, Xiao-yin FU, Pei PEI, Zhen-jie LIAO, et al. Experimental study on culturing *Blastocystis hominis* using the mLES method. *Journal of Parasitic Biology*. 2018;13(03):259–62 + 266.
77. Zhang X, Qiao J, Wu X, Da R, Zhao L, Wei Z. In vitro culture of *Blastocystis hominis* in three liquid media and its usefulness in the diagnosis of blastocystosis. *International Journal of Infectious Diseases*. 2012;16(1):e23–8. <https://doi.org/10.1016/j.ijid.2011.09.012>.
78. He Aijuan Q, Cheng L, Ying Y, Yuanhua C, Xiangren, Zeng Dequan. Comparative study of different detection methods for diagnosing *Blastocystis hominis*. *Journal of Applied Preventive Medicine*. 2008;14(05):309–10.
79. Zhang Hongwei Y, Qiuye H, Lijun Su, Yunpu L, Wen X, Changgui, et al. Survey on asymptomatic *Blastocystis hominis* and its PCR identification. *Journal of Medical Forum*. 2006;27(01):32–3 + 36.
80. Yu Yingfang Wu, Xiuping C, Yanhong Z, Yongnian, Tian Liguang. Using PCR and in vitro culture technique to diagnose a case of *Blastocystis hominis* subtype 3 infection. *Chinese Journal of Parasitology Parasitic Diseases*. 2015;33(03):241.
81. Wang Jiquan L, Tanfo Wu, Yunlian. Report of 2 cases of *Blastocystis hominis*. *New Medicine* 1991(09):474.
82. Yunpu Su, Jinzhong Lu, Guangpei H, Dongfang L. Lu Suhua, Zhang Yali. Two cases of diarrhea caused by *Blastocystis hominis*. *Henan Journal of Preventive Medicine* 1992(01):52.
83. Lin Qiujuan C, Xianfen J, Quansheng Su, Qingping Su, Jinghua. Li Cunhui. Report of 26 cases of *Blastocystis hominis* enteritis in children. *Fujian Medical Journal* 1994(02):70–71.
84. Liao Yuanquan. One case of diarrhea caused by *Blastocystis hominis*. *Chinese Journal of Parasitic Disease Control* 1997(04):74.
85. Wang Weiwei L, Chi Z, Ruilin. A case of *Blastocystis hominis* diseases with purulent blood stool. *Chinese Journal of Zoonoses* 1999(01):70–71.
86. Li L, Daoyin Z, Xiaoyang H, Jinhong S. A case of amoebiasis combined with *Blastocystis hominis* infection. *Journal of Rare Uncommon Diseases*. 2003;10(06):54.
87. Deng-yu LIU, Zuo-chao LU. YUN xiang. A case of infant infected by *Blastocystis hominis*. *Chinese Journal of Parasitology and Parasitic Diseases* 2006(06):432.

88. Tai WP, Hu PJ, Wu J, Lin XC. Six ulcerative colitis patients with refractory symptoms co-infective with *Blastocystis hominis* in China. *Parasitol Res.* 2011;108(5):1207–10. <https://doi.org/10.1007/s00436-010-2164-8>.
89. Zhao Hongying Lu, Lijiang L, Li W, Sheng. Chen Zongbo. A case of *Blastocystis hominis*. *Chinese Journal of New Clinical Medicine.* 2018;11(11):1147–8.
90. He Jianguo J. Jingbo. Screening of anti-*Blastocystis hominis* drugs and the mechanism of action of metronidazole on it. *Journal of Sun Yat-sen University* 1992(03):19–23.
91. Tian Chunlin W, Xiaoling L. Xiaoquan. Observation on the effect of three drugs on *Blastocystis hominis*. *China Tropical Medicine.* 2007;7(03):324–5.
92. Yang LQ, Singh M, Yap EH, Ng GC, Xu HX, Sim KY. In vitro response of *Blastocystis hominis* against traditional Chinese medicine. *J Ethnopharmacol.* 1996;55(1):35–42. [https://doi.org/10.1016/s0378-8741\(96\)01471-7](https://doi.org/10.1016/s0378-8741(96)01471-7).
93. Xu ZHANG, Ji-ying QIAO, Rong ZHANG, Zhi-chao WEI, CAI Juan. In vitro antiprotozoal effects of *Brucea javanica*, *Coptis chinensis*, *Radix pulsatillae* and *Arecae* on *Blastocystis hominis*. *Journal of Tropical Medicine* 2007, 7(11):1044–1047.
94. Ji-ying QIAO, Li-jun LI, Rong ZHANG, Xu ZHANG, Juan CAI, YANG Jun-hua. Antiprotozoal effects of *Oregano oil* and *Brucea javanica* on *Blastocystis hominis* in vitro. *Journal of Parasitic Biology* 2008, 3(06):434–436 + 439.
95. Hongqun L, Wei Q, Ling W, Shuilian Su, Huabin W, Guifeng C, et al. The detection and clinical study of children's repeating bellyache and intestinal protozoan infection. *Journal of Gannan Medical University.* 2010;30(06):876–8.
96. Zhu Jinglin L, Nanhai H, Yaogan H, Xiaoqing L. Jingxiu. 72 cases of clinical observation in pediatric *Blastocystis hominis* disease treatment. *Journal of Gannan Medical University.* 2012;32(06):889–90.
97. Tao Lin J, Haixing Lu, Jeff J, Qunxin T, Guodu Lu. Zuochao. Observation of the effect of two drugs on metronidazole ineffective *Blastocystis hominis*. *Lishizhen Medicine Materia Medica Research.* 2014;25(01):112–4.
98. Qingping Su, Jinghua Su, Manling X, Yihong H. Feng Yi'an, Wang Jiyun et al. Epidemiological investigation, clinical observation and pathogenic mechanism of *Blastocystis hominis*. *Chinese Journal of Zoonoses* 1994(03):41–42 + 65.
99. Yao Fanrong. Study on vitro culture of RPMI1640 culture of *Blastocystis hominis* and experimental animal model in laboratory mice of *Blastocystis hominis* disease. *Master.* Xi'an Jiaotong University; 2003.
100. Dong WANG, Chang-gui XUE, Wen LI, Yun-pu SU, Qiu-ye YAN, Li-jun HE, et al. Experimental study on Kunming mice infected experimentally with *Blastocystis Hominis*. *Henan Medical Research.* 2005;14(04):309–11.
101. Sun Xuning. Study on expressions of TLR2/TLR4 and inflammation-related cytokines in rats infected with *Blastocystis hominis*. *Master.* Guangxi Medical University; 2018.

102. Jing LIU, Jyh-wei SHIN, Pei PEI, Xiao-yin FU, De-jun LIAO, Yun-yu LU, et al. A modified method to infect *Blastocystis hominis* in rats and the pathological changes after infection. Chinese Journal of Parasitology Parasitic Diseases. 2019;37(02):161–6 + 172.
103. YAO Fan-rong QIAO, Ji-ying ZHAO, Yan ZHANG, Xu YANG, Jun-hua. LI Xiao-qi. Experimental Infection of Mice with *Blastocystis hominis*. Chinese Journal of Parasitology Parasitic Diseases. 2005;23(06):444–8.
104. Zhang X. Purification of *Blastocystis hominis* in animal peritoneal cavity and establishment of animal model of *Blastocystis hominis*. Master. Xi'an Jiaotong University; 2011.
105. Li J, Deng T, Li X, Cao G, Li X, Yan Y. A rat model to study *Blastocystis* subtype 1 infections. Parasitol Res. 2013;112(10):3537–41. <https://doi.org/10.1007/s00436-013-3536-7>.
106. Ling-yuan WU, Rui-jia FU, Zuo-chao LU, Li-li TANG, Fan ZHANG, Deng-yu LIU. Expressions and significance of IL-17 and IL-23 in intestinal mucosa of mice infected with *Blastocystis hominis*. Chinese Journal of Schistosomiasis Control. 2012;24(06):676–80 + 740.
107. Cui Yubao C, Lin L. Chaopin. Detection of mIL-2R and T lymphocyte subsets in PBMC of the patients infected by *Blastocystis hominis*. Journal of Tropical Diseases Parasitology. 2003;1(02):85–7.
108. Lin TAO, Hai-xing JIANG, Qun-xin JIN, Guo-du TANG, Ling-yuan WU. LU Zuo-chao. Determination and significance of cytokines in peripheral blood of patients infected with *Blastocystis hominis*. Guangxi Medical Journal. 2014;36(08):1034–6.
109. Qun-xin JIN, Guo-du TANG, Kai-min YU. Pathologic observation of intestinal mucosal lesion and detection of cytokine level in the patients with *Blastocystis hominis* infection. Chinese Journal of Parasitic Disease Control. 2005;18(05):38–40.
110. Yu Kaimin. The relationship between IL-5, eosinophils and intestinal mucosa lesions in the patients with *Blastocystis hominis* infection. Journal of Parasitic Biology. 2011;6(05):351–2 + 360.
111. Yan Y, Su S, Ye J, Lai X, Lai R, Liao H, et al. *Blastocystis* sp. subtype 5: a possibly zoonotic genotype. Parasitol Res. 2007;101(6):1527–32. <https://doi.org/10.1007/s00436-007-0672-y>.
112. Li LH, Zhang XP, Lv S, Zhang L, Yoshikawa H, Wu Z, 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. <https://doi.org/10.1007/s00436-007-0727-0>.
113. Cian A, El Safadi D, Osman M, Moriniere R, Gantois N, Benamrouz-Vanneste S, et al. Molecular epidemiology of *Blastocystis* sp. in various animal groups from two french zoos and evaluation of potential zoonotic risk. PLoS One. 2017;12(1):e0169659. <https://doi.org/10.1371/journal.pone.0169659>.
114. Gazzonis AL, Marangi M, Zanzani SA, Villa L, Giangaspero A, Manfredi MT. Molecular epidemiology of *Blastocystis* sp. in dogs housed in Italian rescue shelters. Parasitol Res. 2019;118(10):3011–7. <https://doi.org/10.1007/s00436-019-06424-5>.
115. Jiménez PA, Jaimes JE, Ramírez JD. A summary of *Blastocystis* subtypes in North and South America. Parasit Vectors. 2019;12(1):376. <https://doi.org/10.1186/s13071-019-3641-2>.

116. Greige S, El Safadi D, Khaled S, Gantois N, Baydoun M, Chemaly M, et al. First report on the prevalence and subtype distribution of *Blastocystis* sp. in dairy cattle in Lebanon and assessment of zoonotic transmission. *Acta Trop.* 2019;194:23–9. <https://doi.org/10.1016/j.actatropica.2019.02.013>.
117. Robertson LJ, Clark CG, Debenham JJ, Dubey JP, Kváč M, Li J, et al. Are molecular tools clarifying or confusing our understanding of the public health threat from zoonotic enteric protozoa in wildlife? *International Journal for Parasitology: Parasites Wildlife.* 2019;9:323–41. <https://doi.org/10.1016/j.ijppaw.2019.01.010>.
118. 10.1016/j.cimid.2018.11.013
Asghari A, Sadraei J, Pirestani M, Mohammadpour I. First molecular identification and subtype distribution of *Blastocystis* sp. isolated from hooded crows (*Corvus cornix*) and pigeons (*Columba livia*) in Tehran Province, Iran. *Comparative immunology, microbiology and infectious diseases* 2019, 62:25–30. <https://doi.org/10.1016/j.cimid.2018.11.013>.
119. Villamizar X, Higuera A, Herrera G, Vasquez AL, Buitron L, Muñoz LM, et al. Molecular and descriptive epidemiology of intestinal protozoan parasites of children and their pets in Cauca, Colombia: a cross-sectional study. *BMC Infect Dis.* 2019;19(1):190. <https://doi.org/10.1186/s12879-019-3810-0>.
120. Rui-Min ZHOU, Su-Hua LI, Ya-Lan ZHANG, Yan DENG, Wei-Qi CHEN, Cheng-Yun YANG, et al. Investigation on human intestinal parasitic diseases in ecological regions of Qinba Mountains in Henan Province in 2015. *Chinese Journal of Schistosomiasis Control.* 2019;31(02):148–54.
121. Yang Y. Investigation on human infection and observing on genetic variability of *Blastocystis hominis* in the south coastal areas of Guangxi province. *Master.* Guangxi Medical University; 2011.
122. Ji-ying QIAO, Xiao-ying LI, Juan CAI, Xu ZHANG, Zhi-chao WEI. LI Ya-qing. Investigation of *Blastocystis hominis* infection in Xi'an city, Shaanxi province. *Journal of Tropical Medicine.* 2011;11(11):1298–300.
123. Li LH, Zhou XN, Du ZW, Wang XZ, Wang LB, Jiang JY, et al. Molecular epidemiology of human *Blastocystis* in a village in Yunnan province, China. *Parasitol Int.* 2007;56(4):281–6. <https://doi.org/10.1016/j.parint.2007.06.001>.
124. Zhang X, Qiao JY, Wu XM, Ma QY, Hu H, Wang J, et al. *Ascaris* spp. and *Capillaria caudinflata* infections in captive-bred crested ibis (*Nipponia nippon*) in China. *Zoo Biol.* 2015;34(1):80–4. <https://doi.org/10.1002/zoo.21191>.
125. Deng L, Yao JX, Liu HF, Zhou ZY, Chai YJ, Wang WY, et al. First report of *Blastocystis* in giant pandas, red pandas, and various bird species in Sichuan province, southwestern China. *Int J Parasitol Parasites Wildl.* 2019;9:298–304. <https://doi.org/10.1016/j.ijppaw.2019.06.007>.
126. Xiao X, Zhou SH, Jiang N, Tian DZ, Zhou ZM, Zhang M, et al. First record of *Leptospira* and *Blastocystis* infections in captive flying squirrels (*Trogopterus xanthipes*) from Enshi County, China. *Acta Trop.* 2019;197:105065. <https://doi.org/10.1016/j.actatropica.2019.105065>.

Tables

Table 1. Institution that have published 5 or more Chinese literatures

Institution	No. documents in the past 30 years	No. literatures in the last 10 years
Guangxi Medical University	60	40
Gannan Medical University	22	6
Xi'an Jiaotong University	21	2
National Institute of Parasitic Diseases, Chinese Center for Disease Control and Prevention	16	14
Henan Center for Disease Control and Prevention	12	2
Fujian Medical University	10	0
Sun Yat-Sen University	8	0
Guangdong Medical University	8	0
Qinghai Institute of Endemic Disease Control	5	0

Table 2. Institution that have published 3 or more English literatures

Institution	No. documents in the past 30 years	No. literatures in the last 10 years
National Institute of Parasitic Diseases, Chinese Center for Disease Control and Prevention	8	6
Northwest A&F University	5	5
Xi'an Jiaotong University	5	4
Harbin Medical University	4	4
Gannan Medical University	3	1

Table 3. Magazines with 5 or more Chinese literatures

Magazine	No. literatures in the past 30 years	No. literatures in the past 10 years
<i>Chinese Journal of Parasitology and Parasitic Diseases</i>	31	14
<i>Chinese Journal of Pathogen Biology</i>	25	7
<i>Chinese Journal of Zoonoses</i>	12	3
<i>Journal of Gannan Medical University</i>	11	6
<i>Chinese Journal of Schistosomiasis Control</i>	9	7
<i>China tropical medicine Parasitic Diseases Foreign Medical Sciences</i>	8	0
<i>Applied Preventive Medicine</i>	6	3
<i>Chinese Journal of Health Laboratory Technology</i>	5	3
<i>Journal of Tropical Medicine</i>	5	2
<i>Journal of Guangxi Medical University</i>	3	2
<i>International Journal of Medical Parasitic Diseases</i>	3	0
<i>Journal of Clinical Pediatrics</i>	3	0
<i>Journal of Tropical Diseases and Parasitology</i>	3	0

Table 4. Prevalence of *Blastocystis sp.* in general population in China

Location	Time	Method of diagnosis	No. examined	No. positive	Prevalence (%)	Subtypes	References
Yunnan	2019	PCR	289	13	4.50	ST1 (3), ST3 (8), ST4 (1), Unknown (1)	[24]
Henan	2019	Iodine	6706	3	0.04		[120]
Yunnan	2016	PCR	319	11	3.44	ST1 (11)	[48]
Guangxi	2013	Improved centrifugal sedimentation with hydrochloric acid and aether/ PCR	497	215	43.26	ST1 (25), ST6 (1), ST1+ST6 (1), Unknown (78)	[22] ^a
Guangxi	2011	Improved centrifugal sedimentation with hydrochloric acid and aether/ PCR	1366	360	26.35	ST1 (12), ST3 (2), ST4 (1), ST6 (1), ST7 (3), ST1+others (6), Unknown (12)	[121] ^b
Shaanxi	2011	Smear/Iodine	781	27	3.46		[122]
Shanghai	2007	PCR	1505	29	1.93	ST1 (6), ST2 (1), ST3 (17), ST6 (1), ST1+ST3 (2), Unknown (2)	[112]
Zhejiang	2007	PCR	170	10	5.88	ST1 (3), ST2 (1), ST3 (6)	[112]
Yunnan	2007	PCR	407	75	18.43	ST1 (22), ST2 (6), ST3 (38), ST1+ST3 (5), ST2+ST3 (1), Unknown (3)	[112]
Yunnan	2007	PCR	239	78	32.6	ST1 (16), ST2 (1), ST3 (55), ST4 (1), ST1+ST2 (1), ST1+ST3 (1), Unknown (3)	[123]

^a 105 samples of positive samples were cultured and PCR tested.

^b 37 of the positive samples were tested by PCR.

Table 5. Prevalence of *Blastocystis* sp. in animals in some provinces

Location	Method of diagnosis	Hosts	No. examined	No. positive	Prevalence (%)	Subtypes (n)	References
Nonhuman primates							
Guangxi	Formalin-ether Sedimentation	Rhesus monkey	150	29	19.33		[72]
Shaanxi	PCR	Chimpanzee and others	127	96	75.59	ST1 (31), ST2 (13), ST3 (12), ST5 (1), ST10 (1), ST13 (37), ST19* (1)	[61]
Birds							
Shaanxi	PCR	Ostrich	9	3	33.3	ST5 (1), ST10 (1), ST20* (1)	[61]
Shaanxi	Smear/Iodine	Crested Ibis	63	6	9.50		[124]
Sichuan	PCR	Black swan / Green peafowl / Ruddy Shelduck	64	7	10.9	ST8 (7)	[125]
		Pigeon / Domestic chicken / Red crowned crane et. al					
Heilongjiang	PCR		185	13	7.00	ST6 (8), ST7 (5)	[62]
Mammals							
Heilongjiang, Liaoning and Jilin	PCR	Reindeer / Raccoon dog / Domestic dog / Brown rat et. al	1080	41	3.8	ST1 (5), ST3 (3), ST4 (13), ST7 (1), ST10 (13), ST13 (4), ST14 (2)	[62]
Heilongjiang	PCR	Sheep	109	6	5.5	ST1 (1), ST5 (1), ST10 (3), ST14 (1)	[69]
Shaanxi	PCR	Goat	789	458	58.00	ST1 (1), ST3 (1), ST4 (9), ST5 (31), ST10 (292), ST14 (123), Unknown (1)	[64]
Shaanxi	PCR	Artiodactyla / Perissodactyla	176	93	52.84	ST1 (1), ST10 (67), ST12 (5), ST14 (17), ST18* (1), ST21* (1), ST22* (1)	[61]
Shaanxi	PCR	Gray kangaroo	11	8	72.2	ST10 (8)	[61]
Heilongjiang	PCR	Cattle	526	54	10.30	ST4 (2), ST5 (1), ST10 (41), ST14 (10)	[65]
Heilongjiang	PCR	Cattle	147	14	9.5	ST3 (2), ST10 (10), ST14 (2)	[69]

Table 5. (continued)

Qinghai	PCR	Yak	1027	278	27.07	ST10 (170), ST12 (38), ST14 (70)	[67]
Shaanxi	PCR	Pig	560	419	74.80	ST1 (15), ST3 (6), ST5 (397), ST10 (1)	[68]
Heilongjiang	PCR	Pig	68	6	8.8	ST5 (6)	[69]
Anhui, Zhejiang et.al	PCR	Cat	299	2	0.8	ST1 (2)	[63]
Hubei	Nested PCR	Flying squirrels	69	21	30.40	ST1 (8), ST3 (4), ST13 (9)	[126]
Sichuan	PCR	Red pandas	23	2	8.7	ST1 (2)	[125]
Sichuan	PCR	Giant panda	81	10	12.30	ST1 (10)	[125]

*Possible novel subtypes

Table 6. Prevalence of mammal *Blastocystis* sp. in different provinces/cities in China

	Heilongjiang	Jilin	Liaoning	Sichuan	Anhui	Jiangsu	Shandong	Shaanxi	Qinghai	Hubei
No. positive	110	8	3	12	48	44	20	978	278	21
No. examined	1682	143	118	104	799	225	120	1584	1027	69
Prevalence (%)	6.54	5.59	2.54	12.5	6.01	19.56	16.67	61.74	27.07	30.43

Figures

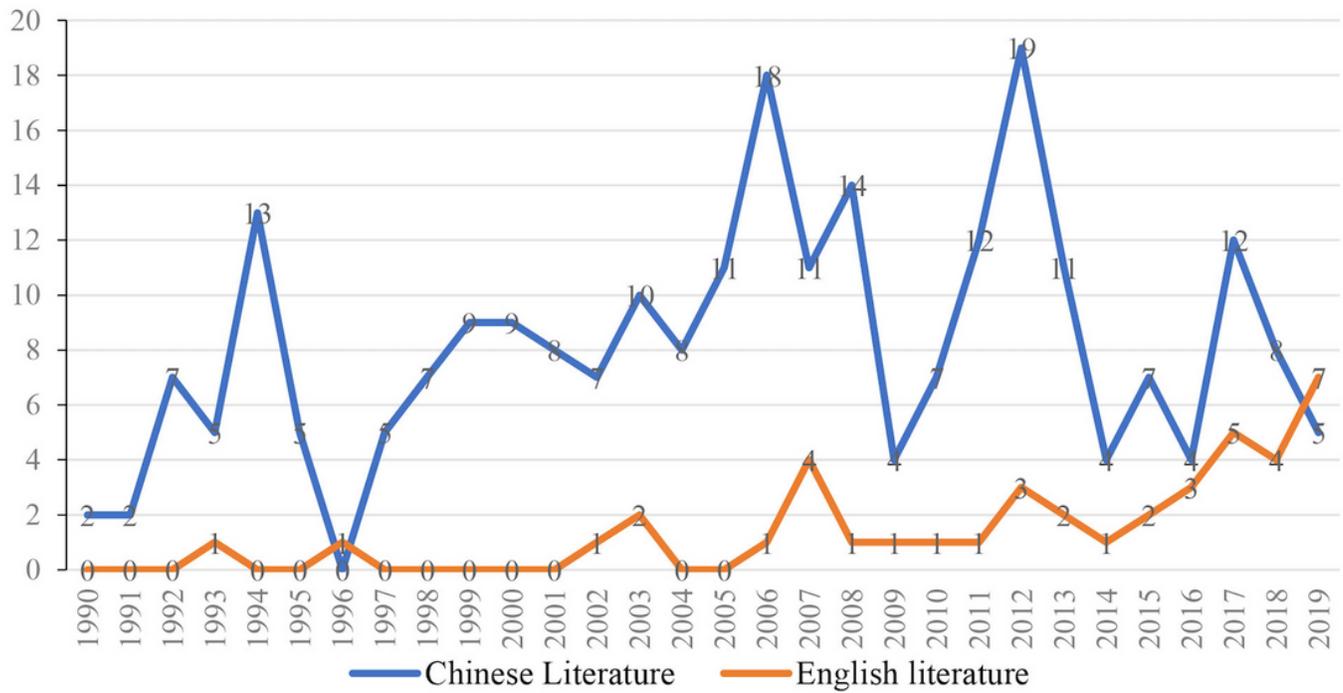


Figure 1

Chinese and English literatures on Blastocystis sp. in China from 1990 to 2019

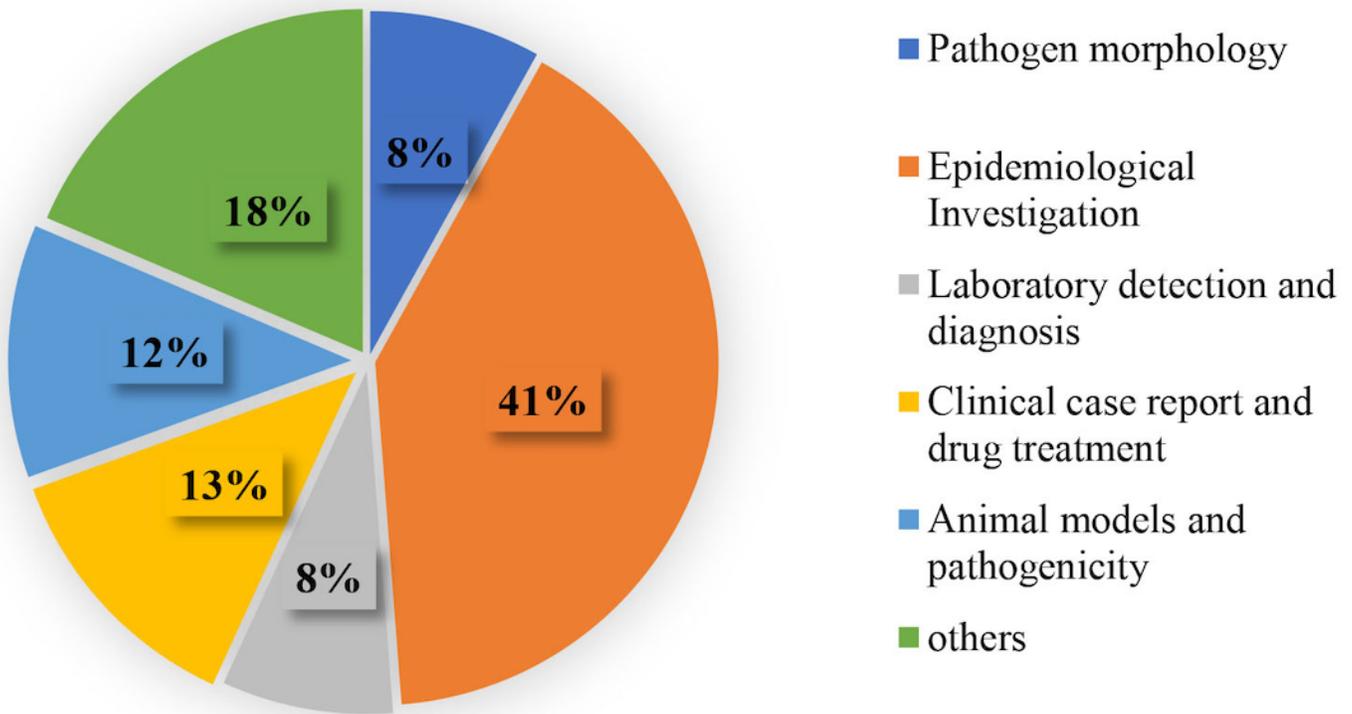


Figure 2

Proportion of different research directions

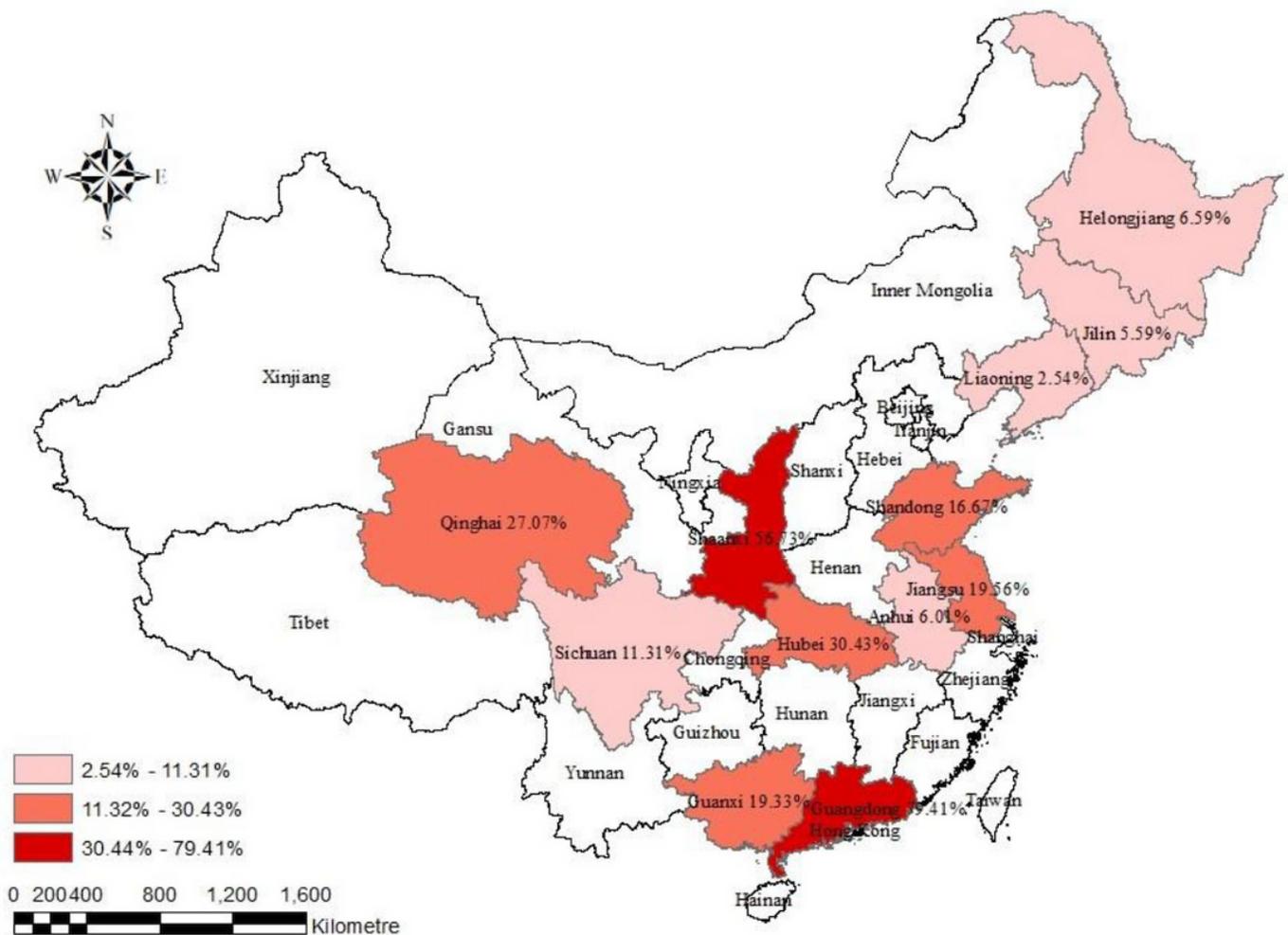


Figure 4

Prevalence of Blastocystis sp. of animal in different provinces in China. Note: The designations employed and the presentation of the material on this map do not imply the expression of any opinion whatsoever on the part of Research Square concerning the legal status of any country, territory, city or area or of its authorities, or concerning the delimitation of its frontiers or boundaries. This map has been provided by the authors.