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REVIEW ARTICLE

Blastocystis in captivated and free-ranging wild animals worldwide: a review

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ABSTRACT

Blastocystis is the most prevalent eukaryotic gastrointestinal symbiont found in humans and animals worldwide. Increased human infection rates are associated with raising concerns about the involvement of the parasite in public health. Over the last decade, the number of linked epidemiology studies has been prudently grown. Microscopy has been used to detect the presence of protozoan and the advent of molecular techniques has made detection easier. However, due to its limited host specificity and zoonotic potential, animals, either livestock or wildlife animals, may serve as a potential reservoir for *Blastocystis* infection transmission. The approach utilised in this study aided in understanding the distribution and prevalence of *Blastocystis* in animals, particularly captivated and free-ranging wild animals worldwide due to increased interest. This review will help comprehend the epidemiological aspects, demographic, subtypes, and the zoonotic potential of *Blastocystis* in wildlife and captive animals.

Keywords: *Blastocystis*; captive; subtype; wildlife; zoonotic.

INTRODUCTION

Blastocystis was first discovered in human faecal samples over a decade ago by Alexeiff (Petrášová *et al*., 2011). It is a ubiquitous anaerobic protist that infects the gastrointestinal tract of both animals and humans (Ahmed & Karanis, 2019). The faecal-oral pathway is the means of spreading this single-celled eukaryote (Tan & Suresh, 2006; Alfellani *et al*., 2013b). Inadequate sanitary conditions by humans or animals could explain the high parasite prevalence seen in impoverished countries compared to developed countries (Ahmed & Karanis, 2018). *Blastocystis* sp. infection is associated with various non-specific intestinal disorders including diarrhoea, stomach pain, skin rash or urticaria, flatulence, vomiting, and constipation (Stensvold *et al*., 2012).

The four most common morphological forms of *Blastocystis* are cystic, vacuolar, granular, and amoeboid. The vacuolar and granular forms are the most common form observed (Boreham & Stenzel, 1993). Significant genetic diversity has been observed within the Stramenopiles group according to a comparative investigation of small subunit rRNA gene sequences (SSU rRNA) (Ahmed & Karanis, 2019). *Blastocystis* sp. subtypes (STs) 1-9 nomenclature was originally introduced in 2007, after which many new subtypes were proposed. By 2013, 17 STs (ST1 to ST17) had been identified across several hosts, each with sufficient genetic variation to be designated as a separate species (Alfellani *et al*., 2013b; Stensvold & Clark, 2020). Currently, a total of 29 subtypes have been proposed. Out of these, the legitimacy of four subtypes (ST18, ST19, ST20, and ST22) is under question due to the possibility that they were created from artefacts which are based on their chimeric appearance (Stensvold & Clark, 2020). The remaining 25 subtypes (ST1 to 17, ST21, ST23-29) have met the currently recommended criteria for unique subtype designations (Maloney & Santin, 2021). Furthermore, ten subtypes namely ST1-9 and ST12 have been discovered in humans with varying occurrence levels (Greige *et al*., 2019).

The article is a compiled study on the distribution and genetic variation of *Blastocystis* in wild and captive wild animals worldwide in the last decade. Important data on the prevalence, distribution and predictors of *Blastocystis* sp. infection and its subtypes in wildlife animals will aid in understanding the host (Andersen & Stensvold, 2016).

MATERIALS AND METHODS

Research articles on the distribution of *Blastocystis* in captivated and free-living wild animals around the world were collected based on search results against Google Scholar, ResearchGate, PubMed and ScienceDirect databases. The keywords used in the search were *Blastocystis*, wildlife, STs, subtypes, zoonotic transmission, zoo animals, captive animals, primates, rodents, artiodactyla, marsupial, birds, carnivora, proboscidea, reptiles, rodentia, perisodactyla, characterisation, life cycle, molecular, genetic diversity and animals. Duplicate articles from all the databases were identified and removed. Additionally, the unclear and confusing articles were also removed. The articles chosen were those written in English and focused on *Blastocystis* in free living wild animals and captivated wild animals in zoological gardens worldwide. Articles reporting both parasitological and molecular methods of detection have also been chosen. Country/region, host, number of positive samples, subtypes of the animal examined, number of animals for each subtype, and references were the variables recorded.

RESULTS AND DISCUSSION

Distribution of *Blastocystis* sp. in captive and wild animals worldwide

A total of 99 selected articles related to *Blastocystis* in captivated wild animals and free-living wild animals from different taxonomic groups namely, non-human primates, artiodactyl, marsupial, birds, carnivora, proboscidea, reptiles, rodentia, and perissodactyl worldwide were reported in this review. These animals were associated with *Blastocystis* sp. infection with ST1, ST2, ST3, ST4, ST6, ST10, ST11, and ST13 being the most recognised subtypes. Mixed subtypes most likely a combination of ST1, ST2, ST3, and ST5 are commonly found in wildlife whereas the least common subtypes are ST15 and ST17. However, ST16 is the only subtype that has not been reported. The most extensive studies on *Blastocystis* in wildlife animals were mostly reported from Australia and China in which high *Blastocystis* diversity was primarily reported from China.

Distribution of *Blastocystis* sp. in different groups of captive and wildlife animals

Non-human primates (NHP)

Studies on *Blastocystis* in non-human primates (NHP) was reported from 27 countries with the prevalence rate ranging from 2% to 100% (Table 1). The pathogenic significance of *Blastocystis* in non-human primates remains largely unknown. To date, China, Australia, and the United Kingdom witnessed the most diverse species being examined. Specifically, 29 groups of animals from China, 19 from Australia and 21 from the United Kingdom were observed. Meanwhile, a study from Tanzania by Petrášová *et al*. (2011) reported a higher number of individuals examined with 206 chimpanzees (*Pan troglodytes*) and 111 vervet monkeys (*Chlorocebus aethiops pygerythrus*) and 49 mantled guereza (*Colobus guereza*). Several different subtypes were identified in these animal hosts with ST1 and ST2 being predominant, followed by ST3. These subtypes were also commonly found in humans. The frequent identification of these subtypes in non-human primates supports suggestions that they may be the reservoir for these subtypes.

Notably, evidence of zoonotic transmission via the faecal-oral route between these animals and humans was shown mainly in animal handlers in zoological gardens as reported by Parkar *et al*. (2010). At the same time, Yoshikawa *et al*. (2009) reported on *Blastocystis* ST2 transmission in monkeys and children in Nepal. Nevertheless, Helenbrook *et al*. (2015) elucidated there was no *Blastocystis* cross-transmission between humans and non-human primates despite close proximity in some instances in north-western Ecuador due to the lack of shared subtypes between humans and monkeys in which *Blastocystis* ST1, ST2, and ST3 were found in human whereas all monkey samples examined were ST8.

Birds

A wide range of bird species has been examined worldwide after a group of primates with the prevalence rate of *Blastocystis* in birds ranging from 2.1% to 100% (Table 2). The studies that examined birds have long been regarded as potential reservoirs of *Blastocystis* sp. transmission (Noël *et al*., 2005). The most studied wild bird population was the ostrich (Roberts *et al*., 2013; Zhao *et al*., 2017; Cian *et al*., 2017; Maloney *et al*., 2020; Deng *et al*., 2021). There were 13 different subtypes found in wild birds worldwide with ST6 being the most reported subtype followed by ST5 and ST7. Therefore, it was believed that these subtypes might be the major reservoirs for the wild birds. It is important to note that the four most common human subtypes namely, ST1-ST4 were not found in wild bird hosts. However, due to the limited studies, the role of birds, specifically wild birds, as hosts and potential reservoirs of these subtypes is unknown.

There is scientific evidence that wild birds are associated with ST1-8, ST10, ST13, ST14, ST20, ST24, ST27 and ST28. Several novel subtypes were reported, such as ST20, which was unique to the ostriches (Zhao *et al*., 2017), whereas ST27 and ST28 were only associated with Garganey and Indian peafowls (Maloney *et al*., 2020). There is no additional subtype reported in Indian peafowls. According to Ramírez *et al*. (2014), ground-dwelling birds are more likely than tree-dwelling birds to be infected with *Blastocystis* due to increased faecal exposure linked with eating behaviour and exposure to exposure parasites prevalent in water. Out of 20 studies on *Blastocystis* in wild birds, only three studies were conducted on free-living wild birds in Iran (Asghari *et al*., 2019) and Malaysia (Yong *et al*., 2008); others were captive wild birds.

Table 1. Studies on *Blastocystis* subtype found in captive and wild non-human primates (NHP) worldwide

Country/ Region	Host	No. of Positive Samples (%)	Subtype (ST)																	Other/ Mixed Subtypes	Condition	Technique	References
			1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17				
	Captivated wild animals																						
Australia	Baboon	2/2 (100)	2																		C	IVC, MOL	Parkar *et al*. (2007)
	Black and white ruffed lemur	2/2 (100)	1				1														C	IVC, MOL	
	Crested macaque	2/2 (100)	2																		C	IVC, MOL	
	Colobus	1/1 (100)																		ND	C	IVC, MOL	
	Orang utan	2/2 (100)	2																		C	IVC, MOL	
	Ring-tailed lemur	1/3 (66.6)	1																		C	IVC, MOL	
	Spider monkey	1/1 (100)																		ND	C	IVC, MOL	
	Vervet monkey	2/2 (100)	1				1														C	IVC, MOL	
	White-cheeked gibbon	2/2 (100)	2																		C	IVC, MOL	
Australia	Hamadryas baboon	3/3 (100)	3																		C	MOL	Parkar *et al*. (2010)
	Silvery gibbon	2/4 (50.0)	2																		C	MOL	
	Sulawesi crested macaque	2/4 (50.0)	2																		C	MOL	
	Sumatran orangutan	4/4 (100)	2	2																	C	MOL	
	White-cheeked gibbon	2/4 (50.0)	2																		C	MOL	
	Ring-tailed lemur	1/4 (25.0)	1																		C	MOL	
	Black and white ruffed lemur	4/4 (100)	4																		C	MOL	
	Tonkean macaque	2/2 (100)	2																		C	MOL	
Australia	Monkey/ *Macaca* sp.	1/1 (100)			1																C	MOL	Roberts *et al*. (2013)
	Chimpanzee/ *Pan troglodytes*	7/10 (70.0)											3							ST1/ST11 (4)	C	MOL	
	Orangutan/ *Pongo abelii*	9/10 (90.0)		9																	C	MOL	
	Gorilla/ *Gorilla gorilla*	10/10 (100)	9	1																	C	MOL	
	François langur/ *Trachypithecus francoisi*	5/6 (83.3)	5																		C	MOL	

China	Cynomolgus macaques/ *Macaca fascicularis*	7/8 (87.5)										NA	C	CM	Zanzani *et al*. (2014)
China	Rhesus macaque/*Maca ca mulatta*	28/29 (96.6)	15	5	7							ST19 (1)	C	MOL	Zhao *et al*. (2017)
	Francois's leaf monkey/ *Presbytis francoisi*	1/1 (100)				1							C	MOL	
	Mandrill/ *Mandrillus sphinx*	1 /4 (25)			1								C	MOL	
	Golden snub-nosed monkey/ *Hinopithecus roxellana*	41/46 (89.1)	4							37			C	MOL	
	De Brazza's monkey/ *Cercopithecus neglectus*	4/5 (80.0)	3					1					C	MOL	
	Hamadryas baboon/ *Papio hamadrayas*	13/23 (56.5)		9	4								C	MOL	
	Chimpanzee/ *Pan troglodytes*	8/10 (80.0)		8									C	MOL	
China	*Macaca* sp.	13/185 (70.27)	7	3	1							ST2/ ST3 ST1/ ST3	C	IVC, MOL	Zhu *et al*. (2020)
China	Red-faced spider monkey	2/4 (50.0)		1	1								C	MOL	Ma *et al*. (2020a)
	De Brazza's monkey	5/5 (100)	1	4									C	MOL	
	Mandrill	9/15 (60.0)	5		4								C	MOL	
	Francois' Black Leaf Monkey	2/3 (66.7)	2										C	MOL	
	Snub-nosed monkey	9/22 (40.9)	6	3									C	MOL	
	Chimpanzee	2/15 (13.3)	1			1							C	MOL	
	Rhesus macaque	6/18 (33.3)		4	2								C	MOL	
	Crab-eating macaque	3/13 (23.1)		2	1								C	MOL	
	Japanese macaque	6/33 (18.8)		5	1								C	MOL	
	White-cheeked gibbon	1/4 (25.0)	1										C	MOL	
	Ring-tailed Lemur	7/16 (43.8)			2	3		2					C	MOL	
	Squirrel monkey	9/30 (30.0)	7			2							C	MOL	

China	Alpacas/ *Vicugna pacos*	87/366 (23.8)					C	MOL	Ma *et al*. (2020b)
China	Tibetan macaque/ *Macaca thibetana*	0/3 (0)					C	MOL	Chen *et al*. (2021)
	Golden monkey/ *Rhinopithecus*	1/2 (50.0)				1	C	MOL	
China	Macaque	6*	6	2	3		C		Zhang *et al*. (2021a)
	Golden monkey	1*	1				C		
	Northern pigtail macaque	1*	1				C		
	Green monkey	2*	2				C		
	Eastern black-and-white colobus	1*	1				C		
	Crab-eating macaque	1*	1				C		
	Japanese macaque	1*	1				C		
	Squirrel monkey	1*	1				C		
	Chimpanzee	2*		1	1		C		
	Orangutan	1*			1		C		
	Gorilla	1*			1		C		
	Patas monkey	2*			2		C		
	Eastern black and white colobus	2*			2		C		
	White-browed monkey	2*			2		C		
	Woolly monkey	8*	2	2	1	3	C	MOL	Scicluna *et al*. (2006)
United Kingdom	Unidentified primate	7*		1	5	1	C	MOL	
	Stump tailed macaque	1*			1		C	MOL	

United Kingdom	Woolly monkey/ *Lagothrix lagotricha*	21*		2	5	1		1 3				C	MOL	Alfellani *et al*. (2013a)
	Allen's swamp monkey/ *Allenopithecus nigroviridis*	4*			4							C	MOL	
	Barbary macaque/ *Macaca sylvanus*	5*			5							C	MOL	
	Mantled guereza/ *Colobus guereza*	3*			2						ND (1)	C	MOL	
	Orangutan/ *Pongo pygmaeus*	12*	4	4	2		2					C	MOL	
	King Colobus/ *Colobus polykomos*	1*			1							C	MOL	
	Diana monkey/ *Cercopithecus diana*	4*	1		2						ND(1)	C	MOL	
	Hamadryas baboon/ *Papio hamadrayas*	2*			2							C	MOL	
	Patas monkey/ *Erythrocebus patas*	5*	1		4							C	MOL	
	Gibbon/*gibbon* sp.	13*	3	2	1		1	5		1		C	MOL	
	Howler monkey/ *Alouatta caraya*	3*						3				C	MOL	
	Leaf monkey/ *Presbytis francoisi*	6*			3		2	1				C	MOL	
	Crested black macaque/	5*			5							C	MOL	

	Macaca nigra												
	Mandrill/ *Madrillus sphinx*	3*	3								C	MOL	
	Collared mangabey/ *Cercocebus torquatus*	1*			1						C	MOL	
	Chimpanzee/ *Pan troglodytes*	13*		4	5	3			1		C	MOL	
	Gorilla/ Gorilla gorilla	4*			1	2				ND (1)	C	MOL	
United Kingdom	Western lowland Gorilla/ *Gorilla gorilla*	16/25 (64)	9	45	8	2					C	MOL	(Betts et al., 2020)
	Javan Gibbon/ *Hylobates moloch*	3/11 (84.6)	18	17	4	4	1		1		C	MOL	
Japan	Orangutan	3/4 (75)									C	IVC., CM	Abe *et al*. (2003)
	Chimpanzee	6/7 (85.7)	6									IVC. CM, MOL	
Japan	Monkey	13*	8	2						ND (3)	C	MOL	Yoshikawa *et al*. (2003a)
Japan	Japanese monkey	2*		2							C	MOL	Yoshikawa *et al*., (2003b)
Japan	Drill/ *Mandrillus leucophaeus*	1*								ND	C	MOL	Yoshikawa *et al*. (2004)
	Siamang/ *Hylobates syndactylus*	1*				1					C	MOL	
	Francois' black leaf monkey/ *Presbytis francoisi*	1*								ND	C	MOL	
	De Brazza's monkey/ *Cercopithecus neglectus*	1*								ND	C	MOL	
	Chimpanzee/ *Pan troglodytes*	1*								ST1, ST5	C	MOL	
	Orangutan/ *Pongo pygmaeus*	1*	1								C	MOL	

	Lion-tailed macaque/ *Macaca silenus*	1*			1							C	MOL	
	Rhesus monkey/ *Macaca mulatta*	1*			1							C	MOL	
Brazil	*Ateles* sp.	1/2 (50.0)				1						C	IVC, MOL	Valença-Barbosa *et al*. (2019)
	Lagothrix lagotricha	1/3 (33.3)										C	IVC, MOL	
	Papio sp.	1/4 (25)		1								C	IVC, MOL	
	Pan troglodytes	1/2 (50)		1								C	IVC, MOL	
	Alouatta sp.	1/3 (33.3)				1						C	IVC, MOL	
	Macaca fuscata	1/3 (33.3)	1									C	IVC, MOL	
	Aotus sp.	2/6 (33.3)	1			1						C	IVC, MOL	
	Macaca mulatta	18/48 (37.5)		9	1	2						C	IVC, MOL	
	Macaca fascicularis	7/27 (25.9)		1	1							C	IVC, MOL	
Brazil	Black howler monkey/ *Alouatta caray*	1/1 (100)				1						C	MOL	Oliveira-Arbex *et al*. (2020)
	Brown howler monkey/ *Alouatta fusca*	1/2 (50)									ND	C	MOL	
	Red howler monkey/ *Alouatta seniculus*	2/2 (100)		1	1	1						C	MOL	
	White-fronted spider monkey/ *Ateles belzebuth*	1/1 (100)		1								C	MOL	
	Brown-headed spider monkey/ *Ateles fusciceps*	1/1 (100)				1						C	MOL	
	Silvery marmoset/ *Callithrix argentata*	1/1 (100)									ND	C	MOL	
	Common Marmoset/ *Callithrix jacchus*	1/1 (100)									ND	C	MOL	

	Hamadryas baboon/ *Papio hamadryas*	1/1 (100)		1						C	MOL	
	Tufted Capuchin monkey/ *Cebus apella*	1/3 (33.3)							ND	C	MOL	
	Woolly monkey/ *Lagothrix lagotricha*	1/3 (33.3)				1				C	MOL	
	Golden-headed lion tamarin/ *Leontopithecus chrysomelas*	1/2 (50.0)							ND	C	MOL	
	Mandrill/ *Mandrillus sphinx*	1/1 (100)		1						C	MOL	
	Guinea baboon/ *Papio papio*	1/2 (50.0)		1						C	MOL	
	Brazilian bare faced tamarim/ *Saguinus bicolor*	1/1 (100)							ND	C	MOL	
	Brown-headed tamarin/ *Saguinus fuscicollis*	1/1 (100)							ND	C	MOL	
	White saddleback tamarin/ *Saguinus melanoleucus*	1/1 (100)							ND	C	MOL	
Spain	*Hapalemur aureus*	1*	1							C	CM, MOL	Santín *et al*. (2011)
	Cercopithecus hamlyni	1*	1							C	CM, MOL	
	Lemur catta	1*			1					C	CM, MOL	
	Mandrillus leucophaeus	1*		1						C	CM, MOL	
	Gorilla gorilla	1*	1							C	CM, MOL	
	Cercocebus atys	1*		1						C	CM, MOL	
	Cercocebus neglectus	1*		1						C	CM, MOL	

France	Brown spider monkey/ *Atelus hybridus*	2*		2								C	MOL	Zhu *et al*. (2020)
	Roloway monkey/ *Cercopithecus roloway*	3*									ST1/ST3(3)	C	MOL	
	Mantled guereza/ *Colobus guereza*	10*	7		1						ST1/ST3(2)	C	MOL	
	Gorilla/ *Gorilla gorilla*	1*		1								C	MOL	
	Gibbon/ *Gibbon sp.*	3*		3								C	MOL	
	Saki/ *Pithecia pithecia*	1*			1							C	MOL	
France	Western lowland gorilla	4/6 (66.7)	1	1			2					C	MOL	Cian *et al*. (2017)
	Orangutan	3/3 (100)					3					C	MOL	
	Chimpanzee	3/3 (100)		1			1					C	MOL	
	Lar gibbon	2/3 (66.7)		1					1			C	MOL	
	Siamang	4/4 (100)			1	1						C	MOL	
	Buff-cheeked gibbon	2/2 (100)	2									C	MOL	
	Southern pig-tailed macaque	3/3 (100)	3		3							C	MOL	
	Mandrill	1/1 (100)	1									C	MOL	
	Owl-faced monkey	1/2 (50.0)	1									C	MOL	
	Roloway monkey	1/1 (100)		1								C	MOL	
	L'Hoest's monkey	1/1 (100)			1							C	MOL	
	De Brazza's monkey	2/2 (100)	1		1							C	MOL	
	Kikuyu black-and-white colobus	2/3 (66.6)	1	1								C	MOL	
	Emperor tamarin	1/3 (33.3)			1							C	MOL	

	Golden-headed lion tamarin	3/4 (75.0)	3									C	MOL	
	White-faced saki	1/1 (100)		1								C	MOL	
	Ring-tailed lemur	5/5 (100)	1	3					1			C	MOL	
	Red ruffed lemur	3/3 (100)	1		1	1	1	1				C	MOL	
	Black-and-white ruffed lemur	2/4 (50.0)			1		1					C	MOL	
	Blue-eyed black lemur	1/1 (100)			1							C	MOL	
Italy	Japanese macaque/ *Macaca fuscata*	1*			1							C	MOL	Alfellani *et al*. (2013b)
	Barbary macaque/ *Macaca Sylvanus*	1*			1							C	MOL	
Italy	Cynomolgus macaques/ *Macaca fascicularis*	85/97 (87.6)	4	14			2			ST1/ST2 (14), ST1/ST3 (3), ST1/ST7 (7), ST2/ST3 (5), ST2/ST5 (1) ST2/ST7 (5), ST3/ST7 (1), ST1/ST2/ ST3 (10), ST1/ST2/ ST7 (5), ST1/ST3/ ST7 (1), ST2/ST3/ ST7 (3), ST1/ST2/ ST3/ST7 (10)	C	MOL	Zanzani *et al*. (2016)

Colombia	Howler monkey/ *Alouatta caraya*	2/2 (100)		2								C	MOL	Ramírez *et al*. (2014)
Denmark	Chimpanzee	20*	1	4	8		7					C	IVC, MOL	Stensvold *et al*. (2009)
	Orang Utan	4*	1	1	2							C	IVC, MOL	
	Gorilla	6*		4	1		1					C	IVC, MOL	
	Siamang	4*	3					1				C	IVC, MOL	
	Mueller's gibbon	1*		1								C	IVC, MOL	
	Golden cheeked gibbon	2*	1		1							C	IVC, MOL	
	Lar gibbon	2*	1					1				C	IVC, MOL	
	Gibbon (unspecified)	2*			1		1					C	IVC, MOL	
	Woolly monkey	16*		1	4	1		10				C	IVC, MOL	
	Diana monkey	1*			1							C	IVC, MOL	
	Barbary macaque	1*			1							C	IVC, MOL	
	Stump-tailed macaque	1*			1							C	IVC, MOL	
	Common marmoset	1*			1							C	IVC, MOL	
	Ring-tailed lemur	6*				2		2	2			C	IVC, MOL	
Bangladesh	Rhesus macaque	20/62 (32.3)	5	3	12							C	IVC, MOL	Li *et al*. (2019)
	Vervet monkey	3/7 (42.9)		1	1					1		C	IVC, MOL	
	Langur	3/5 (60.0)	1							2		C	IVC, MOL,	
	Grey langur	1/2 (50.0)	1									C	IVC, MOL,	
Mexico	Howler monkey/ *Alouatta palliata*	23/59 (39.0)		2								C	MOL	Villanueva-Garcia *et al*. (2017)
Nepal	Rhesus monkeys/ *Macaca mulatta*	10/10 (100)	2	7							ND (1)	C	IVC, MOL	Yoshikawa *et al*. (2009)
Thailand	*Macaca fascicularis*	197/263 (74.9)	34	48	72						MIXED (43)	C	IVC, MOL	Vaisusuk *et al*. (2018)
Malaysia	Non-Human Primates	5/308 (1.62)									NA	C	CM	Adrus *et al*. (2019)
Philippines	Monkey	4*	2	1	5							C	MOL	Rivera (2008)
														Alfellani *et al*. (2013a)

Germany	Howler monkey	2*					2			C	MOL	
	Gray langur	6*	5	1						C	MOL	
	Hamadryas baboon	1*							ND (1)	C	MOL	
	Drill	19*	12		7				ND (4)	C	MOL	
	Mandrill	2*	2							C	MOL	
	Bonobo	4*			4					C	MOL	
	Barbary macaque	7*	1		6					C	MOL	
	Mantled guereza	4*	4							C	MOL	
	Orangutan	1*	1							C	MOL	
	Spider monkey	1*							ND (1)	C	MOL	
	Gorilla	1*		1						C	MOL	
	Gibbon	1*	1							C	MOL	
	Lemur	1*		1						C	MOL	
	Vervet monkey	1*			1					C	MOL	
	Ruffed lemur	1*				1				C	MOL	
Morocco	Barbary macaque/ *Macaca sylvanus*	14*	5	6	3					C	MOL	Alfellani *et al*. (2013a)
Netherlands	Woolly monkey/ *Lagothrix lagotricha*	4*	2	2						C	MOL	Alfellani *et al*. (2013a)
	Red-faced spider monkey/ *Ateles paniscus*	3*	3							C	MOL	
	Grivet / *Chlorocebus aethiops*	2*	2							C	MOL	
	Black mangabey/ *Lophocebus aterrimus*	3*	1	1	1				ST1/ST3 (1)	C	MOL	
	Olive baboons/ *Papio anubis*	1*							ST1/ST3 (1)	C	MOL	
	Crab-eating macaque/ *Macaca fascicularis*	3*	1		2					C	MOL	
	Tamarin/ *Saguinus labiatus*	1*			1					C	MOL	
	Golden lion tamarind/ *Leontopithecus rosalia*	1*	1							C	MOL	

Saint Kitts	Green monkey/ *Chlorocebus sabaeus*	32*	1	2 2	9							C	MOL	Alfellani *et al*. (2013a)
Cameroon	Chimpanzee/ *Pan troglodytes*	25/114 (21.9)									NA	C	MOL	Drakulovski *et al*. (2014)
Peru	Black-headed night monkey/ *Aotus nigriceps*	3/23 (13.0)				3						C	MOL	Helenbrook & Whipps (2021)
Poland	Rhesus macaque	1*			1							C	MOL	Rudzińska *et al*. (2021)
	Chimpanzee	3*		3								C		
	Patas monkey	4*	1	1	2							C		
	Mandrill	4*	3		1							C		
	Javan lutung	5*						5				C		
	Guereza	4*		2				2				C		
	Buff-cheeked gibbon	1*			1							C		
	White-cheeked gibbon	1*			1							C		
	Free-living wild animals													
Senegal	Chimpanzees/ *Pan troglodytes*	33/81 (40.7)	27		1						ST2/ST3, (1) ND (4)	W	MOL	Renelies-Hamilton *et al*. (2019)
Mexico	Black howler monkey/ *Alouatta pigra*	64/166 (38.5)	3	5 9			2					W	MOL	Villanueva-Garcia *et al*. (2017)
South America	Howler monkey host	58/96 (60.4)				47					ND (11)	W	IVC, MOL	Helenbrook *et al*. (2015)
Tanzania	Pan troglodytes	147/206 (71.4)	21									W	MOL	Petrášová *et al*. (2011)
	Chlorocebus aethiops pygerythrus	94/111 (84.7)	5	2	1	2						W	MOL	
	Colobus guereza	41/49 (83.7)	3	1	2	5						W	MOL	

CM - Conventional microscopy, IVC - *In vitro* cultivation, MOL - Molecular technique, NA - Not applicable, ND - Not Defined, C – Captivated wildlife, W – Free-living wildlife, * - This information is not provided as a survey.

Table 2. Studies on *Blastocystis* subtype found in captive and wild birds worldwide

Country/ Region	Host	No of Positive Samples (%)	Subtypes (ST)																	Other/ Mixed Subtypes	Condition	Technique	References
			1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17				
Captivated wild animals																							
Australia	Guinea fowl/ *Numida meleagris*	2/2 (100)							2												C	MOL	Roberts *et al*. (2013)
	Ostrich/ *Struthio camelus*	6/10 (60.0)				6															C	MOL	
	Cassowary/ *Casuarius casuarius*	2/10 (20.0)		2																	C	MOL	
China	Ostrich/ *Struthio camelus*	3 /9 (33.3)					1					1								ST20 (1)	C	MOL	Zhao *et al*. (2017)
China	Pigeon/ *Columba livia*	1/47 (2.1)						1													C	MOL	Wang *et al*. (2018a)
	Red crowned crane/ *Grus japonensis*	6/43 (14.0)						4	2												C	MOL	
China	Common pigeon	4/34 (11.8)								4											C	IVC. CM, MOL	Deng *et al*. (2021)
	Green peafowl	1/12 (8.3)			1																C	IVC. CM, MOL	
	Ostrich	6/19 (31.6)	1				5														C	IVC. CM, MOL	
China	Peafowl	50 (35.0)							9		41										C	MOL	Liu *et al*. (2021)
China	Ostrich	6/429 (1.3)					6														C	MOL	Zhang *et al*. (2021b)
	Blue-eared pheasant	1/429 (0.2)						1													C		
	Peafowl	3/429 (0.6)						3													C		
	Turkey	1/429 (0.2)							1												C		
	Whooper swan	1/429 (0.2)							1												C		
Japan	Japanese quail/ *Coturnix coturnix japonica*	7 *						3	3											ST6/ST7 (1)	C	MOL	Yoshikawa *et al*. (2004)
	Pheasant/ *Phasianus colchicus*	6 *																		ST6/7(1); ND (5)	C	MOL	
Japan	Japanese quail	2 *						1	1												C	MOL	Yoshikawa *et al*. (2004)

Japan	Chinese bamboo partridge	1/1 (100)	1						C	MOL	Abe *et al*. (2002)
	Vulturine guineafowl	1/1 (100)	1						C	MOL	
	Japanese green pheasant	1/1 (100)	1						C	MOL	
	Palawan peacock-pheasant	1/1 (100)						ND	C	MOL	
	Satyr tragopan	1/1 (100)						ND	C	MOL	
	Himalayan monal pheasant	1/1 (100)						ND	C	MOL	
	Great argus pheasant	1/1 (100)						ND	C	MOL	
Japan	Pheasants/ *Phasianus colchicus*	5 *		3				ND (2)	C	MOL	Yoshikawa *et al*. (2004)
	Quails/ *Coturnix coturnix*	9 *		4	5				C		
Brazil	Black-cheeked Lovebird	1/1 (100)				1			C	MOL	Maloney *et al*. (2020)
	Swan goose	1/1 (100)			1				C	MOL	
	Garganey	1/1 (100)						ST7, ST10, ST27, ST28	C	MOL	
	Indian peafowl	2/3 (66.6)						ST27/ST28 (2)	C	MOL	
	Wild Duck	1/1 (100)			1				C	MOL	
	Helmeted Guineafowl	2/2 (100)		1	1				C	MOL	
	Momelanotus	1/1 (100)						ST7, ST14	C	MOL	
	Quail	1/1 (100)						ST6, ST7	C	MOL	
	Mallard	1/1 (100)			1				C	MOL	
	Muscovy ducks	1/1 (100)						ST7/14	C	MOL	
	Pheasant	4/10 (40.0)		2	2				C	MOL	
	Ostrich	2/2 (100)	1					ST5/10/24 (1)	C	MOL	
	Pheasant	4/10 (40.0)		2	2				C	MOL	
Brazil	Lesser Seed Finch/ *Oryzoborus angolensis*	2/35 (5.7)						NA	C	IVC, CM	Marietto-Gonçalves *et al*. (2008)
	Common Peafowl/ *Pavo cristatus*	1/1 (100)						NA	C	IVC, CM	

France	Egyptian fruit bat	1/1 *			1												ST3 (1)	C	MOL	Cian *et al*. (2017)
	Common peafowl	1/2 *															ND	C	MOL	
	American flamingo	1/3 *	1															C	MOL	
	Ostrich	2/2 *					2											C	MOL	
	Greater rhea	3/3 *				2	1											C	MOL	
Colombia	*Passer domesticus*	17*						17										C	CM, MOL	Ramírez *et al*. (2014)
	Thraupis episcopus	6*						6										C	CM, MOL	
	Oryzoborus maximiliani	8*						8										C	CM, MOL	
	Sicalis flaveola	5*						5										C	CM, MOL	
	Petrochelidon pyrrhonota	11*						11										C	CM, MOL	
Spain	Ostrich/ *Struthio camelus*	58*															NA	C	IVC, CM	Ponce Gordo *et al*. (2002)
Spain	Swan goose	3/18 (16.6)															NA	C	IVC, CM	Pérez Cordón *et al*. (2008)
	Galliformes	12/36 (33.3)															NA	C	IVC, CM	
Malaysia	Ostrich/ *Struthio camelus*	37/37 (100)						37										C	MOL	Hemalatha *et al*. (2014)
Free living wild animals																				
China	Blood pheasant	0/1																W	MOL	Chen *et al*. (2021)
	Chinese Monal/ *Lophophorus lhuysii*	0/2																W	MOL	
Iran	Crows/ *Corvus cornix*	64/144 (42.9)													46	13	ND (5)	W	IVC, MOL	Asghari *et al*. (2019)
	Pigeon/ *Columba livia*	67/156 (44.4)													67			W	IVC, MOL	
Malaysia	Large-billed crow	4 (33.96)															NA	W	CM	Yong *et al*. (2008)

CM - Conventional microscopy, IVC - In vitro cultivation, MOL - Molecular technique, NA - Not applicable, ND - Not Defined, C – Captivated wildlife, W – Free-living wildlife, * - This information is not provided as a survey.

Artiodactyl

Deer, boars, camels, alpacas, and other artiodactyla have a prevalence rate ranging from 6.7% to 100% (Table 3). The United Kingdom had the highest prevalence rates concerning the most positively examined samples, even though the samples used in the study were relatively small. The highest number of artiodactyls examined was in Yaks with 278 positive samples out of 1027 conducted in China (Ren *et al*., 2019). Overall, 15 subtypes were found in artiodactyl, namely, ST1, ST2, ST4, ST5, ST7, ST8, ST10-ST15, ST18, ST21, and ST22. *Blastocystis* ST10 was the most discovered *Blastocystis* subtype in cervids, bovids, ovids and camelids, followed by ST5 and ST14 (Zhao *et al*., 2017). The unique and rare subtypes found in these groups are ST8, ST15, ST18, ST21, and ST22 in red deer, camel, alpaca, guanaco, and waterbuck.

The significance of pathogenicity in disease is still unknown. ST18, ST21, and ST22 were discovered to be the novel subtypes of *Blastocystis* infection from waterbuck, alpaca and guanaco. Several studies have been conducted on *Blastocystis* sp. outbreaks in deer with various subtype distributions. In the United Kingdom, red deer were reported to be infected with ST4 and ST10, and muntjac deer have been found infected with ST14 (Cian *et al*., 2017; Betts *et al*., 2018). In China, ST10 has been discovered in fallow deer, sika deer, and white-lipped deer. A rare subtype which is ST13 has been discovered in Java mousedeer in France and mouse deer in the United Kingdom. The distribution of STs in alpacas in China and France revealed that all the isolates were ST10 and ST14 (Abe *et al*., 2002; Zhao *et al*., 2017; Li *et al*., 2020a; Ma *et al*., 2020b). Overall, these findings suggested that alpaca and deer may be *Blastocystis* reservoirs. Meanwhile, domestic yaks' infection status varied significantly depending on their geographical origin in Qinghai Province, China as many factors including wildlife age, varying quantities of samples from various seasons, environmental situations, and altitude variability may have contributed to the disparity in prevalence based on Ren *et al*. (2019). Domestic yaks in Qinghai Province were also found to have the above subtypes indicating a high prevalence. Furthermore, subtype ST12 was discovered in kangaroos and giraffes in Western Australian zoos for the first time by Roberts *et al*. (2013).

Carnivora

Most of the wild carnivore studies were conducted on captive animals rather than wild animals. The prevalence rate ranged from 0.06% to 100% (Table 4). In studies with a limited number of samples, 100% prevalence rate was observed. Compared to other groups of animals, most studies used a small number of samples. ST1, ST2, ST3, ST4, ST5, ST7, ST9, and ST17 are subtypes associated with carnivores, with ST1 and ST3 being the most common subtypes discovered. Several studies on *Blastocystis* in carnivores reported with negative infection in several hosts such as European badger, meerkat, red fox, serval, stoat and many more indicating that they might not be a natural host for zoonotic transmission (Abe *et al*., 2002; Lim *et al*., 2008; Alfellani *et al*., 2013b; Parkar *et al*., 2010; Zhao *et al*., 2017). Others reported that wildlife infections continue to vary with 2.83% in China (Table 4) (Deng *et al*., 2019), 7.5% in the US (Ruaux & Stang, 2014), 2.94% in England (Betts *et al*., 2018), 23.8% in India (Wang *et al*., 2013) and 69.35% in Australia (Duda *et al*., 1998). Only with light-scoping technologies high prevalence rates were recorded (Duda *et al*., 1998) and have been questioned since then. A study by Wang *et al*. (2013) on Indian stray dogs found that various geographical areas have a bearing on diversity. This was mainly infected by temporary and opportunistic ST infection. Others claimed that 99% to 100% of *Blastocystis* infection carnivores are similar to humans by ST (Cian *et al*., 2017).

Rodentia

In comparison to captive rodents, there are fewer studies on *Blastocystis* being conducted on wild rodents (Table 5). Several subtypes were found in rodents namely, ST1-5, ST7, ST8, ST10, ST13, and ST17 with ST4 being the most prevalent subtype (Alfellani *et al*., 2013b; Yoshikawa *et al*., 2016; Betts *et al*., 2018; Farah Haziqah *et al*., 2018; Katsumata *et al*., 2018).

Marsupial

Marsupial studies were scarce with only a few studies carried out on this group of animals worldwide (Table 7). However, *Blastocystis* in marsupial were frequently being reported in Australia (Parkar *et al*., 2007, 2010; Roberts *et al*., 2013). The prevalence rate varies from 0.5% to 100%. *Blastocystis* ST11 was highly reported in marsupial in China (Zhao *et al*., 2017), whereas ST1, ST4, ST8 and ST10 are the common subtypes found in this group worldwide (Parkar *et al*., 2010; Roberts *et al*., 2013; Zhao *et al*., 2017; Cian *et al*., 2017; Li *et al*., 2019).

Proboscidea

To date, studies on proboscidea were lacking with only six studies recorded worldwide. *Blastocystis* sp. was found in elephants from Australia, Netherlands, Belgium, France, and Bangladesh with the prevalence rate varying from 33.3% to 100% (Table 6). It was reported that ST11 was the most common subtype discovered. Nonetheless, ST1 and ST3 were also reported in this animal group.

Perissodactyla

Perissodactyla is among the animal group that received less research attention on genotypic characterisation (Table 8). The prevalence rate ranges from 16.7% to 40%. The most predominant subtype was ST3. Other subtypes identified were ST2, ST3, ST5 and ST10 (Figure 8). The most common perissodactyla being studied was the pony. Recently, Zhang *et al*. (2021) reported the occurrence of *Blastocystis* in a captive wild animal. Pony was found to be the new host for ST2.

Reptiles

There have only been a few conventional studies on *Blastocystis* in reptiles (Table 9) in which no molecular investigations have been conducted in Australia, United Kingdom or China. Most of the studies were conducted in Singapore and the United Arab Emirates. In Malaysia, the only study on genotypic characteristics of *Blastocystis* in reptiles was reported by Mohd Zain *et al*. (2017) who demonstrated *Blastocystis* Clade VIII isolated from a water monitor lizard. The overall number of reptiles examined throughout all the studies was generally smaller. Thus, further molecular studies on reptiles are required to understand better the distribution of subtypes and the evolutionary relationships in Reptilia.

Table 3. Studies on *Blastocystis* in captive and wild artiodactyl worldwide

Country/ Region	Host	No. of Positive Samples (%)	Subtypes (ST)																	Other/ Mixed Subtypes	Condition	Technique	References
			1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17				
Captivated wild animals																							
Australia	Deer/ *Cervus elaphus*	1/50 (2.0)				1															C	MOL	Roberts *et al*. (2013)
	Giraffe/ *Giraffa camelopardalis*	1/6 (1.7)												1							C	MOL	
Australia	Giraffe/ *Giraffa camelopardalis*	5/6 (83.3)												5							C	MOL	Parkar *et al*. (2010)
China	Reindeer / *Rangifer tarandus*	7/104 (6.7)										3			4						C	MOL	Wang *et al*. (2018b)
	Sika deer / *Cervus nippon*	12/82 (14.6)										10				2					C	MOL	
China	Takin/ *Budorcas taxicolor*	28/49 (57.1)										25				2					C	MOL	Zhao *et al*. (2017)
	Alpaca/ *Lama pacos*	12/14 (85.7)										9				2				ST18(1)	C	MOL	
	Guanaco/ *Lama guanicoe*	14/20 (70.0)										13								ST22 (1)	C	MOL	
	Giraffe/ *Giraffa camelopardalis*	2/10 (20.0)										2									C	MOL	
	Bushbuck/ *Tragelaphus scriptus*	8/13 (61.5)										1				7					C	MOL	
	Eland/ *Taurotragus oryx*	6/9 (66.7)										3				3					C	MOL	
	Camel/ *Camelus* sp.	5 /10 (50.0)	1									4									C	MOL	
	Sika deer/ *Cervus nippon*	3/8 (37.5)										3									C	MOL	
	Wapiti/ *Cervus elaphus*	1/3 (33.3)										1									C	MOL	
	Wild yak/ *Bos grunniens*	3/6 (50.0)										2				1					C	MOL	
	Lechwe water buck/ *Kobus leche*	3/3 (10)														2					C	MOL	
	Oryx/ *Oryx gazella*	1/2 (50.0)										1									C	MOL	

	Fallow Deer/ *Dama dama*	1/2 (50.0)				1					C	MOL	
	Waterbuck/ *Kobus ellipsiprymnus*	½ (50.0)								ST21(1)	C	MOL	
	White-lipped Deer/ *Cervus albirostris*	1/1 (100)				1					C	MOL	
China	Yaks/ *Bos grunniens*	278 /1027 (27.1)				170	38	70			C	MOL	Ren *et al*. (2019)
China	Blesbok/ *Damaliscus dorcas*	1/2 (50.0)			1						C	MOL	Li *et al*. (2020a)
	South African oryx/ *Oryx gazella*	1/11 (9.1)			1						C	MOL	
	Red deer/ *Cervus elaphus*	5/2 (40.0)				2					C	MOL	
	Alpaca / *Vicugna pacos*	27/4 (14.8)				1		3			C	MOL	
China	Alpacas/ *Vicugna pacos*	87/366 (23.8)			1 2	39		3 6			C	MOL	Ma *et al*. (2020b)
China	Giraffe	3/429 (0.6)			1	2					C		Zhang *et al*. (2021)
	Sika deer	18/429 (0.4)			6	12					C	MOL	
	White-lipped deer	1/429 (0.2)				1					C	MOL	
	Yak	1/429 (0.2)				1					C	MOL	
	Bactrian camel	2/429 (0.4)				1		1			C	MOL	
China	black bears /*Ursus thibetanus*	45/312 (14.4)	4 5								C	MOL	Ni *et al*. (2021)
	sika deer/ *Cervus nippon*	6/760 (0.8)								ST1, ST14	C	MOL	
United Kingdom	Yak					2					C	IVC, MOL	Betts *et al*. (2018)
	European Bison	3/3 (100)				3					C	IVC, MOL	
	Eurasian Elk	1/2 (50.0)								ST4,10,14	C	IVC, MOL	
	Pygmy Goat	2/2 (100)						1		ST1,10	C	IVC, MOL	
	Red Deer	1/1 (100)								ST4,10(1)	C	IVC, MOL	
	Soay Sheep	1/1 (100)						1			C	IVC, MOL	
	Wild Boar	1/2 (50.0)			1						C	IVC, MOL	
United Kingdom	Wild boar / *Sus scrofa*	2/4 (50.0)			2						C	MOL	Betts *et al*. (2020)
	Red deer/ *Cervus elaphus*	1/3 (33.3)		1		1					C	MOL	
	Red river hog/ *Potamochoerus porcus*	3/6 (50.0)			3						C	MOL	

	Muntjac deer/ *Muntiacus reevesi*	1/1 (100)						1					C	MOL	
	Reindeer/ *Rangifer tarandus*	1/1 (100)					1						C	MOL	
	European Bison/ *Bison bonasus*	3/9 (33.3)					3						C	MOL	
	Bongo/ *Tragelaphus eurycerus*	1/6 (17.0)					1		1				C	MOL	
United Kingdom	Anoa	2/3 (66.7)					1				ND(1)		C	MOL	Alfellani *et al*. (2013a)
	Giraffe	1/4 (25.0)		1									C	MOL	
	Mouse deer	2/2 (100)						2					C	MOL	
	Roe deer	1/2 (50.0)			1								C	MOL	
Denmark	Roe deer	1*					1						C	IVC,MOL	Stensvold *et al*., (2009)
France	Alpaca	1/6 (16.7)					1						C	MOL	Cian *et al*. (2017)
	Java mousedeer	1/1 (100)						1					C	MOL	
	Giraffe	5/6 (83.3)					1		4				C	MOL	
	Common eland	1/1 (100)							1				C	MOL	
	Greater kudu	2/2 (100)					2						C	MOL	
	Bongo	1/1 (100)					1						C	MOL	
	American bison	1/3 (33.3)									ST10,14(1)		C	MOL	
	Blindled wildebeest	1/1 (100)							1				C	MOL	
	Beisa oryx	3/4 (75.0)					3						C	MOL	
	Scimitar-horned oryx	5/5 (100)		1			4						C	MOL	
Bangladesh	Waterbuck	1/7 (14.3)					1						C	IVC, MOL,	Li *et al*., (2019)
	Spotted deer	1/30 (3.3)							1				C	IVC, MOL	
	Gayal	1/4 (25.0)							1				C	IVC, MOL.	
Mauritius	Fallow deer	2/2 (100)					2						C	MOL	Alfellani *et al*., (2013b)
Libya	Camel	47/196 (24.0)	5	5	20		6		3	1	MIXED (7)		C	MOL	Alfellani *et al*. (2013b)
	Gazelle	1/9 (11.1)									MIXED 1)		C	MOL	
Czech Republic	Mouflon	1/5 (20.0)							1				C	MOL	Alfellani *et al*. (2013b)
Brazil	boar	30/39 (76.9)	3	1	14	1							C	IVC, MOL	Valença-Barbosa *et al*. (2019)
Brazil	Marsh Deer/ *Blastocerus dichotomus*	1/1 (100)									NA		C	MOL	Oliveira-Arbex *et al*. (2020)
	Gray Brocket/ *Mazama gouazoubira*	1/1 (100)			1								C	MOL	

	Collared Peccary/ *Pecari tajacu*	1/1 (100)									NA	C	MOL	
Malaysia	Deer	30/100 (30.0)									NA	C	IVC, MOL	Hemalatha *et al*. (2014)
Nepal	Buffalo/ *Bubalus bubalis*	4/19 (21.1)							1		ND(3)	C	MOL	Lee *et al*. (2012)
United States of America	White-tailed deer	71*										C		
Netherlands	Giraffe/ G*iraffa camelopardalis*	12/15 (80.0)					10				NA(2)	C	MOL	Parkar *et al*. (2010)
Belgium	Giraffe/ *Giraffa camelopardalis*	5/5 (100)					4				NA(1)	C	MOL	Parkar *et al*. (2010)
Poland	Wild boar	8*			8							C	MOL	Rudzińska *et al*. (2021)
	Collared peccary	9*			9							C	MOL	
Free living wild animals														
China	Sambar Deer/ *Rusa unicolor*	8/39 (20.5)						3	5			W	MOL	Chen *et al*. (2021)
	Sika deer/ *Cervus nippon*	5/14 (35.7)	4	1								W		
	Long-tailed goral/ *Naemorhedus griseus*	5/17 (29.4)	1		2			2				W		
	Crested deer/ *Elaphodus cephalophus*	4/8 (50.0)						3	1			W		
	Chinese antelope/ *Capricornis milneedwardsii*	1/3 (33.3)							1			W		
	Dwarf musk deer/ *Moschus berezovskii*	0/19 (0)										W		
	Takin/ *Budorcas taxicolor*	0/25 (0)						2	1			W		
	Blue sheep/ *Pseudois nayaur*	3/42 (0.7)										W		
China	Tibetan antelope/ *Pantholops hodgsonii*	30/627 (0.05)				4		2 4	2			W	MOL	Geng *et al*. (2021)
Iran	wild boars/ *Sus scrofa*	3/12 (25.0)									NA	W	CM	Solaymani-Mohammadi *et al*. (2004)

Iran	Wild boar/ *Sus scrofa*	11/25 (44.0)						N/A	W	MOL	Yaghoobi *et al*., (2016)
Iran	Wild boars/ *Sus scrofa*	11/25 (44.0)						NA	W	IVC, MOL	Yaghoobi *et al*. (2016)
Malaysia	Mouse deer	1/4 (25.0)						ND	W	IVC, MOL	Mohd Zain *et al*. (2017)
South Korea	Wild boar/ *Sus scrofa*	45/433 (10.4)			4 5				W	MOL	Lee *et al*. (2020)
Poland	Wild boar/ *Sus scrofa*	1/113 (0.01)			1				W	MOL	Kaczmarek *et al*. (2021)
	Red deer/ *Cervus elaphus elaphus*	2/113 (0.02)	2							MOL	
	European bison/ *`Bison bonasus*	5/113 (0.04)	1	1	2	1			W	MOL	

CM - Conventional microscopy, IVC - *In vitro* cultivation, MOL - Molecular technique, NA - Not applicable, ND - Not Defined, C – Captivated wildlife, W – Free-living wildlife, * - This information is not provided as a survey.

Table 4. Studied on *Blastocystis* in captive and wild carnivora worldwide

Country	Host	No of Positive Samples (%)	Subtypes (ST)																	Other/ Mixed Subtypes	Condition	Technique	References
			1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17				
Captivatd wild animals																							
Australia	Snow Leopard/ *Panthera uncia*	1/6 (1.7)				1															C	MOL	Roberts *et al*. (2013)
China	Raccoon dog/ *Nyctereutes procyonoides*	3/40 (7.5)			3																C	MOL	Wang *et al*. (2018b)
	Arctic fox / Alopex *lagopus*	4/213 (1.9)	2			1			1												C	MOL	
China	Sand badger/*Arctonyx collaris*	1/3 (33.3)														1					C	MOL	
	Leopard cat/*Prionailurus bengalensis*	1/10 (10.0)														1					C	MOL	
China	Northern raccoon	2/429 (0.05)	1	1																	C	MOL	Zhang *et al*. (2021)
	leopard	1/429 (0.02)					1																
United Kingdom	Pine Marten	1/1 (100)	1																		C	IVC, MOL	Betts *et al*. (2018)
United Kingdom	Lynx/ *Lynx lynx*	2/40 (5.0)		1												1					C	IVC, MOL	Betts *et al*. (2020)
	Pine Marten/ *Martes martes*	1/2 (50.0)				1															C		
	Scottish Wild Cat/ *Felis silvestris*	1/13 (8.0)																		ST4,14	C		

France	Cheetah	1/1 (100)		1				C	MOL	Cian *et al*. (2017)
	Grey wolf	1/4 (25.0)			1				MOL	
	Polar bear	1/3 (33.3)			1			C	MOL	
	California sea lion	1/1 (100)	1					C	MOL	
Brazil	Jaguarundi/ *Herpailurus yaguarondi*	1/2 (50.0)					NA	C	MOL	Oliveira-Arbex *et al*. (2020)
	Ocelot/ *Leopardus pardalis*	1/1 (100)					NA	C	MOL	
	Northern Tiger Cat/ *Leopardus tigrinus*	1/1 (100)			1			C	MOL	
	Jaguar/ *Panthera onca*	1/2 (50.0)					NA	C	MOL	
	South American Coati/ *Nasua nasua*	1/4 (25.0)	1				ST1	C	MOL	
	Raccoon/ *Procyon cancrivorus*	1/3 (33.3)					NA	C	MOL	
Free living wild animals										
Poland	Grey wolf/ *Canis lupus*	7/113 (0.06)			1			W	MOL	Kaczmarek *et al*. (2021)

CM - Conventional microscopy, IVC - *In vitro* cultivation, MOL - Molecular technique, NA - Not applicable, ND - Not Defined, C – Captivated wildlife, W – Free-living wildlife, * - This information is not provided as a survey.

Table 5. Studies on *Blastocystis* in captive and wild rodentia worldwide

Country	Host	No of Positive Samples (%)	Subtype (ST)																	Other/ Mixed Subtypes	Condition	Technique	References
			1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17				
Captivated Wild animals																							
China	Patagonian mara/ *Dolichotis patagonum*	3/15 (20.0)					3														C	MOL	Li *et al*. (2020b)
China	Flying squirrels	69/207 (33.3)	2 4		1 2										27						C	MOL	Xiao *et al*. (2019)
China	Chinchilla	4/6 (66.7)																	4		C	IVC, CM, MOL	Deng *et al*. (2021)
	Red giant flying squirrel	1/1 (100)					1														C	IVC, CM, MOL	
	Black great squirrel	1/1 (100)					1														C	IVC, CM, MOL	
United Kingdom	Red Squirrel	1*					1														C	IVC, MOL	Betts *et al*. (2018)
	Water Vole	10*					8													ST1,4,10(2)	C	IVC, MOL	
United Kingdom	Bank vole	1/32 (3.13)						1													C	MOL	Alfellani *et al*. (2013a)
	Wood mouse	1/13 (7.69)			1																C	MOL	
Belgium	Chinchilla	2/5 (40.0)			2																C	MOL	Alfellani *et al*. (2013a)
Poland	Yellow necked mouse	1/1 (100)			1																C	MOL	Alfellani *et al*. (2013a)
Libya	Gundi	1/4 (25.0)																	1		C	MOL	Alfellani *et al*. (2013a)
Japan	Rat	1/1 (100)					1														C	MOL	Yoshikawa *et al*. (2003a)
Brazil	Capybara/ *Hydrochoerus hydrochaeris*	2/23 (8.7)	1							1											C	MOL	Oliveira-Arbex *et al*. (2020)
Brazil	*Rattus rattus*	1/1 (100)			1																C	IVC, MOL	Valença-Barbosa *et al*. (2019)
	Nectomys squamipes	3/3 (100)								1										ND (2)	C	IVC, MOL	
	Akodon cursor	1/2 (50.0)																		ND (1)	C	IVC, MOL	
	Akodon montensis	2/2 (100)																		ND (1)	C	IVC, MOL	

China	Eurasian Red Squirrel	7/72 (9.7)			7						C	MOL	Chai *et al*. (2020)
	Eastern Chipmunk	8/171 (4.7)			8						C	MOL	
	Chinchilla	3/72 (4.2)			2				1		C	MOL	
	Guinea pig	12/90 (13.3)			1 2						C	MOL	
	Chinese Striped Hamster	12/98 (12.2)			1 2						C	MOL	
China	White kangaroo	9/429 (2.1)				5		4			C	MOL	Zhang *et al*. (2021)
France	Norway rat/ *Rattus norvegicus*	1/2 (50.0)			1						C	MOL	Cian *et al*. (2017)
	Capybara/ *Hydrochoerys hydrochaeris*	3/5 (60.0)		1		1				ND (1)	C	MOL	
Maxico	Kangaroo rat/ *Heteromyidae* sp.	4/8 (50.0)			1				2	ND (1)	C	MOL	Martinez-Hernandez *et al*. (2020)
Unites States of America	Brazillian porcupine/ *Coendou prehensilis*	1*								MIXED ST4/ND (1)	C	MOL	Goe *et al*. (2016)
Colombia	Rats/ *Rattus rattus*	3/10 (33.3)		3							C	IVC, MOL	Ramírez *et al*. (2014)
United Arab Emirates	Indian palm squirrel	2/4 (50.0)			2						C	MOL, MOL	AbuOdeh *et al*. (2019)
	Faced squirrel	1/2 (50.0)							1		C	MOL, MOL	
Free living wild animals													
China	Brown rat / *Mus musculus*	4/108 (3.7)			4						W	MOL	Wang *et al*. (2018b)
China	Porcupine/ *Hystrix hodgsoni*	1/7 (14.3)	1								W	MOL	Chen *et al*. (2021)
Indonesia	Polynesian rats/ *Rattus exulans*	11/67 (16.4)			1 1						W	MOL	Katsumata *et al*. (2018)

CM - Conventional microscopy, IVC - *In vitro* cultivation, MOL - Molecular technique, NA - Not applicable, ND - Not Defined, C – Captivated wildlife, W – Free-living wildlife, * - This information is not provided as a survey.

Table 6. Studies on *Blastocystis* in captive and wild proboscidea worldwide

Country	Host	No Positive Samples (%)	Subtype (ST)																Other/ Mixed Subtypes	Condition	Technique	References
			1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17				
Captivated Wild animals																						
Australia	Asian elephant	11/20 (55.0)											11								C	MOL	Roberts *et al*. (2013)
Australia	Asian elephant	9/9 (100)											7							ND (2)	C	MOL	Parkar *et al*. (2010)
Netherlands	Asian elephant	6/14 (42.9)											6								C	MOL	Parkar *et al*. (2010)
Belgium	Asian elephant	1/2 (50.0)											1								C	MOL	Parkar *et al*. (2010)
France	Asian elephant	3/4 (75.0)	3		2																C	MOL	Cian *et al*. (2017)
Bangladesh	Elephant	1/3 (33.3)											1								C	IVC, MOL	Li *et al*. (2019)

CM - Conventional microscopy, IVC - *In vitro* cultivation, MOL - Molecular technique, NA - Not applicable, ND - Not Defined, C – Captivated wildlife, W – Free-living wildlife, * - This information is not provided as a survey.

Table 7. Studies on *Blastocystis* in captive and wild marsupial worldwide

Country	Host	No of Positive Samples (%)	Subtypes (ST)																	Other/ Mixed Subtypes	Condition	Technique	References
			1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17				
Captivated Wild animals																							
Australia	Eastern Wallaroo/ *Macropus robustus*	3/3 (100)				3															C	MOL	Roberts *et al*. (2013)
	Western Grey Kangaroo/ *Macropus fuliginosus*	1/2 (50.0)													1						C	MOL	
	Eastern Grey Kangaroo/ *Macropus giganteus*	3/4 (75.0)				3															C	MOL	
	Red Kangaroo/ *Macropus rufus*	3/4 (75.0)				3															C	MOL	
Australia	Quokka	2/2 (100)													2						C	MOL	Parkar *et al*. (2010)
	Southern hairy nosed wombat	1/1 (100)	1																		C	MOL	
	Western grey kangaroo	1/2 (50.0)												1							C	MOL	
Australia	Chuditch/ *Dasyurus geoffroii*	4/29 (13.8)																		ND(4)	C	IVC, MOL	Parkar *et al*. (2007)
China	Marsupialia Gray kangaroo/ *Macropus fuliginosus*	8 /11 (72.7)											8								C	MOL	Zhao *et al*. (2017)
China	White kangaroo	2/429 (0.5)	2																		C	MOL	Zhang *et al*. (2021)
France	Red-necked wallaby/ *Macropus rufogriseus*	1/1 (50.0)																		ND (1)	C	MOL	Cian *et al*. (2017)
Brazil	Common opossum/ *Didelphis marsupialis*	1/4 (25.0)																		ND (1)	C	MOL	Oliveira-Arbex *et al*. (2020)
Brazil	*Metachirus nudicautatus*	1/1 (100)	1																		C	MOL	
	Didelphis aurita	21/25 (84.0)	4									1								NA (16)	C	MOL	Valença-Barbosa *et al*. (2019)
UK	Macropus rufogriseus / *Diprotodontia Wallaby*	2*											2							NA (1)	C	IVC, MOL	Betts *et al*. (2018)
Colombia	Opossums/ *Didelphis marsupialis*	25/40 (62.5)										2 5									C	IVC, MOL	Ramírez *et al*. (2014)
Poland	Red kangaroo	1*					1														C	MOL	Rudzińska *et al*. (2021)

CM - Conventional microscopy, IVC - *In vitro* cultivation, MOL - Molecular technique, NA - Not applicable, ND - Not Defined, C – Captivated wildlife, W – Free-living wildlife, * - This information is not provided as a survey.

Table 8. Studies on *Blastocystis* in captive and wild perissodactyla worldwide

Country	Host	No of Positive Samples (%)	Subtypes (ST)																	Other/ Mixed Subtypes	Condition	Technique	References
			1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17				
Captivated Wild animals																							
China	Akhal-teke horse/ *Equus caballus*	1/4 (25.0)										1									C	MOL	Zhao *et al*. (2017)
	Pony/ *Pedicularis humilis*	1/6 (16.7)										1									C	MOL	
	Mongolia Wild Ass/ *Equus hemionus pallas*	2/5 (40.0)										1									C	MOL	
China	Pony	2/249 (0.8)		2																	C	MOL	Zhang *et al*. (2021)
France	Common zebra	2/4 (50.0)			1															ND (1)	C	MOL	Cian *et al*. (2017)
	Poitou donkey	2/2 (100)		1	1																C	MOL	
	South American tapir	3/3 (100)			2		1														C	MOL	
United Kingdom	Black rhinoceros	1/1 (100)					1														C	MOL	Alfellani *et al*. (2013b)

CM - Conventional microscopy, IVC - *In vitro* cultivation, MOL - Molecular technique, NA - Not applicable, ND - Not Defined, C – Captivated wildlife, W – Free-living wildlife, * - This information is not provided as a survey.

Table 9. Studies on *Blastocystis* in captive and wild reptiles around the world

Country	Host	No of Positive Samples (%)	Subtypes (ST)																Other/ Mixed Subtypes	Condition	Technique	References
			1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17				
Captivated wild animals																							
France	Green iguana	1/1 (100)																		NA	C	MOL	Cian *et al*. (2017)
	Boa constrictor	1/1 (100)																		NA	C	MOL	
Iran	Cobra snake	1/1 (100)																		NA	C	IVC, CM	Mirzapour *et al*. (2018)
	Phytons Albino	1/1 (100)																		NA	C	IVC, CM	
Singapore	Galapagos tortoise	1*																		NA	C	IVC, CM	Teow *et al*. (1992)
	Starred tortoise	1*																		NA	C	IVC, CM	
	Red-footed tortoise	1*																		NA	C	IVC, CM	
	Mangrove snake	1*																		NA	C	IVC, CM	
	Reticulated python	1*																		NA	C	IVC, CM	
	Copperhed snake	1*																		NA	C	IVC, CM	
	Estuarine crocodile	1*																		NA	C	IVC, CM	
	Rhino iguana	1*																		NA	C	IVC, CM	
United Arab Emirates	African spurred tortoise Centrochel-ys	19*																		NA	C	IVC, CM	AbuOdeh *et al*. (2019)
	Greek tortoise	2*																		NA	C	IVC, CM	
	Mata mata turtle	1*																		NA	C	IVC, CM	

Poland	Leopard tortoise	1*	NA	C		Rudzińska *et al*. (2021)
	Spur-thighed tortoise	1*	NA	C		
	Giant Asian pond turtle	1*	NA	C		
	Radiated tortoise	1*	NA	C		
	African spurred tortoise	1*	NA	C		
Free living wild animals						
Malaysia	Water monitor lizards	1/6 (1.7)	ND (1)	W	IVC, MOL	Mohd Zain *et al*. (2017)

CM - Conventional microscopy, IVC - *In vitro* cultivation, MOL - Molecular technique, NA - Not applicable, ND - Not Defined, C – Captivated wildlife, W – Free-living wildlife, * - This information is not provided as a survey.

CONCLUSIONS

Most *Blastocystis* studies were conducted on wildlife and captive animals in Australia, China, and the United Kingdom. Among the other countries involved were France, Colombia, Brazil, Bangladesh, Netherlands, Belgium, the United Arab Emirates, the United States, Mexico, Indonesia, Japan, Libya, Belgium, Poland, Iran, South Korea, the Czech Republic, Mauritius, Denmark, Malaysia, Spain, Saint Kitts and Nevis, Cameroon, Morocco, Philippines, Germany, Thailand, Tanzania, Nepal, Italy, Senegal, and France. Overall, different geographic regions worldwide were reported to have different subtypes. The geographic region and sample size have an impact on the prevalence rate. Therefore, more *Blastocystis* epidemiological studies are necessary for most countries.

Studies on *Blastocystis* in non-human primates (NHP) were the most commonly being reported with diverse species being examined worldwide as well as higher number of individuals were examined. In contrast, the less frequently wildlife being studied was the reptiles. Thus, further studies are needed to obtain more information on the role of these animal with *Blastocystis* infection.

Detection of human subtypes namely, ST1-ST9 in most group of animals except in reptile indicated that transmission may occur between human and animal. As *Blastocystis* has lack host specificity and variety of wildlife animals were found to be infected with this organism thus *Blastocystis* could serve as a significant reservoir for human infection. Further studies should be expended on wildlife, captive animals, and the interaction in humans transmission are required to identify the zoonotic association and risk factor of this protozoan parasite in those animals.

Conflict of interest statement

On behalf of all authors, the corresponding author states that there is no conflict of interest.

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