Subtype distribution of *Blastocystis* isolates from synanthropic and zoo animals and identification of a new subtype☆

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☆Nucleotide sequence data reported in this paper are available in Genbank under the accession numbers: **FM164412** and **FM164413.**

Abstract

Blastocystis isolates from 56 Danish synanthropic and zoo animals, 62 primates primarily from United Kingdom (UK) collections, and 16 UK primate handlers were subtyped by PCR, sequencing and phylogenetic analysis. A new subtype (ST) from primates and artiodactyls was identified and designated as *Blastocystis* sp. ST 10. STs isolated from non-human primates (n = 70) included ST3 (33%), ST8 (21%), ST2 (16%), ST5 (13%), ST1 (10%), ST4 (4%) and ST10 (3%). A high prevalence of ST8 was seen among primate handlers (25%). This ST is normally very rare in humans, suggesting that acquisition of *Blastocystis* ST8 infections from primates by their handlers had occurred in these cases. Data from published studies of non-human primates, other mammals and birds were collected and interpreted to generate a comprehensive overview on the ST distribution in such animals. On the basis of information on 438 samples, it was found that *Blastocystis* from primates belong mainly to ST1, ST2, ST3, ST5 and ST8, ungulates and dogs mainly ST1, ST2, ST3, ST5 and ST10, rodents ST4, and birds mainly ST6 and ST7. The data indicate moderate host specificity, most clearly exemplified by the fact that STs isolated from avian and non-avian hosts rarely overlap.

Keywords: *Blastocystis*; PCR; Subtypes; Phylogeny; Epidemiology

1. Introduction

Blastocystis is a common single-celled parasite of humans, non-human primates, other mammals, birds, amphibians, reptiles, fish, arthropods and annelids (Stenzel and Boreham, 1996; König and Müller, 1997; Belova and Krylov, 1998; Yoshikawa et al., 2004b, 2007). The parasite exhibits extensive genetic diversity, and on the basis of molecular analysis of the ssrRNA gene, nine distinct subtypes (ST1-ST9) have been identified from humans, non-human primates, other mammals and birds (Noël et al., 2005; Stensvold et al., 2007). *Blastocystis* from non-human sources also comprise isolates that appear to fall outside the genetic range of these nine subtypes, eg. reptilian, amphibian and cockroach isolates (Yoshikawa et al., 2004b, 2007; Stensvold et al., 2007), although they are clearly closely related.

It has been suggested by many authors that some human infections may result from zoonotic transmission of the parasite, but at present this remains unproven. Humans most frequently host ST3 but are also regularly found to carry ST1, ST2 and ST4 (Özyurt et al. 2008). The five other STs (ST5-9) have been isolated only sporadically from humans. Except for this information on humans, little is known about the potential host specificity of *Blastocystis*. Such knowledge is necessary for epidemiological studies aimed at identifying routes of transmission and zoonotic significance, which in turn are important for strategies to control the spread and to increase our understanding of the clinical impact of the parasite.

In recent years, molecular studies have produced a growing body of data on STs of *Blastocystis* isolated from various non-human hosts. The aim of the present study was to identify STs of *Blastocystis* in synanthropic and zoo animals and to generate

hypotheses regarding the distribution and degree of host specificity of STs among non-human *Blastocystis*.

2. Materials and methods

2.1. Danish samples: origin of isolates and PCR

DNA was extracted from faecal samples from a variety of synanthropic and zoo animals at the National Veterinary Institute, Technical University of Denmark (Table 1). All samples were from animals positive for *Giardia* and/or *Cryptosporidium*. None of the samples was examined by in vitro culture for the specific detection of *Blastocystis*. Faecal DNA extraction was performed using the QIAamp DNA Stool Mini Kit (QIAGEN, Hilden, Germany) according to the manufacturer's recommendations.

Samples were screened for *Blastocystis* by PCR at the Statens Serum Institut as previously described (Stensvold et al., 2006) using the primers bl1400ForC and bl1710RevC, which amplify a 310 bp ssrRNA gene fragment, and Extract-N-Amp PCR ReadyMix (Sigma-Aldrich Danmark, Brøndby, Denmark). PCR-positive samples were sequenced as described previously (Stensvold et al., 2006). In cases where sequences indicated the presence of a potential new ST, the primers RD5 and BhRDr (Scicluna et al., 2006) were employed in order to obtain additional ssrRNA gene sequence information for inclusion in phylogenetic analyses.

Two nucleotide sequences amplified by the two different primer sets (Scicluna et al., 2006; Stensvold et al., 2006) representing a novel subtype obtained from a Danish cow (RL056) were submitted to GenBank (accession nos. **FM164412** and **FM164413**).

2.2. United Kingdom samples: origin of isolates and PCR

Non-human primate and monkey handler material was received by the Diagnostic Parasitology Laboratory of the London School of Hygiene and Tropical Medicine from animal facilities and collections for routine parasitological investigation (Table 1). Faecal samples were cultured in Robinson's medium (Clark and Diamond, 2002). *Blastocystis* was harvested from positive cultures and stored in lysis buffer before discontinuing the cultures. Culture lysate DNA was extracted using either a CTAB-based method (Ali et al., 2005) or, more recently, the Gentra Puregene Cell kit (QIAGEN Ltd., Crawley, UK). DNA samples were amplified using primers RD5, BhRDr and BioTaq polymerase (Bioline Ltd., London, UK). PCR products were gel purified and sequenced using the BhRDr primer as previously described (Scicluna et al., 2006).

2.3. Phylogenetic analysis of isolates

Nucleotide sequences were aligned with a selection of 29 previously sequenced *Blastocystis* ssrRNA genes, representing all nine established STs from mammals and birds, and phylogenetic analysis was performed using Bayesian (MrBayes), Maximum Likelihood and Neighbour-joining (PHYLIP) methods as described previously (Scicluna et al., 2006). Pair-wise genetic distances within ST10 and between ST10 and other STs were generated from the 'uncorrected "p" distance matrix' calculated using PAUP*

v.4.0b10 (Swofford, D.L., 2000. PAUP*. Phylogenetic analysis using parsimony (*and other methods). Version 4. Sinauer Associates. Sunderland, Massachusetts, USA).

2.4. Data collection, interpretation and terminology

The references used in the data collection process are listed in Table 2. Different research groups have used distinct molecular methods and terminologies for analysing isolates genetically, hence complicating comparison of results. Original data generated from various studies using different molecular methodologies were standardised to meet the proposed consensus terminology using a recently described algorithm (Stensvold et al., 2007). Not all animal isolates described in the literature have been sequenced and some were characterised only by PCR-restriction fragment length polymorphism (RFLP) or PCR using sequence-tagged-site primers (PCR-STS). However, sequence data and supplementary information published by Abe (2004) enabled interpretation of PCR-RFLP data from some previous studies (Abe et al., 2003a, 2003b, 2003c) and PCR-STS data can also be linked to most of the currently recognised STs. Where some STs were not known at the time of publication, subsequent sequence analyses of such isolates and cross referencing using data from Arisue et al. (2003), Noël et al. (2005) and Stensvold et al. (2007) made it possible to identify STs originally described as 'ND' (not determined). *Blastocystis* has also been reported in reptiles, amphibians, arthropods and annelids, but only a small number of isolates have been sequenced. These were not included in the present study as they appear to represent distinct lineages and are unlikely to represent a zoonotic infection risk for humans.

3. Results

PCR-positive samples from the present study included material from 16 primate handlers, 70 non-human primates, 20 pigs, 25 cattle, two sheep, one deer and one dog (Table 1). The ST distribution of isolates from primate handlers and animals identified in the study is displayed in Table 1. Sequences obtained from isolates from 22 cattle, two lemurs, one deer and one sheep showed relatively low similarity to existing STs when percent identities were examined. These sequences formed a distinct group that clustered together as a separate lineage emerging at the base of the ST4+ST8 clade (Fig. 1) when the 310 bp region was used in phylogenetic analysis; this lineage was interpreted as a novel ST and is here designated as ST10 (Table 1). When the longer sequences obtained using the primers described by Scicluna et al. (2006) were used, ST10 emerged as a sister group to ST8 (Fig. 2). The maximum likelihood bootstrap support for both of these potential relationships was low and the affinities of ST10 must remain unresolved at present. This ST has hitherto not been reported from human infections. Table 3 shows the pair-wise genetic distances within ST10 and between ST10 and other STs.

Table 2 displays the STs of *Blastocystis* infecting 438 animals, including the data from analysis of over 100 isolates in the present study and identifiable ST data from all previously published studies. For comparison, Table 2 also includes the distribution of STs isolated from humans based on 16 major studies (Alfellani, unpublished data).

It can be seen from Table 2 that ST3 is more common in humans than all other STs combined. However, ST1, ST2 and ST4 also occur fairly frequently, whereas ST5 through ST9 occur only sporadically. Hence, the ST distribution among primate handlers

included in the present study was atypical: as expected, ST3 was the predominant ST, seen in 9/16 (56%) of the monkey handlers, but ST8 was the next most common subtype, being seen in four individuals (25%). Two of seven handler samples described previously were also ST8 (Scicluna et al., 2006). Although the numbers are small, since ST8 is very rare in other humans but common in non-human primates, particularly woolly monkeys, and given that the handlers would regularly come into contact with primate faeces in the course of their work, the most likely explanation for this observation is that the handlers acquired the ST8 infections from their charges.

To date, ST4 is the only ST to be isolated from rodents, but the total number of samples (seven) and host species (two) studied is small. This apparent ST restriction in rodents should be viewed with caution until larger studies have been performed. In contrast, ST6 and ST7 predominate in birds, where more samples (35) and host species (eight) have been studied, giving a much stronger indication that a link exists between these STs and avian hosts.

4. Discussion

We believe this is the first study to publish data on *Blastocystis* ST occurrences in non-human hosts in Scandinavia and provides new data from molecular characterisation of 119 animals and 16 primate handlers, adding substantially to the knowledge of *Blastocystis* host specificity. We present a comprehensive and systematic overview of the *Blastocystis* ST distribution in non-human hosts as it is known at the present time. Such

data are essential for an understanding of the host specificity and epidemiology of distinct *Blastocystis* sp. STs.

A novel ST (ST10) was isolated from both primates and ungulates in Denmark. The reason why ST10 has not been identified previously could be due to a geographically restricted distribution. However, given the high frequency of isolation in the present study and the fact that it was isolated from different types of primates and other mammals, it is more likely that some of the primers hitherto employed for *Blastocystis* ST characterisation are unsuitable for the detection of this particular ST. For instance, the R1 primer developed by Böhm-Gloning et al. (1997), which has been used in several studies, anneals to a region of the ssrRNA gene that exhibits sequence variation, and recently it was shown that this primer might preferentially amplify some STs over others (Wong et al., 2008). Indeed, the ssrRNA gene of *Blastocystis* is relatively poorly conserved, causing difficulties in designing sensitive genus-specific primers. In the study by Thathaisong and colleagues (2003), 186 (mainly human) isolates were positive by culture but were negative by PCR, and the isolates may have represented STs that were not amplifiable by the R1 primer. Moreover, many studies (including this one) have used in vitro culture for screening with subsequent extraction of DNA from the cultured isolates. It is not known, however, whether all STs grow equally well in culture; recently, data obtained by Parkar et al. (2007) suggested preferential in vitro amplification of ST2 over ST1. It is possible that ST10 does not grow under culture conditions commonly used and this is why it has not been identified previously.

Since mixed ST infections (MSI) are quite common in humans (Stensvold et al., unpublished data), it is possible that other animals also host MSI, which are not readily

identified by conventional PCR and sequencing. Indeed cultures from several primate samples examined in the UK appeared from the sequence traces to be MSI and were excluded from further analysis. It is suggested that, where possible, DNA should be extracted directly from faeces and that ST-specific primers be developed for PCR analysis to complement the genus-specific primers already in use. Sequencing of genus-specific products will generate data regarding the extent of MSI in non-human hosts. The ST-specific primers might not enable the detection of novel STs, but in combination with the genus-specific data they should identify samples worthy of further investigation.

ST8 has been isolated from humans only rarely (Scicluna et al., 2006; Motazedian et al., 2008; Stensvold et al., 2008) but is common in primate handlers, suggesting that zoonotic spread from primates to primate handlers is responsible for the unexpectedly high prevalence of this ST among these individuals. Zoonotic transmission of *Blastocystis* has been suggested by a plethora of research groups (Snowden et al., 2000; Abe et al., 2003c; Arisue et al., 2003; Thathaisong et al., 2003; Yoshikawa et al., 2003, 2004a; Abe, 2004; Noël et al., 2005; Parkar et al., 2007; Yan et al., 2007; Navarro et al., 2008), yet the extent and nature of this phenomenon remains unclear as the published evidence is equivocal. Given the ubiquity and the host range of *Blastocystis*, our ability to assess the zoonotic potential of *Blastocystis* is dependent on our ability to i) correctly and unambiguously identify STs, ii) detect and differentiate MSI, and iii) understand and analyse possible factors involved in transmission such as transmission sources, transmission vehicles, infectivity of cysts and other stages, contact with faeces or faecally contaminated soil, water and food, coprophagy, and the possibility of animals shedding ingested cysts that are simply passing through the host. In the future, identifying variable

molecular markers (eg. mini- or microsatellites) that differentiate strains within STs will likely prove necessary in positively identifying links between potential animal sources and specific human infections.

Comparing the data in Table 2 with the summary of the data from humans it appears clear that birds usually host ST6 and ST7, but that these are rarely found in mammals, having only been isolated from humans occasionally (Yan et al., 2007; Alfellani, unpublished data; Stensvold, unpublished data). Interestingly, ST9 has so far only been isolated from humans and on very few occasions. ST9 clusters with 'avian' ST6 and ST7, so it is possible that birds are also the normal hosts of this ST. Given their apparent host specificity, it is highly likely that human infections due to such avian STs are of zoonotic origin as was previously suggested by Noël et al. (2005).

The situation in pigs is unclear. Studies seem to fall into two groups – those that find predominantly ST1 (Thathaisong et al., 2003; Navarro et al., 2008) and those that find predominantly ST5 (Abe et al., 2003c;Yoshikawa et al., 2004a; Yan et al., 2007; present study). There appears to be no geographic component to this difference, which at present remains a mystery.

To date, ST4 is the only ST found among rodents and marsupials. This ST has only infrequently been isolated from non-human primates and has not so far been isolated from other mammals; however, in humans ST4 represents approximately 5% of the isolates characterised to date (Table 2). It remains to be established whether contact with rodents poses a risk of transmission to humans of this particular subtype. The high prevalence of ST1-ST3 in humans and other mammals means that differentiating human origins from zoonotic origins of such human infections is not possible at present.

In conclusion, moderate host specificity seems to prevail among *Blastocystis* STs, and the present data corroborate trends from other studies suggesting possible zoonotic transmission of *Blastocystis*, at least of some STs. Future studies should aim to develop high resolution molecular markers for analysing isolates in order to further elucidate the zoonotic potential of the parasite.

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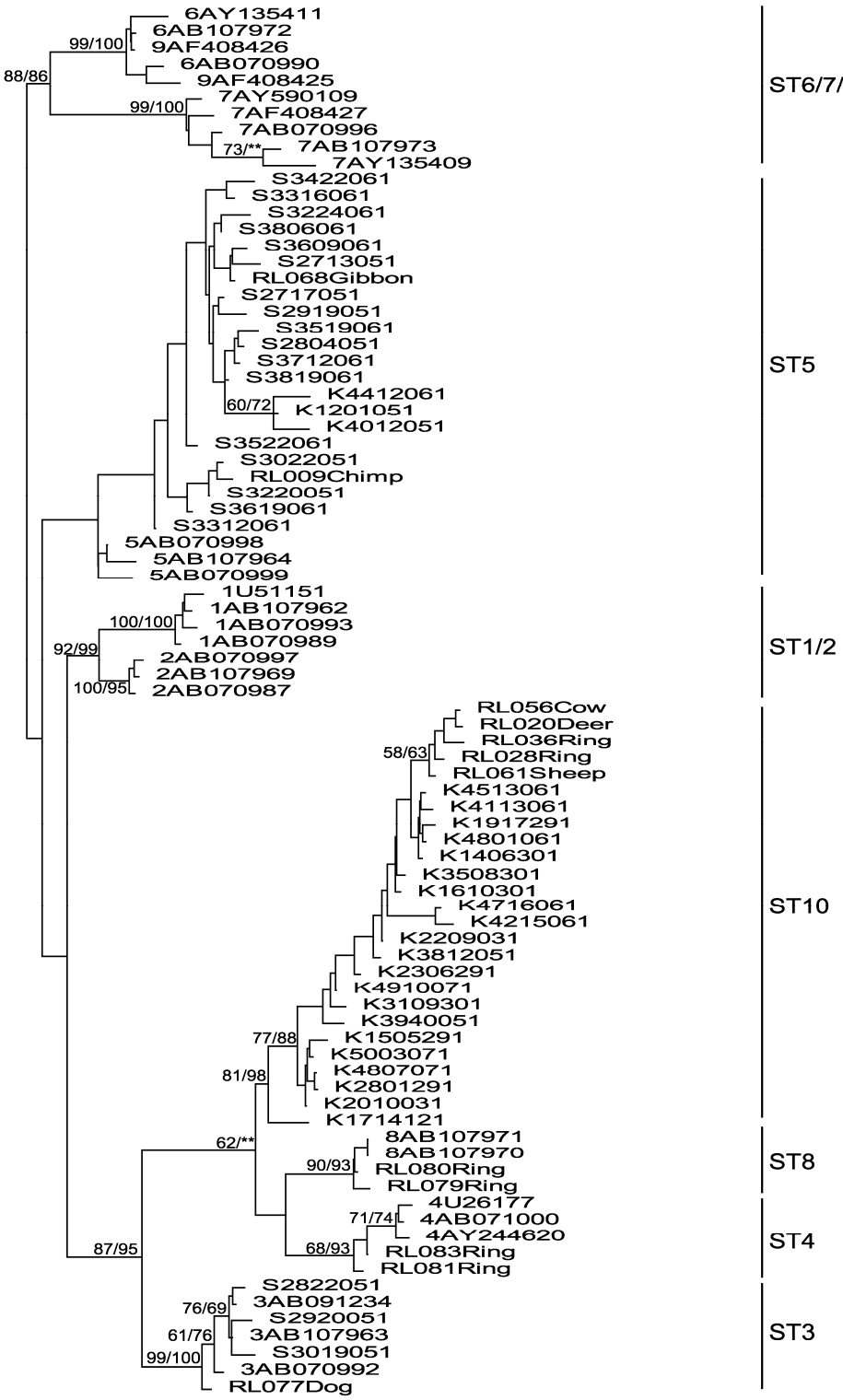
Figure legends

Fig. 1. Phylogenetic tree of the Danish *Blastocystis* sample sequences. The analysis was performed using the 310 bp sequences, which are identified by their sample code. Those starting with S are from pigs, those with K from cattle and most starting with R are from other animals, with the species identity appended (Ring = Ring-tailed lemur). Reference sequences from GenBank have the accession number preceded by the subtype identification. The clade consisting of subtypes 6, 7 and 9 was used as an outgroup. The tree shown is that obtained from the Bayesian analysis with the bootstrap proportions shown being from the Maximum Likelihood analysis (100 replicates) on the left and Neighbor-Joining analysis (1,000 replicates) on the right. The posterior probabilities obtained in the Bayesian analysis were all 1.0. Bootstrap values of less than 50% in both analyses are not shown. Where one analysis gave a value over 50% and the other below 50% the latter is indicated by two asterisks.

Fig. 2. Phylogenetic relationships of *Blastocystis* subtype 10. The analysis was performed using the 'barcode' region (Scicluna et al., 2006). Both samples sequenced (RL056 and K42-09) are cattle. Reference sequences from GenBank have the accession number preceded by the subtype identification. Subtypes 6, 7 and 9 were used as an outgroup. The tree shown is that obtained from the Bayesian analysis with the bootstrap proportions labelled as in Fig. 1. Bootstrap values of less than 50% are not shown. The branch leading to ST7 has been shortened for convenience.



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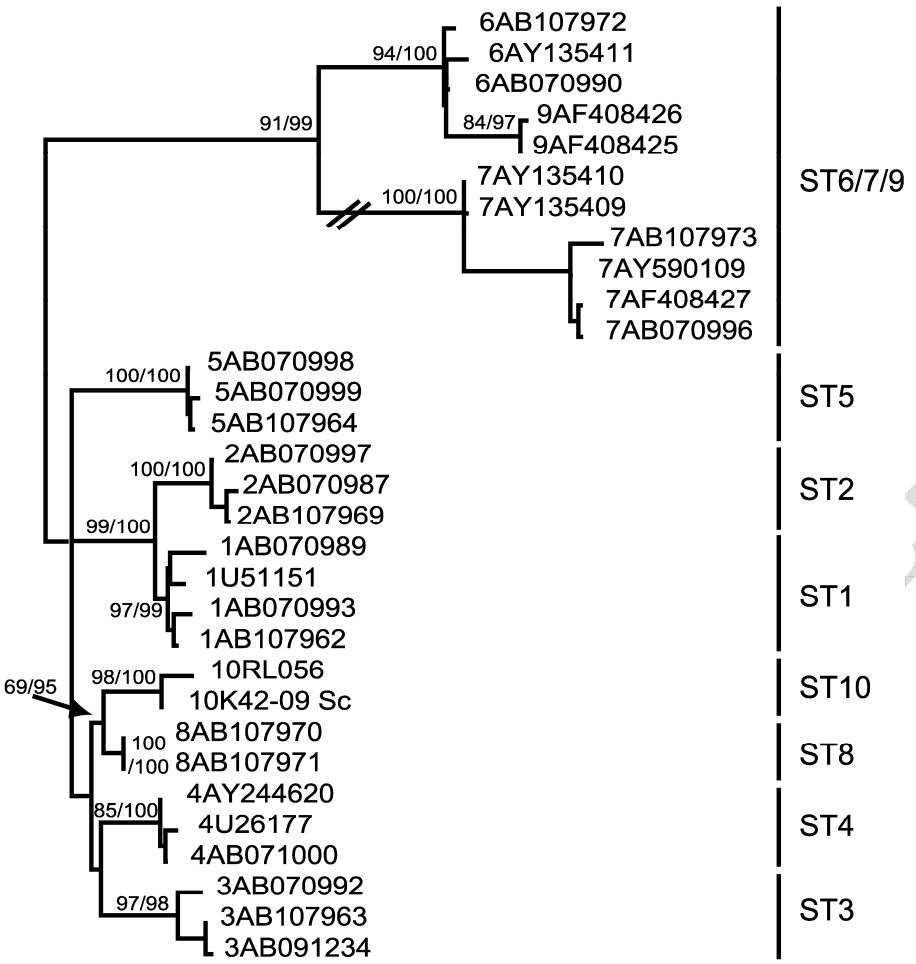
413 Fig.1



414
415
416 Fig.2

417
418 Table 1.
419 *Blastocystis* isolates characterised in the present study[a].
420

Host (common name)	Host (Latin name)	Country of Isolation	Subtype (ST)									
			ST 1	ST 2	ST3	ST4	ST5	ST6	ST7	ST8	ST9	ST10
Humans (primate handlers)	*Homo sapiens*	UK	2	-	9	1	-	-	-	4	-	-
Non-human primates												
Chimpanzee	*Pan troglodytes*	UK	1	4	8	-	6	-	-	-	-	-
		Denmark	-	-	-	-	1	-	-	-	-	-
Orang Utan	*Pongo pygmaeus*	UK	1	1	2	-	-	-	-	-	-	-
Gorilla	*Gorilla gorilla*	UK	-	4	1	-	1	-	-	-	-	-
Siamang	*Hylobates syndactylus*	UK	3	-	-	-	-	-	-	1	-	-
Mueller's gibbon	*Hylobates muelleri*	UK	-	1		-	-	-	-	-	-	-

Golden		UK											-
cheeked	*Hylobates*												
gibbon	*gabriellae*		1	-	1	-	-	-	-	-	_		
Lar gibbon	*Hylobates lar*	UK	1	-		-	-	-	-	1	_	-	
Gibbon		Denmark											-
(unspecified)	*Hylobates* sp.		-	-	-	-	1	-	-	-	_		
		UK	-	-	1	-	-	-	-	-	_	-	
Woolly	*Lagothrix*	UK											-
monkey	*lagotricha*		-	1	4	1	-	-	-	10	_		
Diana	*Cercopithecus*	UK											-
monkey	*diana*		-	-	1	-	-	-	-	-	_		
Barbary	*Macaca*	UK											-
macaque	*sylvanus*		-	-	1	-	-	-	-	-	_		
Stump-tailed	*Macaca*	UK											-
macaque	*speciosa*		-	-	1	-	-	-	-	-	_		
Common	*Callithrix*	UK											-
marmoset	*jacchus*		-	-	1	-	-	-	-	-	_		
Ring-tailed		Denmark											2
lemur	*Lemur catta*		-	-	-	2	-	-	-	2	_		
Unidentified		UK	-	-	2	-	-	-	-	1	_	-	

Other

animals

Pig	*Sus scrofa domestica*	Denmark	-	-	3	-	17	-	-	-	-	-
Cattle	*Bos taurus*	Denmark	-	-	-	-	3	-	-	-	-	22
Sheep	*Ovis aries*	Denmark	-	-	-	-	-	-	-	-	-	1
		UK	-	-	1	-	-	-	-	-	-	-
Roe Deer	*Capreolus capreolus*	Denmark	-	-	-	-	-	-	-	-	-	1
Dog	*Canis lupus familiaris*	Denmark	-	-	1	-	-	-	-	-	-	-

[a]All Danish isolates were from animals that were also positive for *Giardia* and/or *Cryptosporidium*.

Table 2.

Blastocystis subtype distribution identified in non-human primates, other mammals and birds (n = 438).

Host group	*Blastocystis* sp. subtype (ST)											Reference
	ST1	**ST2**	**ST3**	**ST4**	**ST5**	**ST6**	**ST7**	**ST8**	**ST9**	**ST10**	**ST unknown**	
Chimpanzee	1	-	-	-	-	-	-	-	-	-	-	Abe et al. (2003b); Abe (2004)
	1	1	-	-	-	-	-	-	-	-	-	Yoshikawa et al. (2004a)
	1	4	8	-	7	-	-	-	-	-	-	Present study
Gorilla	-	4	1	-	1	-	-	-	-	-	-	Present study
Orang Utan	1	-	-	-	-	-	-	-	-	-	-	Abe et al. (2003b); Abe (2004)
	1	-	-	-	-	-	-	-	-	-	-	Parkar et al. (2007)
	1	-	-	-	-	-	-	-	-	-	-	Yoshikawa et al. (2004a)
	1	1	2	-	-	-	-	-	-	-	-	Present study
Gibbons	-	1	-	-	-	-	-	-	-	-	-	Abe et al. (2003b); Abe (2004)
	2	-	-	-	-	-	-	-	-	-	-	Parkar et al. (2007)
	-	1	-	-	-	-	-	-	-	-	-	Yoshikawa et al. (2004a)
	5	1	2	-	1	-	-	2	-	-	-	Present study
Baboon	2	-	-	-	-	-	-	-	-	-	-	Parkar et al. (2007)
Mandrill/Drill	2	-	-	-	-	-	-	-	-	-	-	Abe et al. (2003b); Abe (2004)

	-	-	-	-	-	-	-	-	-	-	1	Yoshikawa et al. (2004a)
Macaques	1	2	-	-	-	-	-	-	-	-	-	Abe et al. (2003b); Abe (2004)
	1	-	-	-	-	-	-	-	-	-	-	Parkar et al. (2007)
	-	-	1	-	-	-	-	-	-	-	-	Scicluna et al. (2006)
	-	2	-	-	-	-	-	-	-	-	-	Yoshikawa et al. (2004a)
	-	-	2	-	-	-	-	-	-	-	-	Present study
Vervet monkey	1	-	-	-	-	-	-	-	-	-	-	Abe et al. (2003b); Abe (2004)
	1	1	-	-	-	-	-	-	-	-	-	Parkar et al. (2007)
De Brazza's monkey	1	-	-	-	-	-	-	-	-	-	-	Abe et al. (2003b); Abe (2004)
	-	-	-	-	-	-	-	-	-	-	1	Yoshikawa et al. (2004a)
Diana monkey	-	-	1	-	-	-	-	-	-	-	-	Present study
Leaf monkey	1	-	-	-	-	-	-	-	-	-	-	Abe et al. (2003b); Abe (2004)
	-	-	-	-	-	-	-	-	-	-	1	Yoshikawa et al. (2004a)
'Japanese monkey'	-	1	-	-	-	-	-	-	-	-	-	Yoshikawa et al. (1998)
	-	1	-	-	-	-	-	-	-	-	-	Yoshikawa et al. (2003)
Woolly monkey	2	2	1	-	-	-	-	3	-	-	-	Scicluna et al. (2006)
	-	1	4	1	-	-	-	10	-	-	-	Present study
Common marmoset	-	-	1	-	-	-	-	-	-	-	-	Present study

Lemurs	-	-	-	-	-	-	-	1	-	-	-	Abe et al. (2003b); Abe (2004)
	3	1	-	-	-	-	-	-	-	-	-	Parkar et al. (2007)
	-	-	-	2	-	-	-	2	-	2	-	Present study
Unidentified primate	-	1	5	-	-	-	-	1	-	-	-	Scicluna et al. (2006)
	-	-	2	-	-	-	-	1	-	-	-	Present study
Primates Total	**29**	**25**	**30**	**3**	**9**	**-**	**-**	**20**	**-**	**2**	**3**	
Pigs	3	-	1	-	8	-	-	-	-	-	-	Abe et al. (2003c)
	-	-	-	-	1	-	-	-	-	-	-	Arisue et al. (2003)
	122	7	-	-	-	-	-	-	-	-	-	Navarro et al. (2008)
	1	-	-	-	-	-	-	-	-	-	-	Noël et al. (2003)
	-	-	-	-	1	-	-	-	-	-	-	Scicluna et al. (2006)
	20	-	-	-	-	-	-	-	-	-	-	Thathaisong et al. (2003)
	-	-	-	-	16	-	-	-	-	-	-	Yan et al. (2007)
	-	-	-	-	1	-	-	-	-	-	-	Yoshikawa et al. (1998)
	-	-	-	-	1	-	-	-	-	-	-	Yoshikawa et al. (2003)
	4	-	2	-	14	-	-	-	-	-	-	Yoshikawa et al. (2004a)
	-	-	3	-	17	-	-	-	-	-	-	Present study
Pigs Total	**150**	**7**	**6**	**-**	**59**	**-**	**-**	**-**	**-**	**-**	**-**	

Cattle	1	-	2	-	7	-	-	-	-	-	-	Abe et al. (2003c)
	1	-	1	-	6	-	-	-	-	-	-	Yoshikawa et al. (2004a)
	-	-	-	-	3	-	-	-	-	22	-	Present study
Cattle Total	**2**	**-**	**3**	**-**	**16**	**-**	**-**	**-**	**-**	**22**	**-**	
Horse	1	-	-	-	-	-	-	-	-	-	-	Thathaisong et al. (2003)
Deer	-	-	-	-	-	-	-	-	-	1	-	Present study
Sheep	-	-	1	-	-	-	-	-	-	1	-	Present study
Dog	1	3	-	-	-	-	-	-	-	-	-	Parkar et al. (2007)
	-	-	1	-	-	-	-	-	-	-	-	Present study
Horse/Deer/Sheep /Dog Total	**2**	**3**	**2**	**-**	**-**	**-**	**-**	**-**	**-**	**2**	-	
Rat	-	-	-	1	-	-	-	-	-	-	-	Noël et al. (2003)
	-	-	-	3	-	-	-	-	-	-	-	Noël et al. (2005)
	-	-	-	1	-	-	-	-	-	-	-	Yoshikawa et al. (1998)
Guinea pig	-	-	-	1	-	-	-	-	-	-	-	Leipe et al. (1996)
	-	-	-	1	-	-	-	-	-	-	-	Silberman et al. (1996)
Opossum	-	-	-	1	-	-	-	-	-	-	-	Parkar et al. (2007)
Rodent/marsupial Total	**-**	**-**	**-**	**8**	**-**	**-**	**-**	**-**	**-**	**-**	**-**	

Non-primate mammals Total	**154**	**10**	**11**	**8**	**75**	**-**	**-**	**-**	**-**	**24**	**-**	
Duck	-	-	-	-	-	-	1	-	-	-	-	Noël et al. (2003)
Goose	-	-	-	-	-	-	1	-	-	-	-	Abe (2004)
Chicken	-	-	-	-	-	1	-	-	-	-	-	Arisue et al. (2003)
	-	-	-	-	-	-	1	-	-	-	-	Noël et al. (2003)
	-	1	-	-	-	1	-	-	-	-	-	Yoshikawa et al. (2003)
	2	-	-	-	-	-	1	-	-	-	-	Yoshikawa et al. (2004a)
Quail	-	-	-	-	-	-	1	-	-	-	-	Arisue et al. (2003)
	-	-	-	-	-	1	-	-	-	-	-	Yoshikawa et al. (1998)
	-	-	-	-	-	-	1	-	-	-	-	Yoshikawa et al. (2003)
	-	-	-	-	-	4	4	-	-	-	-	Yoshikawa et al. (2004a)
Pheasant	-	-	-	-	-	1	-	1	-	-	2	Abe et al. (2003a)
	1	-	-	-	-	-	1	-	-	-	5	Yoshikawa et al. (2004a)
Guineafowl	-	-	-	-	-	1	-	-	-	-	-	Abe et al. (2003a)
Partridge	-	-	-	-	-	-	1	-	-	-	-	Abe et al. (2003a)
Turkey	-	-	-	-	-	-	1	-	-	-	-	Hess et al. (2006)
	-	-	-	-	-	1	-	-	-	-	-	Noël et al. (2003)
Birds Total	**3**	**1**	**-**	**-**	**-**	**10**	**13**	**1**	**-**	**-**	**7**	

Mammals and birds total	**186**	**36**	**41**	**11**	**84**	**10**	**13**	**21**	**0**	**26**	**10**	
Total all subtypes = 438												
Humans total	**316**	**71**	**577**	**54**	**-**	**28**	**18**	**3**	**2**	**-**	**17**	Alfellani (unpublished)
Total all subtypes =1,086												

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Table 3.

Average pair-wise distances within ST10 and between ST10 and other subtypes based on sequences of the ssrRNA gene region amplified by the primers of (A) Scicluna et al. (2006) or (B) Stensvold et al. (2006).

Blastocystis sp. subtypes (ST)	(A) Pairwise distance (%)	(B) Pairwise distance (%)
ST10/ST10	2.4	0.4
ST10/ST8	5.0	4.1
ST10/ST4	6.6	4.4
ST10/ST3	8.8	6.0
ST10/ST1	9.1	8.2
ST10/ST2	10.1	7.4
ST10/ST5	10.2	6.7
ST10/ST9	12.0	10.3
ST10/ST6	12.0	10.5
ST10/ST7	14.8	11.9