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1 **Subtype distribution of *Blastocystis* isolates from synanthropic and zoo**
2 **animals and identification of a new subtype★**

3

4 C. Rune Stensvold^{a,*}, Mohammed A. Alfellani^b, Sara Nørskov-Lauritsen^a, Katrine Prip^a,
5 Emma L. Victory^b, Charlotte Maddox^c, Henrik V. Nielsen^a, C. Graham Clark^b

6

7 ^a*Department of Bacteriology, Mycology and Parasitology, Statens Serum Institut,*
8 *Artillerivej 5, DK-2300 Copenhagen S, Denmark*

9 ^b*Department of Infectious and Tropical Diseases, London School of Hygiene and*
10 *Tropical Medicine, Keppel Street, London WC1E 7HT, United Kingdom*

11 ^c*Department for Veterinary Diagnostics and Research, National Veterinary Institute, Technical*
12 *University of Denmark, Bülowsvej 27, Copenhagen V, Denmark*

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14 *Corresponding author. Tel.: +45 32 68 36 04; fax: +45 32 68 30 33.

15 *E-mail address: RUN@ssi.dk*

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18 ★Nucleotide sequence data reported in this paper are available in Genbank under the

19 accession numbers: **FM164412** and **FM164413**.

20

21 **Abstract**

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

37

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

39

40 1. Introduction

41 *Blastocystis* is a common single-celled parasite of humans, non-human primates,
42 other mammals, birds, amphibians, reptiles, fish, arthropods and annelids (Stenzel and
43 Boreham, 1996; König and Müller, 1997; Belova and Krylov, 1998; Yoshikawa et al.,
44 2004b, 2007). The parasite exhibits extensive genetic diversity, and on the basis of
45 molecular analysis of the *ssrRNA* gene, nine distinct subtypes (ST1-ST9) have been
46 identified from humans, non-human primates, other mammals and birds (Noël et al.,
47 2005; Stensvold et al., 2007). *Blastocystis* from non-human sources also comprise
48 isolates that appear to fall outside the genetic range of these nine subtypes, eg. reptilian,
49 amphibian and cockroach isolates (Yoshikawa et al., 2004b, 2007; Stensvold et al.,
50 2007), although they are clearly closely related.

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

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

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

65

66

67 **2. Materials and methods**

68

69 *2.1. Danish samples: origin of isolates and PCR*

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

76 Samples were screened for *Blastocystis* by PCR at the Statens Serum Institut as
77 previously described (Stensvold et al., 2006) using the primers b11400ForC and
78 b11710RevC, which amplify a 310 bp *ssrRNA* gene fragment, and Extract-N-Amp PCR
79 ReadyMix (Sigma-Aldrich Danmark, Brøndby, Denmark). PCR-positive samples were
80 sequenced as described previously (Stensvold et al., 2006). In cases where sequences
81 indicated the presence of a potential new ST, the primers RD5 and BhRDr (Sciicluna et
82 al., 2006) were employed in order to obtain additional *ssrRNA* gene sequence
83 information for inclusion in phylogenetic analyses.

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

87

88 *2.2. United Kingdom samples: origin of isolates and PCR*

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

99

100 *2.3. Phylogenetic analysis of isolates*

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

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

109

110 *2.4. Data collection, interpretation and terminology*

111 The references used in the data collection process are listed in Table 2. Different
112 research groups have used distinct molecular methods and terminologies for analysing
113 isolates genetically, hence complicating comparison of results. Original data generated
114 from various studies using different molecular methodologies were standardised to meet
115 the proposed consensus terminology using a recently described algorithm (Stensvold et
116 al., 2007). Not all animal isolates described in the literature have been sequenced and
117 some were characterised only by PCR-restriction fragment length polymorphism (RFLP)
118 or PCR using sequence-tagged-site primers (PCR-STs). However, sequence data and
119 supplementary information published by Abe (2004) enabled interpretation of PCR-RFLP
120 data from some previous studies (Abe et al., 2003a, 2003b, 2003c) and PCR-STs data
121 can also be linked to most of the currently recognised STs. Where some STs were not
122 known at the time of publication, subsequent sequence analyses of such isolates and cross
123 referencing using data from Arisue et al. (2003), Noël et al. (2005) and Stensvold et al.
124 (2007) made it possible to identify STs originally described as 'ND' (not determined).
125 *Blastocystis* has also been reported in reptiles, amphibians, arthropods and annelids, but
126 only a small number of isolates have been sequenced. These were not included in the
127 present study as they appear to represent distinct lineages and are unlikely to represent a
128 zoonotic infection risk for humans.

129

130

131 **3. Results**

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

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

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

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

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

167

168

169 **4. Discussion**

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

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

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

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

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

206 ST8 has been isolated from humans only rarely (Scicluna et al., 2006; Motazedian
207 et al., 2008; Stensvold et al., 2008) but is common in primate handlers, suggesting that
208 zoonotic spread from primates to primate handlers is responsible for the unexpectedly
209 high prevalence of this ST among these individuals. Zoonotic transmission of
210 *Blastocystis* has been suggested by a plethora of research groups (Snowden et al., 2000;
211 Abe et al., 2003c; Arisue et al., 2003; Thathaisong et al., 2003; Yoshikawa et al., 2003,
212 2004a; Abe, 2004; Noël et al., 2005; Parkar et al., 2007; Yan et al., 2007; Navarro et al.,
213 2008), yet the extent and nature of this phenomenon remains unclear as the published
214 evidence is equivocal. Given the ubiquity and the host range of *Blastocystis*, our ability to
215 assess the zoonotic potential of *Blastocystis* is dependent on our ability to i) correctly and
216 unambiguously identify STs, ii) detect and differentiate MSI, and iii) understand and
217 analyse possible factors involved in transmission such as transmission sources,
218 transmission vehicles, infectivity of cysts and other stages, contact with faeces or faecally
219 contaminated soil, water and food, coprophagy, and the possibility of animals shedding
220 ingested cysts that are simply passing through the host. In the future, identifying variable

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

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

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

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

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

249

250

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258 analysis and Dr Jeffrey J. Windsor is thanked for providing the UK sheep sample.

259

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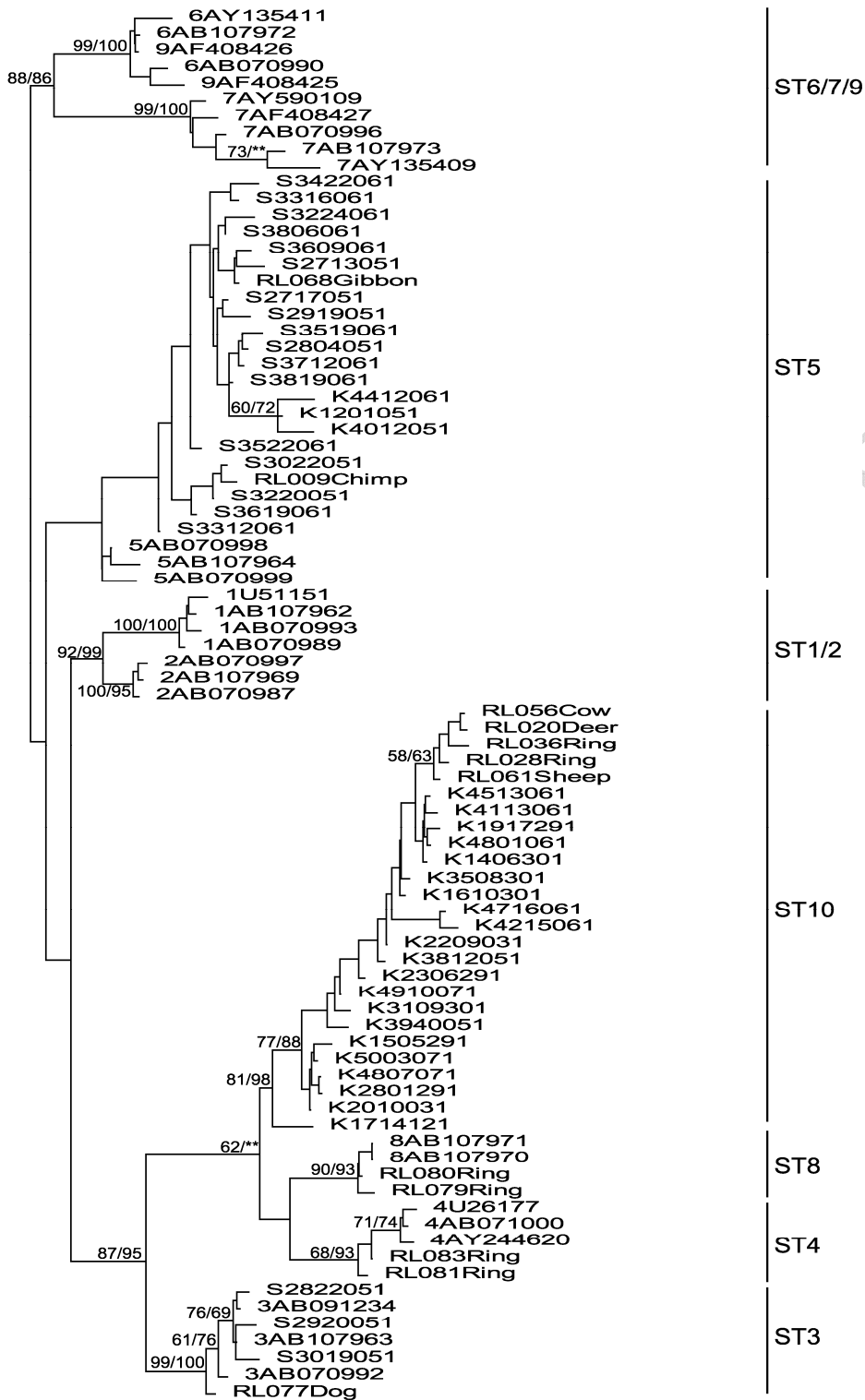
389 **Figure legends**

390

391 Fig. 1. Phylogenetic tree of the Danish *Blastocystis* sample sequences. The analysis was
392 performed using the 310 bp sequences, which are identified by their sample code. Those
393 starting with S are from pigs, those with K from cattle and most starting with R are from
394 other animals, with the species identity appended (Ring = Ring-tailed lemur). Reference
395 sequences from GenBank have the accession number preceded by the subtype
396 identification. The clade consisting of subtypes 6, 7 and 9 was used as an outgroup. The
397 tree shown is that obtained from the Bayesian analysis with the bootstrap proportions
398 shown being from the Maximum Likelihood analysis (100 replicates) on the left and
399 Neighbor-Joining analysis (1,000 replicates) on the right. The posterior probabilities
400 obtained in the Bayesian analysis were all 1.0. Bootstrap values of less than 50% in both
401 analyses are not shown. Where one analysis gave a value over 50% and the other below
402 50% the latter is indicated by two asterisks.

403

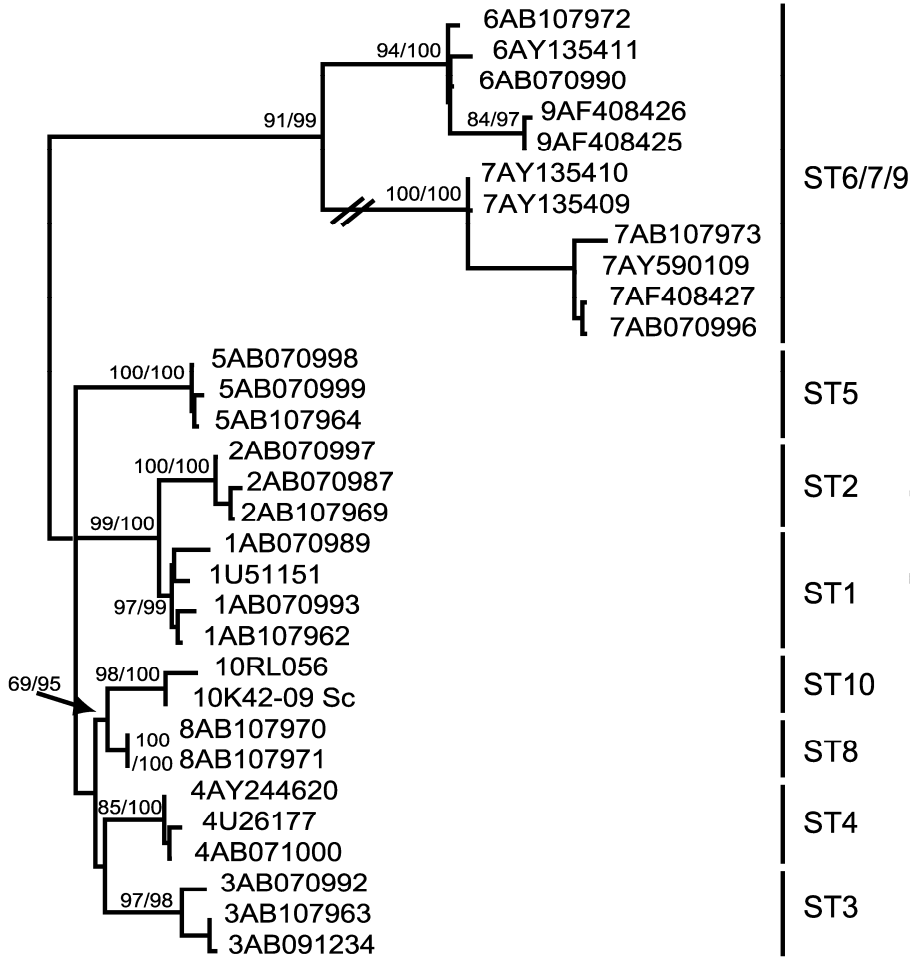
404 Fig. 2. Phylogenetic relationships of *Blastocystis* subtype 10. The analysis was performed
405 using the 'barcode' region (Sciicluna et al., 2006). Both samples sequenced (RL056 and
406 K42-09) are cattle. Reference sequences from GenBank have the accession number
407 preceded by the subtype identification.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



411

412

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)	<i>Homo sapiens</i>	UK	2	-	9	1	-	-	-	4	-	-
Non-human primates												
	<i>Pan</i>	UK										-
Chimpanzee	<i>troglydtes</i>		1	4	8	-	6	-	-	-	-	-
		Denmark	-	-	-	-	1	-	-	-	-	-
	<i>Pongo</i>	UK										-
Orang Utan	<i>pygmaeus</i>		1	1	2	-	-	-	-	-	-	-
	<i>Gorilla</i>	UK										-
Gorilla	<i>gorilla</i>		-	4	1	-	1	-	-	-	-	-
	<i>Hylobates</i>	UK										-
Siamang	<i>syndactylus</i>		3	-	-	-	-	-	-	1	-	-
Mueller's gibbon	<i>Hylobates</i> <i>muelleri</i>	UK	-	1	-	-	-	-	-	-	-	-

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

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

421

422 ^aAll Danish isolates were from animals that were also positive for *Giardia* and/or423 *Cryptosporidium*.

424 Table 2.
 425 *Blastocystis* subtype distribution identified in non-human primates, other mammals and birds ($n = 438$).
 426

Host group	<i>Blastocystis</i> 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												

427

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428 Table 3.

429 Average pair-wise distances within ST10 and between ST10 and other subtypes based on

430 sequences of the *ssrRNA* gene region amplified by the primers of (A) Scicluna et al.

431 (2006) or (B) Stensvold et al. (2006).

432

<i>Blastocystis</i> 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

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