Parasitology Research (2019) 118:3011–3017
https://doi.org/10.1007/s00436-019-06424-5

PROTOZOOLOGY - ORIGINAL PAPER

Molecular epidemiology of *Blastocystis* sp. in dogs housed in Italian rescue shelters

Alessia Libera Gazzonis[1] · **Marianna Marangi**[2] · **Sergio Aurelio Zanzani**[1] · **Luca Villa**[1] · **Annunziata Giangaspero**[2] · **Maria Teresa Manfredi**[1]

Received: 19 February 2019 / Accepted: 7 August 2019 / Published online: 15 August 2019

Abstract

Blastocystis is a ubiquitous protozoan with a wide range of hosts. In humans, its presence has been associated with gastrointestinal disorders, although its role as a pathogen still needs to be elucidated. Until now, 17 *Blastocystis* subtypes (STs) have been identified, with ST1–ST4 the most commonly found in humans. Among domestic animals, the same STs reported in humans have been detected in dogs. An epidemiological survey on dog kennels was carried out to evaluate the prevalence of *Blastocystis* and the STs involved. Overall, 99 faecal samples were collected from the rescue shelters. *Blastocystis* detection was performed through conventional barcoding PCR targeting the 1800-bp SSU-rDNA, followed by sequencing and phylogenetic analysis. *Blastocystis* DNA was found in 21 faecal samples (21.2%), and all samples were successfully sequenced and identified as ST3 in a unique monophyletic group. The presence of *Blastocystis* was reported for the first time in dogs from Italy, with the identification of ST3, the subtype most commonly found in humans.

Keywords *Blastocystis* · Subtypes · Dog · Zoonosis · Molecular epidemiology · Genotyping

Introduction

Blastocystis (stramenopiles) is a gastrointestinal protozoan occurring in humans and in a wide range of animals worldwide (Tan 2008). Because of its high genetic diversity and since its taxonomy still needs to be clarified, a consensus on terminology indicated *Blastocystis* sp. as the most appropriate nomenclature for those isolated in mammal and avian species (Stensvold et al. 2007). The transmission is via the faecal-oral route (Stensvold and Clark 2016) as well as through contaminated water or food (Angelici et al. 2018; Caradonna et al. 2017; Lee et al. 2012; Leelayoova et al. 2004, 2008). In humans, *Blastocystis* has been linked to various gastrointestinal symptoms and is involved in several chronic gastrointestinal illnesses (Boorom et al. 2008; Stensvold et al. 2009a); nevertheless, its presence in the gut of healthy individuals suggests the role of *Blastocystis* as a commensal microorganism (part of a healthy microbiome) (Chabé et al. 2017). Indeed, *Blastocystis* is one of the most frequently detected microeukaryotes in epidemiological surveys, with worldwide distribution. Prevalence values differ according to geographical regions, with higher values mostly in developing countries (Tan 2008). Incorrect hygiene measures and waste disposal management, contact with animals, and consumption of contaminated food or water are considered enhancers of the risk of transmission (Clark et al. 2013; Tan 2008). In addition to humans, *Blastocystis* could be involved in intestinal disorders in other mammals. *Blastocystis* has been proposed as a pathogen-causing diarrhoea in laboratory and zoo animals (Goe et al. 2016; Zanzani et al. 2014a), and evidence of *Blastocystis* pathogenicity has also been highlighted in some experimental animals, i.e. chickens and rats (Elwakil and Hewedi 2010; Moe et al. 1997; Phillips and Zierdt 1976). Moreover, *Blastocystis* was isolated in a dog showing intestinal symptoms (Chapman et al. 2009) as well as in cattle and swine (Pakandl 1991; Quílez et al. 1995). A high variability in

Section Editor: Panagiotis Karanis

✉ Alessia Libera Gazzonis
alessia.gazzonis@unimi.it

[1] Department of Veterinary Medicine, Università degli Studi di Milano, Milan, Italy

[2] Department of Science of Agriculture, Food and Environment, University of Foggia, Foggia, Italy

prevalence rates among animals has been reported; however, large-scale epidemiological surveys are still limited (Tan 2004).

A variety of methods for the laboratory diagnosis of *Blastocystis* have been described, including direct microscopical visualization with Lugol's iodine or permanent trichrome staining of fixed faecal smears. However, microscopical identification can be very challenging due to the high morphological polymorphism of *Blastocystis* and resulting in the requirement for trained personnel (Stensvold and Clark 2016). The use of molecular tools is highly recommended due to their high sensitivity and specificity (Roberts et al. 2011). Currently, the "barcode region" (Scicluna et al. 2006) within the small subunit rDNA (SSU-rDNA) is considered the most informative sequence, due to its use in both *Blastocystis* detection and more importantly genotyping, i.e. identification of the genetic diversity of this protozoan (Stensvold 2013). Indeed, based on the analyses of polymorphisms across the SSU-rDNA, at least 17 subtypes (STs) have been identified in mammals and birds, with a certain degree of host specificity (Stensvold and Clark 2016). Humans have been reported to harbour mainly ST1–ST4, whereas ST5–ST9 and ST12 have been isolated only sporadically (Ramírez et al. 2016; Stensvold et al. 2007). The four most common STs in humans (ST1–ST4) have also been detected in other host species, including primates, hoofed mammals, rodents and even birds (Alfellani et al. 2013). Subtypes ST5–ST8 are considered potentially zoonotic but are rarely detected in humans, being more commonly found in other hosts: ST5 in hoofed animals, ST6 and ST7 in birds, and ST8 in nonhuman primates (Stensvold and Clark 2016).

Thus, according to the molecular data, it turns out that several animal species harbour potentially zoonotic STs. Data concerning the STs of *Blastocystis* in dogs are limited in the European scenario; in this species, STs commonly detected in humans (ST1 and 2) have been previously identified (Osman et al. 2015; Ramírez et al. 2014; Wang et al. 2013), and cases with the presence of the same STs in owners and dogs have been described (Belleza et al. 2016; Nagel et al. 2012). To add data both on the circulation of *Blastocystis* in this animal species and on the subtypes involved, we carried out an epidemiological study in a population of shelter dogs by a comprehensive approach.

Material and methods

Study design and sampling

From September 2015 to March 2016, faecal samples of 99 dogs housed in six shelters (average number of sampled dog per shelter—19) located in Northern Italy (Lombardy region, 45°35′08″ N, 9°55′49″ E) were collected and included in the study. Six shelters were selected based on their collaboration to participate in the project and compliance. One shelter (D) was managed by the local health authority, while the other by nonprofit associations. Among selected shelters, there was no contact between dogs in different kennels as they were both geographically distant (minimum distance—5 km) and due to biosecurity rules; the dogs only came out of the shelters once they were adopted. In addition, there were no common staff or volunteers between shelters, and the visits of potential adopters were regulated in terms of time and access to the buildings. All the shelters were fenced, with no contact between dogs and wild animals. Apparently, healthy dogs were randomly recruited among the housed animals; pure breeds and cross-breeds of different sizes, and animals of age varying from 3 months to 14 years were included in the study. Fresh faecal samples were collected and transported refrigerated to the laboratory and then processed within 24 h; an aliquot was stored at − 20 °C until molecular analysis. The following data concerning shelter management were recorded: number of hosted dogs; total area and available surface for cages and fenced areas; structure of cages (floor and slope); and procedures and frequency of cleaning of cages and fenced areas.

DNA extraction, PCR amplification and sequencing

Genomic DNA was extracted from each faecal sample of approximately 200 mg using the QiaAMP DNA Stool Mini Kit (Qiagen, Hilden, Germany) according to the manufacturer's instructions and then eluted in 200 μL AE buffer. Primers RD5 (5′-ATCTGGTTGATCCTGCCAGT-3′) and BhRDr (5′-GAGCTTTTTAACTGCAACAACG-3′) were used to amplify a fragment of approximately 600 base pairs (bp) within the 1800 bp SSU-rDNA of *Blastocystis* (Scicluna et al. 2006). PCR mix contained 10 μL of Phire Reaction Buffer 5X (Thermo Scientific, USA), 0.4 μL of dNTPs (200 μM) (Qiagen, USA), 1 μL of primer pairs (10 μM), 0.4 μL of Phire Hot Start II DNA Polymerase 1 U (Thermo Scientific, USA) and 5 μL (approximately 100 ng) of genomic DNA per reaction according to the manufacturer's protocol. PCR was performed as follows: denaturation at 98 °C for 30 s, followed by 98 °C for 5 s, 59 °C for 30 s and 72 °C for 1 min for 35 cycles, and finally 72 °C for 2 min. Two negative controls (PCR water) were included in each PCR run. PCR products were run on 1.2% agarose gel, and positive samples were purified with Exonuclease I (EXO I) and Thermosensitive Alkaline Phosphatase (FAST AP) (Fermentas) enzymes according to the manufacturer's instructions.

Purified PCR products were directly sequenced in both directions using the ABI PRIMS BigDye Terminator v. 3.1 Cycle Sequencing Kit (Applied Biosystems, Foster City, CA, USA) with the same primers as the abovementioned PCR procedures, according to the manufacturer's instructions. Sequencing was performed on an ABI PRISM 3130 Genetic Analyzer (Applied Biosystems, USA), chromatograms were evaluated by using the Finch TV software and primer regions, and bad quality regions were removed. Once sequences were cleaned up, each sequence was compared with the *Blastocystis* homologous nucleotide sequences available in GenBank databases using the BLASTN program (https://blast.ncbi.nlm.nih.gov/). Finally, the sequences corresponding to *Blastocystis* SSU-rDNA gene portion were gathered in a FASTA file and aligned each other using the ClustalW implementation of BioEdit software v7.0.5, and the alignment was adjusted manually, if necessary.

Genotyping and phylogenetic analysis

To attribute the subtypes, obtained sequences were aligned with the SSU-rDNA *Blastocystis* sequences representing subtypes ST1–17 using BioEdit software v7.0.5. Phylogenetic analyses of the obtained sequences and the homologous sequences from GenBank were performed using the maximum likelihood method in Mega v7.0.9. The tree was rooted using a *Blastocystis lapemi* sequence as the outgroup (accession number: AY590115). Bootstrap confidence values for the branching reliability were calculated with 10,000 replicates.

Data analysis

A sample was considered positive for *Blastocystis* if it had a positive PCR assay and if sequencing confirmed *Blastocystis* identity. Prevalence (*P*) and 95% confidence interval (95% CI) were calculated, considering overall data and within

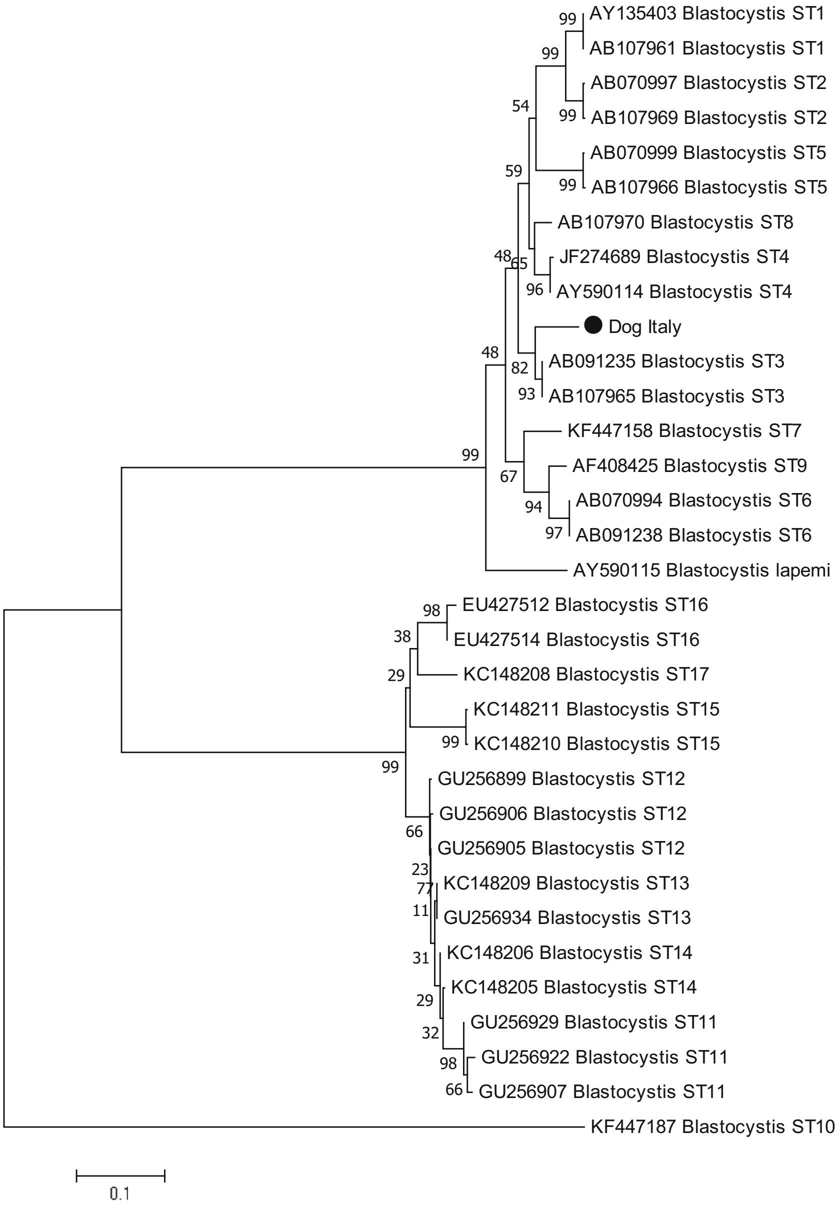

Fig. 1 Phylogenetic topology from the maximum likelihood analysis based on the partial SSU-rDNA sequence of *Blastocystis* isolates. The sequence of *Blastocystis* obtained in the present study (black circle) was analysed in comparison with the sequences available in GenBank for the *Blastocystis* subtypes ST1–17 detected genetically on the basis of the same gene and were reported with their accession numbers. The sequence of *Blastocystis lapemi* (accession no. AY590115) was used as the outgroup. GenBank Accession Number MK770357 and sample identification number of sequence from the present study are reported

selected categories. A univariate logistic regression analysis assessed the association between the presence of *Blastocystis* and the following individual variables: age (< 1 year, ≥ 1 year), sex, size (small, medium, large) and shelter (A, B, C, D, E and F). Variables showing a *P* value < 0.1 were entered in a multivariate logistic regression model, which was developed using a backward procedure until retained variables were significant (*P* value < 0.05). Statistical analysis was performed using SPSS (version 19.0; SPSS, Chicago, IL).

Results

Out of 99 examined faecal samples, 21 (*P* = 21.2%, 95% CI = 14.41–30.26) were proven to be positive for *Blastocystis* by PCR (expected fragment of approximately 600 bp of the SSU-rDNA). With sequencing, high-quality sequences were obtained for all the samples, and no intraspecific nucleotide variations were detected between any of the investigated *Blastocystis* sequences (100% identity); therefore, just one sequence was used for phylogenetic analysis. The tree indicated that the sequence clusters with the *Blastocystis* ST3 sequences in a monophyletic group and that this clade is distant from the other *Blastocystis* subtypes (Fig. 1). The obtained sequence was submitted to GenBank database under accession number MK770357.

Individual data regarding dogs that scored positive for *Blastocystis* are outlined in Table 1. *Blastocystis* DNA was found in both young (21.4%) and adult animals (21.2%) and in both females (18.9%) and males (22.6%). Concerning dog size, *Blastocystis* DNA was more commonly found in large (31.7%) breeds than in small (25%) or medium (9.5%) breeds. Any association between the presence of *Blastocystis* and considered individual variables (age, sex, size, shelter) was not found by univariate logistic regression analysis (Table 2). Although no significant difference was found, prevalence values varied from 18.2 to 37.5% according to the shelters. Only shelter E housed all negative dogs among those examined. Shelter management was similar among the investigated rescue shelters; nevertheless, it is worth noting the differences in the buildings hosting the dogs. The number of hosted dogs ranged from 50 to 150 individuals, while the total available area varied from 2000 to 35,000 square metres (sq. m). Dogs spent most of the day in the housing areas, consisting of cages, and they were taken for approximately 1–3 h a day to socialize in the fenced areas. The whole available surface varied significantly among the shelters. Shelter A had the lowest available surface area, with a total housing area of 700 sq. m and a single fenced area of 200 sq. m in common to all dogs. In the other shelters, on the contrary, the surface available to cages and fenced areas ranged from 750 to 2500 sq. m and from 1000 to 4800 sq. m, respectively. Shelter D, in accordance with hygienic sanitary procedures, did not allow dogs to access common fenced areas. Cages were structurally similar in investigated shelters, with cement floors or mixed with tiles;

Table 1 Data of dogs harbouring *Blastocystis* sp.

Sample	Shelter	Age[†]	Gender	Breed	Size
1	A	12 y	Male	Mixed	Large
2	A	9 y	Female	Mixed	Large
3	A	3 y	Male	Dogo Argentino	Large
4	A	7 y	Male	Doberman	Large
5	A	12 y	Male	German shepherd	Large
6	A	7 y	Male	Mixed	Large
7	B	11 y	Female	Rottweiler	Large
8	B	2 y	Female	Mixed	Large
9	B	9 y	Male	Rottweiler	Large
10	C	9 y	Male	Mixed	Large
11	C	8 y	Female	Mixed	Small
12	D	10 y	Male	Pekingese	Small
13	D	3 m	Male	Mixed	Small
14	D	3 m	Male	Cane Corso	Large
15	D	7 y	Female	Beauceron	Large
16	D	4 y	Male	Labrador Retriever	Medium
17	D	11 y	Male	Mixed	Small
18	F	10 y	Male	Bullmastiff	Large
19	F	11 m	Female	Mixed	Medium
20	F	4 y	Male	Labrador Retriever	Medium
21	F	10 y	Female	Mixed German shepherd	Medium

[†] *y* years, *m* months

Table 2 Results of univariate logistic regression analysis of variables associated with the presence of *Blastocystis* sp. in shelter dogs

Variable category	Positive/examined	% (95% CI[*])	*P* value
Age			0.983
< 1 year	3/14	21.4 (7.57–47.59)	
≥ 1 year	18/85	21.2 (13.84–31.01)	
Sex			0.667
Male	14/62	22.6 (13.95–34.4)	
Female	7/37	18.9 (9.48–34.21)	
Size			0.058
Small	4/16	25 (10.18–49.5)	
Medium	4/42	9.5 (3.76–22.06)	
Large	13/41	31.7 (19.57–46.99)	
Shelter			0.815
A	6/16	37.5 (18.48–61.36)	
B	3/11	27.3 (9.74–56.56)	
C	2/11	18.2 (5.14–47.7)	
D	6/27	22.2 (10.61–40.75)	
E	0/12	0 (0–24.25)	
F	4/22	18.2 (7.31–38.51)	

[*] *95% CI* 95% confidence interval

all cages also had a slope facilitating the cleaning procedures, except in shelter A. Finally, cleaning procedures were similar among shelters; however, in shelter A, the disinfection of the cages was performed only once a week, while it was daily in the others. In addition, fenced areas had similar cleaning protocols for manure removal: cleaning was performed daily in shelters B, E and F, while only weekly in shelters A and C. Data on facilities and on hygienic procedures of each shelter are provided in Table 3.

Discussion

Data obtained in the present study provided the first report in Italy on the presence of *Blastocystis* in canine faecal samples. Overall, a prevalence of 21.2% was found, which was higher than that previously reported in dogs from other European countries by molecular analysis, which ranged from 0% in Spain and Greece (Paulos et al. 2018; Spanakos et al. 2011) to 3.4% in France (Osman et al. 2015). Nevertheless, the abovementioned studies examined only owned or stray dogs. Indeed, shelter dogs seem to be more at risk of harbouring *Blastocystis* than owned dogs: in a survey carried out in the USA, *Blastocystis* was detected in 9.7% of examined shelter dogs while any positivity to *Blastocystis* was not found in owned dogs (Ruaux and Stang 2014). Similarly, Wang et al. (2013) reported *Blastocystis* only in two shelter dogs out of 80 examined from both owned and shelter dogs from the city of Brisbane. In owned dogs, higher prevalence values of *Blastocystis* ranging from 14.5 to 37% were found in developing countries (Belleza et al. 2016; Ramírez et al. 2014). In the present study, *Blastocystis* was detected in five out of six examined shelters, suggesting the spread and maintenance of *Blastocystis* in the environment, with prevalence values varying from 18.2 to 37.5%, regardless of individual characteristics (i.e. age, sex and size). Differences in the prevalence of *Blastocystis* may be associated with the hygiene level achievable in shelters, despite the daily care of personnel and volunteers. All examined shelters had hygienic protocols comprising a first cleaning with only water and a subsequent disinfection both of cages and fenced areas, with variable timing also depending on the availability of personnel. Indeed, apart from shelter D, managed by the local health authority, the other shelters were handled by nonprofit associations, primarily with voluntary personnel. In addition, facilities were often undersized for the number of animals housed and were built with material that did now not allow for easy and complete disinfection. Indeed, shelter A, the one with the highest prevalence of *Blastocystis*, showed less efficient hygienic measures, with the cleaning of fenced areas and disinfection of cages performed only weekly. In addition, shelter A presented the worst facilities, without a sloping floor in the cages, which favours water stagnation and makes cleaning difficult and less efficient, and the lowest surface available per animal considering both cages and fenced areas. In contrast, *Blastocystis* was not detected in shelter E, which showed the largest available surface both for cages and fenced areas, suggesting a greater environmental dispersion of the pathogen and therefore a lower risk for dogs to come into contact with it.

In dogs investigated in the present survey, only ST3 was detected. Considering data available from Europe on *Blastocystis* in dogs, ST3 has been previously detected in a dog from Denmark (Stensvold et al. 2009b), while in dogs from France, ST2 and ST10 were reported (Osman et al. 2015). Differences in the spread of STs among geographical areas were reported concerning *Blastocystis* in humans (Alfellani et al. 2013); similarly, it could be inferred that the same differences may exist also for *Blastocystis* STs in dogs. Indeed, considering isolates from non-European countries, more STs have been detected in dogs, including the zoonotic ST1–ST6 and ST10, which are commonly reported in cattle (Belleza et al. 2016; Ruaux and Stang 2014; Wang et al. 2013).

In Italy, molecular studies in human samples showed the occurrence of ST1–ST8, with ST3 as the most prevalent

Table 3 Data of shelters included in the survey on *Blastocystis* sp. detection

Shelter id	Number of *Blastocystis* positive/examined dogs (%)	Number of hosted dogs	Total area (sq. m)	Cages				Fenced area[§]	
				Total housing area (sq. m)	Cage floor	Slope	Cleaning frequency (water/disinfectant)	Total area (sq. m)	Cleaning frequency (water)
A	6/16 (37.5%)	80	3000	700	Cement	No	Daily /weekly	200	Weekly
B	3/11 (27.3%)	90	6000	870	Cement/tiles	Yes	Daily/daily	2130	Daily
C	2/11 (18.2%)	50	4000	800	Cement	Yes	Daily/daily	1000	Weekly
D	6/27 (22.2%)	60	2000	800	Cement	Yes	Daily/daily	–	–
E	0/12 (0%)	100	35,000	2500	Cement	Yes	Daily/daily	4800	Daily
F	4/22 (18.2%)	150	3500	750	Cement	Yes	Daily/daily	1000	Daily

[§] Shelter D does not allow dogs in fenced area according to hygienic sanitary procedures subtype (Mattiucci et al. 2016; Meloni et al. 2011). Moreover, a survey on nonhuman primates (*Macaca fascicularis*) raised in Italy in breeding facilities confirms the spread of ST3, together with the other zoonotic STs (ST1, ST2, ST5 and ST7), suggesting the potential risk for researchers and keepers of acquire zoonotic *Blastocystis* STs (Zanzani et al. 2016). Indeed, in humans, ST3 is the *Blastocystis* subtype with the highest prevalence worldwide and probably represents the human species-specific ST (Noël and Dufernez 2005); animals harbouring ST3 may thus reflect environmental contamination by humans, confirming the zoonotic potential of animals for *Blastocystis* human infections.

Thus, the results obtained in the present survey endorse the role of dogs in the maintenance of this zoonotic protozoan parasite. With 1,850,587 owned pets registered in the Lombardy region (https://www.anagrafecaninalombardia.it at 20/12/2018), in the study area and generally in northern Italy, the number of owned pets is increasing. As a consequence, sanitary problems linked to dogs as a reservoir of zoonotic parasitic infections as well as to urban faecal contamination should not be neglected, as a study already highlighted in the same area (Zanzani et al. 2014c, c).

Conclusions

Blastocystis showed a high diffusion in shelter dogs from Northern Italy, even if the importance of this protozoan as an intestinal pathogen of dog has been not clarified until now. On the other hand, the high prevalence recorded in the present study confirms the importance of dogs in the maintenance and spread of *Blastocystis*, with the record of ST3, typical of humans. Concerning the zoonotic potential of *Blastocystis* sp., although a higher occurrence of this parasite has been registered among people handling animals than among those without contact with animals (Belleza et al. 2016; Nagel et al. 2012; Parkar et al. 2010; Wang et al. 2014), further studies are necessary to increase the knowledge of the role of dogs as a reservoir for humans. Although *Blastocystis* detection is not a component of routine parasitological examination, it is desirable to add it to the parasitological screening of shelter dogs, particularly prior to the transfer of the dog/s in a new family, to minimize the risk of transmission for the adopting family members and for other pets. In this view, the use of this efficient molecular tool may help fill in the gaps in the knowledge of this intriguing and still not well-understood protozoan.

Acknowledgements Authors are grateful to the shelters' personnel and volunteers for their help in the sample collection.

Compliance with ethical standards The collection of biological samples from live animals was performed in the respect of animal welfare according to current legislation. The study was conducted with the approval of each of the involved shelter and the Institutional Animal Care and Use Committee of Università degli Studi di Milano (Permission OPBA_34_2017).

Conflict of interest The authors declare that they have no conflict of interest.

References

Alfellani MA, Stensvold CR, Vidal-Lapiedra A, Onuoha ES, Fagbenro-Beyioku AF, Clark CG (2013) Variable geographic distribution of *Blastocystis* subtypes and its potential implications. Acta Trop 126: 11–18. https://doi.org/10.1016/j.actatropica.2012.12.011

Angelici MC, Nardis C, Scarpelli R, Ade P (2018) Blastocystis hominis transmission by non-potable water: a case report in Italy. New Microbiol 41:173–177

Belleza MLB, Reyes JCB, Tongol-Rivera PN, Rivera WL (2016) Subtype analysis of *Blastocystis* sp. isolates from human and canine hosts in an urban community in the Philippines. Parasitol Int 65: 291–294. https://doi.org/10.1016/j.parint.2016.02.009

Boorom KF, Smith H, Nimri L, Viscogliosi E, Spanakos G, Parkar U, Li LH, Zhou XN, Ok UZ, Leelayoova S, Jones MS (2008) Oh my aching gut: irritable bowel syndrome, *Blastocystis*, and asymptomatic infection. Parasit Vectors 1:40. https://doi.org/10.1186/1756-3305-1-40

Caradonna T, Marangi M, Del Chierico F, Ferrari N, Reddel S, Bracaglia G, Normanno G, Putignani L, Giangaspero A (2017) Detection and prevalence of protozoan parasites in ready-to-eat packaged salads on sale in Italy. Food Microbiol 67:67–75. https://doi.org/10.1016/j.fm.2017.06.006

Chabé M, Lokmer A, Ségurel L (2017) Gut protozoa: friends or foes of the human gut microbiota? Trends Parasitol 33:925–934. https://doi.org/10.1016/j.pt.2017.08.005

Chapman S, Thompson C, Wilcox A et al (2009) What is your diagnosis? Rectal scraping from a dog with diarrhea. Vet Clin Pathol 38:59–62. https://doi.org/10.1111/zph.12522

Clark CG, van der Giezen M, Alfellani MA, Stensvold CR (2013) Recent developments in *Blastocystis* research. Adv Parasitol 82:1-32. doi.org/10.1016/B978-0-12-407706-5.00001-0

Elwakil HS, Hewedi IH (2010) Pathogenic potential of *Blastocystis* hominis in laboratory mice. Parasitol Res 107:685–689. https://doi.org/10.1007/s00436-010-1922-y

Goe AM, Heard DJ, Easley JR, Weeden AL, Childress AL, Wellehan JF (2016) *Blastocystis* sp. and *Blastocystis ratti* in a Brazilian porcupine (*Coendou prehensilis*) with diarrhea. J Zoo Wildl Med 47:640–644. https://doi.org/10.1638/2015-0093.1

Lee IL, Tan TC, Tan PC, Nanthiney DR, Biraj MK, Surendra KM, Suresh KG (2012) Predominance of *Blastocystis* sp. subtype 4 in rural communities, Nepal. Parasitol Res 110:1553–1562. https://doi.org/10.1007/s00436-011-2665-0

Leelayoova S, Rangsin R, Taamasri P, Naaglor T, Thathaisong U, Mungthin M (2004) Evidence of waterborne transmission of *Blastocystis hominis*. Am J Trop Med Hyg 70:658–662

Leelayoova S, Siripattanapipong S, Thathaisong U, Naaglor T, Taamasri P, Piyaraj P, Mungthin M (2008) Drinking water: a possible source of *Blastocystis* spp. subtype 1 infection in schoolchildren of a rural community in central Thailand. Am J Trop Med Hyg 79:401–406

Mattiucci S, Crisafi B, Gabrielli S, Paoletti M, Cancrini G (2016) Molecular epidemiology and genetic diversity of *Blastocystis* infection in humans in Italy. Epidemiol Infect 144:635–646. https://doi.org/10.1017/S0950268815001697

Meloni D, Sanciu G, Poirier P, El Alaoui H, Chabé M, Delhaes L, Dei-Cas E, Delbac F, Luigi Fiori P, Di Cave D, Viscogliosi E (2011)

Molecular subtyping of *Blastocystis* sp. isolates from symptomatic patients in Italy. Parasitol Res 109:613–619. https://doi.org/10.1007/s00436-011-2294-7

Moe KT, Singh M, Howe J, Ho LC, Tan SW, Chen XQ, Ng GC, Yap EH (1997) Experimental *Blastocystis* hominis infection in laboratory mice. Parasitol Res 83:319–325. https://doi.org/10.1007/s004360050256

Nagel R, Cuttell L, Stensvold CR, Mills PC, Bielefeldt-Ohmann H, Traub RJ (2012) *Blastocystis* subtypes in symptomatic and asymptomatic family members and pets and response to therapy. Intern Med J 42: 1187–1195. https://doi.org/10.1111/j.1445-5994.2011.02626.x

Noël C, Dufernez F (2005) Molecular phylogenies of *Blastocystis* isolates from different hosts: implications for genetic diversity, identification of species, and zoonosis. J Clin Microbiol 43:348–355. https://doi.org/10.1128/JCM.43.1.348

Osman M, Bories J, El Safadi D, Poirel MT, Gantois N, Benamrouz-Vanneste S, Delhaes L, Hugonnard M, Certad G, Zenner L, Viscogliosi E (2015) Prevalence and genetic diversity of the intestinal parasites *Blastocystis* sp. and *Cryptosporidium* spp. in household dogs in France and evaluation of zoonotic transmission risk. Vet Parasitol 214:167–170. https://doi.org/10.1016/j.vetpar.2015.09.015

Pakandl M (1991) Occurrence of *Blastocystis* sp. in pigs. Folia Parasitol (Praha) 38:297–301

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. https://doi.org/10.1016/j.vetpar.2009.12.032

Paulos S, Köster PC, de Lucio A, Hernández-de-Mingo M, Cardona GA, Fernández-Crespo JC, Stensvold CR, Carmena D (2018) Occurrence and subtype distribution of *Blastocystis* sp. in humans, dogs and cats sharing household in northern Spain and assessment of zoonotic transmission risk. Zoonoses Public Health 65:993–1002. https://doi.org/10.1111/zph.12522

Phillips BP, Zierdt CH (1976) *Blastocystis* hominis: pathogenic potential in human patients and in gnotobiotes. Exp Parasitol 39:358–364. https://doi.org/10.1016/0014-4894(76)90039-4

Quílez J, Sánchez-Acedo C, Clavel A, Causapé AC (1995) Occurrence of *Blastocystis* sp. in cattle in Aragón, northeastern Spain. Parasitol Res 81:703–705. https://doi.org/10.1007/BF00931851

Ramírez JD, Sánchez LV, Bautista DC, Corredor AF, Flórez AC, Stensvold CR (2014) *Blastocystis* subtypes detected in humans and animals from Colombia. Infect Genet Evol 22:223–228. https://doi.org/10.1016/j.meegid.2013.07.020

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. https://doi.org/10.1016/j.meegid.2016.03.017

Roberts T, Barratt J, Harkness J, Ellis J, Stark D (2011) Comparison of microscopy, culture, and conventional polymerase chain reaction for detection of *Blastocystis* sp. in clinical stool samples. Am J Trop Med Hyg 84:308–312. https://doi.org/10.4269/ajtmh.2011.10-0447

Ruaux CG, Stang BV (2014) Prevalence of *Blastocystis* in shelter-resident and client-owned companion animals in the US Pacific Northwest. PLoS One 16;9(9):e107496. https://doi.org/10.1371/journal.pone.0107496

Scicluna SM, Tawari B, Clark CG (2006) DNA barcoding of *Blastocystis*. Protist 157:77–85. https://doi.org/10.1016/j.protis.2005.12.001

Spanakos G, Papadogiannakis E, Kontos V, Menounos P, Velonakis E, Koutis C, Vakalis N (2011) Molecular screening for *Blastocystis* sp. in canine faecal samples in Greece. J Hell Vet Med Soc 62:216–220. https://doi.org/10.3760/cma.j.issn.0366-6999.20122801

Stensvold CR, Suresh GK, Tan KSW, Thompson RC, Traub RJ, Viscogliosi E, Yoshikawa H, Clark CG (2007) Terminology for *Blastocystis* subtypes - a consensus. Trends Parasitol 23:93–96. https://doi.org/10.1016/j.pt.2007.01.004

Stensvold CR, Lewis HC, Hammerum AM, Porsbo LJ, Nielsen SS, Olsen KE, Arendrup MC, Nielsen HV, Mølbak K (2009a) *Blastocystis*: unravelling potential risk factors and clinical significance of a common but neglected parasite. Epidemiol Infect 137:1655–1663. https://doi.org/10.1017/S0950268809002672

Stensvold CR, Alfellani MA, Nørskov-Lauritsen S, Prip K, Victory EL, Maddox C, Nielsen HV, Clark CG (2009b) Subtype distribution of *Blastocystis* isolates from synanthropic and zoo animals and identification of a new subtype. Int J Parasitol 39:473–479. https://doi.org/10.1016/j.ijpara.2008.07.006

Stensvold CR (2013) Comparison of sequencing (barcode region) and sequence-tagged-site PCR for *Blastocystis* subtyping. J Clin Microbiol 51:190–194. https://doi.org/10.1128/JCM.02541-12

Stensvold CR, Clark CG (2016) Current status of *Blastocystis*: a personal view. Parasitol Int 65:763–771. https://doi.org/10.1016/j.parint.2016.05.015

Tan KSW (2004) *Blastocystis* in humans and animals: new insights using modern methodologies. Vet Parasitol 126:121–144. https://doi.org/10.1016/j.vetpar.2004.09.017

Tan KSW (2008) New insights on classification, identification, and clinical relevance of *Blastocystis* spp. Clin Microbiol Rev 21:639–665. https://doi.org/10.1128/CMR.00022-08

Wang W, Cuttell L, Bielefeldt-Ohmann H, Inpankaew T, Owen H, Traub RJ (2013) Diversity of *Blastocystis* subtypes in dogs in different geographical settings. Parasit Vectors 6:1–5. https://doi.org/10.1186/1756-3305-6-215

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 Southeast Queensland, Australia, and Cambodia. Vet Parasitol 203:264–269. https://doi.org/10.1016/j.vetpar.2014.04.006

Zanzani SA, Epis S, Bandi C, Manfredi MT (2014a) What is your diagnosis? Fecal smear stained with Lugol's solution and Giemsa from a cynomolgus macaque (*Macaca fascicularis*) presenting with liquid diarrhea. Vet Clin Pathol 43:293–294. https://doi.org/10.1111/vcp.12128

Zanzani SA, Di Cerbo AR, Gazzonis AL, Genchi M, Rinaldi L, Musella V, Cringoli G, Manfredi MT (2014b) Canine fecal contamination in a metropolitan area (Milan, North-Western Italy): prevalence of intestinal parasites and evaluation of health risks. Sci World J 132361: 1–6. https://doi.org/10.1155/2014/132361

Zanzani SA, Gazzonis AL, Scarpa P, Berrilli F, Manfredi MT (2014c) Intestinal parasites of owned dogs and cats from metropolitan and micropolitan areas: prevalence, zoonotic risks, and pet owner awareness in northern Italy. Biomed Res Int 696508. https://doi.org/10.1155/2014/696508

Zanzani SA, Gazzonis AL, Epis S, Manfredi MT (2016) Study of the gastrointestinal parasitic fauna of captive non-human primates (*Macaca fascicularis*). Parasitol Res 115:307–312. https://doi.org/10.1007/s00436-015-4748-9