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PROTOZOOLOGY - REVIEW

Current status of *Blastocystis* sp. in animals from Southeast Asia: a review

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Abstract

Blastocystis is the most frequently observed eukaryotic gastrointestinal symbiont in humans and animals. Its low host specificity and zoonotic potential suggest that animals might serve as possible reservoirs for transmission. The prevalence and subtype distributions of *Blastocystis* sp. in animal populations in Southeast Asia, a hotspot for zoonotic diseases, are reviewed. Recommendations for future research aimed at understanding the zoonotic role of Blastocystis are also included. Seven countries have, so far, reported *Blastocystis* infection in various animals, such as livestock, poultry, companion animals, and non-human primates. Pigs were the most studied animals, and there were records of 100% prevalence in pigs, cattle, and ostriches. Using polymerase chain reaction (PCR)-based approaches, twelve *Blastocystis* sp. subtypes (STs), namely ST1, ST2, ST3, ST4, ST5, ST6, ST7, ST8, ST9, ST10, ST12, and ST14 have been recognised infecting animals of Southeast Asia. ST1 and ST5 were the most frequently identified, and Malaysia observed the most diverse distribution of subtypes. Further investigations on *Blastocystis* sp. in various animal hosts, using adequate sample sizes and uniform detection methods, are essential for a better understanding of the distribution of this organism. Detailed genome studies, especially on STs shared by humans and animals, are also recommended.

Keywords *Blastocystis* · Distribution · Prevalence · Subtypes · Animals · ASEAN

Introduction

Blastocystis sp. is a ubiquitous intestinal protistan parasite found in a wide range of animals, including humans (Tan 2004; Chandrasekaran et al. 2014). It is an anaerobic protist (Skotarczak 2018), and a member of the Stramenopiles branch of Eukarya, a complex and heterogeneous evolutionary assemblage of heterotrophic and photosynthetic protozoa (Silberman et al. 1996). *Blastocystis* is a polymorphic organism with four forms commonly described in literature, namely the vacuolar, granular, amoeboid, and cyst forms (Tan et al. 2002). The transmission is faecal-oral, and it commonly inhabits its host's large intestine (Tan 2008). Despite being the most widely encountered eukaryotic gastrointestinal symbiont in humans and animals (Adao and Rivera 2018), and having been described since the early 1900s, there have only been a handful of significant advances in the understanding of *Blastocystis* biology over the last decade (Tan 2008).

One of the primary drivers of *Blastocystis* ubiquity is its genetic diversity (Nieves-Ramirez et al. 2018). Based on the phylogeny of their small subunit ribosomal RNA (SSU rRNA) gene, at least 17 subtypes (STs, ST1-ST17) have been identified in a broad host range including humans, other mammals, birds, reptiles, and insects (Alfellani et al. 2013, Stensvold et al. 2012). Many of these subtypes are common to humans and animals; however, ST9 is exclusively isolated from humans (Ahmed and Karanis 2018). Currently, humans can host ten STs (STs 1–9, and ST12); nine of the ten STs have been reported in both humans and animals, hence the likelihood of zoonotic transmission (Clark et al. 2013; Mohammad et al. 2018a; Stensvold et al. 2020).

Due to the low host specificity and zoonotic potential of *Blastocystis*, it has been suggested that animals might serve as large potential reservoir for transmitting infection (Ahmed and Karanis 2018). The recommendation that intimate

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associations between man and animals could aid transmission of parasites has, in turn, prompted investigations on the prevalence of *Blastocystis* in animals from domestic environments; which would, thereby, enrich the understanding of the transmission of *Blastocystis* (Chuong et al. 1996). Besides, awareness on the role of wildlife in the transmission of pathogens of human health importance has grown in Asia since the emergence of diseases such as the Nipah virus, severe acute respiratory syndrome, and the avian influenza (Lee et al. 2008). These have, consequently, led to an upsurge in investigations on the epidemiology of *Blastocystis* sp. in several animal groups around the world, including Southeast Asia.

Zoonoses are a rising concern of Southeast Asia, a diverse region experiencing rapid social, economic, and demographic transformation (Bordier and Roger 2013). Together with agricultural practices, these factors have made this region a hotspot for zonotic diseases (Coker et al. 2011). The objective of this article was to review studies on *Blastocystis* sp. in this region in order to provide a clearer knowledge of its distribution in different animal hosts across Southeast Asia and to provide informed recommendations on the direction for future research including those which could ultimately lead to the understanding of the zoonotic character of *Blastocystis*.

Methods

Databases including Google Scholar, PubMed, and ScienceDirect were searched for articles reporting on the presence of *Blastocystis* in animals throughout countries of Southeast Asia. The following keywords were used: *Blastocystis*, STs, subtypes, distribution, epidemiology, prevalence, molecular, intestinal parasites, genetic diversity, characterization, and animals. Articles, written in English, from which samples were obtained in countries belonging to the Association of South-Eastern Asian Nations (ASEAN) and in which identification of the parasite was by either or both parasitological and molecular methods were used for this review. A total of 47 articles were, thus, found for seven ASEAN countries. Information extracted included the country, host animal, number of samples, number of samples positive for *Blastocystis*, subtype(s) identified, method(s) used for identification, and number of samples per subtype.

Results

In the last decade, more studies have emerged in Southeast Asia giving a clearer picture on the status and genetic diversity of *Blastocystis* sp. in wild animals, poultry and other birds, livestock, reptiles, arthropods, and companion animals. The 47 articles that met the inclusion criteria were studies from Cambodia, Indonesia, Malaysia, Philippines, Thailand, Singapore, and Vietnam (summarised in Table 1). Most studies were clustered in Malaysia, Indonesia, and Thailand with foci in livestock, poultry, and wildlife. Detection methods mainly employed were the gold standard methods for *Blastocystis* which included conventional microscopy, in vitro culture, and Polymerase chain reaction (PCR)-based approaches. The prevalence of *Blastocystis* infection and the subtypes identified varied among the different taxonomic groups. The genetic diversity of *Blastocystis* sp. in different animals from Southeast Asia is described in Table 2.

Distribution of *Blastocystis* sp. across ASEAN countries

Although the occurrence of *Blastocystis* sp. in animals has been documented in seven countries, only five of them have reported infection in a wide range of animals. Studies on animal samples from Cambodia and Vietnam reported *Blastocystis* only in pigs and dogs and in pigs only, respectively. Studies from Indonesia, Malaysia, Philippines, and Thailand showed the presence of *Blastocystis* in livestock animals and non-human primates. The presence of infection in poultry was indicated by researches from Indonesia, Malaysia, and the Philippines.

Thus far, *Blastocystis* ST1, ST2, ST3, ST4, ST5, ST6, ST7, ST8, ST9, ST10, ST12, and ST14 have been recognised in animals across Southeast Asia. The most frequently identified subtypes were ST1 and ST5, with the least-observed subtypes being ST8 and ST9. The most widespread subtype was ST5, as it was found present in six of the seven countries where *Blastocystis* had been studied in animals. ST2 was identified in five countries, while ST9, a subtype considered unique to humans, and ST8 were detected in Malaysia only. ST12 was identified in Thailand alone. Malaysia witnessed the most diverse distribution of subtypes; a total of ten subtypes, ST1–ST10, were observed. Singapore and Vietnam had the least number of studies, and in each country, only one subtype was identified. While Malaysia had the highest numbers of ST1, ST4, ST6, and ST7, the highest numbers of ST2, ST3, and ST5 were from Thailand.

Distribution of *Blastocystis* sp. in animal hosts

Artiodactyla

The prevalence of infection in cattle, goats, pigs, deer, sheep, and guar ranged from 14.43 to 100% (Table 1). The highest prevalence rates were found in pigs and cattle (with records of 100%), which were also the most sampled. High prevalence of *Blastocystis* infection in pigs and cattle have been documented in other parts of the world (Abe et al. 2002; Masuda et al. 2018; Mokhtar and Youssef 2018; Moura et al. 2018; Greige et al. 2019). Nevertheless, Fayer et al. (2012) and

Table 1 Summary of published studies/reports on *Blastocystis* sp. in animal hosts in Southeast Asia

Host	Location	Sample size	Number of positive samples (%)	Subtypes (STs) identified	Technique	References
Artiodactyla						
Pig	Cambodia	73	33 (45.2)	ST5	Sequencing	Wang et al. 2014
	Indonesia	93	81 (87.1)	ST1, ST2, ST5, ST7	In vitro cultivation, sequencing	Yoshikawa et al. 2016
	Malaysia	10	10 (100)	NA	In vitro cultivation	Hemalatha et al. 2014
	Philippines	12	12 (100)	ST1, ST5	In vitro cultivation, RFLP	Rivera and Tan 2005
	Philippines	2	2 (100)	ST1, ST2	In vitro cultivation, sequencing	Rivera 2008
	Philippines	99	20 (20.2)	ST1, ST5, ST7, Mixed	In vitro cultivation, sequencing	Adao et al. 2016
	Philippines	122	47 (38.5)	NA	Formalin-ether sedimentation, in vitro cultivation	De La Cruz et al. 2016
	Thailand	26	25 (96.1)	ST1	In vitro cultivation, RFLP	Thathaisong et al. 2003
	Thailand	102	32 (31.37)	ST1, ST3, ST12, ST14	Sequencing	Sanyanusin et al. 2017
	Thailand	90	32 (35.55)	ST1, ST3, ST5	Sequencing	Pintong et al. 2018
	Thailand	87	40 (45.98)	ST1, ST5	Sequencing	Udonsom et al. 2018
	Vietnam	12	12 (100)	ST5	Sequencing	Alfellani et al. 2013
Guar	Malaysia	10	3 (30)	NA	In vitro cultivation	Hemalatha et al. 2014
Goat	Malaysia	236	73 (30.9)	ST1, ST3, ST6, ST7	STS analysis	Tan et al. 2013
	Malaysia	20	13 (65)	NA	In vitro cultivation	Hemalatha et al. 2014
	Malaysia	31	8 (25.81)	ST4, ST8, ST10	Sequencing	Noradilah et al. 2017
	Philippines	6	1 (16.7)	ST14	In vitro cultivation, sequencing	Adao et al. 2016
	Thailand	38	36 (94.74)	ST10, ST12, ST14	Sequencing	Udonsom et al. 2018
Cattle	Indonesia	500	72 (14.43)	NA	Sedimentation, modified Fulleborn's floatation	Hastutiek et al. 2019
	Indonesia	100	100 (100)	NA	Sedimentation, sucrose flotation	Susana et al. 2019
	Indonesia	108	108 (100)	ST10	In vitro cultivation, STS analysis, sequencing	Suwanti et al. 2020
	Malaysia	29	10 (34.5)	NA	In vitro cultivation	Hemalatha et al. 2014
	Malaysia	3	1 (33.33)	ST10	Sequencing	Mohammad et al. 2018a
	Thailand	42	21 (50)	ST10, ST12	Sequencing	Udonsom et al. 2018
Sheep	Malaysia	38	22 (57.9)	NA	In vitro cultivation	Hemalatha et al. 2014
Host	Location	Sample size	Number of positive samples (%)	Subtypes (STs) identified	Technique	References
Artiodactyla						
Deer	Malaysia	14	4 (28.6)	NA	In vitro cultivation	Hemalatha et al. 2014
Mouse-deer	Malaysia	4	1 (25)	Unknown	In vitro cultivation, sequencing	Mohd Zain et al. 2017
Deer	Malaysia	100	30 (30)	ST10	Sequencing	Mohammad et al. 2018b
Perissodactyla						
Horse	Thailand	8	1 (12.5)	ST1		

Table 1 (continued)

Host	Location	Sample size	Number of positive samples (%)	Subtypes (STs) identified	Technique	References
					In vitro cultivation, RFLP	Thathaisong et al. 2003
Insecta						
Cockroach	Singapore	10	8 (80)	NA	In vitro cultivation	Zaman et al. 1993
	Singapore	4	4 (100)	NA	In vitro cultivation, sequencing	Yoshikawa et al. 2007
	Malaysia		10%	NA	In vitro cultivation	Suresh et al. 1997
	Malaysia	30	3 (10)	NA	In vitro cultivation	Chuong et al. 1996
	Malaysia	151	61 (40.4)	ST3	In vitro cultivation, sequencing	Farah Haziqah et al. 2017
Carnivora						
Dog	Cambodia	80	1 (1.3)	ST2	Sequencing	Wang et al. 2013
	Malaysia	84	40 (47.62)	ST1, ST3, ST4, ST8, 10	Sequencing	Noradilah et al. 2017
	Philippines	145	21 (14.5)	ST1, ST2, ST3, ST4, ST5	In vitro cultivation, STS analysis, sequencing	Belleza et al. 2016
	Thailand	3	3 (100)	ST5	In vitro cultivation, sequencing	Parkar et al. 2007
	Thailand	189	5 (2.6)	NA	In vitro cultivation	Leelayoova et al. 2009
	Thailand	13	1 (7.69)	ST3	Sequencing	Udonsom et al. 2018
Cat	Indonesia	90	48 (53.33)	NA	Sedimentation	Patagi et al. 2018
	Malaysia	60	12 (20)	ST1	Sequencing	Farah Haziqah et al. 2018a
Aves						
Duck	Philippines	31	3 (9.6)	ST7, *B. pythoni*	In vitro cultivation, Sequencing	Adao et al. 2016
	Malaysia	20	8 (40)	ST1, ST2, ST3, ST7	Sequencing	Noradilah et al. 2017
Swan	Malaysia	20	7 (35)	ST1, ST3	Sequencing	Noradilah et al. 2017
Ostrich	Malaysia	37	37 (100)	ST6	In vitro cultivation, STS analysis	Chandrasekaran et al. 2014
	Malaysia	37	37 (100)	NA	In vitro cultivation	Hemalatha et al. 2014
Host	Location	Sample size	Number of positive samples (%)	Subtypes (STs) identified	Technique	References
Aves						
Large-billed crow	Malaysia	106	4 (3.77)	NA	Formol-ether concentration	Lee et al. 2008
Chicken	Indonesia	38	13 (34.2)	ST7	In vitro cultivation, Sequencing	Yoshikawa et al. 2016
	Malaysia	107	27 (25.23)	NA	In vitro cultivation	Farah Haziqah et al. 2014
	Malaysia	104	27 (26)	ST1, ST3, ST6, ST7, ST9	Sequencing	Noradilah et al. 2017
	Malaysia	15	1 (6.67)	ST6	Sequencing	Mohammad et al. 2018a
	Malaysia	179	47 (26.27)	ST1, ST6, ST7, ST8	In vitro cultivation, Sequencing	Farah Haziqah et al. 2018b
	Philippines	8	8 (100)	ST2, ST3	In vitro cultivation, RFLP	Rivera and Tan 2005
	Philippines	1	1 (100)	ST6	In vitro cultivation, Sequencing	Rivera 2008
	Philippines	34	5 (14.7)	ST7, Mixed		

Table 1 (continued)

Host	Location	Sample size	Number of positive samples (%)	Subtypes (STs) identified	Technique	References
					In vitro cultivation, Sequencing	Adao et al. 2016
Primates						
Macaques	Indonesia	88	38 (43)	NA	Concentration	Jones-Engel et al. 2004
Pig-tailed macaques	Malaysia	8	1 (12.5)	NA	Direct wet mount, sedimentation	Lim et al. 2008
Macaques	Philippines	50	5 (10)	NA	Formol-ether concentration	Casim et al. 2015
	Thailand	628	263 (41.87)	ST1, ST2, ST3	In vitro cultivation, sequencing of SSU rRNA gene	Vaisusuk et al. 2018
Orangutans	Indonesia	262	36 (13.7)	NA	Sodium acetate-acetic acid-formalin-concentration (SAFC)	Labes et al. 2010
	Malaysia	3	1(33.33)	NA	Direct wet mount, sedimentation	Lim et al. 2008
	Malaysia	10	5 (50)	NA	In vitro cultivation	Hemalatha et al. 2014
Monkey	Philippines	4	4 (100)	ST1, ST2, ST3	In vitro cultivation, sequencing	Rivera 2008
Non-human primates	Malaysia	308	5 (1.62)	NA	Floatation, sedimentation, opportunistic necropsy	Adrus et al. 2019
Rodentia						
Rat	Indonesia	77	10 (13)	ST4	In vitro cultivation, sequencing	Yoshikawa et al. 2016
	Indonesia	98	6 (6)	NA	Wet smear	Prasetyo 2016
	Malaysia	95	48 (51)	NA	Floatation	Premaalatha et al. 2017
Host	Location	Sample size	Number of positive samples (%)	Subtypes (STs) identified	Technique	References
Rodentia						
Rat	Malaysia	290	133 (45.9)	ST1, ST4, ST5, ST7	In vitro cultivation, sequencing	Farah Haziqah et al. 2018c
	Singapore	3	3 (100)	ST7	In vitro cultivation, sequencing	Noel et al. 2005
Reptilia						
Sea snake	Singapore	1	1 (100)	NA	In vitro cultivation	Teow et al. 1991
Sea snake	Singapore	1	1 (100)	Unknown	In vitro cultivation, sequencing	Noel et al. 2005
Crocodile	Singapore	1	1 (100)	NA	In vitro cultivation	Teow et al. 1992
Iguana	Singapore	1	1 (100)	NA	In vitro cultivation	Teow et al. 1992
Python	Singapore	1	1 (100)	Unknown	In vitro cultivation, sequencing	Noel et al. 2005
Snake	Singapore	20	3 (15)	NA	In vitro cultivation	Teow et al. 1992
Lizard	Singapore	1	1 (100)	Unknown	In vitro cultivation, sequencing	Noel et al. 2005
House lizard	Malaysia	30	2 (6.67)	NA	In vitro cultivation	Chuong et al. 1996
	Malaysia		7%	NA	In vitro cultivation	Suresh et al. 1997
Water monitor lizard	Malaysia	6	1 (1.6)	Unknown	In vitro cultivation, sequencing	Mohd Zain et al. 2017
Tortoise	Singapore	3	3 (100)	NA	In vitro cultivation	Teow et al. 1992

STS, sequence-tagged site; *RFLP*, restriction fragment length polymorphism; *Blastocystis* subtype equivalents of RFLP results were obtained from Stensvold et al. (2007) and Wang et al. (2018a)

Wang et al. (2018a) respectively described infection rates as low as 19.15% and 9.5% in cattle.

Eleven subtypes of *Blastocystis* have been identified in Artiodactyla in ASEAN countries: ST1, ST2, ST3, ST4, ST5, ST6, ST7, ST8, ST10, ST12, and ST14 (Table 2). Most common in pigs were ST1 and ST5, which is similar to reports from Stensvold et al. (2009) and Alfellani et al. (2013). Although ST5 has been isolated in other mammalian animals, including man, pigs are referred to as the main reservoir of this subtype and a possible source of infection to man (Wang et al. 2018a). Comparable with the findings of Stensvold et al. (2009), Alfellani et al. (2013), Cian et al. (2017), and Greige et al. (2019); ST10 was quite common in cattle, goat, and deer of ASEAN countries. The frequent identification of ST10 in cattle supports suggestions that Bovidae may be the natural host for this subtype (Masuda et al. 2018). The absence of ST10 in human populations, however, suggests that cattle play a negligible role as zoonotic reservoirs of *Blastocystis* sp. (Greige et al. 2019). A predominance of *Blastocystis* ST1, ST6, and ST7 was reported in goats in Malaysia, by Tan et al. (2013), with no reports on STs beyond ST7. In this study, PCR amplification was carried out using sequenced-tagged site (STS) primers that aimed to detect ST1–ST7; this could have led to positive samples for ST8 upwards being missed out. Aside from ST10 and ST14, Song et al. (2017) mentioned the presence of ST1, ST3, ST4, ST5, and ST7 in goats in China; Alfellani et al. 2013 also reported ST3 and ST7 in goats in Libya, while Mokhtar and Youssef 2018 identified ST1 and ST4 in Egypt. The presence of ST1, ST3, and ST4 (major subtypes in humans) suggests that goats may have a role in the transmission of *Blastocystis* to man. The regular occurrence of ST1 and ST5 in pigs, and of ST10 in cattle, goats, and deer irrespective of the country of study, could indicate the absence of geographic limitation in the distribution of these STs.

Perissodactyla

In Southeast Asia, the presence of *Blastocystis* in this group of animals is rare. The only such report to date is a 12.5% prevalence in horses associated with ST3 (Thathaisong et al. 2003). ST3 has been identified as the major subtype in Perissodactyla of French zoos (Cian et al. 2017). Hemalatha et al. 2014, however, described the absence of *Blastocystis* in faecal samples from horses in Malaysia; Mokhtar and Youssef also reported the same from Egypt in 2018.

Carnivora

With more studies describing the presence of *Blastocystis* in dogs than cats (Table 1), prevalence in Carnivora ranged from 1.3 to 100%. The absence of infection in dogs and cats was also reported (Chuong et al. 1996; Hemalatha et al. 2014; Farah Haziqah et al. 2018a; Mohammad et al. 2018a). Both presence and absence of *Blastocystis* in dogs and cats have been documented in other regions.

Prevalence of 2.5% and 24% were described in pet and pound dogs from Brisbane, Australia, and stray dogs in India, respectively (Wang et al. 2013). However, Duda et al. (1998) had recorded a much higher prevalence (70.8%) in Brisbane pound dogs using light microscopy only in 1998. Wang et al. (2013) attributed a likely reason for this difference to be an improved standard of care and hygiene of the pound dogs compared with that of 1998. In 1998, Duda et al. had suggested that in vitro culture conditions were not optimal for the growth of *Blastocystis* sp. from dogs and cats. In their analysis, faecal samples positive for *Blastocystis* from dogs and cats by wet mount were cultured in parallel with a human isolate of *Blastocystis*. MEM (minimal essential medium, Gibco BRL) failed to support the culture of *Blastocystis* sp. from dogs and cats but did for the human isolate. And while the growth of *Blastocystis* sp. from cats was unsuccessful, growth from dog samples appeared slower and less consistent than the growth of *Blastocystis* from human on inspissated egg slant media. Interestingly, Farah Haziqah et al. (2018a) reported a zero prevalence of *Blastocystis* sp. in 82 dogs and 180 cats by in vitro cultivation in modified Jones' medium, but 20% (12/60) from these cats turned out to be positive when screened by DNA barcoding. Their in vitro study showed that viable cells or cysts were destroyed under extremely acidic conditions similar to the pH in the gastrointestinal tract of carnivorous animals. They resolved that gastrointestinal pH is an important determinant of *Blastocystis* viability and consequently influences the epidemiology of infection within avian, mammalian, and human hosts (Farah Haziqah et al. 2018a).

Studies from Japan, Egypt, and Spain using agar-slant medium, STS primers, and sequencing respectively did not detect *Blastocystis* infection in dogs and cats (Abe et al. 2002; Mokhtar and Youssef 2018; and Paulos et al. 2018). Carnivores, including dogs and cats, screened in the UK, France, and Croatia by sequencing were also reported negative for *Blastocystis* infection (Alfellani et al. 2013). Conversely, a research in 2018 by Moura et al. showed 0% and 2.6% *Blastocystis* infection in pet cats and dogs respectively by sedimentation technique; these samples were found negative when screened by PCR-STS. It is suggested that dogs are not natural hosts for *Blastocystis* but rather are transiently and opportunistically infected with a diversity of STs (Wang et al. 2013) and do not play a significant role as natural reservoirs of human infection (Paulos et al. 2018).

Dogs and cats in ASEAN countries have been indicated to carry ST1-ST5, ST8, and ST10. Stensvold et al. identified ST3 in a dog in Denmark in 2009, ST1–ST3 were also described in several members of the order Carnivora in French zoos by Cian et al. (2017).

Table 2 Subtype distribution of *Blastocystis* sp. in different animals from Southeast Asia

Host	Location	Number of subtype observations	Subtype (ST)															Technique	References
			ST1	ST2	ST3	ST4	ST5	ST6	ST7	ST8	ST9	ST10	ST12	ST14	Mixed ST	Unknown ST	*B. pythoni*		
Artiodactyla																			
Pig	Philippines	12	1	-	-	-	11	-	-	-	-	-	-	-	-	-	-	RFLP	Rivera and Tan 2005
	Philippines	2	1	1	-	-	-	-	-	-	-	-	-	-	-	-	-	Sequencing	Rivera 2008
	Thailand	20	20	-	-	-	-	-	-	-	-	-	-	-	-	-	-	RFLP	Thathaisong et al. 2003
	Vietnam	12	-	-	-	-	12	-	-	-	-	-	-	-	-	-	-	Sequencing	Alfellani et al. 2013
	Cambodia	33	-	-	-	-	20	-	-	-	-	-	-	-	13	-	-	Sequencing	Wang et al. 2014
	Philippines	20	10	-	-	-	7	-	2	-	-	-	-	-	1	-	-	Sequencing	Adao et al. 2016
	Indonesia	73	12	8	-	-	73	-	8	-	-	-	-	-	59	-	-	Sequencing	Yoshikawa et al. 2016
	Thailand	32	9	-	1	-	-	-	-	-	-	-	3	19	-	-	-	Sequencing	Sanyanusin et al. 2017
	Thailand	32	6	-	1	-	25	-	-	-	-	-	-	-	-	-	-	Sequencing	Pintong et al. 2018
	Thailand	40	2	-	-	-	38	-	-	-	-	-	-	-	-	-	-	Sequencing	Udonsom et al. 2018
Goat	Malaysia	73	44	-	8	-	-	30	30	-	-	-	-	-	30	-	-	STS analyses	Tan et al. 2013
	Philippines	1	-	-	-	-	-	-	-	-	-	-	-	1	-	-	-	Sequencing	Adao et al. 2016
	Malaysia	8	-	-	-	5	-	-	-	1	-	2	-	-	-	-	-	Sequencing	Noradilah et al. 2017
	Thailand	36	-	-	-	-	-	-	-	-	-	2	6	3	-	25	-	Sequencing	Udonsom et al. 2018
Cattle	Thailand	21	-	-	-	-	-	-	-	-	-	2	4	-	-	15	-	Sequencing	Udonsom et al. 2018
	Malaysia	1	-	-	-	-	-	-	-	-	-	1	-	-	-	-	-	Sequencing	Mohammad et al. 2018a
	Indonesia	20	-	-	-	-	-	-	-	-	-	20	-	-	-	-	-	STS analyses, sequencing	Suwanti et al. 2020
Mouse-deer	Malaysia	1	-	-	-	-	-	-	-	-	-	-	-	-	-	1	-	Sequencing	Mohd Zain et al. 2017
Deer	Malaysia	12	-	-	-	-	-	-	-	-	-	12	-	-	-	-	-	Sequencing	Mohammad et al. 2018b
Perissodactyla																			
Horse	Thailand	1	1	-	-	-	-	-	-	-	-	-	-	-	-	-	-	RFLP	Thathaisong et al. 2003
Insecta																			
Cockroach	Malaysia	2	-	-	2	-	-	-	-	-	-	-	-	-	-	1	-	Sequencing	Farah Haziqah et al. 2017
	Singapore	4	-	-	-	-	-	-	-	-	-	-	-	-	-	4	-	Sequencing	Yoshikawa et al. 2007
Carnivora																			
Dog	Thailand	3	-	-	-	-	3	-	-	-	-	-	-	-	-	-	-	Sequencing	Parkar et al. 2007
	Cambodia	1	-	1	-	-	-	-	-	-	-	-	-	-	-	-	-	Sequencing	Wang et al. 2013
	Philippines	23	1	2	4	3	3	-	-	-	-	-	-	-	3	10	-	STS analyses	Belleza et al. 2016
	Malaysia	40	8	-	22	5	-	-	-	4	-	1	-	-	-	-	-	Sequencing	Noradilah et al. 2017
	Thailand	1	-	-	1	-	-	-	-	-	-	-	-	-	-	-	-	Sequencing	Udonsom et al. 2018
Cat	Malaysia	12	12	-	-	-	-	-	-	-	-	-	-	-	-	-	-	Sequencing	Farah Haziqah et al. 2018a
Aves																			
Duck	Philippines	3	-	-	-	-	-	-	2	-	-	-	-	-	-	-	1	Sequencing	Adao et al. 2016
	Malaysia	8	3	1	3	-	-	-	1	-	-	-	-	-	-	-	-	Sequencing	Noradilah et al. 2017
Swan	Malaysia	7	3	-	4	-	-	-	-	-	-	-	-	-	-	-	-	Sequencing	Noradilah et al. 2017
Chicken	Philippines	8	-	1	4	-	-	-	-	-	-	-	-	-	-	3	-	RFLP	Rivera and Tan 2005
	Philippines	1	-	-	-	-	-	1	-	-	-	-	-	-	-	-	-	Sequencing	Rivera 2008
	Philippines	5	-	-	-	-	-	-	4	-	-	-	-	-	1	-	-	Sequencing	Adao et al. 2016
	Indonesia	8	-	-	-	-	-	-	8	-	-	-	--	-	-	-	-	Sequencing	Yoshikawa et al. 2016
	Malaysia	27	3	-	7	-	-	2	12	-	3	-	-	-	-	-	-	Sequencing	Noradilah et al. 2017
	Malaysia	10	1	-	-	-	-	2	5	2	-	-	-	-	-	37	-	Sequencing	Farah Haziqah et al. 2018c
	Malaysia	1	-	-	-	-	-	1	-	-	-	-	-	-	-	-	-	Sequencing	Mohammad et al. 2018a
Ostrich	Malaysia	37	-	-	-	-	-	14	-	-	-	-	-	-	-	23	-	STS	

Table 2 (continued)

Host	Location	Number of subtype observations	Subtype (ST)													Technique	References
			ST1	ST2	ST3	ST4	ST5	ST6	ST7	ST8	ST9	ST10	ST12	ST14	Mixed ST	Unknown ST	*B. pythoni*		
Primates																			Chandrasekaran et al. 2014
Monkey	Philippines	4	2	1	1	-	-	-	-	-	-	-	-	-	-	-	-	Sequencing	Rivera 2008
Macaques	Thailand	197	34	48	72	-	-	-	-	-	-	-	-	-	9	34	-	Sequencing	Vaisusuk et al. 2018
Rodentia																			
Rat	Singapore	3	-	-	-	-	-	-	3	-	-	-	-	-	-	-	-	Sequencing	Noel et al. 2005
	Indonesia	9	-	-	-	9	-	-	-	-	-	-	-	-	-	-	-	Sequencing	Yoshikawa et al. 2016
	Malaysia	47	2	-	-	43	1	-	1	-	-	-	-	-	-	-	-	Sequencing	Farah Haziqah et al. 2018b
Reptilia																			
Lizard	Singapore	1	-	-	-	-	-	-	-	-	-	-	-	-	-	1	-	Sequencing	Noel et al. 2005
Sea snake	Singapore	1	-	-	-	-	-	-	-	-	-	-	-	-	-	1	-	Sequencing	Noel et al. 2005
Python	Singapore	1	-	-	-	-	-	-	-	-	-	-	-	-	-	1	-	Sequencing	Noel et al. 2005
Water monitor lizard	Malaysia	1	-	-	-	-	-	-	-		-	-	-	-	-	1	-	Sequencing	Mohd Zain et al. 2017

STS, sequence-tagged site; *RFLP*, restriction fragment length polymorphism; I subtype equivalents of RFLP results were obtained from Stensvold et al. (2007) and Wang et al. (2018a)

Aves

Blastocystis has been isolated from chicken, ostrich, duck, swan, and crow in Southeast Asia; all birds examined were from domestic environments. While chickens were the most studied, a prevalence of 100% was reported in ostriches and a range from 3.8 to 40% in the other birds (Table 1). These figures are comparable with records from Australia (Stenzel et al. 1994), Brazil (Bergamo do Bomfim and Machado do Couto 2013; Zanetti et al. 2020), Iran (Asghari et al. 2019), and Lebanon (Greige et al. 2018). In Malaysia, the prevalence of *Blastocystis* infection among free-range chickens was reported to be significantly higher than that of barn-reared chickens due to exposure of free-range chickens by their scavenging habits to environmental contamination (Farah Haziqah et al. 2014).

ST6 and ST7 were the predominant Avian STs in Southeast Asia; however, ST1, ST2, ST3, ST8, and ST9 were also identified. This finding is consistent with reports from Stensvold et al. (2009), Alfellani et al. (2013), Ramírez et al. (2014), Mokhtar and Youssef (2018), Greige et al. (2018), Wang et al. (2018b), and Deng et al. (2019). It is important to note the presence of ST9, a supposedly human subtype, in chicken in Malaysia, as reported by Noradilah et al. (2017).

Insecta

The presence of *Blastocystis* has been documented in cockroaches in Southeast Asia with a prevalence rate ranging from 10 to 80% (Table 1), and a significantly higher infection in nymphs than in adults reported by Farah Haziqah et al. (2017). So far, the only identified *Blastocystis* subtype in cockroaches in this region is ST3 by Farah Haziqah et al. (2017). Cian et al. (2017) have reported the presence of ST1–3 in roaches and locust, while ST4 was described in cockroaches by Valença-Barbosa et al. (2019). The observation of STs 1–4 is worrisome as cockroaches are ubiquitous, and these STs are the main subtypes infecting man, indicating that cockroaches could serve as a potential source of human infection.

Rodentia

Rats have been described as positive for *Blastocystis* infection, with ST1, ST4, ST5, and ST7 identified. These subtypes have also been reported in rodents elsewhere (Yoshikawa et al. 2004; Cian et al. 2017, Wang et al. 2018b; Betts et al. 2020), emphasizing the high prevalence of zoonotic ST4 within the rodent population.

Reptilia

Although house lizards, water monitor lizards, crocodiles, snakes, iguana, and tortoises have presented with

Blastocystis infection with varying frequency of infection, studies have yet to identify the STs in them.

Primates

Blastocystis infection has been found in orangutan, macaque, and monkeys. The occurrence was 50% and below, and only ST1, ST2, and ST3 were identified in macaque and monkey. These STs have been documented for macaque, monkeys, and other primates in Europe (Scicluna et al. 2006; Stensvold et al. 2009; Cian et al. 2017, Betts et al. 2020) and Brazil (Valença-Barbosa et al. 2019). ST4, ST5, and ST8 have also been identified in monkeys elsewhere (Yoshikawa et al. 2004; Scicluna et al. 2006; Stensvold et al. 2009, Valença-Barbosa et al. 2019).

Conclusion

An upsurge in the studies of *Blastocystis* infection in animals has been observed in Southeast Asia over the last decade. Molecular methods for detecting this organism have also been adopted, leading to the identification of subtypes available in various animal groups, thereby improving knowledge of *Blastocystis* sp. epidemiology.

Studies on the occurrence of *Blastocystis* sp. in at least one animal group have been carried out in seven of the eleven ASEAN countries. The majority of these studies have been from Malaysia, with livestock and poultry animals being the most examined. Twelve *Blastocystis* sp. subtypes: ST1, ST2, ST3, ST4, ST5, ST6, ST7, ST8, ST9, ST10, ST12, and ST14 have been identified in Southeast Asia. ST5 was the most dominant of them and was isolated mostly from pigs. Next to ST5 is ST1, which contrarily, has been found across many animal groups. Most of the subtypes identified were those commonly reported in man, an indication of the possibility of animal-to-man transmission. Common practice in Southeast Asia is the keeping of cats and dogs as companion animals, and intensive farming of livestock and poultry for cheap protein sources. In the course of handling and grooming their animals and during meat processing, pet owners and pig and poultry farmers may be at risk for *Blastocystis* infection. Generally, these animal handlers are encouraged to engage in good hygiene practices to reduce this risk.

Molecular techniques employed in the detection of subtypes varied from the use of restriction fragment length polymorphism (RFLP) to sequence-tagged site (STS) primers and then to the sequencing of partial or full SSU rDNA genes. For the prevalence of *Blastocystis* sp. in various animal groups and human populations to be accurately depicted, there is a need for uniformity in the diagnostic techniques employed in reported surveys.

In studies that considered for the exhibition of symptoms, animals were either reported as not showing symptoms or showed symptoms that did not correlate with infection. Nonetheless, the consistent presence of particular subtypes in livestock and poultry (ST5 in pigs, ST6 and 7 in poultry, and ST10 in cattle) is worth exploring. Studies are needed regarding the potential impact of *Blastocystis* on the well-being and productivity of infected animals. Also to be investigated is the impact of *Blastocystis* on the composition of gut microbiota and pH (and vice versa) in different animal hosts. Results from such investigations could provide insight into why infection with *Blastocystis* is rare in certain species, such as cats, dogs, and horses, and the host specificity of STs in general.

It is encouraged that additional investigation on *Blastocystis* sp. in diverse animal hosts and from other parts of Southeast Asia is carried out to provide a richer representation of the epidemiology of *Blastocystis* in this region. The use of adequate sample sizes in these studies is important. Overall, long-term studies are required to establish the incidence of *Blastocystis* in humans and animals in the same populations at the same time. The aim will be to assess whether these animals contaminate the food and water, thus transmitting *Blastocystis* infection to humans; this will help to confirm the actual risk of zoonotic transmission. Detailed genome studies, especially on STs shared by humans and animals, are also recommended to show the degree of similarity or variation within these STs.

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