

## Molecular prevalence and subtype distribution of *Blastocystis* sp. in Asia and in Australia

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### ABSTRACT

*Blastocystis* is a prevalent protozoan parasite reported in humans, animals, and environmental samples. Over the past decade, numerous studies have investigated the prevalence and subtype distribution of *Blastocystis* sp. alongside with its genetic and biochemical features. However, studies on subtype distribution of this protozoan in humans, animals, and environmental samples represent the potential transmission routes. In this review, we evaluated studies performed in Asian countries and in Australia to provide an overview of environmental factors on the prevalence and subtype distribution of *Blastocystis* sp. among humans, animals, and the environment.

**Key words:** Asia, Australia, *Blastocystis*, distribution, zoonotic transmission

### HIGHLIGHTS

- *Blastocystis* sp. is a prevalent protozoan reported in humans, animals, and the environment.
- Subtype distribution represents the potential transmission routes.
- The prevalence and the distribution pattern of subtypes vary from the east to the west countries in Asia.

### INTRODUCTION

*Blastocystis* is an intestinal, anaerobic protozoan parasite, which can be isolated from humans, animals, and the environment (Parija & Jeremiah 2013; Stensvold & Clark 2016). *Blastocystis* sp. is mostly reported from both developed and developing countries (Bart *et al.* 2013; Scanlan *et al.* 2016), and is thought to be correlated with unfavorable sanitation conditions (Javanmard *et al.* 2018; Oliveira-Arbex *et al.* 2018). Numerous epidemiological studies have highlighted the global distribution of *Blastocystis* sp., with high prevalence of this protozoan infection in developing countries (Abdulsalam *et al.* 2013; El Safadi *et al.* 2014; Poulsen *et al.* 2016).

Apart from anthroponotic transmission, contaminated food and water resources, as well as intimate contact with animals, appear to be the main alternative routes of infection (Ahmed & Karanis 2018; Greige *et al.* 2018; Javanmard *et al.* 2019). The distribution, pathogenicity and genetic diversity of this protozoan have been highlighted using a variety of molecular and biochemical approaches (Mohammad Rahimi *et al.* 2019). Multiple lineages have been described based on different typing techniques. According to the latest classification, molecular diversity throughout a ~600-bp fragment of the small subunit ribosomal RNA (SSU rRNA) gene has led to the description of at least 23 separated subtypes (ST) (Stensvold & Clark 2020). Subtypes 1–9 and ST12 (Ramirez *et al.* 2016) have been reported in humans with subtype ST1-4 being the most common (Stensvold & Clark 2016).

Because the same *Blastocystis* subtypes have been detected in humans and animals, zoonotic transmission is most likely a significant mode of infection (Betts *et al.* 2018, 2020; Greige *et al.* 2018; Rezaei Riabi *et al.* 2018; Li *et al.* 2019a). Numerous studies reported *Blastocystis* sp. and its subtypes in animals, environment, and humans from Asian countries. Three molecular techniques have been used to classify *Blastocystis* sp. subtypes, including RFLP (restricted fragment length

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polymorphism), STS (subtype specific sequence-tagged site), and PCR sequencing along with real-time PCR coupled with high resolution melting (HRM) curve analysis (Mohammad Rahimi *et al.* 2019).

In this review, we provide a detailed overview of *Blastocystis* sp. reported in Australia and Asian countries concerning prevalence and various subtypes. Therefore, PubMed, Scopus and Web of Science (ISI) were searched up to the year 2020 with the following keywords and their combinations: 'Blastocystis', 'STs', 'subtypes', 'Molecular epidemiology', 'Name of each Asian country', 'Australia', 'Human', 'Animal' and 'Water'. Titles, abstracts, and full-text articles were assessed to evaluate the eligibility of articles that includes the author, year of publication, sample size, host/source, country, subtyping method, and prevalence of *Blastocystis* sp. and its subtypes were extracted. Accordingly, non-related subjects, review articles, systematic and meta-analysis, non-English language, and congresses were excluded. The prevalence data was only retrieved from studies which employed molecular methods for detection of this protist. Extracted subtyping data, the relevant accession numbers, and used primers were checked for each paper to evaluate the validity and reliability of generated data. The data from Australia and New Zealand were also included in this study due to the closeness of Australia and New Zealand to East Asian countries.

## RESULTS

All the subtyping results extracted from each study were treated based on the consensus terminology table provided by Stensvold *et al.* (2007) (Table 1); however, the original data provided by each paper together with the year of study, author, sample size, hosts, the method of study, and reported subtypes are included in supplementary Table S1. In total, 99 papers met the criteria and were included in this study. The available data were collected on the prevalence of *Blastocystis* sp. (Figure 1(a) and 1(b)) and its subtypes in humans (Figure 2), animals (Figures 3 and 4), and environmental samples from Asia, Australia and New Zealand (supplementary Table S1).

### Burden of *Blastocystis* sp. in Asian countries

#### China

In China, *Blastocystis* sp. infections have been reported from humans with a prevalence rate of 3.86–32.6%, with an average prevalence of 11.62% (Yan *et al.* 2006; Li *et al.* 2007a, 2007b; Zhang *et al.* 2017, 2019; Gong *et al.* 2019). The average prevalence is lower than reported from the Philippines (49.1%), Indonesia (34.25%) and Thailand (22.31%), located in East and Southeast Asia. Subtyping characterized ST1–7 from human cases, while ST3 seems to be the most prevalent subtype reported from China (61.17%) followed by ST1 (28.41%) and ST2 (5.08%). Interestingly, this result is similar to that reported from North and South America, which demonstrated a high prevalence of ST3 followed by ST1 and ST2. Reports from North and South American countries suggested ST3 as the predominant subtype (Jimenez *et al.* 2019). Moreover, a systematic review and meta-analysis performed by Deng *et al.* (2019a), on the prevalence and subtype distribution of *Blastocystis* sp., confirmed that ST3 was the most prevalent subtype in China.

The prevalence of *Blastocystis* sp. reported from animals in China was between 3 and 74.8%, with an average of 22.58%. The highest and lowest prevalences were identified from pigs (74.8%) and goats (0.3%). Pigs, cattle, sheep, goats, birds, canines, rodents, alpacas, non-human primates, pandas, and deer were demonstrated to harbor *Blastocystis* sp. (Yan *et al.* 2007; Song *et al.* 2017a, 2017b; Zhao *et al.* 2017; Zhu *et al.* 2017; Li *et al.* 2018, 2019b; Wang *et al.* 2018; Deng *et al.* 2019b; Ren *et al.* 2019; Xiao *et al.* 2019). Subtyping techniques detected ST1–7, ST10, ST12–ST14 from animal samples while ST10 (37.91%) followed by ST5 (24.7%) were the frequently reported subtypes mostly from cattle and pigs, respectively.

#### Thailand

Studies in Thailand show that the prevalence of *Blastocystis* sp. in human populations ranges from 5.2 to 40.6% with an average prevalence of 22.31%. This prevalence rate is close to that reported from countries such as Malaysia (19.25%) and Nepal (25.2%). The prevalence rate of *Blastocystis* sp. in Thailand was also significantly higher than that reported from Singapore (3.3%) and lower than that in the Philippines (49.1%) and Indonesia (34.25%). Extracted subtyping results show the presence of ST1–7 and also reports of unknown subtypes from Thailand. ST1 (52.2%) was the major reported subtype followed by ST3 (36.6%), ST4 (6.5%), and ST2 (4.9%) (Jantermtor *et al.* 2013; Thathaisong *et al.* 2013; Pipatsatitpong *et al.* 2015; Popruk *et al.* 2015; Sanpool *et al.* 2015; Palasuwan *et al.* 2016; Yowang *et al.* 2018; Srichaipon *et al.* 2019). In contrast with most of the studies in Asian countries, and those from other continents, ST3 was not the predominant subtype. The subtype distribution

**Table 1** | Prevalence rate and subtype distribution of *Blastocystis* sp. in Asian countries

Country	Molecular method	Host	(%) <sup>a</sup>	(%) <sup>b</sup>	<i>Blastocystis</i> subtypes															Predominant subtype	
					ST1	ST2	ST3	ST4	ST5	ST6	ST7	ST8	ST9	ST10	ST11	ST12	ST13	ST14	ST17		Unknown
Thailand	Barcoding sequencing, RFLP, Non-barcoding sequencing, STS	Human	5.2–40.6	22.31	✓	✓	✓	✓	✓	✓	✓	-	-	-	-	-	-	-	-	ST3	
		Environment (Water)	5.9–20	12.95	✓	✓	✓	-	-	✓	-	-	-	-	-	-	-	-	-	-	ST3
		Animal (dog, cat, pig, cattle, sheep, goat, primate, horse)	-	40	✓	-	✓	-	✓	-	-	-	-	✓	-	✓	-	✓	-	-	ST3
China	Non-barcoding sequencing, Barcoding sequencing, STS	Human	3.86–32.6	11.62	✓	✓	✓	✓	✓	✓	✓	-	-	✓	-	✓	-	✓	-	ST3	
		Animal (pig, cattle, sheep, goat, non-human primate, deer, panda, alpaca, rodent, canine, bird)	3–74.8	22.58	✓	✓	✓	✓	✓	✓	✓	✓	-	-	✓	-	✓	-	✓	-	ST10
Philippines	Non-barcoding sequencing, STS, Barcoding sequencing	Human	15.3–82.9	49.1	✓	✓	✓	✓	-	✓	✓	-	-	-	-	-	-	-	-	ST3	
		Environment (wastewater)	-	15	✓	✓	-	-	-	-	-	-	-	-	-	-	-	-	-	-	ST1
		Animal (dog, bird, pig, and monkey)	-	15.8	✓	✓	✓	-	-	✓	✓	-	-	-	-	-	-	-	-	✓	ST2
Indonesia	STS, Barcoding sequencing	Human	29.9–33.8	31.85	✓	✓	✓	-	-	✓	-	-	-	-	-	-	-	-	-	ST1	
		Animal (pig, bird, and rodent)	50	-	✓	✓	-	✓	-	✓	-	-	-	-	-	-	-	-	-	-	ST2
Malaysia	STS, Barcoding sequencing, Non-barcoding sequencing	Human	9.17–40.3	24.73	✓	✓	✓	✓	-	✓	-	-	-	-	-	-	-	-	-	ST3	
		Environment (water)	100	100	✓	✓	✓	✓	-	-	-	✓	-	✓	-	-	-	-	-	-	ST3
		Animal (canines, sheep, goats, deer, pigs, and cockroaches)	6.3–33.33	23.95	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	-	-	-	-	-	ST1
Nepal	STS	Human	25.6–26.1	25.85	✓	-	✓	-	-	✓	✓	-	-	-	-	-	-	-	-	ST6	
		Environment (water)	-	100	✓	-	-	-	-	✓	-	-	-	-	-	-	-	-	-	-	ST1
		Animal (cattle, buffaloes, goat, monkey, and pig)	-	15.4	✓	-	-	-	-	✓	✓	-	-	-	-	-	-	-	-	✓	ST7
Singapore	RFLP	Human	-	3.3	-	-	✓	-	-	-	-	-	-	-	-	-	-	-	-	ST3	
Cambodia	Barcoding sequencing	Human	-	55.23	✓	✓	✓	-	-	-	-	-	-	-	-	-	-	-	-	ST3	
		Animal (pig)	-	23.25	-	✓	-	-	✓	-	-	-	-	-	-	-	-	-	-	-	ST5
Japan	STS, RFLP, Non-barcoding sequencing	Human	-	0.5	✓	✓	✓	✓	-	✓	-	-	-	-	-	-	-	-	-	ST3	
		Animal (cattle, rodent, and pig)	-	39.1	✓	✓	✓	✓	-	-	-	-	-	✓	-	-	-	✓	-	-	ST14

(Continued.)

**Table 1** | Continued

Country	Molecular method	Host	(%) <sup>a</sup>	(%) <sup>b</sup>	Blastocystis subtypes															Predominant subtype	
					ST1	ST2	ST3	ST4	ST5	ST6	ST7	ST8	ST9	ST10	ST11	ST12	ST13	ST14	ST17		Unknown
South Korea	Barcoding sequencing	Animal (non-human primate, herbivores, and carnivores)	-	31.8	√	√	√	-	√	-	-	-	-	√	-	-	-	√	-	-	ST5
India	Barcoding sequencing	Human	12.27–57.9	24	√	-	√	-	-	-	-	-	-	-	-	-	-	-	-	-	ST3
		Animal (dog)	-	24	√	-	-	√	√	√	-	-	-	-	-	-	-	-	-	-	-
Bangladesh	Barcoding sequencing	Animal <sup>c</sup>	-	15.5	√	√	√	-	-	-	-	-	√	√	-	√	√	-	-	-	ST3
Saudi Arabia	STS	Human	-	16.7	√	√	√	-	√	-	√	-	-	-	-	-	-	-	-	-	ST3
UAE	Barcoding sequencing	Human	-	44.4	√	√	√	-	-	-	-	-	-	-	-	-	-	-	-	-	ST3
		Animal (cattle, sheep, reptiles, rabbits, rodents)	-	20.2	√	√	√	-	-	-	-	-	√	√	-	√	√	√	-	-	ST10
Qatar	Non-barcoding sequencing	Human	-	71.1	√	√	√	-	-	-	-	-	-	-	-	-	-	-	-	-	ST3
Iran	Barcoding sequencing, Non-barcoding sequencing, STS, RFLP	Human	-	27	√	√	√	-	√	√	√	-	-	-	-	-	-	-	-	√	ST3
		Animal (cattle)	-	9.6	-	√	√	-	√	-	-	-	-	-	-	-	-	-	-	-	ST2
Lebanon	Non-barcoding sequencing,	Human	-	39	√	√	√	-	-	√	-	-	-	-	-	-	-	-	-	-	ST3
		Animal (chickens and cattle)	-	47.6	√	√	√	-	√	√	√	-	-	√	-	-	-	-	-	√	ST6
Australia	Barcoding sequencing, Non-barcoding sequencing, NGS	Human	-	55.1	√	√	√	√	√	√	√	√	-	-	-	-	-	-	-	-	ST3
		Environment (wastewater)	-	61.53	√	-	-	√	-	√	-	√	-	-	-	-	-	-	-	√	ST4
		Animal (bird, canines, deer, pig, non-human primates, kangaroos, elephants, leopards and giraffes)	-	34.83	√	√	√	√	√	√	√	√	-	-	-	√	√	√	-	-	-

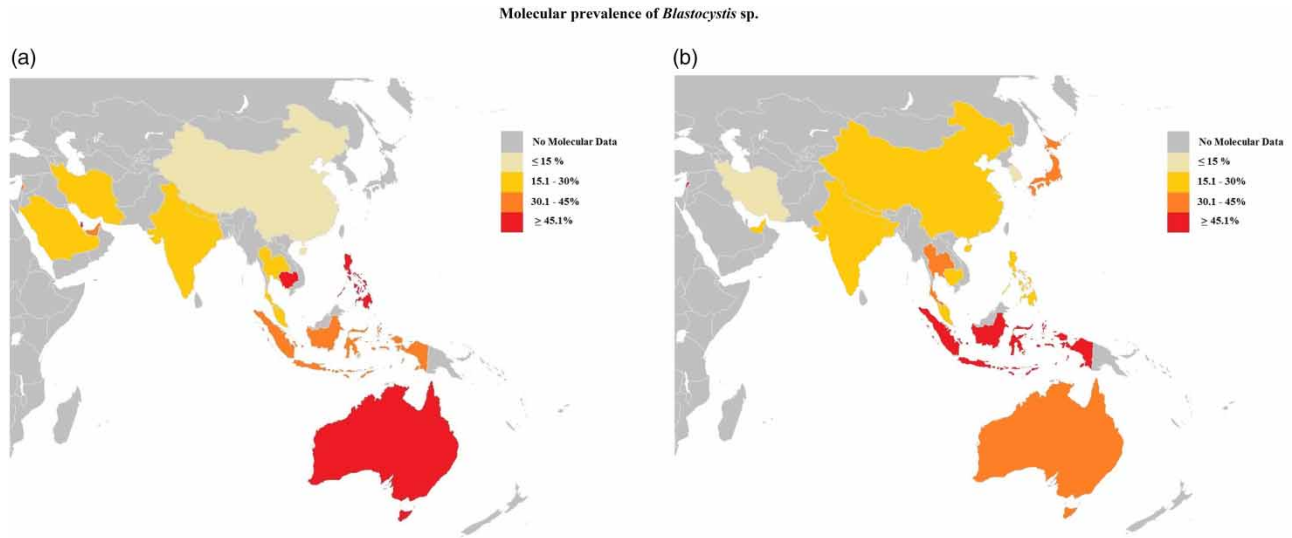
All subtypes are translated based on consensus terminology suggested by Stensvold *et al.* (2007).

Abbreviations: RFLP (restricted fragment length polymorphism); STS (subtype specific sequence-tagged site); NGS: next generation sequencing; ST: subtype.

<sup>a</sup>Prevalence range.

<sup>b</sup>Average prevalence.

<sup>c</sup>There was no separation based on the type of animals.



**Figure 1** | The prevalence of *Blastocystis* sp. in Asian countries and Australia based on molecular studies. (a) In humans and (b) animals.

reported from this county is similar to the subtype distribution in Indonesia (Yoshikawa *et al.* 2016; Kesuma *et al.* 2019). ST4 seems to be the other most frequently reported subtype.

The overall prevalence of *Blastocystis* sp. from animals in Thailand was 40%. *Blastocystis* sp. in animals was reported from dogs, pigs, cattle, sheep, goats, primates and horses, of which the highest and lowest prevalence were identified from pigs (76.4%) and dogs (2.6%), respectively. Subtyping identified ST1, ST3, ST5, ST10, ST12, ST14, and unknown subtypes. The most common subtype was ST3 (31.14%) followed by ST5 (20.6%), ST2 (15.7%), unknown subtype (13.11%) and ST1 (13.75%) (Thathaisong *et al.* 2003; Pintong *et al.* 2018; Udonsom *et al.* 2018; Vaisusuk *et al.* 2018). The absence of evidence of ST4 from animal sources illustrates the high likelihood of anthroponotic transmission of ST4.

### Philippines

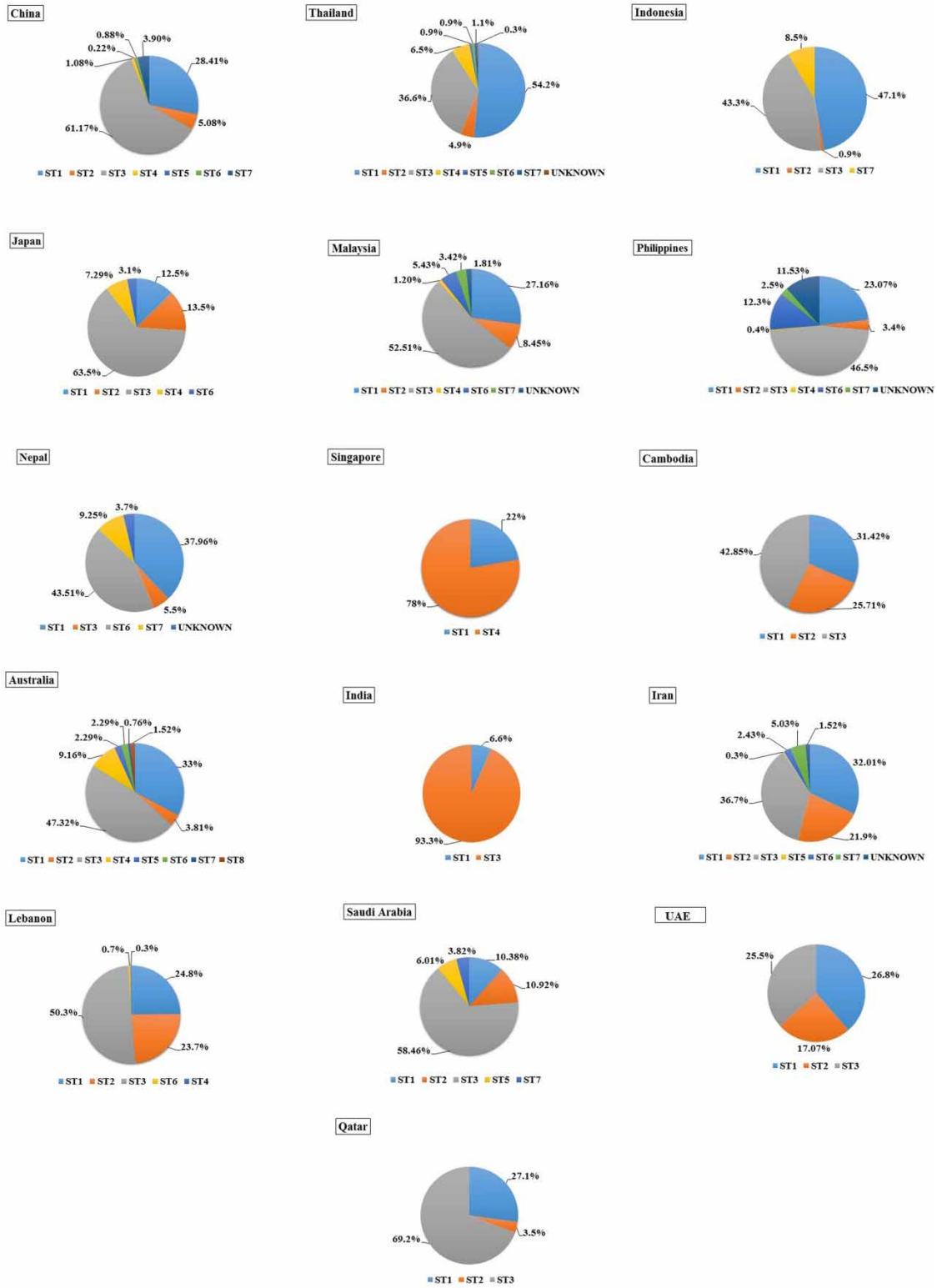
The prevalence rate of *Blastocystis* sp. in human carriers varied from 15.3 to 82.9% with an average of 49.1%. Although the number of studies investigating the prevalence of *Blastocystis* sp. in human cases is low, the prevalence rate of this protist in the Philippines is significantly higher than that reported from countries such as Singapore, Thailand, and China. Subtyping analysis revealed the presence of ST1–4, ST6, ST7, and unknown subtypes of which ST3 (46.51%) was the predominant subtype followed by ST1 (23.07%), ST6 (12.3%), ST2 (3.4%), ST7 (2.5%), and ST4 (0.4%). Moreover, 11.53% of the subtyped samples were from 19 subtypes, suggesting high diversity of the barcoding region of the SSU rRNA gene (Adao *et al.* 2016; Belleza *et al.* 2016). The high prevalence of subtypes of birds like ST6 and ST7 suggests the risk of zoonotic transmission, particularly from birds (Rezaei Riabi *et al.* 2018).

The prevalence rate of *Blastocystis* sp. among animals was reported to be 15.8%. *Blastocystis* sp. was found in dogs, birds, pigs, and monkeys (Rivera 2008; Belleza *et al.* 2016). The subtypes reported from animals were ST1–3, ST6, ST7 and unknown subtypes. Unknown subtypes (34.48%) was the most common, followed by ST2 and ST3 (17.24% for each one), ST6 (13.7%), ST1 (10.34%), and ST7 (6.89%) (Rivera 2008; Belleza *et al.* 2016).

### Indonesia

There are few studies on the prevalence of *Blastocystis* sp. in humans or animals in Indonesia. The prevalence in humans was reported to be as high as 29.9% (Yoshikawa *et al.* 2016) and 33.8% (Kesuma *et al.* 2019). Molecular characterization of isolated *Blastocystis* sp. from humans illustrates the majority are ST1 (47.1%) followed by ST3 (43.3%), ST7 (8.5%), and ST2 (0.9%). Such a high prevalence of *Blastocystis* sp. ST7 from humans in Indonesia may illustrate the probability of zoonotic transmission of *Blastocystis* sp. from bird sources. This pattern of transmission is similar to that reported from the Philippines, where ST6 and ST7 have been responsible for 14.7% of total *Blastocystis* sp. isolated from humans (Adao *et al.* 2016; Belleza *et al.* 2016).

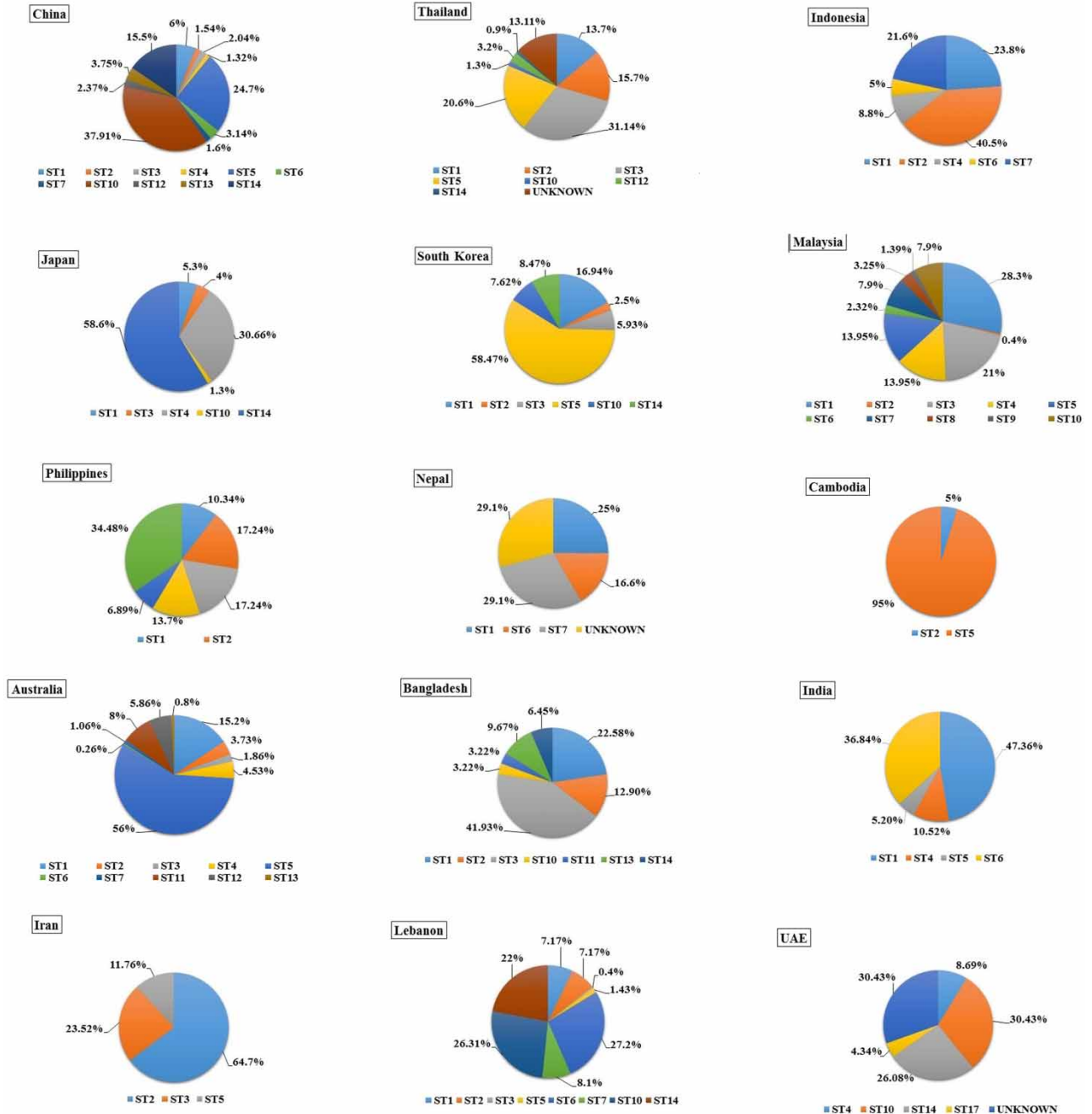
*Blastocystis* sp. subtypes in humans



**Figure 2** | Distribution of *Blastocystis* sp. subtypes in humans. The presented percentages are based on consensus terminology provided by Stensvold *et al.* (2007).



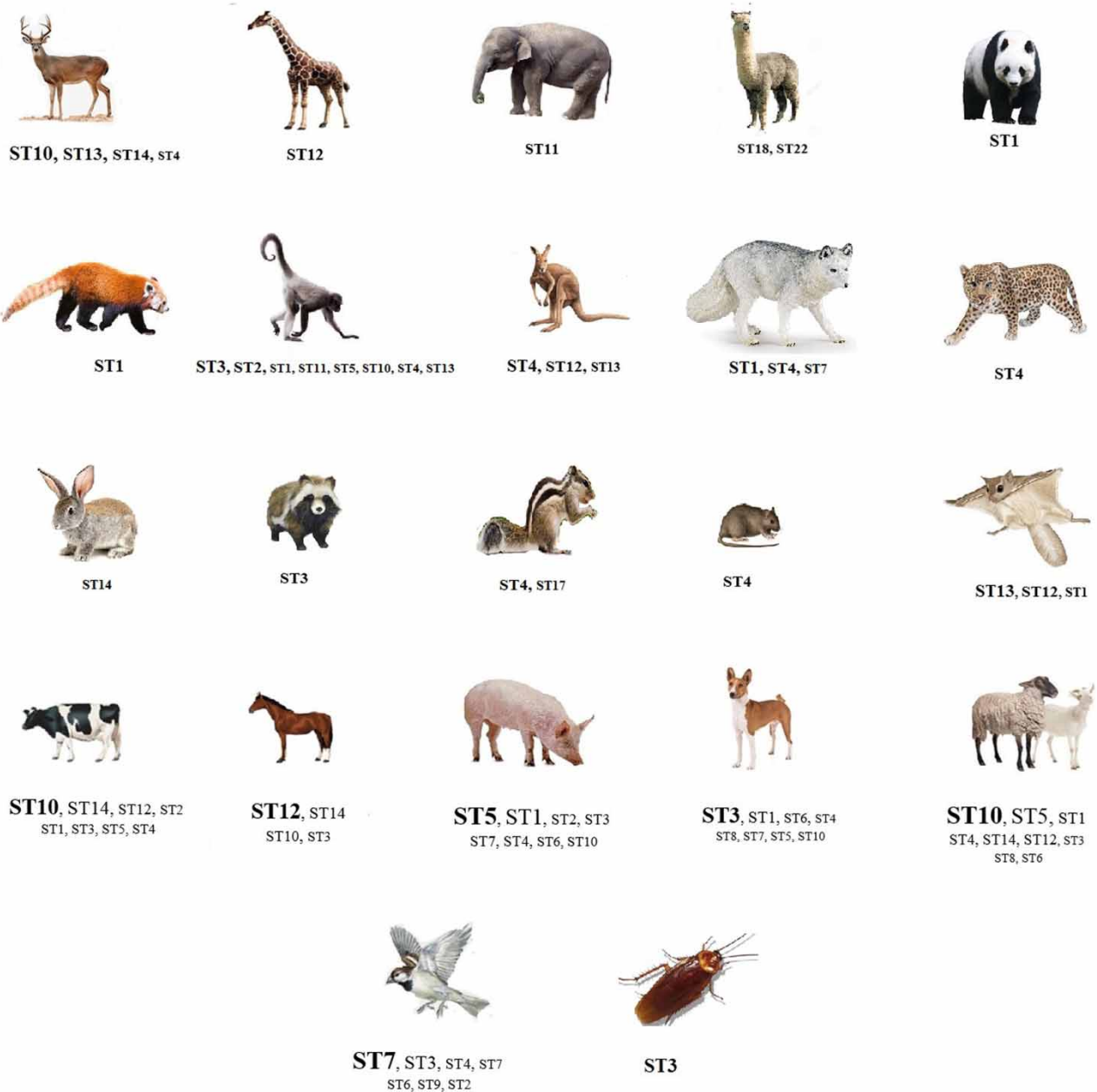
*Blastocystis* sp. subtypes in animals



**Figure 3** | Distribution of *Blastocystis* sp. subtypes in animals. The presented percentages are based on consensus terminology provided by Stensvold *et al.* (2007).

The prevalence of *Blastocystis* sp. in animals in Indonesia was reported to be as high as 50%. Accordingly, *Blastocystis* sp. was reported from pigs, birds, and rodents. The subtype analysis revealed ST2 (40.5%), ST1 (23.8%), ST7 (21.6%), ST4 (8.8%), and ST6 (5%) to be the prevalent subtypes reported from animals (Yoshikawa *et al.* 2016). Apparently, the lack of reports of ST3 and high prevalence of ST7 in animals signify the importance of zoonotic transmission from bird sources (Yoshikawa *et al.* 2016).

## Studied animals and detected subtypes



**Figure 4** | The reported subtypes of *Blastocystis* sp. in different animals from Asian countries and Australia. The font size shows predominance of the subtypes.

### Malaysia

The mean prevalence of *Blastocystis* sp. reported from humans in Malaysia was 19.25% with a range of 9.17–40.3%. Based on the subtype distribution of *Blastocystis* sp., ST1–4 and ST6 have been reported from humans, of which ST3 (52.51%) is the major subtype. ST1 (27.16%), ST2 (8.45%), ST6 (5.43%), ST7 (3.42%), and ST4 (1.2%) were the following prevalent subtypes. There are several reports of *Blastocystis* sp. with unknown subtypes (Tan *et al.* 2009, 2013; Ragavan *et al.* 2015; Nithyamathi *et al.* 2016; Mohammad *et al.* 2017; Noradilah *et al.* 2017b).



The prevalence of *Blastocystis* sp. from animals in Malaysia ranged from 6.3 to 33.33% with an average of 23.95%. *Blastocystis* sp. was reported from canines, sheep, goats, deer, pigs, and cockroaches. Based on the consensus terminology, ST1–10 has been reported from animals in this country. ST1 (28.3%) and ST2 (0.46%) were the most and the least prevalent subtypes reported from animals (Tan *et al.* 2013; Noradilah *et al.* 2017a; Farah Haziqah *et al.* 2018a, 2018b; Mohammad *et al.* 2018a, 2018b, 2018c).

### Nepal

Reports of *Blastocystis* sp. from Nepal are rare. The pooled prevalence in humans seems to be 25.85%. Yoshikawa *et al.* (2009) demonstrated that 25.6% of children with gastrointestinal symptoms carried *Blastocystis* sp. Lee *et al.* (2012a) reported 26.1% of participants were positive for *Blastocystis* sp. Almost all of the studies in Nepal used the STS subtyping technique for molecular characterization and they reported ST4 as the predominant subtypes in humans; after consensus terminology they were converted to ST6. Accordingly, ST6 (43.51%) followed by ST1 (37.96%), ST7 (9.25%) and ST3 (5.5%) were reported as the detected subtypes in humans in Nepal (Yoshikawa *et al.* 2009; Lee *et al.* 2012a). The majority of ST6 from human cases in Nepal may result from the type of molecular analyses and techniques which were employed to determine the subtype distribution in this country.

The prevalence of *Blastocystis* sp. in animals, including cattle, buffaloes, goats, monkeys, and pigs, conducted by Lee *et al.* (2012b) were shown to be 15.4%, while in the study performed by Yoshikawa *et al.* (2009) all 10 (100%) rhesus monkeys harbored *Blastocystis* sp. Molecular analyses of *Blastocystis* subtypes in animals reported the majority of ST7 (29.1%) followed by ST1 (25%), and ST6 (16.6%). An unknown subtype was reported as 3.7 and 29.1% in humans and animals, respectively. Lee *et al.* (2012b) revealed that all water samples were positive for *Blastocystis* sp. ST6. The high prevalence of ST6 in human samples, animals and water samples suggest zoonotic waterborne transmission of *Blastocystis* sp. in Nepal.

### Singapore

The prevalence rate of *Blastocystis* sp. reported from Singapore was 3.3%, which seems to be significantly lower than that reported from other Southeast Asian countries. Little data has been generated from this country. In a single study conducted by Wong *et al.* (2008), the results of molecular analysis of the *Blastocystis*-positive samples showed the ST3 subtype among samples.

### Cambodia

Based on a study performed with Cambodian villagers in close contact with pigs, 55.23% harbored *Blastocystis* sp. In addition, ST1-3 was characterized among *Blastocystis*-positive samples with a majority of ST3 (42.85%) (Wang *et al.* 2014). The molecular analysis of *Blastocystis* sp. was performed in dogs and pigs, where the prevalence rate was reported as approximately 23.25%. Only one of 80 samples (1.3%) was diagnosed positive for *Blastocystis* sp. ST2 was the reported subtype (Wang *et al.* 2013), while of 73 pigs, 45.2% were positive for *Blastocystis* ST5 (Wang *et al.* 2014).

### Japan

There are no molecular epidemiology studies indicating the prevalence of *Blastocystis* sp. in Japan, although a study published by Horiki *et al.* (1997) reported the prevalence of 0.5% for this protist in healthy Japanese subjects using a stool examination technique. However, subtyping of *Blastocystis* sp. was performed in this country on a number of positive samples. Since the majority of *Blastocystis*-positive subtyping in Japan was carried out using STS primers, translation of the reported subtypes based on the consensus terminology may provide a different view of the subtype distribution in Japan. Accordingly, ST1–4 and ST6 among human samples were the reported subtypes in Japan. The most common was ST3 (Yoshikawa *et al.* 2000; Kaneda *et al.* 2001).

Studies on cattle, rodents, and pigs represent the prevalence rate of 39.1% of *Blastocystis* sp. in Japan. As a result, subtyping analysis showed the presence of ST1 (5.3%), ST3 (4%), ST4 (30.66%), ST10 (1.3%), and ST14 (58.6%) among the samples (Abe *et al.* 2003; Iguchi *et al.* 2007; Katsumata *et al.* 2018; Masuda *et al.* 2018). The subtype analysis according to the consensus terminology indicated a lack of ST5 among pig samples in Japan. In addition, the presence of ST4 from rodents supports previous studies around the world, which signified the high prevalence of this subtype in rodents. The distribution pattern of *Blastocystis* sp. subtypes in animals and humans demonstrated the high and low prevalence of ST3 in humans and animals (63.5 vs. 4%), highlighting the probability of anthroponotic transmission of *Blastocystis* sp. in Japanese subjects.

### South Korea

There are no studies reporting the prevalence of *Blastocystis* sp. among human subjects in South Korea. The prevalence of *Blastocystis* sp. in animals was reported to be 6.7% using molecular approaches. Subtyping indicated ST5 (58.47%) as the

major subtype followed by ST1 (16.94%), ST14 (8.47%), ST10 (7.62%), ST3 (5.93%), and ST2 (2.5%) (Lee *et al.* 2018; Paik *et al.* 2019; Lee *et al.* 2020). The subtyping analysis showed that ST5 was the only subtype obtained from the examined wild boars. However, ST1–3 and 5 were characterized from pigs with ST5 being the most common. The reports of ST5 from pigs and cattle suggest the probability of human cases harboring this subtype in South Korea.

### Bangladesh

*Blastocystis* sp. and the distribution of its subtypes were investigated among captive wildlife in Bangladesh (Li *et al.* 2019a). The prevalence rate of this protist among non-human primates, herbivores, and carnivores was reported as: 31.8, 4.9, and 0%, respectively. Additionally, ST1 (16.94%), ST2 (12.9%), ST3 (41.93%), ST10 (3.22%), ST11 (3.22%), ST13 (9.67%) and ST14 (6.45%) were characterized and ST3 was the predominant subtype (Li *et al.* 2019a). However, studies on *Blastocystis* sp. and its subtypes in humans in Bangladesh are absent.

### India

Although there are cases of *Blastocystis* sp. from humans and animals, there is a paucity of prevalence studies of *Blastocystis* sp. and its subtypes using molecular analysis. Two separate studies suggest the prevalence of *Blastocystis* sp. in humans to be 12.27% (Pandey *et al.* 2015) and 26% (Das *et al.* 2016). However, in a recent study, the prevalence of *Blastocystis* sp. was reported to be 57.9% in patients with visceral leishmaniasis using a metagenomics approach, which was significantly higher than that previously reported from apparently healthy subjects in this country (Lappan *et al.* 2019). Therefore, an average prevalence rate of 24% is estimated in India. Subtyping results revealed that ST1 (6.6%) and ST3 (93.3%) were the prevalent subtypes in human cases with the majority being ST3. There is not enough generated data to propose or establish a distribution pattern of *Blastocystis* sp. in India. The prevalence of *Blastocystis* sp. in dogs in India was reported as 24%. ST1 (47.36%) was the major subtype followed by ST6 (36.84%), ST4 (10.52%), and ST5 (5.2%).

### Arabic Peninsula

There are few studies on the prevalence of subtype distribution of *Blastocystis* sp. alongside the Persian Gulf and almost all of them were carried out on foreign workers or visitors. A prevalence of 44.4% has been reported in apparently healthy human subjects, who were referred to a medical center due to mandatory pre-employment tests in the UAE. In this study, ST1 (26.8%), ST2 (17.07%), and ST3 (25.5%) were reported (AbuOdeh *et al.* 2016). Abu-Madi *et al.* (2015) reported a prevalence of *Blastocystis* sp. among foreign immigrants in Qatar and this was found to be 71.1% and three human-prevalent subtypes ST1 (27.1%), ST2 (3.5%), and ST3 (69.2%) were reported. The prevalence rate of 16.7% was reported from Saudi Arabia in two other studies (Mohamed *et al.* 2017a, 2017b). ST1 (10.38%), ST2 (10.22%), ST3 (58.46%), ST5 (6.01%), and ST7 (3.82%) were characterized among samples from Saudi Arabia (Mohamed *et al.* 2017a, 2017b).

The prevalence of *Blastocystis* sp. amongst animals in the UAE was reported to be 20.2%. *Blastocystis* sp. was reported from cattle, sheep, reptiles, rabbits, and rodents. The most prevalent subtype was ST10 (30.43%) followed by ST14 (26.08%), ST4 (8.69%), and ST17 (4.34%). Furthermore, 30.43% of samples were of unknown subtypes. There are no molecular data on *Blastocystis* sp. and its subtypes from Yemen, Oman, and Bahrain, other Arabic Peninsula countries.

### Iran

There have been several studies on the prevalence of *Blastocystis* sp. in humans in Iran and molecular epidemiology studies estimated the prevalence rate as 27%. Based on the consensus terminology, six subtypes ST1 (32.01%), ST2 (21.9%), ST3 (36.7%), ST5 (0.3%), ST6 (2.43%), and ST7 (5.03%), together with unknown subtypes (1.52%), have been reported (Motazedian *et al.* 2008; Moosavi *et al.* 2012; Badparva *et al.* 2014; Azizian *et al.* 2016; Beiromvand *et al.* 2017; Jalallou *et al.* 2017; Khademvatan *et al.* 2017, 2018; Mirjalali *et al.* 2017; Rezaei Riabi *et al.* 2017, 2018; Salehi *et al.* 2017; Piranshahi *et al.* 2018; Mardani Katakai *et al.* 2019; Taghipour *et al.* 2019). The subtype distribution in Iran shows that ST1–3 were the most prevalent subtypes (Alinaghizade *et al.* 2017). The high subtype diversity in human subjects in Iran suggests different potential sources of infection for *Blastocystis* sp. For instance, in a study performed by Rezaei Riabi *et al.* (2018), living near a chicken factory was proposed as the reason of carrying ST7 by a symptomatic subject.

Reports of *Blastocystis* sp. in animals from Iran are rare. Badparva *et al.* (2015) reported the prevalence rate 9.6% of *Blastocystis* sp. from cattle using STS primers that after converting based on consensus terminology, ST2 (64.7%), ST3 (23.52%), and ST5 (11.76%) were the characterized subtypes. Javanmard *et al.* (2019) investigated the occurrence of subtype distribution of *Blastocystis* sp. in wastewater samples and they were characterized as ST2, ST6 and ST8 among the samples.

The presence of ST5–ST7 in human subjects, animals, and environmental samples suggests a probability of zoonotic transmission of *Blastocystis* sp. together with anthroponotic transmission in Iran.

### Lebanon

The prevalence of *Blastocystis* sp. in human subjects in Lebanon was reported to be about 39%. The molecular typing suggested ST3 (50.37%) as the most common subtype followed by ST2 (23.7%), ST1 (24.81%), and ST6 (0.74%) (El Safadi *et al.* 2013; Osman *et al.* 2015; Greige *et al.* 2018, 2019). The prevalence in animals was reported as high as that in humans with an average of 47.6%. *Blastocystis* sp. was detected in chickens and cattle and ST1 (7.17%), ST2 (7.17%), ST3 (0.47%), ST5 (1.43%), ST6 (27.2%), ST7 (8.1%), ST10 (26.31%), and ST14 (22%) were characterized (Greige *et al.* 2018, 2019). The low prevalence of human-common subtypes 1–3 among animal samples suggests the high probability of anthroponotic transmission for *Blastocystis* sp. in human cases despite the reports of ST6 (an avian subtype) in humans.

### Burden of *Blastocystis* sp. in Australia and New Zealand

#### Australia

The pooled prevalence of *Blastocystis* sp. in Australia was approximately 55.1%. The subtyping analysis of *Blastocystis* sp. in Australia represented a high subtype diversity suggesting the high potential of zoonotic transmission and a broad range of animal hosts responsible for human infection. In this regard, ST1 (33%), ST2 (3.81%), ST3 (47.32%), ST4 (9.16%), ST5 (2.29%), ST6 (2.29%), ST7 (0.76%), and ST8 (1.52%) were reported from humans in Australia (Parkar *et al.* 2010; Nagel *et al.* 2012; Roberts *et al.* 2013b; Wang *et al.* 2014). The subtype distribution pattern of *Blastocystis* sp. is similar to that reported in the Philippines, Thailand, and European countries (Belleza *et al.* 2015; Adao *et al.* 2016; El Safadi *et al.* 2016; Palasuwan *et al.* 2016; Udonsom *et al.* 2018; Yowang *et al.* 2018).

The average prevalence of *Blastocystis* sp. in animals was 34.83%. The occurrence of this protist was investigated among farm animals together with a broad variety of wild and zoo animals. The presence of *Blastocystis* sp. was reported from birds, canines, deer, pigs, non-human primates, kangaroos, elephants, leopards and giraffes. ST1 (15.2%), ST2 (3.73%), ST3 (1.86%), ST4 (4.53%), ST5 (56%), ST6 (0.26%), ST7 (1.06%), ST11 (8%), ST12 (5.86%), and ST13 (0.8%) were characterized among the samples (Parkar *et al.* 2007, 2010; Roberts *et al.* 2013a; Wang *et al.* 2014). The high diversity among reported subtypes is most probably due to high diversity among studied hosts.

#### Countries without molecular data on *Blastocystis* sp.

Although the presence of *Blastocystis* sp. was reported from most Asian countries, there are countries in which no reports of *Blastocystis* sp. and its subtypes are available. For example, there are no reports of this protist in Asian countries of the commonwealth of independent states (CIS) such as Turkmenistan, Uzbekistan, Tajikistan, Kazakhstan, and Kirgizstan. There are no molecular data from some countries in the south of Asia including Pakistan and Afghanistan. In addition, the prevalence of *Blastocystis* sp. and its subtypes are not reported from Mongolia or countries from the Middle East such as Iraq, Syria, Kuwait, and Palestine. There are no reliable studies on *Blastocystis* sp. in New Zealand. It may be possible that the prevalence and subtype distribution of *Blastocystis* sp. could be similar to that reported from Australia.

#### *Blastocystis* sp. in environmental samples: the risk of waterborne transmission

There are few reports of *Blastocystis* sp. and its subtypes from environmental samples. However, the prevalence of the protozoan among almost all of them was high. In addition, the human-prevalent subtypes of *Blastocystis* sp. were reported from all of them, highlighting the probability of waterborne transmission of this eukaryote. In a study conducted by Leelayoova *et al.* (2008) in Thailand, one-fifth of drinking water samples was detected to be positive for *Blastocystis* sp. subtype 1. In Nepal, the presence of *Blastocystis* sp. was confirmed in all four water samples collected from two rivers. Out of these samples, ST1 and ST6 were found to be between 100 and 75% of samples, respectively (Lee *et al.* 2012b). The prevalence of *Blastocystis* sp. in river water samples was also evaluated in Malaysia and from 51 *Blastocystis*-positive samples, ST1–4, ST8, and ST10 with the majority of ST3 characterized (Noradilah *et al.* 2016).

The presence of *Blastocystis* sp. in wastewater was also investigated. In the Philippines, Banaticla & Rivera (2011) checked the presence of *Blastocystis* sp. and its subtypes in wastewater treatment plants and ST1 and ST2 were the identified subtypes. In addition, in Iran, the barcoding region of *Blastocystis* sp. was amplified among five out of 12 wastewater samples of which ST2, ST6, and ST8 were identified among 2, 2, and 1 samples, respectively (Javanmard *et al.* 2019). In a recent study by Zahedi *et al.* (2019), a next generation sequencing (NGS) approach was employed to identify 18S ribosomal RNA (rRNA)

of Eukarya, particularly pathogenic fecal protists, among wastewater treatment plants (WWTPs) at different stages (influent, intermediate, and effluent) and characterized *Blastocystis* sp. among eight out of 26 samples. In addition, ST1–4, ST6 and ST8 were recognized. The presence of *Blastocystis* sp. was also reported from water samples collected from different rivers, as well as the Black Sea in Turkey (Koloren *et al.* 2018).

The presence of *Blastocystis* sp. cysts in the water samples indicates the possibility of fecal contamination of the water resources by humans or animals. It was proposed that *Blastocystis* sp. not only remains alive in water with temperatures of 4 and 25 °C, but also it seems that it may resist conventional chlorine treatment (Ahmed & Karanis 2018). Several confirmed and most probable waterborne outbreaks due to *Blastocystis* sp. have been reported from Nepal, Italy, China, and Morocco (Karanis *et al.* 2007; Baldursson & Karanis 2011; Frealle *et al.* 2015). Furthermore, there are reports demonstrating transmission of *Blastocystis* sp. from drinking water to humans (Leelayoova *et al.* 2004; Lee *et al.* 2012b; Anuar *et al.* 2013; Angelici *et al.* 2018). Climate conditions, human activities, socioeconomic conditions, and a water crisis, were suggested to be factors which increased the risk of waterborne transmission of protozoan parasites including *Blastocystis* sp. (Ahmed & Karanis 2018; Ahmed *et al.* 2018; Javanmard *et al.* 2018).

## CONCLUSIONS

ST1–3, particularly ST1 and ST3, are the most prevalent subtypes among human subjects in Asian countries. Therefore, it seems that like the reports of subtype distribution from other continents, ST1–3 are the most prevalent subtypes in the world. Interestingly, although the actual source of ST4 in Asian countries is unclear, the reports of this subtype in humans in East Asian countries are higher than the west Asian countries.

Among the potentially zoonotic subtypes, ST6 and ST7, two prevalent avian subtypes, were frequently reported from humans in most East Asian countries together with Iran and Lebanon, two West Asian countries, suggesting the probable role of birds in zoonotic transmission of *Blastocystis* sp. in Asia. In addition, human-prevalent subtypes, ST1–4, were recognized in animals highlighting the probability of zoonotic transmission of this protozoan.

The distribution of *Blastocystis* sp. in Asian countries suggests that the prevalence and the distribution pattern of subtypes vary from the east to the west countries. In other words, the climate probably affects the transmission cycle of this eukaryote. It seems that the prevalence of this protozoan is not only affected by socioeconomic conditions, but also a tropical climate may increase the prevalence rate of *Blastocystis* sp. The example for this observation could be concluded from epidemiological studies, which were performed in the countries around the Persian Gulf. Here, the prevalence of *Blastocystis* sp. was found to be high in immigrant workers, who came from South East Asian countries to the UAE and Qatar. Evidence indicates the high prevalence of *Blastocystis* sp. in western developed countries with a Mediterranean climate. Therefore, apart from climate, culture and lifestyle may also play important roles in the distribution and prevalence of *Blastocystis* sp. In addition, the potential role of gut microbiome on the prevalence and subtype distribution of *Blastocystis* sp. in the human gut should be considered. Several studies in Asia found the human infecting subtypes of *Blastocystis* sp. in water. These water resources were used for irrigation, recreational purposes, and drinking water production. *Blastocystis* sp. appeared resistant in some water and environmental conditions. It is most likely that contamination of water sources is from animal feces. The importance of the waterborne capability of *Blastocystis* sp. and possible waterborne outbreaks due to this protist should not be ignored.

One of the most important issues in true estimation of the prevalence of *Blastocystis* sp. is the methodology of detection. Actually, in most of the epidemiological studies on intestinal parasites, the focus has not been on *Blastocystis* sp. and the prevalence of this protist was reported by parasitological tests, in addition to other parasites. Therefore, the true prevalence rate of this protist is higher than the current estimation. On the other hand, it should be considered that molecular techniques show only the presence of *Blastocystis* sp. DNA in a sample; therefore, molecular methods such as PCR detect not only live *Blastocystis* sp., but also dead parasites and/or genome of the protist, both of which may affect the true estimation of *Blastocystis* sp. in a sample.

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## AUTHOR CONTRIBUTIONS

Conceived and designed the experiments: HM PK MRZ. Analyzed the data: SN HM. Data validation: HM PK. Wrote the paper: HM PK PJ. All authors read and approved the final version of the manuscript.

## DATA AVAILABILITY STATEMENT

All relevant data are included in the paper or its Supplementary Information.

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