Epidemiology of Blastocystis sp. infection from 1990 to 2019 in China

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Research Article

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Abstract

Background: Blastocystis is ubiquitous presence in animals and humans worldwide and has a high level genetic diversity. The aim of this study was to conduct a summary of Blastocystis prevalence, subtypes (STs) in humans and animals in China and depict their distribution.

Methods: We searched for the articles related to epidemiology of Blastocystis in humans and animals throughout China which published from January 1, 1990, to July 1, 2019 in the following databases: PubMed, China National Knowledge Infrastructure (CNKI) and Wanfang database. The keywords were Blastocystis and one of the following ones: STs, subtypes, distribution, epidemiology, prevalence, infection, molecular, geographic, intestinal parasites, genetic diversity and characterization.

Results: In recent years, various molecular epidemiological studies have been carried out in some provinces/regions of China to identify subtypes of Blastocystis. 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 infection in animal were also conducted in some provinces. A variety of animals were investigated including pigs, cattle, sheep, yak, giant panda, and Crested ibis (Nipponia nippon), et al., with the main subtypes of ST1–ST8, ST10, ST12–ST14.

Conclusion: In recent years, some provinces/regions in China have conducted various molecular epidemiological studies to identify the Blastocystis subtypes. It is important to focus on new subtypes and mixed subtypes of infection, while increasing data on ribosomal alleles. We encourage the scientific community to start research on humans and surrounding animals (including domestic and wild animals) to better understand the possibility of Blastocystis transmission between humans and animals. We call for action among researchers studying intestinal parasitic diseases (Blastocystis), start drawing the subtype of Blastocystis and increase the subtype related to its clinical symptoms.

1 Background

Blastocystis is widely distributed throughout the world. It is an anaerobic intestinal parasite that can infect humans and a variety of animals [1, 2]. Blastocystis is the most common intestinal protozoa in human fecal specimens, which probably due to its rapid propagation and survival ability in different hosts such as humans and animals. Similar to the transmission route of some intestinal protozoans, the route of Blastocystis both in humans and animals is via fecal-oral transmission, such as through contaminated water and food [3]. Based on gene analysis of small subunit of the ribosomal RNA (SSU-rRNA), wide genetic diversity is observed within Blastocystis, and multiple subtypes (STs) have been reported [4]. Currently, at least 17 subtypes are known, of which ST1 to ST8 and ST12 have been reported in humans [5, 6]; ST9 was exclusively identified in humans; and ST10–ST17 were identified only in animals [7]; novel subtypes are still being discovered [8]; and mixed-subtype infections of Blastocystis occurs [9]. ST1–ST4 commonly occur in humans, but ST4 is only reported in European region [10].

Clinical manifestations of Blastocystis are very diverse, including acute or chronic diarrhea, abdominal pain, nausea, anorexia, bloating, fatigue and flatulence [11, 12], along with allergy [13]. The prevalence of Blastocystis is high. Obviously, this parasite has a certain impact on human health, but its role in human health and disease is still uncertain [14]. A high rate of asymptomatic carriers exist in Blastocystis infections, and it is still uncertain whether the clinical symptoms of Blastocystis infection are related to a specific subtype or several subtypes of Blastocystis, or whether it is colonized by multiple parasites, causing the pathogenicity in Blastocystis under strong debate [2, 3]. But, Clark proposed that different subtypes may have different pathological potentials [15]. Kaneda et al. suggested that ST1, ST2, and ST4 may be related to gastrointestinal symptoms [16]. Blastocystis ST1 is regarded as a pathogenic subtype associated with irritable bowel syndrome diarrhea (IBS-D) [17]. Blastocystis ST3 is considered to be virulent, which not only increases the pathogenicity of this parasite, but also increases the level of IgE in the serum, thereby causing allergies [13]. Jimenez et al. [3] proposed that the pathogenicity of Blastocystis is still controversial for many reasons, for example, the high proportion of asymptomatic carriers, host susceptibility, differences in intestinal microbiota and different pathogenic potentials of different Blastocystis subtypes. The prevalence of Blastocystis is high in some areas of China, which is mainly attributed to use of earthen toilets or manure pits, contact with animal, drinking unboiled water directly and poor hygiene [18, 19]. Blastocystis can colonize the intestines of humans, domestic animals (cattle, sheep, goats and pigs) and wild animals, which has been observed in many provinces in China. Therefore, we describe herein our summary of the studies published about epidemiology of Blastocystis in humans and animals throughout China, and aim to depict the prevalence of Blastocystis in different provinces, display the distribution of Blastocystis subtypes among different hosts (humans and animals).

2 Methods

Search strategy

Geographically, the study domain was restricted to research in China. Studies reported in English and Chinese were selected. The information in the article includes whether Blastocystis and epidemiology (infection status and/or genotype) in humans or animals are mentioned.

Both PubMed and China National Knowledge Infrastructure (https://www.cnki.net/) and Wanfang (http://www.wanfangdata.com.cn/index.html) database were used to find potentially eligible articles. The articles about epidemiology of Blastocystis were searched in humans and animals throughout China, which were published from January 1, 2010, to July 31, 2019. In the search, we included keywords Blastocystis and one of the following keywords: STs, subtypes, distribution, epidemiology, prevalence, infection, molecular, geographic, intestinal parasites, genetic diversity and characterization. Duplicate studies from the three databases were removed. We excluded conference abstract papers, case reports, case series and review articles. For the same survey, multiple results may be published in different forms, and we select articles with complete results. A total of 215 articles were found, and only 82 of them met the above criteria (Fig. 1). More details of the 82 articles were displayed in Additional file Table E1, including title, author, journal.
Information extraction and analysis

Two investigators independently screened abstracts, full-text articles, performed data extraction. Data were extracted by the first investigator from the included articles, evaluated by the second investigator and final evaluation was conducted by the third investigator. The characteristics were extracted from each study, including the province of the samples, number of samples, number of samples positive for Blastocystis, host, detection method, subtype identification, number of samples per subtype, title, first author and year of publication. We extracted relevant information from each article that met the inclusion criteria of this study.

According to the different detection methods of Blastocystis in the study, the overall infection rate in China and the infection rate in each province were calculated, and revealed the distribution of subtypes in different provinces in China. At the same time, we could determine the distribution of subtypes in humans and different kinds of animals.

3 Results

Distribution of investigation of Blastocystis infection

For the study of Blastocystis infection in humans, we divide the humans into the general population, students, children, hospitalized or outpatients, diarrhea cases, and people with HIV or tuberculosis and other diseases. We have summarized the infection of Blastocystis in humans and animals, shown in Table 1 and Table 2.

Risk factors of Blastocystis infection in humans

The risk factors of Blastocystis infection are diverse. Several studies have shown that not washing hands after going to the toilet, drinking unboiled tap water, eating outside for a long time, raising poultry or livestock, low immune function, poor nutritional status, female, body mass index < 19, anemia and barefoot working in farm are the risk factors that cause Blastocystis infection [21, 28, 29, 32, 44, 45]. In addition, suffering from some underlying diseases such as Helicobacter pylori infection and hepatitis B are also risk factors for Blastocystis infection [46].

Infection status and subtypes of Blastocystis in general population

In general population, the infection status and subtype distribution of Blastocystis in China are shown in Table 1. The infection rate of Blastocystis in the population has regional differences. For example, surveys in different regions of Guangxi found that the infection rate of Blastocystis in Bama Yao Autonomous County was 43.26%, and the infection rate in Beihai and Qinzhou was 36.35% [24, 25]. The methods used in the two studies were the same and the subjects were the local general population. Yan et al. identified that the subtype of Blastocystis in humans was ST1–ST3. ST3 was the main subtype (40.0%), followed by ST1 (37.1%) the mixed subtype of ST1 and ST3 accounted for 14.3%, and unknown subtypes have also been found [47].

Blastocystis infection in students and children

There are few studies focus on Blastocystis infection in students. The infection rate of Blastocystis in primary and university students from Jiangxi was 1.1% and 10.09%, respectively [48, 49]. The prevalence of Blastocystis in college students in Guangxi was 14.93% [50]. Another study in Guangxi found that infection subtypes include ST1, ST3, ST4, ST6 and ST7, among which ST3 was the main subtype (32.08%) [51].

A study found that children in Jiangxi Province have a higher infection rate (35.9%) and have symptoms such as diarrhea and recurrent abdominal pain [52]. The infection rate of children with diarrhea in Yunnan (3.1%) and Fujian (8.94%) was lower than that in Jiangxi [53, 54]. Cao et al. performed fecal microscopy on children in Shenzhen Children's Hospital and found that the infection rate of Blastocystis was low (0.4%) [55].

Infection status and subtype of Blastocystis in inpatients or outpatient without distinction of disease

Some investigations related to Blastocystis infections were conducted in the hospitals, and the subjects were hospitalized or outpatients. These patients were randomly selected from the hospital. The infection rate of patients in the First Affiliated Hospital of Guangxi Medical University was 22.78%, and the infection rate was significant different in gender. The disease of these patients was not clear [56]. The study found that the infection rate of Blastocystis in hospitals in Nanning, Guangxi has little change over time. The prevalence of patients were 16.27% and 16.77%, respectively in 2005 and 2013 [57].

Blastocystis infection and its subtypes in patients with diarrhea

Blastocystis was one of the common pathogens in patients with diarrhea. The main manifestations of patients with Blastocystis include abdominal pain or diarrhea, followed by fatigue and anorexia. Some researchers have found that these gastrointestinal symptoms may be related to colitis [58]. Diarrhea patients have different infection rates in different seasons, and the subtype was mainly ST1 [23, 59]. There are few studies on the association between the subtypes of Blastocystis and clinical manifestations, some of which are related to the subtypes of Blastocystis in patients with diarrhea (Table 1).

Blastocystis infection in patients with underlying diseases

There are few studies on the co-infection of Blastocystis and underlying diseases (HIV patients, tuberculosis patients, cancer patients and chronic disease patients). The prevalence, clinical manifestation and subtypes of Blastocystis in patients with HIV/AIDS, pulmonary TB, cancer and chronic diseases show in Table 1. Blastocystis and HIV co-infection were studied in Yunnan, Anhui and Guangxi provinces. Studies have suggested that Blastocystis infection increases the level of IL-2 in HIV-infected persons, changes the Th1/Th2 balance, and accelerates the conversion of HIV infection to AIDS [60]. In China, ST12 infection was first detected in AIDS patients in 2018 [28]. There was no difference in the infection of Blastocystis in tuberculosis patients (6.2%) and the healthy group
(7.6%) [44]. The detection of cancer patients found that the infection rate of Blastocystis in lung, stomach, colorectal cancer patients was higher than that of other cancer patients, and the infection rate of cancer patients was significantly higher than that of the general population (malignant tumors: 43.24%, non-malignant tumors: 22.59%, 19.70% of the general population) [61]. The symptoms of diarrhea in cancer patients may be related to ST1 [30]. The infection rate of Blastocystis in patients with chronic disease was 18.29%, and clinical symptoms such as abdominal pain, diarrhea, and vomiting may occur [32].

**Distribution and infection of Blastocystis in animals**

There is a list of the distribution and genetic diversity of Blastocystis in different animals including non-human primates, birds, and mammals in China. Some provinces have performed genotyping of animal Blastocystis (Table 2). There are significant differences in the prevalence of Blastocystis in pigs in different regions and age groups [34]. The infection rate of Blastocystis in cats was low. Among the 346 cats surveyed in six provinces, only 2 cats were found to be infected with Blastocystis in Lu'an, Anhui Province [42]. Among animal Blastocystis isolates, the potential zoonotic subtype including ST1, ST3 and ST5 accounts for 38.5% [38]. At the same time, insects could also be infected, and the body surface and digestive tract of cockroach and housefly can be infected with Blastocystis [62].

**4 Discussion**

Based on the above information, we found that the infection of Blastocystis in different populations and regions is different. The infection rate of primary school students was lower than that of college students [48, 49]. It may be that under the management of parents and teachers, primary school students have developed good hygiene habits, such as washing hands frequently. The investigations of Blastocystis infection in hospitalized patients found that the infection rate in Guangxi was higher than that in other regions. This may be due to the help of doctors after local residents developed symptoms such as diarrhea, or different detection methods used in the study. The specific reasons need to be studied in depth [63]. Studies on patients with diarrhea have shown that ST1 is related to clinical symptoms such as diarrhea and has potential pathogenicity. The main subtype of diarrhea patients is ST1, and the main subtype of asymptomatic Blastocystis infection is ST3 [23, 30, 45]. CD4+ cell count ≤ 500 cells/μL, and an HIV-RNA viral load > 50 copies/ml were the influencing factors of Blastocystis infection in HIV-seropositive individuals [64].

Considering the subtype of Blastocystis in humans and animals, this study found that ST1–ST7 and ST12 were present in the sample of humans in China [28, 63, 65], of which a large number were typed most frequently as ST1, ST2 and ST3 [21, 51, 23, 66], including mixed subtypes of ST1 and ST3, ST1 and ST2, and ST2 and ST3 [20], followed by other subtypes in minor percentages. Foreign studies have found that ST1–ST9 of Blastocystis can infect humans [67]. However, it has not been found that ST8 and ST9 can infect humans in China. This study found that ST1–ST8 and ST10, as well as ST12–ST14 were present in the sample of animals in China (Table 2). Some foreign studies have found that ST1–ST7 can infect animals [67]. However, ST9, ST11, ST15–ST17 have not been found to infect animals in China. In China, ST10 predominates in animal infection [39, 40, 41, 68, 69], followed by ST5 [34]. This is different from some foreign studies. An Italian study found that the subtype of Blastocystis infection in dogs was ST3 [70]. The subtype of Blastocystis infection in animals from the United States was mainly ST8 (20.6%), followed by ST6 (17.3%) and ST5 (15.9%) [3]. Because ST1–ST8 subtypes can infect both humans and animals, there are studies abroad to explore the possibility of the transmission of Blastocystis between humans and animals. In 2019, a report in Lebanon believed that Blastocystis has a potential risk of transmission from livestock to its contacts. The study found that ST1, ST2, ST3 infected in cows and people who have been in contact with cows, and the sequence of ST3 is exactly the same between cows and their contacts [71]. Few studies conducted in this research field in China, and there is a lack of corresponding data. The most recent epidemiological data of Blastocystis and its subtypes are limited to reports from a few provinces in China [40, 53, 59], and most of these reports come from research conducted by certain institutions. We are considering summarizing the distribution of Blastocystis and its subtypes in various provinces in China. However, there are no data available in many provinces/regions. The fact that most of the research comes from a few provinces may be related to the fact that there are more investigators and enough attention. This shows that in underrepresented provinces, more investigators and more attention are needed to infer the true distribution of Blastocystis in different regions of China.

There are several limitations in our study. Firstly, most of our included studies were cross-sectional studies where selection bias may have occurred, for example, many research subjects are selected from hospitals, schools, etc. Secondly, the detection methods of Blastocystis in each study are different, and the primers and primer lengths are different when performing polymerase chain reaction (PCR) detection. Thirdly, some provinces have conducted fewer studies or only conducted studies in a certain urban area, and the majority of province no research on Blastocystis has been conducted, so some data are less representative. Finally, in addition to real differences, the possible reasons for the different infection rates among different regions may be attributed to the large time span of this study (1990–2019) and the possible differences in the results of the researcher's identification through microscopy.

**5 Conclusions**

In recent years, various molecular epidemiological studies have been conducted in some provinces/regions of China to identify the subtypes of Blastocystis. This study believes that it is important to focus on new subtypes and mixed subtypes of infection, while increasing data on ribosomal alleles. In addition, the relationship between Blastocystis subtypes and clinical symptoms should be studied. Finally, we should pay attention to the people and surrounding animals (including domestic and wild animals) to better explore the possibility and means of transmission of Blastocystis between humans and animals.

**Abbreviations**

STs, subtype; CNKI, China National Knowledge Infrastructure; HIV, human immunodeficiency virus; SSU-rRNA, Small Subunit Ribosomal RNA; PCR, Polymerase chain reaction.
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.

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Authors’ contributions
C-QN and L A performed the statistical analysis and drafted the paper. C-QN and L-GT conceived and designed the study. L A and L-GT critically reviewed the paper. H-ZH and C-JH modification to the paper. All authors read and approved the final version of the paper.

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Correspondence: Li-Guang Tian

References


Tables

Table 1 Infection and characteristics of *Blastocystis* in humans
<table>
<thead>
<tr>
<th>No.</th>
<th>Author, year, reference number</th>
<th>Study area (years of the survey)</th>
<th>Study design</th>
<th>Age range / mean or median</th>
<th>Sex (n)</th>
<th>Participants</th>
<th>No. positive (prevalence)</th>
<th>Method of diagnosis</th>
<th>Primer sequence (amplifi)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.</td>
<td>Li LH et al., 2007, [20]</td>
<td>Shanghai (2006)</td>
<td>Cross-sectional observational study</td>
<td>2-96 years / median</td>
<td>NA</td>
<td>1505 people living in villages</td>
<td>29 (1.93%)</td>
<td>PCR</td>
<td>NA</td>
</tr>
<tr>
<td>2.</td>
<td>Li LH et al., 2007, [20]</td>
<td>Eryuan county, Yunnan (2005)</td>
<td>Cross-sectional observational study</td>
<td>5-57 years / median</td>
<td>NA</td>
<td>407 people from villages</td>
<td>75 (18.43%)</td>
<td>PCR</td>
<td>NA</td>
</tr>
<tr>
<td>3.</td>
<td>Li LH et al., 2007, [20]</td>
<td>Yongjia county, Zhejiang (2006)</td>
<td>Cross-sectional observational study</td>
<td>4 months to 90 years / median</td>
<td>NA</td>
<td>170 in-patients</td>
<td>10 (5.88%)</td>
<td>PCR</td>
<td>NA</td>
</tr>
<tr>
<td>5.</td>
<td>Zhou Ruimin et al., 2019, [22]</td>
<td>Rural permanent population in Henan (2019)</td>
<td>Cross-sectional observational study</td>
<td>1-101 years / 38.95 years</td>
<td>Male (3191), female (3515)</td>
<td>6706 residents of Qinhuang Mountains Ecological Zone</td>
<td>3 (0.04%)</td>
<td>Iodine</td>
<td>NA</td>
</tr>
<tr>
<td>6.</td>
<td>ZHANG Shun-xian et al., 2016, [23]</td>
<td>Kunming, Yunnan (2014-2015)</td>
<td>Case-control study</td>
<td>Diarrhea cases (1121): 0-6 months (180), 6-12 months (263), 1-2 years (269), 2-5 years (132), 5-65 years (244), &gt; 65 years (27)</td>
<td>Diarrhea cases (1121): male (559), female (562), Non-diarrhea cases (319): male (165), female (154).</td>
<td>1121 diarrhoea cases and 319 non-diarrhoea cases from hospitals</td>
<td>Diarrhoea cases: 47 (4.19%), non-diarrhoea cases: 11 (3.44%)</td>
<td>PCR</td>
<td>Forward primer (5′ - GGAAGACCTCCTCTGACGA reverse primer (R): 5′ - GTCAAAAATGAAAGCAG ATCACGCTCTAATCCTCTATCGGCCACTTCTCTCAAT - TAGATTTGTGTTGGTGTTAGAT)</td>
</tr>
<tr>
<td>7.</td>
<td>He Shanzhan, 2013, [24]</td>
<td>Bama Yao autonomous county, Guangxi (2011)</td>
<td>Cross-sectional observational study</td>
<td>2-80 years / median</td>
<td>Male (253), female (244).</td>
<td>497 local residents</td>
<td>215 (43.26%)</td>
<td>Improved acid ether centrifugal sedimentation / PCR**</td>
<td>Forward primer (F): 5′ - GGAAGACCTCCTCTGACGA reverse primer (R): 5′ - GTCAAAAATGAAAGCAG ATCACGCTCTAATCCTCTATCGGCCACTTCTCTCAAT - TAGATTTGTGTTGGTGTTAGAT</td>
</tr>
<tr>
<td>8.</td>
<td>Yang Yan, 2011, [25]</td>
<td>Beihai City and Qinzhou City, Guangxi (2010)</td>
<td>Cross-sectional observational study</td>
<td>6 months to 91 years</td>
<td>Male (702), female (664).</td>
<td>1366 rural permanent population</td>
<td>360 (36.35%)</td>
<td>Improved acid ether centrifugal sedimentation / PCR*</td>
<td>Forward primer (F): 5′ - GGAAGACCTCCTCTGACGA reverse primer (R): 5′ - GTCAAAAATGAAAGCAG ATCACGCTCTAATCCTCTATCGGCCACTTCTCTCAAT - TAGATTTGTGTTGGTGTTAGAT</td>
</tr>
<tr>
<td>10.</td>
<td>Tian LG et al., 2012, [27]</td>
<td>Fuyang city, Anhui (2008)</td>
<td>Case-control study</td>
<td>HIV positives: 6-65 years / mean 42.8 years, HIV negative individuals: 6-65 years / mean 41.5 years</td>
<td>HIV positives: 143 males and 159 females, 303 HIV negative Individuals: 144 males and 159 females</td>
<td>302 HIV positives, 303 HIV negative Individuals</td>
<td>HIV positives: 49 (16.23%), HIV negative individuals: 67 (22.11%)</td>
<td>In vitro culture</td>
<td>NA</td>
</tr>
<tr>
<td>11.</td>
<td>Teng Xuejiao et al., 2018, [28]</td>
<td>Tengchong city, Yunnan (2016-2017)</td>
<td>Cross-sectional observational study</td>
<td>10-74 years / mean 40.4 years</td>
<td>HIV positives: male (157), female (167)</td>
<td>324</td>
<td>12 (3.70%)</td>
<td>PCR</td>
<td>Forward primer: 5′-GGAATATC-3′ reverse primer: 5′-ACTAGGAATTCTCGTTC</td>
</tr>
<tr>
<td>12.</td>
<td>Li XX et al., 2015, [29]</td>
<td>Gushi County, Henan (2012)</td>
<td>Cross-sectional observational study</td>
<td>≤ 60 years old (153), &gt; 60 years old (188) / median 62 years</td>
<td>Male (249), female (120)</td>
<td>369 Patients with pulmonary TB (PTB) undergoing anti-Mycobacterium tuberculosis treatment</td>
<td>22 (5.96%)</td>
<td>In vitro culture</td>
<td>NA</td>
</tr>
<tr>
<td>No.</td>
<td>Authors et al., Year</td>
<td>Location</td>
<td>Study Type</td>
<td>Age Range</td>
<td>Gender</td>
<td>Number of Patients</td>
<td>Conditions</td>
<td>Proportion</td>
<td>Test Method</td>
</tr>
<tr>
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<td>13.</td>
<td>Zhang W et al., 2017, [30]</td>
<td>Harbin city, Heilongjiang (2016-2017)</td>
<td>Cross-sectional observational study</td>
<td>25 to 84 years</td>
<td>Male (220), female (161)</td>
<td>381 cancer patients: lung (80), stomach (88), colorectal (49), liver (47), esophagus (29), breast (28), hematologic (22); other types of cancer (28)</td>
<td>27 (7.09%)</td>
<td>PCR</td>
<td>NA (600 bp)</td>
</tr>
<tr>
<td>14.</td>
<td>HU Ying et al., 2015, [31]</td>
<td>Nanning city, Guangxi (2013-2014)</td>
<td>Cross-sectional observational study</td>
<td>5 to 79 years</td>
<td>Male (378), female (305)</td>
<td>683 tumor patients: Digestive system (228), respiratory system (189), urinary system (128), nervous system (87), other tumor patients (31)</td>
<td>Smear: 29 (4.25%), improved acid ether centrifugal sedimentation: 83 (12.15%)</td>
<td>Smear/improved acid ether centrifugal sedimentation</td>
<td>NA</td>
</tr>
<tr>
<td>15.</td>
<td>HU Ying et al., 2017, [32]</td>
<td>Nanning city, Guangxi (2016)</td>
<td>Cross-sectional observational study</td>
<td>&lt; 42 years old (310), ≥ 42 years old (603)</td>
<td>Male (367), female (546)</td>
<td>913 patients with chronic diseases</td>
<td>Smear: 30 (3.29%), improved acid ether centrifugal sedimentation: 137 (15.01%)</td>
<td>Smear/improved acid ether centrifugal sedimentation</td>
<td>NA</td>
</tr>
</tbody>
</table>

* Number of symptomatic cases among patients infected with Blastocystis at China-Myanmar border

** 105 samples of positive samples were cultured and PCR tested

a 37 of the positive samples were tested by PCR

NA means not available

Table 2 Infection and characteristics of Blastocystis in animals in China
<table>
<thead>
<tr>
<th>No.</th>
<th>Author, reference number</th>
<th>Study area (years of the survey)</th>
<th>Hosts (age or gender)</th>
<th>No. positive</th>
<th>Prevalence (%)</th>
<th>Method of diagnosis</th>
<th>Sequence of primers (Product size)</th>
<th>Subtypes (a)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.</td>
<td>Nong Ziju et al., 2012, [33]</td>
<td>Guangxi (2010)</td>
<td>150 Rhesus monkeys (2.5–4 years)</td>
<td>29</td>
<td>19.33</td>
<td>Improved acid ether centrifugal sedimentation</td>
<td>NA</td>
<td>NA</td>
</tr>
<tr>
<td>4.</td>
<td>Deng L, et al., 2019, [36]</td>
<td>Sichuan (2017-2018)</td>
<td>81 giant pandas (&lt; 1.5 years: 4, 1.5-5.5 years: 23, &gt; 5 years: 54, Male 31, female 50, 23 red pandas, 64 birds</td>
<td>Giant pandas: 10/81, red pandas: 2/23, birds: 7/64</td>
<td>PCR</td>
<td>Forward primer: 5'-GGAGGTA GTGACAAATAATC-3', reverse primer: 5'-TGCTTCGCAGCTTG TTACATC-3', (600 bp)</td>
<td>NA</td>
<td>Giant pandas: ST1 (10), red pandas: ST1 (2), birds: ST8 (7)</td>
</tr>
<tr>
<td>8.</td>
<td>Zhu W, et al., 2017, [40]</td>
<td>Daqing city, Qiqihar city, Harbin city, Heilongjiang (2013-2014)</td>
<td>526 cattle: ≤ 3 months (n = 69), 3–12 months (n = 61), and aged &gt; 12 months (n = 66)</td>
<td>Cattle:54/526, Daqing city: 9/140, Qiqihar city: 0/190, Harbin city: 45/196</td>
<td>Cattle (10.27), Daqing city: 6.43, Qiqihar city: 0.00, Harbin city: 22.96</td>
<td>Nested PCR</td>
<td>RDI5 (5'-GGGATCCGATCTCTCCG CCGTCCAGTA-3') and RDI6 (5'-GGAGGTAAGTGGACAAATAATC-3') (1780 bp), 2F (55), GGAGGTAAGTGGACAAATAATC-3', reverse primer: 5'-TGCTTCGCAGCTTG TTACATC-3', (600 bp)</td>
<td>DAQ1 (3), ST14 (6). Harbin city: ST3 (24), ST5 (1), ST10 (38), ST14 (4)</td>
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<td>10.</td>
<td>Li W et al., 2019, [42]</td>
<td>Zhejiang, Anhui, Shanghai, Jiangsu, Shandong, Jiangxi (2015-2018)</td>
<td>346 cats (151 males and 195 females: ≤ 12 months (60) and &gt; 12 months (286)</td>
<td>2/346, Blastocystis was only observed in Lu’an, Anhui (22.2%, 2/9)</td>
<td>PCR</td>
<td>NA</td>
<td>ST1 (2)</td>
<td></td>
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<tr>
<td>11.</td>
<td>Xiao X et al., 2019, [43]</td>
<td>Enshi county, Hubei (2017)</td>
<td>69 flying squirrels (3 fecal samples per animal)</td>
<td>Fecal samples: 63/207, flying squirrels 21/69</td>
<td>30.43</td>
<td>Nested PCR</td>
<td>RDI5: 5'-GGAGGTAAGTGGACAAATAATC-3', reverse primer: 5'-TGCTTCGCAGCTTG TTACATC-3', (600 bp)</td>
<td>ST1 (24), ST3 (12), ST13 (27)</td>
</tr>
</tbody>
</table>
Possible novel subtypes
NA means not available

Figures

Figure 1
Flow diagram, literature search and screening process