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Molecular Subtyping of *Blastocystis* from Diverse Animals in the United Arab Emirates

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The contribution of *Blastocystis* from non-human hosts to zoonotic transmission is only partly known. The objective of this study was to determine the distribution of *Blastocystis* genetic subtypes in different animal species in United Arab Emirates. A total of 114 stool samples were tested using PCR of the small subunit (SSU) rRNA gene and sequence analysis. Twenty-three *Blastocystis*-positive samples were identified. The following detection rates were observed: cattle, 22.7%; sheep, 63.6%; rabbits, 33.3%; rodents, 37.5%; reptiles, 21.2%. Four subtypes were identified in this study; ST4, ST10, ST14, and ST17; ST10 was isolated from sheep and cattle, corroborating previous data indicating that these are natural hosts for this subtype. Cases of mixed subtype colonization were also detected. Conspicuously, we found ST14 in rabbits. The discovery of ST17 in a squirrel indicates a novel host for this subtype. Furthermore, the discovery of ST4 in rodents suggests that these may serve as reservoir for human *Blastocystis* ST4 colonization. Six tortoises and one iguana were positive for *Blastocystis.* In conclusion, this is the first report of *Blastocystis* infection in various animals in the UAE. Apart from ST4, no potentially zoonotic subtypes were detected.

Key words: *Blastocystis*; subtypes; sequencing; animals; United Arab Emirates.

Introduction

Blastocystis is an enteric parasitic protist found in humans, other mammals, birds, reptiles, and others animals (Clark et al. 2013; Yoshikawa et al. 2004a,b). With a cyst stage involved in faecal-oral

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transmission of the parasite, the extent to which human-human, human-animal and animal-human transmission occurs remains under scrutiny (Parkar et al. 2010; Stensvold et al. 2012a,b).

While the pathogenic potential of Blastocystis in humans remains uncertain, many carriers probably remain asymptomatic or exhibit slight intestinal discomfort including acute or chronic diarrhea, abdominal pain, flatulence, vomiting, constipation, and irritable bowel syndrome (Jimenez-Gonzalez et al. 2012; Tan et al. 2010). Reported prevalence rates vary according to geographical region but are generally higher in developing countries than in developed ones possibly due to differences in the standards of hygiene, waste disposal, exposure to animals and consumption of contaminated food or water (Clark et al. 2013).

Remarkable genetic diversity exists within the genus, and several distinct ribosomal lineages, the so-called 'subtypes (ST)' have been identified in mammalian and avian hosts. Consequently, *Blastocystis* from such hosts was recently classified into 17 STs (Alfellani et al. 2013a,b). Among the 17 STs, nine (ST1 to ST9) have been reported in humans with varying prevalence across the globe (Alfellani et al. 2013a,b; Cian et al. 2017; Clark et al. 2013). Moreover, ST12 was recently reported in a human host (Ramírez et al. 2016). With the exception of ST9 found only in humans until now, the remaining STs reported in humans have also been reported in non-human hosts (Alfellani et al. 2013a,b; Parkar et al. 2007, 2010; Ramirez et al. 2014; Santin et al. 2011; Stensvold et al. 2009; Yoshikawa et al. 2016a,b). Most *Blastocystis* from amphibian and reptilian hosts are distinct and do not form part of the acknowledged subtype system.

Several studies on the prevalence and subtyping of *Blastocystis* in different groups of animals from different parts of the globe have provided evidence suggesting limited zoonotic potential of this parasite. Nevertheless, a higher prevalence of the parasite among animal handlers compared with individuals not normally in contact with animals was reported in several publications (Lee et al. 2012; Parkar et al. 2010; Rajah-Salim et al. 1999; Rivera 2008; Stensvold et al. 2009; Tan 2008; Yoshikawa et al. 2009). Moreover, successful experimental infections of chickens and rats with human isolates have demonstrated the possibility of transmission of the parasite between human and animal hosts (Ajjampur and Tan 2016; Cian et al. 2017; Iguchi et al. 2007; Růžková et al. 2018).

Substantial data on the molecular epidemiology, transmission and subtype distribution of *Blastocystis* is available for a number of different animal groups from various regions across the globe (Alfellani et al. 2013a,b; Cian et al. 2017; Parkar et al. 2007, 2010; Petrasova et al. 2011; Roberts et al. 2013; Stensvold et al. 2009; Wang et al. 2014). We previously reported a *Blastocystis* prevalence of 44.4% among asymptomatic individuals in UAE; subtypes 1, 2, and 3 were the only ones detected (no ST4 was seen) (AbuOdeh et al. 2016). However, there has been no report of *Blastocystis* in animals in UAE. Given the understanding that additional epidemiological data is needed to identify potential animal reservoirs of human cases of *Blastocystis* colonisation, the objective of the present study was to carry out molecular characterization of *Blastocystis* isolated from animal faecal samples in order to increase our knowledge of the epidemiology and host specificity of this parasite in the UAE.

Results

Of the 114 samples tested (Table 1), 23 (20.2%) were *Blastocystis*-positive as confirmed by sequencing, while 91 (79.8%) were negative. The following positivity rates were recorded across the various animal groups: cattle, 5/22 (22.7%); sheep, 7/11 (63.6%); rabbits, 1/3 (33.3%); rodents, 3/8 (37.5%); tortoises, 6/21 (28.5%); iguana, 1/1 (100%). In the case of rodents, only members of the family Sciuridae were positive for *Blastocystis* (3/6; 50%); two *Funambulus palmarum* and one *Rhinosciurus laticaudatus*.

Subtypes identified in the study comprised ST4, ST10, ST14 and ST17 as evidenced by BLAST calls in the NCBI database and phylogenetic analysis (Fig. 1). In the case of cattle, the finding of ST10 in three of the five animals was confirmed by high (98%) bootstrap support (Fig. 1). For the remaining two samples (6P and 7P), assignment of subtype was less straightforward. By BLAST queries, the top hit for the two sequences was MF186665, which is a sequence from an elk assigned to ST5 by Betts et al. (2018) with 99% coverage and 97.39% identity. Higher identity (98.78%) but lower coverage (93%) was seen for MH201330, which is a sequence obtained from Korean cattle stool, assigned by the authors of the study to ST14. In the phylogenetic tree (Fig. 1), 6P and 7P cluster together, but in a subclade of their own in the clade comprising subtypes 5, 12, 13, and 14. While the support for this clade is relatively high (91%; Fig. 1), the resolution within the clade is poor in our analysis, which is why the subtype could not be

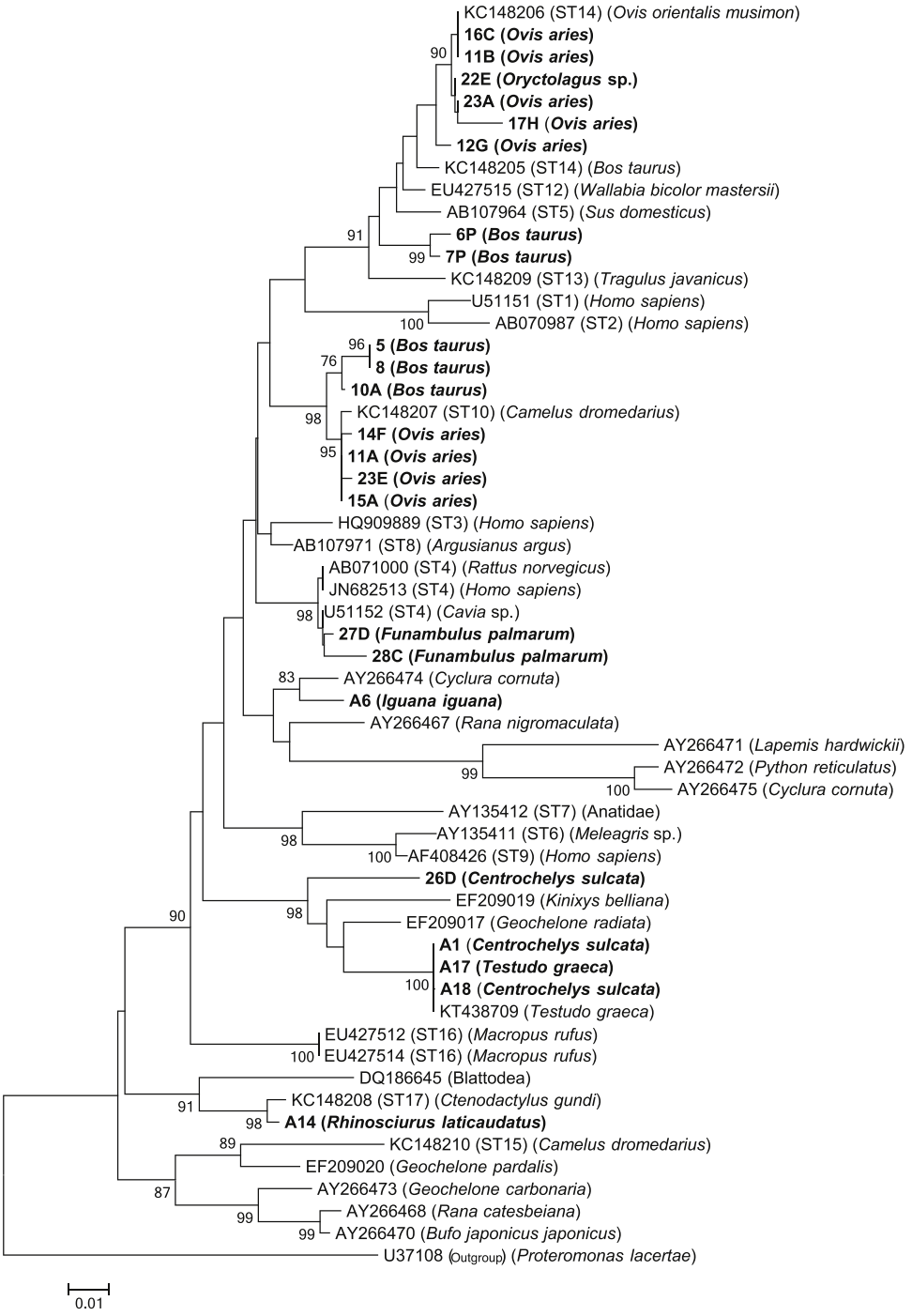

Figure 1. Evolutionary relationships of *Blastocystis* SSU rRNA sequences. Sequences obtained by barcoding in the present study were phylogenetically analysed with reference sequences used by Alfellani et al. (2013a,b) and indicated in bold font. *Proteromonas lacertae* was used as outgroup. For reference *Blastocystis* sequences, subtype numbers are given in parentheses where applicable. Apart from ST11 (sequence not available for analysis for this part of the gene), all subtypes from ST1 to ST17 were included. For all *Blastocystis* sequences, the Latin name of the host from which they were identified is indicated. The evolutionary history was inferred using the Neighbor-Joining method. The evolutionary distances were computed using the Kimura 2-parameter method and are in the units of the number of base substitutions per site. Only bootstrap values higher than 75 are shown. The analysis involved 57 nucleotide sequences. All ambiguous positions were removed for each

Table 1. A total number of 114 samples were collected for this UAE study.

Host	Scientific Name; Numbers sampled
Artiodactyla	
Cow	*Bos taurus; 22*
Sheep	*Ovis aries; 11*
Goat	*Capra hircus; 6*
Aves	
Domestic duck	*Anas platyrhynchos domesticus; 2*
Quail	*Coturnix coturnix; 2*
Falcon	*Falco cherrug; 1*
Partridge	*Ammoperdix heyi; 1*
Peacock	*Pavo cristatus; 1*
Pigeon	*Oena capensis; 3*
Parrot	*Psittacula krameri; 2*
Chicken	*Gallus gallus; 6*
Carnivora	
Domestic cat	*Felius catus; 2*
Dog	*Canis familiaris; 1*
Mongolian ferret	*Mustela putorius furo; 1*
Lagomorpha	*Oryctolagus* spp.; 3
Rabbit	
Perciformes	
Fish	*Oreochromis niloticus*; 9
Rodentia	
Indian palm squirrel	*Funambulus palmarum; 4*
Mongolian chinchilla	*Chinchilla lanigera; 1*
Shrew-faced squirrel	*Rhinosciurus laticaudatus; 1*
White squirrel	*Sciurus carolinensis; 1*
Golden hamster	*Mesocricetus auratus; 1*
Squamata	
Burmese python	*Python bivittatus;3*
Rainbow snake	*Farancia erytrogramma; 1*
Common boa	*Boa constrictor; 1*
Iguana	*Iguana iguana; 1*
Chameleon	*Chamaeleo zeylanicus; 1*
Bearded dragon	*Pogona vitticeps; 1*
Milk snake	*Lampropeltis triangulum; 1*
Common house gecko	*Hemidactylus frenatus; 2*
Testudinidae	
African spurred tortoise	*Centrochelys sulcata; 19*
Greek tortoise	*Testudo graeca; 2*
Mata mata turtle	*Chelus fimbriata; 1*

called with certainty for 6P and 7P. However, the edited alignment used for the phylogeny included only ~400 bp, and this is probably explaining the low resolution in this part of the tree.

As for the sheep *Blastocystis* sequences, subtypes ST10 and ST14 were found. Mixed subtypes were detected in two of the sheep (ST10 and ST14).

In one of the three rabbit samples, we identified a ST14 sequence, clustering with the ST14 sequences from sheep (Fig. 1). In the case of rodents, we identified ST4 in two Indian palm squirrels, and ST17 in a shrew-faced squirrel (Fig. 1; Table 2).

Interestingly, in the case of reptilian hosts, six tortoises (five African and one Greek) and one iguana were positive for *Blastocystis.* Four sequences from tortoises (A1, A17, A18, and 26D) were used in the phylogeny (Fig. 1). All four clustered in a tortoise-specific *Blastocystis* clade verified by high (98%) bootstrap support. Three of these sequences (A1, A17, and A18) clustered together in a subclade shared by other tortoise sequences from GenBank (100% bootstrap support). Sequence 26D clustered separately (Fig. 1). The remaining two tortoise sequences (A10 and A11) were excluded from the phylogenetic analysis because of relatively limited sequence length in the case of sample A10 and poor quality of the A11 sequence. No sequence-positive samples were found in goats, birds, fish, dog, cat, and members of the squamata and carnivora orders.

Discussion

To our knowledge, this is the first report on molecular characterization of *Blastocystis* in animals in the UAE. Of the 23 samples sequencing positive for *Blastocystis*, 21 represented colonization by a single strain (as evidenced by Sanger sequences of both cloned and non-cloned PCR products), whereas mixed colonization (two STs) was observed in two samples, for which PCR products had been subject to by cloning. Due to financial constraints, we were not able to clone all PCR products and therefore could not confirm whether they reflected single or mixed subtype colonisation. Subtyping resulted in the identification of ST4, ST10, ST14 and ST17.

sequence pair. There was a total of 407 positions in the final dataset. Evolutionary analyses were conducted in MEGA7 (Kumar et al. 2016). Prior to the phylogenetic analysis, the multiple sequence alignment was manually edited to remove major gaps and areas of ambiguous alignment.

Table 2. Subtype results from sequencing of *Blastocystis* positive samples.

Sample ID	Common name of host	Latin name of host	Subtype	Accession number
5	Cow	*Bos taurus*	10	MH807179
6P	Cow	*Bos taurus*	UT	MN263292
7P	Cow	*Bos taurus*	UT	MN263293
8	Cow	*Bos taurus*	10	MH807180
10A	Cow	*Bos taurus*	10	MH807181
*11A	Sheep	*Ovis aries*	10	MH807182
11B			14	MH807184
12G	Sheep	*Ovis aries*	14	MH807185
14F	Sheep	*Ovis aries*	10	MH807186
15A	Sheep	*Ovis aries*	10	MH807187
16C	Sheep	*Ovis aries*	14	MH807188
17H	Sheep	*Ovis aries*	14	MH807189
*23E	Sheep	*Ovis aries*	10	MH807183
23 A			14	MH807191
22E	Rabbit	*Oryctolagus* spp.	14	MH807190
27D	Indian palm squirrel	*Funambulus palmarum*	4	MH807193
28C	Indian palm squirrel	*Funambulus palmarum*	4	MH807194
A14	Shrew-faced Squirrel	*Rhinosciurus laticaudatus*	17	MN263294
26D	African spurred tortoise	*Centrochelys sulcata*	NA	MH807192
A1	African spurred tortoise	*Centrochelys sulcata*	NA	MH807195
A6	Iguana	*Iguana iguana*	NA	MH807196
A10	African spurred tortoise	*Centrochelys sulcata*	NA	MH807197
A11	African spurred tortoise	*Centrochelys sulcata*	NA	Poor quality to be deposited and assigned an accession number
A17	Greek Tortoise Syria	*Testudo graeca*	NA	MH807198
A18	African spurred tortoise	*Centrochelys sulcata*	NA	MH807199

Note: An asterisk indicates mixed infections detected in the sample.
UT: untypable.
NA: subtype terminology not applicable.

The study did not aim to determine the exact prevalence of *Blastocystis* across the animal groups sampled; for this purpose, more sensitive methods, such as real-time PCR (Stensvold et al. 2012b), would be appropriate. Therefore, the positivity rates obtained among the different species are the minimum positive rates. However, since the method used in the present study has been used in numerous other studies (Betts et al. 2018; Lee et al. 2018; Parkar et al. 2010; Roberts et al. 2013; Wang et al. 2018a,b; Zhu et al. 2017), it appears relevant and pertinent to compare the positivity rate across the sampled animal groups with rates reported by others using conventional PCR and sequencing.

A total of five confirmed *Blastocystis*-positive samples were obtained from 22 cow fecal samples tested, indicating a detection rate of at least 22.7%, and five SSU rRNA gene sequences were obtained from the cow samples. Analysis of the three ST10 sequences of *Blastocystis* (Fig. 1) showed high identity (99.84%) to MF186678 in GenBank (not included in Fig. 1), which is a ST10 sequence found in bison, whereas the two remaining sequences showed highest identity to *Blastocystis* sequences from cattle from a Korean study (MH201330); the authors of the Korean study assigned this sequence to ST14; nevertheless, the phylogenetic analysis performed in the Korean study did not include ST12 and ST13 in their analysis; and still, the bootstrap support for assigning MH201330 to ST14 in the Korean study was only 90%. Although both sequences were obtained from cloned PCR products, we did not suspect that the sequences were reflecting chimeras, since they were almost identical to each other and to sequences identified by others. Longer sequences are probably needed to enable assignment of a subtype to this sequence with confidence. The overall detection rate in cattle

in the present report was somewhat similar to those reported in the United Kingdom (22.6%) and the USA (19.2%) (Alfellani et al. 2013a,b; Fayer et al. 2012). On the other hand, far lower detection rates were observed in recent studies in Korea (6.7%), Iran (9.6%), and China (10.3%) (Lee at al. 2018, Badparva et al. 2015; Zhu et al. 2017).

Regarding sheep, a detection rate of 63.6% was observed in our study. Contrasting data were communicated from recent analyses in China (5.5% and 6%), respectively (Li et al. 2018; Wang et al. 2018a,b). Both ST10 and ST14 were detected in sheep, which is in agreement with other reports (Betts et al. 2018; Li et al. 2018; Stensvold et al. 2009; Wang et al. 2018a,b). However, unlike Wang et al. (2018a,b), Li et al. (2018) and Stensvold et al. (2009), we could not detect the potentially zoonotic subtypes ST1, ST3 and ST5 among our positive samples. Furthermore, mixed infections (ST10 and ST14) were detected in two sheep samples. Sequences confirmed as ST14 (clones 11B, 12, 23A, 16, 17) were highly identical to LT594969 (99% identity) from a cow in Mosul, Iraq.

We opted for cloning for the identification of mixed subtype colonization over direct sequencing of the PCR product (Alfellani et al. 2013b,c; Roberts et al. 2013; Stensvold 2013; Stensvold et al. 2012a,b), which tends to select for the dominant subtype in cases of mixed subtype carriage. Despite the fact that most of the animals screened exhibited a single subtype, we confirmed the presence of mixed infections in two sheep harbouring both ST10 and ST14. In a similar study where cloning was employed, Betts et al. (2018) confirmed the presence of mixed colonization with more than one subtype (four subtypes) in an elk (Betts et al. 2018). Furthermore, while every attempt was made to ensure that the samples collected were from different animals it is also probable that the detection rates and the mixed subtypes observed in the present survey were a result of contamination with other samples or the environment. It is therefore difficult to conclude that the revealed subtypes were from one animal since many sheep of the same species were housed in the same farm. Thus, along with the recent findings of mixed subtype carriage in an elk (an artiodactyl) by Betts et al. (2018), and our detection of two subtypes in sheep (which is also an artiodactyl), the speculation put forward by Betts and colleagues regarding whether the presence of multiple infections is probably the norm in certain animals demands further investigation. When cloning is applied, the risk of obtaining chimeric sequences exists, and resulting DNA sequences should therefore be scrutinized and analysed carefully before conclusions are made.

Like others, we show that synanthropic artiodactyls tend to demonstrate a preponderance of subtypes 10 and 14 no matter which part of the globe they originate from (Alfellani et al. 2013a; Betts et al. 2018). Moreover, we did not find ST1, ST2, ST3 and ST4, which account for more than 90% of human *Blastocystis* carriage, in any of the assayed artiodactyls in the present study. A previous study in Libya reported none to very low detection rates in livestock with ST2, ST1 and ST3 (0%, 7% and 9%), respectively (Alfellani et al. 2013a). More samples must be screened and characterized from the artiodactyls order before ascertaining their potential for zoonotic transmission especially among animal handlers and individuals living around farms. And, if subtypes usually found in humans are found in such animals, SSU rRNA gene allele and maybe even MLST allele profiling should be performed to enable detection of cryptic host specificity (Stensvold et al. 2012a). Thus, screening large numbers of cattle would therefore allow us to give a more accurate measure of the detection rate of potentially zoonotic subtypes in livestock in UAE.

As regards the rabbits in the study, three samples were screened for the presence of *Blastocystis*. Sample 22 (Clone 22E) was the only one (33.3%) shown to be positive for the parasite, with ST14 as the only subtype identified (99.84% identical to LT594969). To our knowledge, only three studies have reported molecular data on *Blastocystis* in rabbits (Alfellani et al. 2013a; Roberts et al. 2013; Wang et al. 2018a,b), and the only study reporting the presence of *Blastocystis* was the Chinese study by Wang et al. (2018a,b) in which ST4 was the only subtype detected. The number of rabbit samples collected in our survey was very limited and since very few epidemiological surveys have been conducted to date among this animal group it is too early to draw any conclusions on the subtypes infecting this order of mammals.

The number of samples collected from rodents was also limited; however, the overall detection rate with *Blastocystis* in the five rodent species studied here was 37.5% (3/8). Many recent reports showed evidence of the existence of ST4 in rodents (Alfellani et al. 2013a; Betts et al. 2018; Haziqah et al. 2018; Katsumata et al. 2018; Yoshikawa et al. 2016a,b). ST4 is common in humans, at least in Europe, and the discovery of ST4 in the two Indian palm squirrels in the present study adds support to the growing amount of data that suggests that rodents may serve as reservoir for human *Blas-*

tocystis infection (Haziqah et al. 2018). It should be noted, however, that in an earlier study, we found no evidence of ST4 in humans in the UAE (AbuOdeh et al. 2016). Unlike in Europe where ST4 is frequently found in humans, ST4 has rarely been reported in studies from South America, China, Malaysia, Middle East, Africa and North America (Alfellani et al. 2013b; Ben Abda et al. 2017; Haziqah et al. 2018; Poirier et al. 2011; Ramírez et al. 2016; Wang et al. 2018a,b). It was proposed by Clark et al. (2013) that a recent emergence of ST4 into the human population in Europe (which is plausible due to the limited intra-subtype genetic diversity seen within this particular subtype) could explain the heterogeneous geographical dissemination of this ST across the human population. Ramírez et al. (2016) suggested a possible host-pathogen interaction or genetic background that impeded ST4 to colonize individuals in South America.

Since we used cloning prior to sequencing, it makes little sense to try and query the sequences at the *Blastocystis* online database in order to obtain allele (i.e., genotype) information, the sequences in the *Blastocystis* online database reflecting mainly sequences obtained by Sanger sequencing of non-cloned PCR products.

The observation of ST17 in the shrew-faced squirrel in the present survey suggests a novel host for this subtype (Table 2). So far, ST17 has only been found in a gundi (Alfellani et al. 2013a).

Many reports on reptilian *Blastocystis* have appeared from various regions (Teow et al. 1991; Yoshikawa et al. 2016a). An overall detection rate of 21.2% (7/33) was observed in the two reptile groups, Testudinata (6/21, 28.5% [African spurred tortoises, 5/19 (26.3%); Greek Tortoise, 1/2 (50%)]), and Squamata (Iguana, 1/11 [9.1%] was observed. Unlike a French study where a 66.7% (2/3) detection rate was reported in Testudinata (Cian et al. 2017), our study reported a significantly lower rate of detection (28.5%). Five of the six African spurred tortoise SSU rRNA gene sequences showed 90%–99% identity to sequences in GenBank. The four tortoise sequences included in the phylogenetic analysis were seen to exhibit monophyly (Fig. 1). Interestingly, the *Blastocystis* sequences from the Greek tortoise (also known as Spur-thighed tortoise) clustered, with maximum bootstrap support, with *Blastocystis* sequences form the African tortoises, implying that they may be infected with the same strain; alternatively, this might be a result of contamination with feces from the other species. Intriguingly, the two Spur-thighed tortoises reported in a French study (KR259510 and KR259511) (Cian et al. 2017) were not called upon blasting our isolate on the GenBank, the reason potentially being that these two sequences have limited length in comparison to our isolate and the genetic diversity seen across the shared region appears substantial.

The detection rate among the Squamata samples tested in the present study was 1/11 (9.1%). This is much less than that reported by Cian et al. (2017) in which 2/12 (16.7%) was reported in a French study from animals in two zoos. The only iguana (*Iguana iguana*) included in the present study was found to be positive for *Blastocystis* sp. among the other Squamata species tested. This is similar to the French survey where *Blastocystis* sp. was isolated from one of the two (1/2) iguana samples (Green iguana, *Iguana iguana*). Therefore, together with previous studies, this report indicates that *Blastocystis* sp. is common in reptiles (Cian et al. 2017; Suresh et al. 1997; Teow et al. 1992; Yoshikawa et al. 2016a). Additional sampling and preferably sequencing of the near-complete SSU rRNA genes is needed to ascertain the level of genetic diversity in reptilian hosts in greater detail.

Conclusion

This is the first report of *Blastocystis* infection in various animals in the UAE. Of the 104 fecal samples tested, 20.2% were confirmed positive for *Blastocystis* spp. Subtypes 10 and 14 were identified for the first time in synanthropic artiodactyls in UAE, corroborating previous studies that these artiodactyl members are natural hosts for these subtypes. We also detected ST4 in rodents, supporting earlier surveys suggesting that rodents may act as reservoir for human *Blastocystis* colonization. No other potentially zoonotic subtypes were detected in the present study. Another important finding in the study was the detection of ST14 in a rabbit. Regarding the reptilian samples, *Blastocystis* was relatively common, exhibiting some degree of diversity. Further subtyping of *Blastocystis* in various animal groups and humans in the UAE is warranted to answer the question about zoonotic transmission of *Blastocystis* in this country in greater detail.

Methods

Sample collection and pre-DNA extraction storage: Fresh fecal samples were obtained from various animal species across the UAE and kept in leak-proof containers without preservative. They were transported without delay to the Uni-

versity of Sharjah, UAE and stored at −20 °C until submitted to DNA extraction and PCR. The sampling covered a broad range of animals, and a total of 114 samples were collected for the study (Table 1). Samples were collected from cows, sheep, and goats housed in local farm pens in AlAin (a major city in the emirate of Abu Dhabi, UAE). For the other types of animals, feces as fresh as possible was collected from cages in the animals and pets' municipal market in the Sharjah emirate. Fecal samples were collected from animals secluded in separate cages or pens and care was taken to reduce the risk of sample contamination.

DNA extraction, PCR, cloning and analysis of DNA sequences: DNA was extracted from all stool samples using the Bioline stool DNA Kit (London, UK) as per the manufacturer's recommendations and stored at −20 °C until analyzed. The 600-bp barcoding region of the SSU rRNA gene of *Blastocystis* was amplified using the primers BhRDr and RD5 (Scicluna et al. 2006; Stensvold 2013). Briefly, the amplification conditions consisted of 30 cycles of 1 min each at 94 °C, 59 °C and 72 °C, with an additional two-minute final extension. PCR products were separated on 1.2% agarose gels stained with ethidium bromide. Positive and negative (DNA replaced by water) controls were included with each batch of samples analyzed.

PCR products (600 bp) of positive samples was excised and retrieved from the gel using QIAquick® Gel Extraction kit from Qiagen (Valencia, California, USA). Of the 23 PCR-positive samples, 18 PCR products were cloned into pDrive cloning plasmid using the QIAGEN® PCR Cloning kit (Valencia, California, USA), and then plasmids were transformed by heat shock into *E. coli* DH5α chemocompetent (MAX Efficiency® DH5α™ Competent) cells (Invitrogen, Carlsbad, CA, USA), all procedures according to the manufacturers' instructions. After an incubation period of 24 h, several separate colonies from each plate were randomly picked and grown in LB broth in the presence of 100 μg/mL ampicillin. Plasmids were purified using a QIAprep® Spin Miniprep Kit (Qiagen, Valencia, California, USA). Prior to sequencing, plasmids were analyzed for the presence of the 600-bp PCR product insert using *EcoRI* restriction digestion. Sequencing was performed using the ABI 3730XL sequencer in McLab (Molecular Cloning Laboratories; San Francisco, CA, USA). The primers T7 forward and Sp6 reverse were used for sequencing. For all the remaining isolates, amplified PCR products were separated by agarose gel and fragments visualized under UV light after staining with ethidium bromide. The size of the DNA product was confirmed using a 100-bp ladder, and PCR products were purified using PureLink Quick PCR Purification Kit from Invitrogen (Carlsbad, CA, USA) according to the manufacturer's instructions, and sent for sequencing in a commercial sequencing facility (Inqaba Biotec, Pretoria, South Africa).

Subtyping and phylogenetic analysis of *Blastocystis* isolates: PCR products were sequenced on both strands, and the resulting sequences assembled online using online software (available at http://www.prabi.fr/). CLC Main Workbench 6 (Qiagen, Redwood City, California, USA) was also used for sequence end trimming and editing, in addition to contig assembly. Sequences were aligned with sequences covering the "barcoding region" of SSU rRNA gene retrieved from GenBank to provide representatives of the known subtypes of Blastocystis. For phylogenetic analysis of the reptiles, SSU rRNA gene sequences were analyzed together with an outgroup sequence (Proteromonas lacertae, Genbank accession no. U37108), and the evolutionary relationship between taxa was inferred using MEGA7 software (Kumar et al. 2016). To perform DNA sequence alignment, we used the Clustal W algorithm in MEGA7, with default parameters. Phylogenetic analysis was performed using Neighbor-Joining method implemented in MEGA7. Selected reference subtype sequences and *Blastocystis* sequences available from the GenBank were included in the construction of the phylogenetic tree. The SSU rRNA gene sequences obtained in this study were deposited in GenBank under accession numbers MH807179- MH807199 and MN263292-MN263294.

Appendix A. Supplementary Data

Supplementary material related to this article can be found, in the online version, at doi:https://doi.org/10.1016/j.protis.2019.125679.

References

AbuOdeh R, Ezzedin S, Samie A, Stensvold CR, ElBakri A (2016) Prevalence and subtype distribution of *Blastocystis* in healthy individuals in Sharjah, United Arab Emirates. Infect Genet Evol **37**:158–162

Ajjampur SSR, Tan KSW (2016) Pathogenic mechanisms in *Blastocystis* spp. —Interpreting results from *in vitro* and *in vivo* studies. Parasitol Int **65**:772–779

Alfellani MA, Taner-Mulla D, Jacon AS, Imeede CA, Yoshikawa H, Stensvold CR, Clark CG (2013a) Genetic diversity of *Blastocystis* in livestock and zoo Animals. Protist **164**:497–509

Alfellani MA, Stensvold CR, Vidal-Lapiedra A, Onuoha ES, Fagbenro-Beyioku AF, Clark CG (2013b) Variable geographic distribution of *Blastocystis* subtypes and its potential implications. Acta Tropica **126**:11–18

Badparva E, Sadraee J, Kheirandish F (2015) Genetic Diversity of *Blastocystis* isolated from cattle in Khorramabad, Iran. Jundishapur J Microbiol **8**:e14810

Ben Abda I, Maatoug N, Ben Romdhane R, Bouhelmi N, Zallegua N, Aoun K, Viscogliosi E, Bouratbine A (2017) Prevalence and Subtype Identification of *Blastocystis* sp. in Healthy Individuals in the Tunis Area, Tunisia. Am J Trop Med Hyg **96**:202–204

Betts EL, Gentekaki E, Thomasz A, Breakell V, Carpenter AI, Tsaousis AD (2018) Genetic diversity of *Blastocystis* in non-primate animals. Parasitology **145**:1228–1234

Cian A, El Safadi D, Osman M, Moriniere R, Gantois N, Benamrouz-Vanneste S, Delgado-Viscogliosi P, Guyot K, Li LL, Monchy S, Noël C, Poirier P, Nourrisson C, Wawrzyniak I, Delbac F, Bosc S, Chabé M, Petit T, Certad G, Viscogliosi E (2017) Molecular epidemiology of *Blastocystis* sp. in various animal groups from two French zoos and evaluation of potential zoonotic risk. PLoS ONE **6**:e0169659

Clark CG, van der Giezen M, Alfellani MA, Stensvold CR (2013) Recent developments in *Blastocystis* research. Adv Parasitol **82**:1–32

Fayer R, Santin M, Macarisin D (2012) Detection of concurrent infection of dairy cattle with *Blastocystis*, *Cryptosporidium*, *Giardia* and *Enterocytozoon* by molecular and microscopic methods. Parasitol Res **111**:1349–1355

Haziqah F, Mohd Zain SN, Chandrawathani P, Premaalatha B, Mohd Khairul Nizam MK, Arutchelvan R, Suresh K (2018) Genetic diversity of rodent *Blastocystis* sp. from Peninsular Malaysia. Trop Biomed **35**:586–592

Iguchi A, Ebisu A, Nagata S, Saitou Y, Yoshikawa H, Iwatani S, Kimata I (2007) Infectivity of different genotypes of human *Blastocystis hominis* isolates in chickens and rats. Parasitol Int **56**:107–112

Jimenez-Gonzalez DE, Martinez-Flores WA, Reyes-Gordillo J, Ramirez-Miranda ME, Arroyo-Escalante S, Romero-Valdovinos M, Stark D, Souza-Saldivar V, Martinez-Hernandez F, Flisser A, Olivo-Diaz A, Maravilla P (2012) *Blastocystis* infection is associated with irritable bowel syndrome in a Mexican patient population. Parasitol Res **110**:1269–1275

Katsumata M, Yoshikawa H, Tokoro M, Mizuno T, Nagamoto T, Hendarto J, Asih PBS, Rozi IE, Kimata I, Takami K, Syafruddin D (2018) Molecular phylogeny of *Blastocystis* isolates from wild rodents captured in Indonesia and Japan. Parasitol Res **117**:2841–2846

Kumar S, Stecher G, Tamura K (2016) MEGA7: Molecular Evolutionary Genetics Analysis Version 7. 0 for bigger datasets. Mol Biol Evol **33**:1870–1874

Lee H, Lee SH, Seo MG, Kim HY, Kim JW, Lee YR, Kim JH, Kwon OD, Kwak D (2018) Occurrence and genetic diversity of *Blastocystis* in Korean cattle. Vet Parasitol **15**:70–73

Lee LL, Chye TT, Karmacharya BM, Govind SK (2012) *Blastocystis* sp. : waterborne zoonotic organisms, a possibility? Parasit Vectors **5**:130

Li WC, Wang K, Gu Y (2018) Occurrence of *Blastocystis* sp. and *Pentatrichomonas hominis* in sheep and goats in China. Parasit Vectors **11**:93

Parkar U, Traub RJ, Kumar S, Mungthin M, Vitali S, Leelayoova S, Morris K, Thompson RC (2007) Direct characterization of *Blastocystis* from faeces by PCR and evidence of zoonotic potential. Parasitology **134**:359–367

Parkar U, Traub RJ, Vitali S, Elliot A, Levecke B, Robertson I, Geurden T, Steele J, Drake B, Thompson RC (2010) Molecular characterization of *Blastocystis* isolates from zoo animals and their animal-keepers. Vet Parasitol **169**:8–17

Petrasova J, Uzlikova M, Kostka M, Petrzelkova KJ, Huffman MA, Modry D (2011) Diversity and host specificity of *Blastocystis* in syntopic primates on Rubondo Island, Tanzania. Int J Parasitol **41**:1113–1120

Poirier P, Wawrzyniak I, Albert A, El Alaoui H, Delbac F, Livrelli V (2011) Development and evaluation of a real-time PCR assay for detection and quantification of *Blastocystis* parasites in human stool samples: prospective study of patients with hematological malignancies. J Clin Microbiol **49**:975–983

Rajah-Salim H, Suresh KG, Vellayan S, Mak JW, Khairul AA, Init I, Vennila GD, Saminathan R, Ramakrishnan K (1999) *Blastocystis* in animal handlers. Parasitol Res **85**:1032–1033

Ramirez JD, Sanchez LV, Bautista DC, Corredor AF, Florez AC, Stensvold CR (2014) *Blastocystis* subtypes detected in humans and animals from Colombia. Infect Genet Evol **22**:223–228

Ramírez JD, Sánchez A, Hernández C, Flórez C, Bernal MC, Giraldo JC, Reyes P, López MC, García L, Cooper PJ, Vicuña Y, Mongi F, Casero RD (2016) Geographic distribution of human *Blastocystis* subtypes in South America. Infect Genet Evol **41**:32–35

Rivera WL (2008) Phylogenetic analysis of *Blastocystis* isolates from animal and human hosts in the Philippines. Vet Parasitol **156**:178–182

Roberts T, Stark D, Harkness J, Ellis J (2013) Subtype distribution of *Blastocystis* isolates from a variety of animals from New South Wales, Australia. Vet Parasitol **196**:85–89

Růžková J, Květoňová D, Jirků M, Lhotská Z, Stensvold CR, Parfrey LW, Jirků Pomajbíková K (2018) Evaluating rodent experimental models for studies of *Blastocystis* ST1. Exp Parasitol **191**:55–61

Santin M, Gomez-Munoz MT, Solano-Aguilar G, Fayer R (2011) Development of a new PCR protocol to detect and subtype *Blastocystis* spp. from humans and animals. Parasitol Res **109**:205–212

Scicluna SM, Tawari B, Clark CG (2006) DNA barcoding of *Blastocystis*. Protist **157**:77–85

Stensvold CR (2013) Comparison of sequencing (barcode region) and STS PCR for *Blastocystis* subtyping. J Clin Microbiol **51**:190–194

Stensvold CR, Alfellani M, Clark CG (2012a) Levels of genetic diversity vary dramatically between *Blastocystis* subtypes. Infect Genet Evol **12**:263–273

Stensvold CR, Ahmed UN, Andersen LO, Nielsen HV (2012b) Development and evaluation of a genus-specific, probe-based, internal-process-controlled real-time PCR assay for sensitive and specific detection of *Blastocystis* spp. J Clin Microbiol **50**:1847–1851

Stensvold CR, Alfellani MA, Norskov-Lauritsen S, Prip K, Victory EL, Maddox C, Nielsen HV, Clark CG (2009) Subtype distribution of *Blastocystis* isolates from synanthropic and zoo animals and identification of a new subtype. Int J Parasitol **39**:473–479

Suresh K, Mak JW, Chuong LS, Ragunathan T (1997) Sac-like pouches in *Blastocystis* from the house lizard *Cosymbotus platyurus*. Parasitol Res **83**:523–525

Tan KS (2008) New insights on classification, identification, and clinical relevance of *Blastocystis* spp. Clin Microbiol Rev **21**:639–665

Tan KS, Mirza H, Teo JD, Wu B, Macary PA (2010) Current Views on the Clinical Relevance of *Blastocystis* spp. Curr Infect Dis Rep **12**:28–35

Teow WL, Zaman V, Ng GC, Chan YC, Yap EH, Howe J, Gopalakrishnakone P, Singh M (1991) A *Blastocystis* species from the sea-snake, *Lapemis hardwickii* (Serpentes: Hydrophiidae. Int J Parasitol **21**:723–726

Teow WL, Ng GC, Chan PP, Chan YC, Yap EH, Zaman V, Singh M (1992) A survey of *Blastocystis* in reptiles. Parasitol Res **78**:453–455

Wang W, Owen H, Traub RJ, Cuttell L, Inpankaew T, Bielefeldt-Ohmann H (2014) Molecular epidemiology of *Blastocystis* in pigs and their in-contact humans in South-

east Queensland, Australia, and Cambodia. Vet Parasitol **203**:264–269

Wang J, Gong B, Yang F, Zhang W, Zheng Y, Liu A (2018a) Subtype distribution and genetic characterizations of *Blastocystis* in pigs, cattle, sheep and goats in northeastern China's Heilongjiang Province. Infect Genet Evol **57**:171–176

Wang J, Gong B, Liu X, Zhao W, Bu T, Zhang W, Liu A, Yang F (2018b) Distribution and genetic diversity of *Blastocystis* subtypes in various mammal and bird species in northeastern China. Parasit Vectors **11**:522

Yoshikawa H, Koyama Y, Tsuchiya E, Takami K (2016a) *Blastocystis* phylogeny among various isolates from humans to insects. Parasitol Int **65**:750–759

Yoshikawa H, Morimoto K, Wu Z, Singh M, Hashimoto T (2004a) Problems in speciation in the genus *Blastocystis*. Trends Parasitol **20**:251–255

Yoshikawa H, Yoshida K, Nakajima A, Yamanari K, Iwatani S, Kimata I (2004b) Fecal-oral transmission of the cyst form of *Blastocystis hominis* in rats. Parasitol Res **94**:391–396

Yoshikawa H, Tokoro M, Nagamoto T, Arayama S, Asih PBS, Rozi IE, Syafruddin D (2016b) Molecular survey of *Blastocystis* sp. from humans and associated animals in an Indonesian community with poor hygiene. Parasitol Int **65**: 780–784

Yoshikawa H, Wu Z, Pandey K, Pandey BD, Sherchand JB, Yanagi T, Kanbara H (2009) Molecular characterization of *Blastocystis* isolates from children and rhesus monkeys in Kathmandu, Nepal. Vet Parasitol **160**:295–300

Zhu W, Tao W, Gong B, Yang H, Li Y, Song M, Lu Y, Li W (2017) First report of *Blastocystis* infections in cattle in China. Vet Parasitol **246**:38–42