Molecular Epidemiology and Subtype Distributions of *Blastocystis* Sp. in Humans and Non-Human Primates

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Abstract: *Blastocystis* sp. is an intestinal protozoan that could infect human and many other non-human mammal hosts worldwide. It is estimated that there are currently more than one billion infections in humans, however the importance of public health is still remain controversy. In the recent years, various molecular epidemiological studies have been applied in the world to identify subtypes of *Blastocystis* sp., due to its remarkable genetic diversity. *Blastocystis* sp. isolates from humans and animals have been divided into 22 subtypes (STs) based on the phylogeny of small subunit rRNA genes (SSU rDNA). In this review, we investigated the molecular epidemiology and assessed the subtype distributions of *Blastocystis* sp. in humans and non-human primates (NHPs). These findings will update the epidemiology and subtype of *Blastocystis* sp., and it will help to understand the current subtypes and zoonotic potential of *Blastocystis* sp..

Keywords: *Blastocystis* Sp, Epidemiology, Subtype, Zoonotic Potential, Humans, Non-Human Primates.

INTRODUCTION

Blastocystis sp. is a common anaerobic intestinal eukaryotes that found in humans and many other mammal animals, which reported that it related to urticarial and some intestinal diseases such as diarrhea and abdominal discomfort [1,2]. *Blastocystis* sp., as an obligate eukaryotic microorganism of the gut microbiota, has more than 1 billion individuals worldwide [3,4]. The spread is mainly via fecal-oral route or indirect contact, resulting in a higher prevalence rate in some places with poor sanitary conditions. As well as, it reported that some certain subtypes has a high prevalence rate in Irritable Bowel Syndrome (IBS) patients. To date, some studies have shown that *Blastocystis* sp. also has a higher infection rate in asymptomatic patients, which has led to some controversies about the pathogenicity and clinical manifestations of this parasite [5,6].

Since *Blastocystis* sp. was first discovered in humans in 1911, it was named *Blastocystis* hominis [7]. In the following decades, a large number of *Blastocystis* infections were identified in humans and animals by microscopic examination and in vitro culture techniques [8,9,10]. With the development of molecular detection and sequencing technology in the past two decades, it was found that has extensive genetic diversity and infects many other animals, and re-named as *Blastocystis* sp.. It was divided into several different subtypes (STs) by molecular subtyping of the SSU-rDNA, the most subsequent researchers followed the consensus nomenclature [11,12].

Herein, we reviewed the published literature that employed PCR-based detection to re-evaluate the epidemiology and subtyping of *Blastocystis* sp. in humans and non-human primates (NHPs), and it provide a data of the *Blastocystis* subtypes circulating in worldwide.

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DETECTION METHODS FOR *BLASTOCYSTIS* SP

Blastocystis sp. from humans and animals have been reported to be similar in microscopic morphology [13]. Although many morphological forms had been described, the significance of most is still not clear. The four recognized forms of *Blastocystis* sp. are the cyst, vacuolar, granular, and amoeboid forms, and the central vacuole forms is the most common form in laboratory culture and stool samples, but how the different forms transitioned is not yet known [14]. At present, there are still conflicting in the classification of *Blastocystis* sp. at the species level, since some researchers now believe that *Blastocystis* sp. is a common constituent of the healthy gut microbiota and it is associated with higher bacterial richness [4,15]. Before 2006, microscopy and vitro culture has been the most widely used method for identification of *Blastocystis* sp. [16]. Previously, the detection of *Blastocystis* sp. was most relied on staining techniques and in vitro culture, which lacks of information on pathogenicity of different STs of *Blastocystis* sp. between different hosts. Diagnosis of *Blastocystis* sp. in fecal samples is difficult due to its small size and polymorphism, requiring important professional knowledge and requires a lot of expertise [17].

Recently, molecular tools have been increasingly used for the identification, subtyping, zoonotic potential assesment for *Blastocystis* sp.. Molecular detection methods include conventional PCR and real-time PCR (qPCR) based on its small subunit rRNA genes (SSU rDNA), followed by Sanger sequencing, and PCR amplification with subtype-specific sequence-tagged-site (STS) sequence [18,19,20,21]. Although researchers have developed a large number of molecular detection methods, it has not been standardized [20]. A SYBR green qPCR which used the SSU rRNA gene for detection of *Blastocystis*-specific DNA, has proven to be the most sensitive detection method so far, and subsequent subtyping was performed by melting curve analysis [22].

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BLASTOCYSTIS SP. IN HUMANS

Blastocystis sp. infections in humans have been documented worldwide, involving 56 countries, mainly in Asia and Europe (Table **1)**. Globally, the average prevalence of *Blastocystis* sp. infections in humans were 24.54% (14181/57781) by molecular identification (Table **1**). The average infection rate of *Blastocystis* sp. in humans varies across continents, with the highest rate of 52.46% in North America [23,24,25,26], and the Oceania with the lowest infection rate at 21.53% [27,28].

Thus far, ten subtypes (ST1-ST9, ST12) have been reported in humans. In general, subtypes ST1 and ST3 represent the most widely distributed subtypes in humans (Fig **1**). However, there are some differences by regions, indicating that an association between subtypes and human hosts. In Europe, the subtypes of *Blastocystis* sp. are mainly ST1-ST4 in humans, while in Asia and Africa are mainly ST1-ST3 (Fig **1**). Data are scarce from Oceania: in Australia, ST1 (31.84%) and ST3 (44.84%) were detected in humans, and ST2, ST4-ST8 with a lower infection rate [27,28,29,30,31]. However, *Blastocystis* sp. ST12 was only identified in two reports in China and Bolivia [32,33].

In some countries of Asia, Africa, South America and North America, children and immunocompromised patients seem to have a higher risk of *Blastocystis* sp. infection than the general population [34,35]. *Blastocystis* sp. had a high prevalence of infection (74.2%) among 1-12 years old childrenwere reported worldwide [23,36,37,38,39,40,41]. In Europe, *Blastocystis* sp. infection is mostly reported in travelers and immigrants, it found that some subtypes in Europe may be related to immigration and tourism, such as ST1 [29, 42, 43, 44]. In addition, a study in Italy found that mixed subtypes of *Blastocystis* sp. and co-infection with other parasites are common in humans, especially for Dientamoeba fragile [44]. Interestingly, a rare ST5 subtype in humans was found in piggery staff who were in close contact with pigs, which suggests that close contact may increase the risk of zoonotic transmission of *Blastocystis* sp. [30]. Although local subtype distribution may significantly varied, 90–95% of human infections could be attributed to one of the ST1-ST3, with a predominance of ST3 (Fig. **2a**).

BLASTOCYSTIS SP. IN NHPS

Blastocystis sp. has been isolated from NHPs all over the world, including China, Bangladesh, Thailand, Japan, Tanzania, Mexico, Spain and other countries, and most of the documents were concentrated in Asia (Table **2**). The average molecular prevalence of *Blastocystis* sp. infection was 45.14% (1379/3055) in NHPs worldwide. *Blastocystis* sp. infection rates in NHPs vary across the world (1.75%-100%), and it seems has a higher prevalence in China, particularly in macaques [45,46,47]. Stensvold[48] found *Blastocystis* sp. ST8 has a high prevalence (4/16, 25.00%) among primate handlers, which is very rare in humans, indicating that close contact will increase the risk of transmission. Similarly, ST1-ST3 infection in zoo animals and staff were also reported in southeastern Brazil and the sequences were highly consistent, highlighting that these STs have zoonotic potential [49]. However, it was not confirmed in other reports [29,50]. The pathogenicity of *Blastocystis* sp. in NHPs is still controversial, since most of the reported infections are asymptomatic individuals [29,51]. In general, non-human primates seem to be more susceptible than humans for *Blastocystis* sp..

At the subtype distribution, a total of 13 subtypes including ST1-ST5, ST7-ST10, ST13, ST15, ST17, ST19 have been identified in NHPs (Fig. **2b**). The subtypes with higher prevalence are ST1, ST2, and ST3, which are similar to the subtypes that infect humans. Interestingly, *Blastocystis* sp. ST9 reported in previous studies that only infects humans has also been identified in NHPs [52]. And a more recent study showed that ST17, which is rare in non-human primates, has a higher infection rate in squirrel monkeys, and this is the first time that *Blastocystis* sp. has been identified in squirrel monkey [47]. In two previous studies in China, *Blastocystis* sp. appears to have more subtype diversity in wild NHPs than captive ones [46,52]. The role of NHPs in the zoonotic transmission of *Blastocystis* sp. is still unclear, and more further investigations is needed to clarify this mystery in the future.

PHYLOGENY OF *BLASTOCYSTIS* SP.

Based on the lengths of complete sequences small subunit rRNA (SSU rRNA) gene, human and animal *Blastocystis* sp. isolates have been divided into 22 subtypes (STs, ST1-ST17, ST22, ST23-ST26), four new STs (ST18-ST21) were also proposed, however their validity is controversial, it is likely to be molecular chimaeras and further data is needed to confirm [13]. At present, the commonly used subtype identification methods of *Blastocystis* sp. are mainly through sequencing of SSU-rDNA Polymerase chain reaction (PCR) products and PCR with subtype-specific sequence-tagged-site (STS) diagnostic primer (Table **3**) [53,54]. In terms of suitability and sensitivity, SSU rDNA-based subtyping of *Blastocystis* sp. is unquestionably the best method of choice [53]. This barcode region is amplified by a broad eukaryotic specific primer RD5 and a stricter specific primer BhRDr in ~600 bp at the 5’-end of the SSU rDNA gene [17]. For *Blastocystis* STs, there are 22 genetically distinct strains recognized, ten of which (ST1-ST9, ST12) have been found in humans [55]. More than 90% of human infections belong to *Blastocystis* sp. ST1-ST3, except for ST9, the rest STs found in humans are also found in other animals [3,56,57].

Blastocystis sp. was previously considered as a fungus, and it was identified as an unusual Stramenopiles until 1996[58], since this group is named for the straw-like tubular hairs on the flagella. The *Blastocystis* sp. is usually a multinucleated, spherical cell with a diameter of about 5-10 microns and has typical eukaryotic characteristics, and the isolates no significant morphological differences in different hosts [59].

So far, the full-length SSU rDNA gene sequence of *Blastocystis* sp. ST1-ST17 has been obtained, except for ST11. Coincidentally, the missing region of ST11 is the barcode region used for subtype identification, which leads to a new subtype proposed based on the novel barcode sequence, may be the missing region of ST11 [13]. Thence the criterion for identification of new subtypes is that 80% of the SSU rRNA genes have been sequenced and the difference between the SSU rRNA genes of the previously sequenced complete blastocysts by more than 4% can be designated as a new subtype [59]. In this paper, a phylogenetic tree was produced using barcode regions of SSU rDNA gene sequences obtained from currently known *Blastocystis* sp. (ST1-ST17) in which significant ribosomal RNA gene diversity and host specificity among *Blastocystis* sp. isolate has been found (Fig. **3**). Although the authenticity of ST18-ST26 is still controversial, the next new subtype is proposed to be named ST27 according to the proposed Guidelines for the *Blastocystis* sp. Subtypes [13].

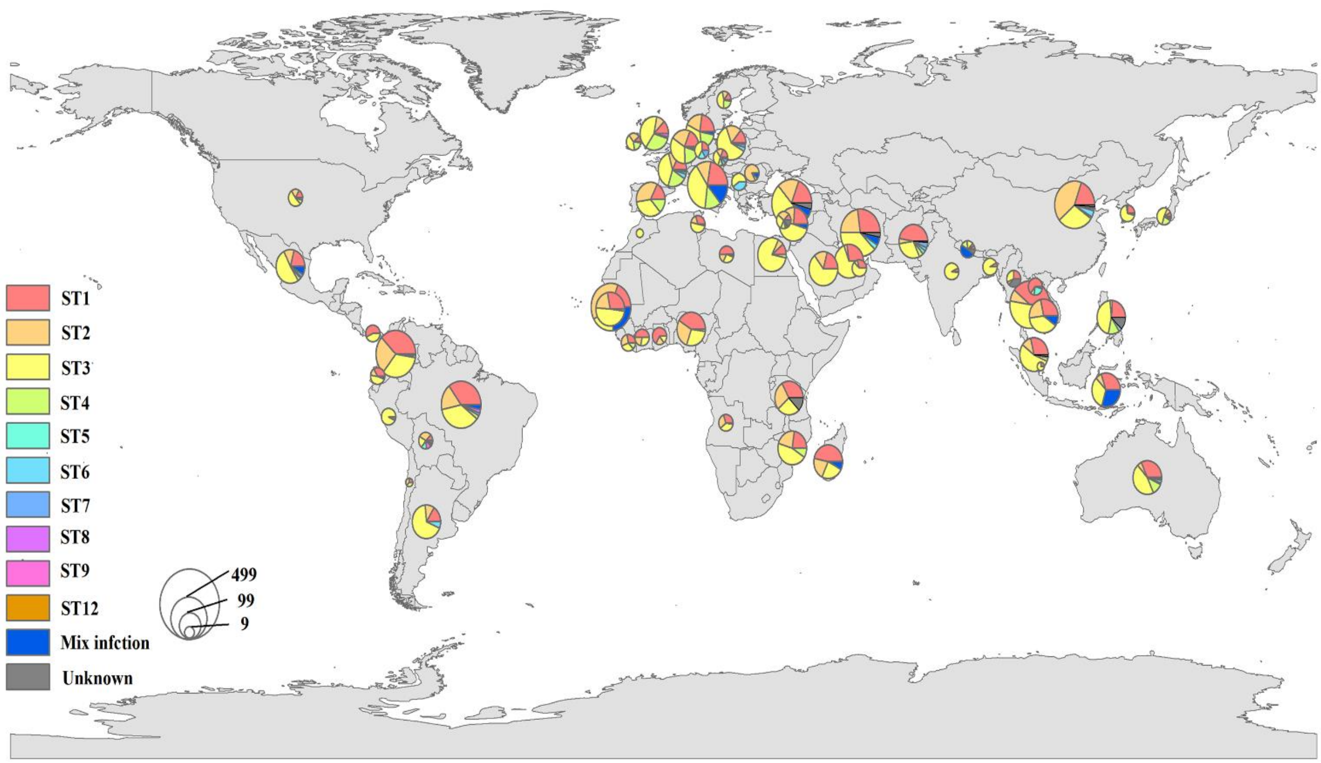

Figure 1: Epidemiology and Subtype di stribution of *Blastocystis* sp. in humans, as determined by molecular methods. The size of the circle represents the number of *Blastocystis* sp. subtypes identified in the region.

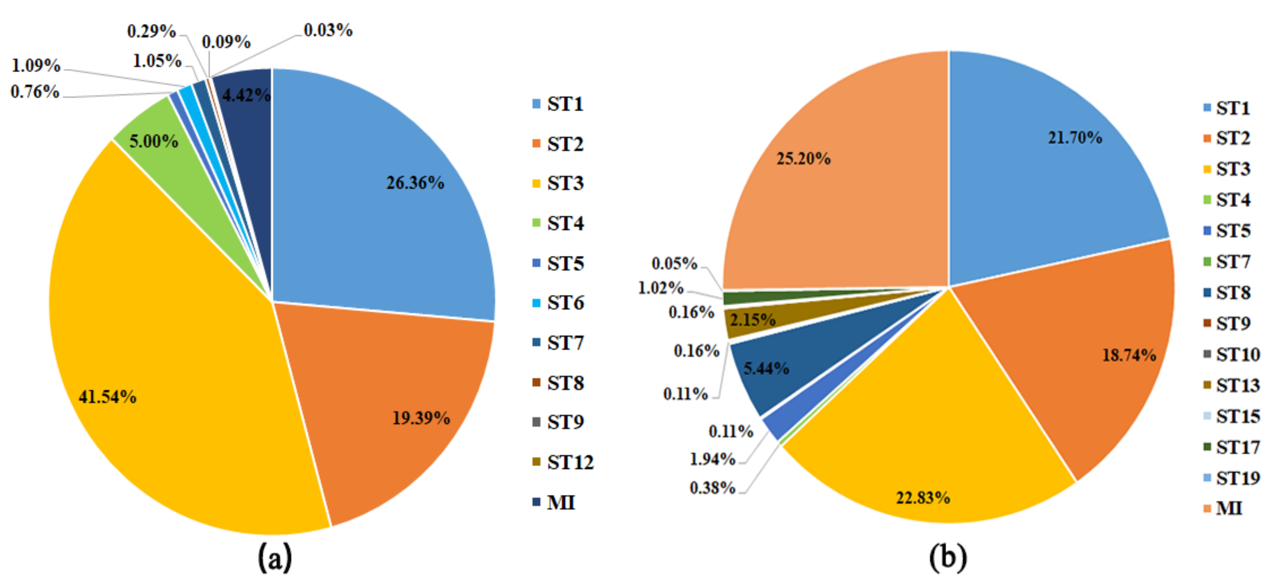

Figure 2: Distributions of *Blastocystis* sp. subtype in humans and non-human primates. a) *Blastocystis* sp. subtype distributions in humans; b) *Blastocystis* sp. subtype distributions in non-human primates.

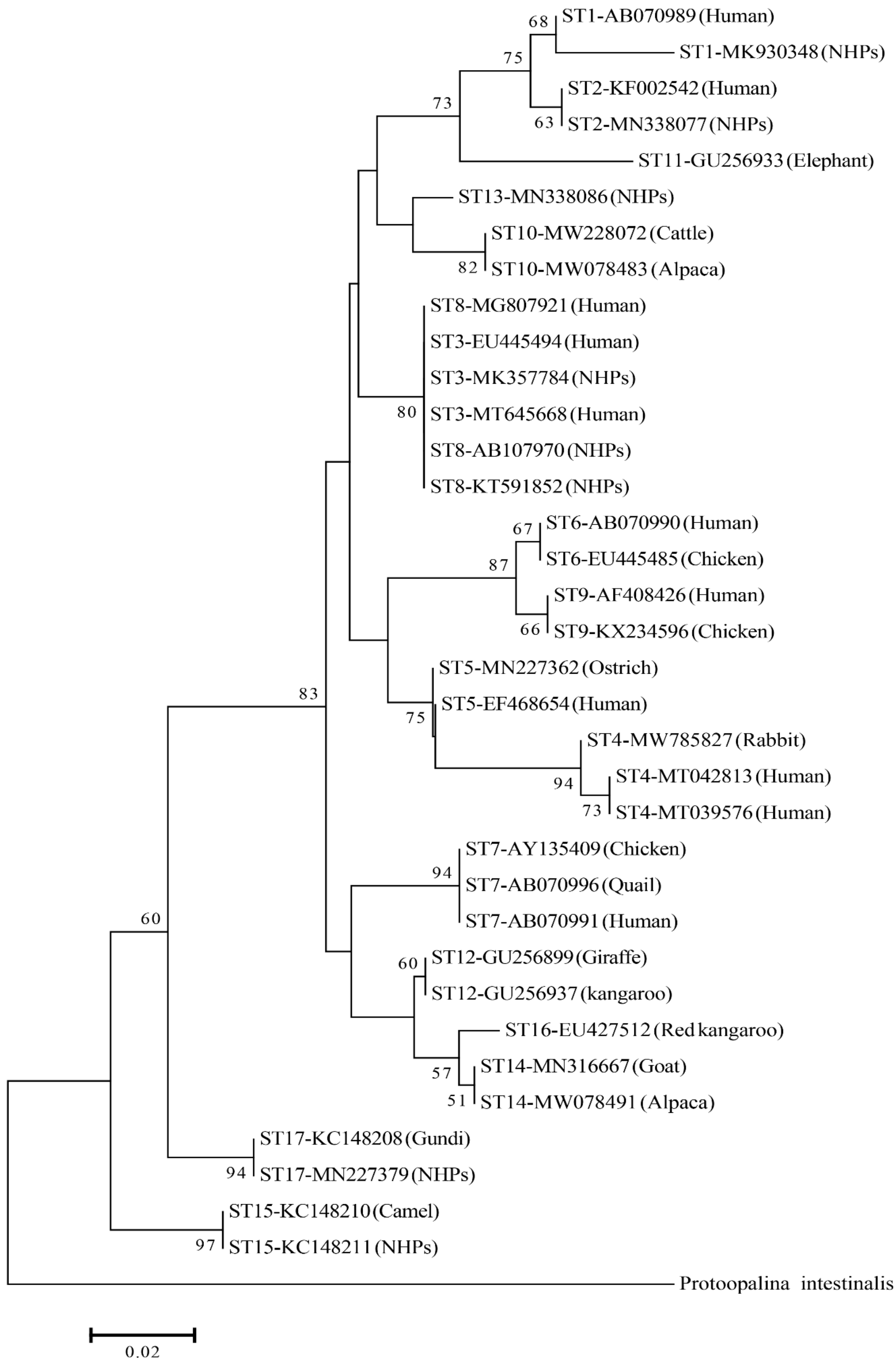

Figure 3: Phylogenetic relationships among of barcode regions of small subunit rRNA gene (SSU rDNA) sequences of *Blastocystis* sp. subtypes (ST) between 1 and 17. The tree shown was analysed using the neighbor-joining method, and the trees by the Kimura-2-parameter model. The numbers on the branches are percent bootstrapping values from 1000 replicates, with values > 50% shown in the tree.

Table 1: Prevalence, geographic and subtybe distribution of *Blastocystis* sp. in humans.

Country	Sample origin	No. positive/No. examined	Subtype(n)	References
Asia				
China	Adult patients	3/126	ST5(3)	Zhu et al., 2020
China	Human volunteers	390/1118	ST2(389) ST5(1)	Ma et al., 2020
China	Kindergarten children	87/609	ST1(38) ST2(8) ST3(41)	Qi et al., 2020
China	Primary students	71/466	ST1(10) ST3(18) ST6(32) ST7(11)	Ning et al., 2020
China	Individuals	48/507	ST1(16) ST2(1) ST3(24) ST4(7)	Deng et al., 2020
China	Hospitalized patients	21/198	ST1(2) ST3(8) ST6(10) ST7(1)	Kang et al., 2019
China	HIV-positive patients	12/324	ST1(3) ST3(2) ST4(3) ST7(3) ST12(1)	Teng et al., 2018
China	Cancer Patients	27/381	ST1(12) ST3(15)	Zhang et al., 2017
China	Diarrhea patients	47/1121	ST1(46) ST2(1)	Zhang et al., 2016
China	Humans	3/3	ST5(2) Mixed ST3/ST5(1)	Yan et al., 2007

China	Village individuals	78/239	ST1(16) ST2(1) ST3(55) ST4(1) Mixed ST1/ST2(1) Mixed ST1/ST3(1) Unknown(3)	Li et al., 2007a
China	Humans	192/2321	ST1(47) ST2(9) ST3(116) ST4(1) ST6(1) Mixed ST1/ST2(1) Mixed ST1/ST3(8) Mixed ST2/ST3(1) Unknown(8)	Li et al., 2007b
China	Yao people	13/289	ST1(3) ST3(8) ST4(1) Unknown(1)	Gong et al., 2019
Myanmar	Wa people	16/172	ST1(4) ST3(5) ST4(1) Unknown(6)	
Iran	Patients	152/1878	ST1(4) ST2(7) ST3(10) ST7(1)	Salehi et al., 2021
Iran	Patients	10/100	ST1(2) ST2(5) ST6(1)	Sharifi et al., 2020
Iran	Astrointestinal symptomatic and asymptomatic patients	68/864	ST1(21) ST2(14) ST3(11)	Delshad et al., 2020

Iran	Patients	45/184	ST1(8) ST2(10) ST3(10) Mixed ST1/ST3(1) Unclassified(13)	Shirvani et al., 2020
Iran	Children	36/864	ST1(20) ST2(10) ST3(6)	Niaraki et al., 2020
Iran	Patients	239/1383	ST1(2) ST2(6) ST3(16)	Bahrami et al., 2020
Iran	Cancer children and adolescents	24/200	ST1(5) ST2(8) ST3(9) ST7(2)	Asghari et al., 2020
Iran	Tuberculosis patients	13/161	ST1(7) ST2(5) ST3(1)	Taghipour et al., 2019
Iran	Gastrointestinal patients and healthy individuals	85/354	ST1(8) ST2(8) ST3(25)	Mardani Kataki et al., 2019
Iran	*Blastocystis*-positive symptomatic/asymptomatic patients	55/55	ST1(13) ST2(14) ST3(25) ST6(2) ST7(1)	Rezaei Riabi et al., 2018
Iran	HIV-positive patients	51/286	ST1(11) ST2(6) ST3(29) ST6(2) Mixed ST1/ST3(3)	Piranshahi et al., 2018

Iran	Patients	69/481	ST1(11) ST2(3) ST3(20) ST4(1) ST5(4) Mixed ST1/ST3(3) Mixed ST1/ST4(3) Mixed ST3/ST4(5)	Khademvatan et al., 2018
Iran	Human	146/618	ST1(5) ST2(5) ST3(14)	Salehi et al., 2017
Iran	IBD patients	9/69	ST1(1) ST3(7)	Mirjalali et al., 2017
Iran	Healthy individuals	35/166	ST1(14) ST2(12) ST3(9)	Mirjalali et al., 2017
Iran	IBS patients	24/122	ST1(9) ST3(12) ST4(2) ST5(1)	Khademvatan et al., 2017
Iran	Healthy individuals	21/122	ST1(8) ST3(9) ST4(1) ST5(3)	Khademvatan et al., 2017
Iran	Diarrheic and Non-diarrheic Patients	63/300	ST1(21) ST2(23) ST3(19)	Jalallou et al., 2017
Iran	Patients	55/282	ST1(9) ST2(20) ST3(25)	Beiromvand et al., 2017
Iran	Diarrheic and non-diarrheic patients	58/58	ST1(18) ST2(21) ST3(19)	Alinaghizade et al., 2017
Iran	IBS patients	26/80	ST1(3) ST2(5) ST3(8) Unknown(10)	Azizian et al., 2016

Iran	Symptomatic and asymptomatic individuals	100/420	ST1(21) ST3(25) ST5(21) Mixed infections(33)	Moosavi et al., 2012
Lebanon	Syrian refugees	195/306	ST1(43) ST2(31) ST3(89)	Khaled et al., 2021
Lebanon	Slaughterhouse staff members and Hospital patients	65/100	ST1(8) ST2(11) ST3(35) ST6(2)	Greige et al., 2018
Malaysia	Migrant workers	22/220	ST1(8) ST2(2) ST3(12)	Sahimin et al., 2020
Malaysia	Humans	45/243	ST1(14) ST2(7) ST3(24)	Mohammad et al., 2018
Malaysia	Subjects	191/473	ST1(63) ST2(27) ST3(98) ST4(3)	Noradilah et al., 2017
Malaysia	Schoolchildren	186/1760	ST1(42) ST2(13) ST3(101) ST4(13) ST5(6) Unknown(7)	Nithyamathi et al., 2016
Korea	Diarrheal and non-diarrheal Human	29/324	ST1(7) ST2(1) ST3(20) Multiple infections(1)	Kim et al., 2020
Thailand	Diabetes Mellitus Patients	16/130	ST1(4) ST3(12)	Popruk et al., 2020
	Non- Diabetes Mellitus human	9/100	ST1(2) ST3(6) ST4(1)	

Thailand	Students	416/1025	ST1(130) ST2(42) ST3(244)	Srichaipon et al., 2019
Thailand	Children students	17/357	ST1()	Ruang-areerate et al., 2019
Thailand	Asymptomatic adults	41/178	ST1(7) ST2(1) ST3(28) ST4(1) ST6(1) ST7(3)	Yowang et al., 2018
Thailand	Individuals who lived near pig farms	10/154	ST1(4) ST3(2) ST5(4)	Pintong et al., 2018
Thailand	Villagers	13/220	ST2(1) ST3(11) ST6(1)	Palasuwan et al., 2016
Thailand	Hospitalized Patients	20/20	ST1(4) ST3(12) ST6(2) ST7(2)	Sanpool et al., 2015
Thailand	Villagers	77/207	ST1(24) ST2(11) ST3(41) ST4(1)	Popruk et al., 2015
Thailand	Child care center children and staff members	35/258	ST1(3) ST2(4) ST3(28)	Pipatsatitpong et al., 2015
Thailand	Orphans	44/331	ST1(9) ST3(33) Unknown(2)	Boondit et al., 2014
Thailand	Home for Girls	118/370	ST1(112) ST2(4) ST6(2)	Thathaisong et al., 2013
Thailand	Patients	56/562	ST1(12) ST3(32) ST6(2) ST7(10)	Jantermtor et al., 2013
Thailand	Schoolchildren	126/675	ST1(53) ST2(15)	Leelayoova et al., 2008

Thailand	Patients	4/4	ST1(1) ST3(1) ST4(1) Mixed ST1/ST3(1)	Yoshikawa et al., 2004
Lao PDR	Asymptomatic individuals	32/60	ST1(16) ST3(2) ST5(6) ST7(1)	Sanpool et al., 2017
Cambodia	Villagers	116/210	ST1(33) ST2(27) ST3(45) Mixed infections(11)	Wang et al., 2014
Turkey	Patients	11/67	ST1(2) ST2(2) ST3(2)	Sarzhanov et al., 2021
Turkey	Cancer Patients	29/201	ST1(3) ST2(5) ST3(6) Mixed ST1/ST2(1)	Mulayim et al., 2021
Turkey	Pregnant women	14/100	ST1(3) ST2(2) ST3(9)	Malatyali et al., 2020
Turkey	Hemodialysis patients	8/84	ST1(1) ST2(2) ST3(4)	Gulhan et al., 2020
Turkey	Urticarial patients	16/133	ST1(4) ST2(1) ST3(11)	Aydin et al., 2019
Turkey	Human volunteers	11/35	ST1(2) ST2(4) ST3(5)	Karasartova et al., 2018
Turkey	School-aged Children	35/468	ST1(11) ST2(9) ST3(12) ST7(1)	Sankur et al., 2017

Turkey	Children	115/303	ST1(12) ST2(4) ST3(20) ST4(5) Mixed ST1/ST3(3) Mixed ST1/ST2(1) Mixed ST2/ST3(1)	Dogan et al., 2017
Turkey	Patients	8/37	ST1(1) ST2(4) ST3(3)	Yildiz et al., 2016
Turkey	Cancer patients	25/232	ST1(5) ST2(4) ST3(13) Unknown ST(3)	Yersal et al., 2016
Turkey	Rural and urban population	28/56	ST1(10) ST2(5) ST3(7) ST4(3) ST5(1) ST6(1) ST7(1)	Koltas and Eroglu, 2016
Turkey	Post-traumatic splenectomized patients	6/30	ST1(3) ST3(3)	Karasartova et al., 2016
Turkey	Ulcerative colitis patients	12/150	ST1(2) ST2(1) ST3(8) ST7(1)	Coskun et al., 2016
Turkey	Diarrhea and without diarrhea patients	43/350	ST1(6) ST2(3) ST3(12) ST4(5) ST6(1) ST7(5) Mixed infections(11)	Korkmaz et al., 2015

Turkey	Positive *Blastocystis* patients	44/61	ST1(9) ST2(13) ST3(17) Mixed ST1/ST3(4) Mixed ST1/ST2(1)	Ertug et al., 2015
Turkey	Symptomatic patients	94/617	ST1(13) ST2(11) ST3(42) ST6(1) ST7(1) Mixed ST2/ST3(2) Unknown(24)	Dagci et al., 2014
Turkey	Positive *Blastocystis* patients	25/25	ST1(9) ST2(6) ST3(10)	Eroglu and Koltas, 2010
Turkey	Asymptomatic and symptomatic patients	32/32	ST2(3) ST3(29)	Eroglu et al., 2009
Turkey	Symptomatic and asymptomatic individuals	66/203	ST1(10) ST2(9) ST3(38) Mixed ST1/ST2(2) Mixed ST1/ST3(7)	Dogruman-Al et al., 2009
Turkey	Symptomatic and asymptomatic individuals	87/87	ST1(8) ST2(12) ST3(66) ST4(1)	Ozyurt et al., 2008
Turkey	Symptomatic and asymptomatic patients	92/286	ST1(17) ST2(20) ST3(51) Mixed ST1/ST3(2) Mixed ST2/ST3(2)	Dogruman-Al et al., 2008

North Cyprus	Volunteers	64/230	ST1(8) ST2(18) ST3(20) ST4(7) ST6(3) ST7(2) non-ST(6)	Seyer et al., 2017
Indonesia	Primary School Children	18/141	ST1(5) ST3(10) ST4(1)	Sari et al., 2018
Indonesia	Children	147/492	ST1(40) ST2(2) ST3(29) Mixed ST1/ST3(31) Mixed ST1/ST2(6) Mixed ST2/ST3(8) Mixed ST1+ST2/ST3(2)	Yoshikawa et al., 2016
Indonesia	Immigrant workers	28/128	ST1(3) ST2(9) ST3(13)	Lee et al., 2013
Saudi Arabia	Patients	133/1262	ST1(19) ST2(7) ST3(107)	Mohamed et al., 2017a
Saudi Arabia	Cancer patients and non-cancer patient	50/218	ST1(19) ST2(20) ST3(11)	Mohamed et al., 2017b
Lebanon	Schoolchildren	157/249	ST1(35) ST2(39) ST3(64) mixed infection(19)	Osman et al., 2016
Lebanon	Lebanese symptomatic and asymptomatic patients	36/220	ST1(11) ST2(12) ST3(12) ST4(1)	El Safadi et al., 2013
India	Irritable Bowel Syndrome Patients	65/250	ST1(6) ST3(59)	Das et al., 2016

India	Healthy individuals	27/220	ST1(No description) ST3(No description) Mixed ST1/ST3(2)	Pandey et al., 2015
Philippines	Humans	195/1271	ST1(44) ST2(6) ST3(81) ST4(29) ST5(8) Unknown(27)	Belleza et al., 2016
Philippines	Asymptomatic individuals	29/35	ST1(9) ST3(19) ST4(1)	Adao et al., 2016
Philippines	Human infect with *Blastocystis* sp.	5/5	ST1(1) ST3(4)	Rivera, 2008
United Arab Emirates	Healthy individuals	59/133	ST1(11) ST2(3) ST3(23)	AbuOdeh et al., 2016
Qatar	Immigrant workers	114/608	ST1(31) ST2(4) ST3(79)	Abu-Madi et al., 2015
Pakistan	IBS-D patients and healthy control	134/315	ST1(87) ST2(10) ST3(49) ST4(8) ST5(7) ST6(6) ST7(10) Unknown(2)	Yakoob et al., 2010
Pakistan	Humans	10/10	ST1(2) ST3(7) ST4(1)	Yoshikawa et al., 2004
Nepal	Children	21/82	ST1(4) ST2(4) ST3(12)	Yoshikawa et al., 2009

Nepal	Rural communities	63/241	ST1(2) ST4(12) Mixed ST1/ST4(33) Mixed ST2/ST4(3) Mixed ST1/ST2/ST4(5) Unkonwn(8)	Lee et al., 2012
Singapore	Hospital patients	9/276	ST1(2) ST3(7)	Wong et al., 2008
Bangladesh	Symptomatic and asymptomatic patients	26/26	ST1(2) ST3(24)	Yoshikawa et al., 2004
Japan	Patients	50/50	ST1(4) ST2(5) ST3(26) ST4(11) ST7(2) Unknown(2)	Yoshikawa et al., 2004
South America				
Argentina	Asymptomatic patients	65/65	ST1(11) ST2(10) ST3(41) ST6(3)	Ramirez et al., 2016
Argentina	Patients	67/270	ST1(10) ST2(4) ST3(48) ST6(5)	Casero et al., 2015
Brazil	Transplant candidates	24/150	ST1(9) ST2(3) ST3(11) ST7(1)	Silva et al., 2020

Brazil	Patients with diabetes mellitus	34/99	ST1(13) ST2(4) ST3(12) ST6(1) ST7(1) ST8(3)	Melo et al., 2021
	Control group	23/76	ST1(5) ST2(5) ST3(10) ST6(1) ST8(2)	
Colombia	Human	94/100	ST1(47) ST2(31) ST3(16)	Potes-Morales et al., 2020
Colombia	Human	336/649	ST1(48) ST2(21) ST3(43) ST8(1) ST9(3)	Higuera et al., 2020
Colombia	Indigenous Children	252/284	ST1(59) ST2(62) ST3(70) ST4(6) ST6(1) non-identified subtype(5)	Sanchez et al., 2017
Colombia	Children	256/2026	ST1(55) ST2(50) ST3(142) ST4(2) ST6(5) ST7(2)	Ramirez et al., 2017
Colombia	Asymptomatic patients	181/181	ST1(82) ST2(47) ST3(33) ST4(7) ST6(4) ST7(7) Novel-ST(1)	Ramirez et al., 2016

Colombia	Human	125/125	ST1(70) ST2(40) ST3(15)	Ramirez et al., 2014
Colombia	Human	12/12	ST1(4) ST2(3) ST3(4) Mixed ST1/ST2(1)	Santin et al., 2011
Chile	Irritable Bowel Syndrome (IBS) patients	8/37	ST1(2) ST2(3) ST4(3)	Pena et al., 2020
Brazil	Human	11/20	ST1(3) ST2(1) ST3(3) ST4(3) ST7(1)	Melo et al., 2020
Brazil	Human	11/20	ST1(3) ST2(1) ST3(3) ST4(3) ST7(1)	de Melo et al., 2020
Brazil	School children	41/549	ST1(15) ST2(9) ST3(16) Mixed ST1/ST3(1)	Oishi et al., 2019
Brazil	Patients with urticaria	31/56	ST1(7) ST2(5) ST3(8) ST4(6) ST6(1) Mixed ST1/ST3(1)	Melo et al., 2019
Brazil	Volunteers	216/766	ST1(37), ST2(16), ST3(42) ST4 (3), ST6 (1) ST8 (3)	Segui et al., 2018
Brazil	Daycare children, workers and household	76/161	ST1(31) ST2(4) ST3(19) ST7(3)	Oliveira-Arbex et al., 2018

Brazil	Inhabitants of rural valleys	164/294	ST1(23) ST2(23) ST3(29) ST4(3) ST8(6) non-identified subtype(1)	Barbosa et al., 2018
Brazil	Urban community humans	64/64	ST1(23) ST2(4) ST3(27) ST4(1) Mixed ST1/ST3(9)	Valenca Barbosa et al., 2017
Brazil	Asymptomatic patients	22/22	ST1(2) ST2(7) ST3(8) ST7(1) ST8(2) Novel-ST(2)	Ramirez et al., 2016
Brazil	Individuals	67/126	ST1(23) ST2(7) ST3(29) ST6(2) ST7(4) Mixed ST1/ST3(1) Mixed ST3/ST7(1)	David et al., 2015
Brazil	Members of the indigenous Tapirapé ethnic group	66/382	ST1(27) ST2(21) ST3(11) Mixed ST1/ST2(5) Mixed ST1/ST3(1) Mixed ST2/ST3(1)	Malheiros et al., 2011
Bolivia	Asymptomatic patients	40/40	ST2(13) ST3(12) ST5(8) ST12(3) Novel-ST(4)	Ramirez et al., 2016

Bolivia	Rural Population	127/223	ST1(8) ST2(2) ST3(3)	Macchioni et al., 2016
Bolivia	Children	43/268	ST2(4) ST9(8)	Macchioni et al., 2015
Ecuador	Asymptomatic patients	25/25	ST1(3) ST3(21) Novel-ST(1)	Ramirez et al., 2016
Ecuador	Human	44/55	ST1(21) ST2(10) ST3(12) Mixed ST1/ST3(1) Mixed ST1/ST2(2)	Helenbrook et al., 2015
Peru	Asymptomatic patients	13/13	ST3(12) Novel-ST(1)	Ramirez et al., 2016
North America				
Panama	Children (1 to 12years old)	49/66	ST1(28) ST3(21)	Perea et al., 2020
Mexico	University students	95/202	ST1(14) ST2(8) ST3(28) ST4(16) ST5(5) ST7(12) Mixed ST1/ST3(6)	Perez et al., 2020
Mexico	Human	124/182	ST1(9) ST2(14) ST3(84) Mixed ST1/ST3(9) Mixed ST2/ST3(5) Mixed ST1/ST2/ST3(3)	Rojas-Velazquez et al., 2019
Mexico	Volunteers	109/182	ST1(7) ST2(11) ST3(54)	Rojas-Velazquez et al., 2018

Mexico	Unrelated *Blastocystis* sp. carriers	77/80	ST1(30) ST2(7) ST3(40)	Villegas-Gomez et al., 2016
Mexico	*Blastocystis* sp.-infected children	47/47	ST1(24) ST2(11) ST3(9) ST7(1)	Villalobos et al., 2014
Mexico	HIV-infected patients	8/27	ST1(4) ST3(4)	Sanchez-Aguillon et al., 2013
Mexico	Irritable bowel syndrome patient	20/90	ST1(2) ST2(1) ST3(5) Mixed ST1/ST3(11) Mixed ST1/ST2(1)	Jimenez-Gonzalez et al., 2012
USA	Adults and children	10/139	ST1(2) ST2(3) ST3(5)	Scanlan et al., 2016
USA	Human	1/1	ST4(1)	Santin et al., 2011
USA	Human	5/19	ST1(1) ST2(1) ST3(1) ST4(1) ST8(1)	Whipps et al., 2010
USA	Patients	9/21	ST1(1) ST3(6)	Jones et al., 2009
Europe				
Spain	Zookeepers	2/19	ST1(2)	Koster et al., 2021
Spain	Children	197/1512	ST1(37) ST2(59) ST3(35) ST4(30) ST8(1)	Muadica et al., 2020
Spain	Human	63/179	ST1(7) ST2(33) ST3(9) ST4(4)	Paulos et al., 2018
Spain	Human	1/1	ST4(1)	Santin et al., 2011

Spain	Diarrhea patients	51/51	ST3(48) ST4(1) ST5(1) Unknown(1)	Dominguez-Marquez et al., 2009
Serbia	Gastrointestinal disorders patients	10/10	ST3(6) ST6(4)	Suli et al., 2021
Czech Republic	Asymptomatic individuals	70/288	ST1(13) ST2(11) ST3(25) ST4(7) ST5(1) ST6(5) ST7(6) ST8(1) Mixed(1)	Lhotska et al., 2020
Italy	Patients	142/192	ST1(41) ST2(23) ST3(57) ST4(17) ST7(4)	Gabrielli et al., 2020
Italy	HIV-positive patients	39/156	ST1(12) ST2(3) ST3(20) ST4(4)	Sulekova et al., 2019
Italy	Autochthonous and immigrant patients	258/509	ST1(51) ST2(17) ST3(59) ST4(13) Mixed(80)	Piubelli et al., 2019
Italy	HIV-positive patients	39/156	ST1(12) ST2(3) ST3(20) ST4(4)	Fontanelli Sulekova et al., 2019
Italy	Patients	189/195	ST1(29) ST2(26) ST3(86) ST4(41) ST6(6) ST8(1)	Mattiucci et al., 2016

Italy	Symptomatic patients	30/30	ST1(2) ST2(5) ST3(13) ST4(6) Mixed ST1/ST3(1) Mixed ST2/ST3(1) Mixed ST2/ST8(1) Mixed ST3/ST7(1)	Meloni et al., 2011
Italy	A 41-year-old Italian man	1/1	ST1(1)	Angelici et al., 2018
Holland	Travellers	174/479	ST1(29) ST2(38) ST3(54) ST4(41) ST6(6) ST7(2) Mixed(4)	van Hattem et al., 2019
Holland	Patients	107/442	ST1(23) ST2(23) ST3(43) ST4(12) ST6(1) ST7(1)	Bart et al., 2013
Poland	Colorectal cancer patients	12/95	ST1(2) ST2(1) ST3(9)	Sulzyc-Bielicka et al., 2021
	Volunteers	2/76	ST1(1) ST3(1)	
Poland	Travellers	122/122	ST1(16) ST2(24) ST3(72) ST6(4) ST7(4) Mixed ST1/ST3(2)	Rudzinska et al., 2019
Poland	Immunodeficiency patients	3/249	ST2(1) ST3(2)	Bednarska et al., 2018

Poland	Diagnosed patients	23/26	ST1(3) ST2(2) ST3(15) ST6(2) ST7(1)	Kaczmarek et al., 2017
France	Patients	62/643	ST1(11) ST2(8) ST3(27) ST4(8) ST6(8)	Menu et al., 2019
France	Patients	143/788	ST1(28) ST2(18) ST3(61) ST4(28) ST6(3) ST7(3) Mixed infection(2)	El Safadi et al., 2016
France	Immunodeficiency patients	27/186	ST1(1) ST2(1) ST3(4) ST4(17) ST6(1) ST7(3)	Petrasova et al., 2011
France	Patients	40/40	ST1(8) ST2(4) ST3(20) ST3(4) ST7(1) Mixed ST1/ST3(3)	Souppart et al., 2009
Ireland	Infant	3/59	ST2(1) ST3(1) ST4(1)	Scanlan et al., 2018
Ireland	Healthy adults	59/105	ST1(7) ST2(14) ST3(25) ST4(12) ST8(1)	Scanlan et al., 2014

Sweden	Travellers	15/35	ST1(2) ST2(2) ST3(5) ST4(5) ST8(1)	Forsell et al., 2017
Sweden	Patients	63/68	ST1(10) ST2(9) ST3(30) ST4(13) ST7(1)	Forsell et al., 2012
Romania	IBS and colitis patients	49/49	ST2(15) ST4(1) Mixed ST1/ST2(2)	Matiut and Hritcu, 2015
UK	Patients	271/271	ST1(34) ST2(26) ST3(114) ST4(85) ST5(2) ST6(1) ST7(4) ST8(5)	Alfellani et al., 2013
UK	Primate handlers	16/16	ST1(2) ST3(9) ST4(1) ST8(4)	Stensvold et al., 2009
Denmark	Patients	56/153	ST1(21) ST2(14) ST3(16) ST4(5)	Stensvold et al., 2012
Denmark	Acute diarrhea patients	25/444	ST1(1) ST2(4) ST3(1) ST4(19)	Stensvold et al., 2011
Denmark	Aatients	99/1000	ST1(20) ST2(15) ST3(39) ST4(16) ST6(1) ST8(1) Mixed infection(7)	Rene et al., 2009

Denmark	Suspected enteroparasitic disease patients	28/107	ST1(5) ST2(9) ST3(13) ST4(1)	Parkar et al., 2007
Germany	Patients	12/12	ST1(3) ST3(5) ST5(2) ST7(2)	Yoshikawa et al., 2004
Africa				
Ghana	HIV positive outpatients and HIV negative blood donors	22/192	ST1(14) ST2(4) ST3(3)	Di Cristanziano et al., 2019
Madagascar	Patients and volunteers	171/265	ST1(80) ST2(36) ST3(42) Mixed infection(13)	Greigert et al., 2018
Egypt	IBS patients	22/115	ST1(4) ST3(18)	El-Badry et al., 2018
Egypt	Iron deficiency anemic versus non-anemic patients	78/226	ST1(13) ST3(65)	El Deeb and Khodeer, 2013
Egypt	Urticarial patients and control volunteers	37/104	ST3(37)	Zuel-Fakkar et al., 2011
Egypt	Urticarial patients	54/54	ST3(54)	Hameed et al., 2011
Egypt	Symptomatic patients	20/20	ST1(3) ST2(12) ST3(4) Mixed ST1/ST3(1)	Souppart et al., 2010
Egypt	Gastrointestinal symptomatic patients	44/85	ST1(8) ST2(4) ST3(24) ST4(8)	Hussein et al., 2008
Angola	School children population	90/351	ST1(23) ST2(23) ST3(27) ST5(1) ST7(1)	Dacal et al., 2018

Mozambique	Asymptomatic school children	154/807	ST1(35) ST2(35) ST3(70) ST4(14)	Muadica et al., 2021
Cote d'Ivoire	Patients	64/110	ST1(32) ST2(14) ST3(18)	D'Alfonso et al., 2017
Tunisia	Healthy Individuals	68/524	ST1(18) ST2(10) ST3(31) ST4(1) ST7(1)	Ben Abda et al., 2017
Nigeria	Children	167/199	ST1(51) ST2(42) ST3(33) ST7(1)	Poulsen et al., 2016
Nigeria	Patients	22/47	ST1(10) ST3(9) ST4(3)	Alfellani et al., 2013
Tanzania	Patients	106/174	ST1(36) ST2(28) ST3(27) ST7(1) Unknown(14)	Forsell et al., 2016
Tanzania	Human	8/8	ST1(1) ST2(3) ST3(2)	Petrasova et al., 2011
Morocco	Associated Appendicular Peritonitis in a Child	1/1	ST3(1)	Frealle et al., 2015
Senegal	Healthy school children	588/731	ST1(113) ST2(226) ST3(107) Mixed(135)	Khaled et al., 2020
Senegal River Basin	Children	93/93	ST1(29) ST2(21) ST3(51) ST4(2)	El Safadi et al., 2014
Libya	Patients	38/150	ST1(19) ST2(3) ST3(15) ST7(1)	Alfellani et al., 2013

Liberia	Children	25/43	ST1(7) ST2(7) ST3(8) ST4(3)	
Libya	Outpatients	55/380	ST1(23) ST2(11) ST3(8) Mixed infections(3)	Abdulsalam et al., 2013
Oceania				
Australia	Piggery staff	30/36	ST1(11) ST3(16) ST5(3)	Wang et al., 2014
Australia	Patients	98/513	ST1(28) ST2(5) ST3(40) ST4(12) ST6(3) ST7(1) ST8(2)	Roberts et al., 2013
Australia	Symptomatic patients	11/11	ST1(4) ST3(3) ST4(1) Mixed ST1/ST4(1) Mixed ST3/ST4(1) Mixed ST4/ST6(1)	Nagel et al., 2012
Australia	Patients	92/513	ST1(28) ST2(6) ST3(41) ST4(12) ST6(3) ST8(1)	Roberts et al., 2011

Table 2: Prevalence, geographic and subtybe distribution of *Blastocystis* sp. in NHPs.

Country	**Sample origin**	**No. positive/No. examined**	**Subtype(n)**	**References**
Asia				

China	Zoo non-human primates	39/128	ST1(13) ST2(6) ST3(1) ST17(19)	Deng et al., 2021
China	Golden Monkeys	18/37	ST1(7) ST2(8) ST3(3)	Ma et al., 2020a
China	Macaque	13/185	ST1(7) ST2(2) ST3(1) Mixed ST1/ST3(1) Mixed ST2/ST3(1)	Zhu et al., 2020
China	*Cercopithecus neglectus*	5/5	ST1(1) ST2(4)	Ma et al., 2020b
	Trachypithecus francoisi	2/3	ST1(2)	
	Mandrillus sphinx	9/15	ST1(5) ST3(4)	
	Ateles paniscus	2/4	ST2(1) ST3(1)	
	Lemur catta	7/16	ST3(2) ST5(3) ST9(2)	
	Rhinopithecus spp.	9/22	ST1(6) ST2(3)	
	Macaca mulatta	6/18	ST2(4) ST3(2)	
	Saimiri sciureus	9/30	ST1(7) ST5(2)	
	Nomascus leucogenys	1/4	ST1(1)	
	Macaca fascicularis	3/13	ST2(2) ST3(1)	
	Macaca fuscata	6/33	ST2(5) ST3(1)	
	Pan troglodytes	2/15	ST1(1) ST5(1)	
China	Zoo non-human primates	2/114	ST2 (1) ST4 (1)	Li et al., 2020a

China	Macaques	235/505	ST1(148) ST2(110) ST3(226) Mixed ST1/ST2(72) Mixed ST1/ST3(143) Mixed ST2/ST3(102) Mixed ST1/ST2/ST3(70)	Li et al., 2020b
China	*Macaca mulatta*	28/29	ST1(15) ST2(5) ST3(7) ST19(1)	Zhao et al., 2018
	Presbytis francoisi	1/1	ST5(1)	
	Mandrillus sphinx	1/4	ST3(1)	
	Hinopithecus roxellana	41/46	ST1(4) ST13(37)	
	Cercopithecus neglectus	4/5	ST1(3) ST10(1)	
	Papio hamadrayas	13/23	ST1(9) ST3(4)	
	Pan troglodyte	8/10	ST2(8)	
China	*Macaca fascicularis*	85/97	ST1(4) ST2(14) ST7(2) Mixed ST2+ST1(14) Mixed ST2+ST3(5) Mixed ST2+ST7(5) Mixed ST3+ST1(3) Mixed ST5+ST2(1) Mixed ST7+ST1(7) Mixed ST7+ST3(1) Mixed ST1+ST2+ST3(10) Mixed ST1+ST2+ST7(5) Mixed ST2+ST3+ST3(3) Mixed ST1+ST3+ST7(1) Mixed ST1+ST2+ST3+ST7(10)	Zanzani et al., 2016
Bangladesh	Zoo NHPs	27/85	ST1(7) ST2(4) ST3(13) ST13(3)	Li et al., 2019

Thailand	Long-tailed macaques	197/628	ST1(34) ST2(48) ST3(72) Mixed infections(9)	Vaisusuk et al., 2018
Nepal	Rhesus monkeys	10/10	ST1(2) ST2(4) Mixed ST1/ST2(3) Unknown(1)	Yoshikawa et al., 2009
Philippines	Monkeys	4/4	ST1(2) ST2(1) ST3(1)	Rivera, 2008
Japan	Monkeys	5/5	ST1(1) ST5(3) Mixed ST1/ST5(1)	Yoshikawa et al., 2004
Japan	Primates	2/12	ST1(2)	Abe et al., 2003
Africa				
Senegal	Chimpanzees	47/48	ST1(47)	Menu et al., 2021
Congo	Gorilla	19/19	ST1(11) ST2(8)	
Tanzania	Syntopic primates	155/290	ST1(29) ST2(3) ST3(3) ST5(7)	Petrasova et al., 2011
Ghana	Black-and-white colobus	3/10	ST2(3)	Teichroeb et al., 2009
South America				
Ecuador	Mantled howler monkey	58/96	ST8(58)	Helenbrook et al., 2015
Colombia	*Alouatta* spp.	2/2	ST4(2)	Ramirez et al., 2014
North America				
Mexico	Wild howler monkeys	87/225	ST1(4) ST2(80) ST8(3)	Villanueva-Garcia et al., 2017
Europe				
Spain	Non-human primates	23/51	ST1(9) ST3(8) ST8(6)	Koster et al., 2021

France	Gorilla	8/9	ST1(2) ST2(2) ST3(4)	Menu et al., 2021
Spain	Primate	7/7	ST1(1) ST2(2) ST3(3) Mixed ST1/ST2/ST3(1)	Santin et al., 2011
UK	Non-human primates	106/106	ST1(12) ST2(12) ST3(43) ST4(1) ST5(10) ST8(22) ST15(3)	Alfellani et al., 2013
UK	Non-human primates	62/62	ST1(7) ST2(11) ST3(23) ST4(1) ST5(7) ST8(13)	Stensvold et al., 2009
Denmark	Non-human primates	8/8	ST4(2) ST5(2) ST8(2) ST10(2)	

Table 3: Primer pairs for identification of *Blastocystis* sp. Subtypes.

Target organism	**Primer name**	**Sequence(5'-3')**	**Amplicon size (bp)**	**References**
***Blastocystis* sp.**	BhRDr	GAGCTTTTTAACTGCAACAACG	600	Scicluna et al., 2006
	RD5	ATCTGGTTGATCCTGCCAGT		
	Blast 505–532	GGAGGTAGTGACAATAAATC	500	Santín et al., 2011
	Blast 998–1017	TGCTTTCGCACTTGTTCATC		
	BLF	CGAATGGCTCATTATATCAGTT	260	Menounos et al., 2008
	BLR	TCTTCGTTACCCGTTACTGC		
	SR1F	GCTTATCTGGTTGATCCTGCCAGTAGT	1800	Yoshikawa et al., 2000
	SR1R	TGATCCTTCCGCAGGTTCACCTA		
***Blastocystis* sp.**	BL18SPPF1	AGTAGTCATACGCTCGTCTCAAA	320~342	Poirier et al., 2011
	BL18SR2PP	TCTTCGTTACCCGTTACTGC		

***Blastocystis* sp ST1**	ST1F	TAATACATGAGAAAGTCCTCTGG	336	Wang et al., 2013
	ST1R	CTCAATCCAATTGCAAGAC		
	SB82F	TCTTGCTTCATCGGAGTC	462	Yoshikawa et al., 1998
	SB82R	CCTTCTCGCAGTTCTTTATC		
	SB83F	GAAGGACTCTCTGACGATGA	351	
	SB83R	GTCCAAATGAAAGGCAGC		
	ST1F	CATGAGAAAGTCCTCTGGTGAGG	1500	Yoshikawa and Iwamasa, 2016
	ST1R	CATCCTTTTACAGACAACCAATCTC		
***Blastocystis* sp ST2**	ST2F	GCTCGTAGTTGAAGTGAGGGAGTG	203–204	Wang et al., 2013
	ST2R	ATCGAACACGAAAGCCTTCAATC		
	SB155F	ATCAGCCTACAATCTCCTC	650	Yoshikawa et al., 1998
	SB155R	ATCGCCACTTCTCCAAT		
	ST2F	CATGAGTAAAGTCCCGTWGGGA	1500	Yoshikawa and Iwamasa, 2016
	ST2R	CCCTTTTACAGTTCATTCGCCTA		
***Blastocystis* sp ST3**	ST3F	GATTGAGAACAACGTACAAACC	339–341	Wang et al., 2013
	ST3R	ATGAACCAAACCAATCAAATAC		
	SB227F	TAGGATTTGGTGTTTGGAGA	526	Yoshikawa et al., 2000
	SB227R	TTAGAAGTGAAGGAGATGGAAG		
	SB228F	GACTCCAGAAACTCGCAGAC	473	
	SB228R	TCTTGTTTCCCCAGTTATCC		
	SB229F	CACTGTGTCGTCATTGTTTTG	631	
	SB229R	AGGGCTGCATAATAGAGTGG		
	ST3F	CATGYWHDWDGTCTTGTAGACTGCA	1200	Yoshikawa and Iwamasa, 2016
	ST3R	GTCCCTCTAAGAGGATTCCTATAGA		
***Blastocystis* sp ST4**	ST4F	TTGAAGTGAACTTGGRTTGATGTG	1050	Yoshikawa and Iwamasa, 2016
	ST4R	AWYAAYTCCGYAAAGAATYAMTATATCA		
	SB332F	GCATCCAGACTACTATCAACATT	338	Yoshikawa et al., 2000
	SB332R	CCATTTTCAGACAACCACTTA		
***Blastocystis* sp ST5**	ST5F	GTAAATGACCAATTCTTATTAC	106	Wang et al., 2013
	ST5R	GTGGGATGAAGTATATGATG		
	SB340F	TGTTCTTGTGTCTTCTCAGCTC	704	Yoshikawa et al., 2003
	SB340R	TTCTTTCACACTCCCGTCAT		
	ST5F	GCTAATACATGAMTAAATCCTCAACTTC	1200	Yoshikawa and Iwamasa, 2016
	ST5R	CGTATAGTGTCCCTCTAAGAAGTATACA		

***Blastocystis* sp ST6**	ST6F	TGGATGAGTATTTGARWTSAATTTTCG	1050	Yoshikawa and Iwamasa, 2016
	ST6R	CCCAGATACWMAAACGTATCCG		
	SB336F	GTGGGTAGAGGAAGGAAAACA	317	Yoshikawa et al., 2003
	SB336R	AGAACAAGTCGATGAAGTGAGAT		
***Blastocystis* sp ST7**	ST7F	TGGGBAACCAKTAAAGAAGTAC	1340	Yoshikawa and Iwamasa, 2016
	ST7R	TCCCAAGTCTATCGACTCAGA		
	SB337F	GTCTTTCCCTGTCTATTCTGCA	487	Yoshikawa et al., 2003
	SB337R	AATTCGGTCTGCTTCTTCTG		
***Blastocystis* sp ST8**	ST8F	GAATGAAAACCAGTAGACTTAGTCTATTCG	1480	Yoshikawa and Iwamasa, 2016
	ST8R	CTCTATTCCTTTTACAGACTAGAAAC		
***Blastocystis* sp ST9**	ST9F	RAGAATGTCAAATTCTTGTAAAMTARTC	1030	Yoshikawa and Iwamasa, 2016
	ST9R	CCCAGATACWMAAACGTATCCG		

CONCLUSIONS

This review described the prevalence, subtype distributions and genetic characterizations of *Blastocystis* sp. in human and NHPs. *Blastocystis* sp. has been widely described in humans and NHPs worldwide. This review provides an up-to-date overview of the epidemiology and subtype distribution of *Blastocystis* sp. in humans and NHPs. The infection rates vary from region to region, and species to species, however, the factors that account for these differences are a mystery. With the explosive research of molecular epidemiology in the past ten years, molecular sequences data on *Blastocystis* sp. subtypes in humans and NHPs has become more abundant. Although most of the *Blastocystis* sp. subtypes that infect NHPs have also been reported in humans, the zoonotic risk of *Blastocystis* sp. transmission between NHPs and humans is still need more data to verify. In addition, the host specificity of *Blastocystis* sp. and its pathogenic potential are unclear, so further research is needed to focus on these problems.

SEARCH STRATEGY

In the section of “Molecular epidemiology and subtype distribution of *Blastocystis*,” the database of PubMed, Web of Science, and China National Knowledge Infrastructure were searched, up to 1 May 2021, by using the following search strategy:((((epidemiology) OR molecular) OR subtype)) AND *Blastocystis*, no language restriction was set, then screening the literatures one by one, and the literature that uses molecular detection methods to identify the subtype of *Blastocystis* into the candidate. The epidemiology data were extracted from the literatures were listed in the Table **1,2**.

ETHICAL STANDARDS

Not applicable.

DECLARATION OF COMPETING INTEREST

The authors declare that they have no competing interest

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