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GENETICS, EVOLUTION, AND PHYLOGENY - SHORT COMMUNICATION

Molecular characterization of *Blastocystis* in cattle in Turkey

Zeynep Erdem Aynur[1] · Özgür Güçlü[1,2] · İbrahim Yıldız[3] · Hüseyin Aynur[4] · Hatice Ertabaklar[3] · Bülent Bozdoğan[1,5] · Sema Ertuğ[3]

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Abstract

Blastocystis genus exist in a wide variety of hosts, including humans, birds, insects, annelids, amphibians, fish, and mammals. PCR-based molecular diagnostic methods have been successfully used to detect *Blastocystis* spp. in feces, and small subunit ribosomal ribonucleic acid (SSU rRNA) gene-based subtyping is the preferred method for diagnosis. There has been discussion about the subtypes of *Blastocystis* spp. which has been detected so far. To date, 26 different subtypes have been reported. The aim of this study was to determine the existence and diversity of *Blastocystis* spp. in cattle. In our study, a total of 80 stool samples were collected from cows and calves at 13 different farms in Burdur and one farm in Aydın. Using molecular method, a total of 9 samples out of 80 samples were found to be positive (11.25%) for *Blastocystis*. As a result of sequence analysis of *Blastocystis* positive samples, the subtype 14 was detected on seven samples, while in the other two samples, *Blastocystis* subtype 10 was identified. The ST10 and ST14 subtypes are commonly reported in animals but not isolated from human. Our analyses showed genetic differences among *Blastocystis* subtypes. Our study is the first *Blastocystis* subtyping study from cattle in Turkey.

Keywords *Blastocystis* · Cattle · SSU rRNA · Turkey

Blastocystis is one of the most common parasites found in fecal samples of human and animals (Cian et al. 2017). The pathogenicity of *Blastocystis* is not yet clear and may vary depending on the subtype (ST) of parasite, and the immune status of the patient (Cirioni et al. 1999; Elwakil and Hewedi 2010). This parasite may cause various intestinal pathologies especially irritable bowel syndrome (Wawrzyniak et al. 2013), and more grave infections among immune-compromised HIV and cancer patients (Lepczyńska et al. 2017).

Transmission of infection occurs through fecal-oral way, by ingestion of food and water contaminated by *Blastocystis* cysts from infected patients (Leelayoova et al. 2008). Human in contact with animals have higher risk of getting *Blastocystis* infection (Parkar et al. 2010). Recent-year studies indicated presence of *Blastocystis* among domestic farm animals like bovine, pigs, and pets like cats and dogs and wild animals such as pheasants, ducks, and primates in Japan as well as UK and European countries (Abe et al. 2002; Cian et al. 2017; Osman et al. 2015).

Recently, PCR-based molecular diagnostic methods have been successfully used to determine *Blastocystis* in feces, and SSU rRNA gene-based subtyping is the preferred method for diagnosis (Wawrzyniak et al. 2013). Wide genetic diversity is observed within *Blastocystis*. There has been discussion about the subtypes of *Blastocystis* which has been detected so far. Currently, a total of 26 subtypes have been identified and submitted to Genbank (https://www.ncbi.nlm.nih.gov/genbank/) (Maloney et al. 2018). *Blastocystis* subtypes from ST1 to ST9 and ST12 have been reported in humans at varying prevalence levels (Ramírez et al. 2016; Stensvold and Clark 2016). The subtypes between ST1 and ST8 are the subtypes with zoonotic potential (Cian et al. 2017). ST1 is isolated from human and other mammals, while ST2 from primates and pigs, ST4 from rodents, ST5 from cows and pigs, and ST6

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✉ Özgür Güçlü
oguclu@adu.edu.tr

[1] Recombinant DNA and Recombinant Protein Research Center (REDPROM), Adnan Menderes University, Aydın, Turkey

[2] Department of Plant and Animal Production, Sultanhisar MYO, Adnan Menderes University, Aydın, Turkey

[3] Department of Parasitology, Faculty of Medicine, Adnan Menderes University, Aydın, Turkey

[4] Yore Veterinary Clinic, Aydın, Turkey

[5] Department of Medical Microbiology, Faculty of Medicine, Adnan Menderes University, Aydın, Turkey

and ST7 from birds. The most commonly isolated ST3 in epidemiological studies has been reported as the only subtype of human origin. The less known ST8 was isolated from monkeys, humans, and pheasants, while ST9 was rarely isolated from humans (Stensvold et al. 2009; Tan 2008).

Studies conducted in different parts of the world showed that ST10 was the most common type detected on cattle along with ST1, ST3, ST5, ST6, and ST14. Until recently no genetic study has been conducted on *Blastocystis* isolated from cattle in Turkey. The aim of this study was to determine the existence and diversity of *Blastocystis* in cattle in different cities of Turkey.

In our study, a total of 80 different stool samples were collected from cows and calves under veterinary control after the spontaneous defecation at 13 distinct farms in Burdur and one farm in Aydın between April 2017 and November 2017. Burdur is a city in south of Turkey and Aydın is located in west part of Turkey. Only one sample was taken from each animal. The range of animal age was from 4 weeks to 4 years. All samples were delivered to the laboratory following cold chain rules.

Genomic DNA isolation was performed with Qiagen Stool mini kit (Qiagen, Hilden, Germany) from 200 mg fecal sample according to the manufacturer's instructions. The *Blastocystis* SSU-rDNA gene was amplified with the primers RD5 and BhRDr in a single PCR (Scicluna et al. 2006). The PCR products were subjected for examination by 2% agarose gel electrophoresis analysis and then observed in UV gel imaging system. The positive PCR products of *Blastocystis* were purified and sequenced by Macrogen Inc. All sequences were compared with 26 reference ST sequences in GenBank (http://www.ncbi.nlm.nih.gov/BLAST) (Maloney et al. 2018). The multiple-sequence alignments were done with ClustalW facility in Bioedit 7.2.6 (Hall 1999) using the default parameters. The neighbor-joining analyses were performed using genetic distances from the Kimura-2 parameter by MEGA 7.0 (Tamura et al. 2013) software. The reliability of cluster formation was evaluated using a bootstrap analysis of 1000 iterations.

Using molecular method, a total of 9 (11.25%) samples out of 80 samples were found to be positive for *Blastocystis* (Table 1) and these samples were sequenced. The SSU rRNA gene sequences of these identified subtypes were recorded in the database with access numbers GenBank MH125194-MH125197 and MH817024-MH817028.

Blastocystis is a protozoon that has been widely studied in recent years; there is still more to investigate about the epidemiology of this parasite with many unknowns for transmission and importance of animal reservoirs. Although epidemiology of human *Blastocystis* isolates are studied in Turkey, no study was done about epidemiology of these parasite from animal especially cattle. In present work, we studied molecular epidemiology of *Blastocystis* from cattle and found that, two of the cattle *Blastocystis* isolates were ST10 while remaining seven were ST14 according to the BLAST analyses in GenBank (Fig. 1). While 39 polymorphic sites were detected, nucleotide and haplotype diversity of these genotypes was 0.069 and 0.5, respectively. The mean ratio of transitions to transversions was 12:1. Tajima's D test of neutrality was not significant ($D = 0.112$, $P > 0.10$), indicating that the sequences

Table 1 Subtypes and prevalence of *Blastocystis* spp. detected from cattle in the world

Country	Number of subtypes	*Blastocystis* prevalence (positive/no. of examined)	References
Japan	ST1(1), ST3(1), ST6(6)	8/8 (100%)	Yoshikawa et al. 2004
Japan	ST1 (1), ST3 (2)	3/8 (37.5%)	Yoshikawa et al. 2003
Japan	ST1(1), ST3(2), ST5(7)	10/39 (26%)	Abe et al. 2003
Nepal	ST6 (1)	1/6 (16.7%)	Lee et al. 2012
Denmark	ST5 (3), ST10 (22)	25/25 (100%)	Stensvold et al. 2009
Libya	ST5 (2), ST10 (6), ST14 (2), mixed type (5)	15/36 (41.7%)	Alfellani et al. 2013
Colombia	ST1 (12), ST3 (8)	20/25 (80%)	Ramirez et al. 2014
England	ST1 (1), ST5 (1), ST10 (3), mixed type (2)	7/31 (22.6%)	Alfellani et al. 2013
Iran	ST5 (9), ST3 (2), ST6 (2), ST3 + ST5 (2), unknown (4)	19/196 (9.6%)	Badparva et al. 2015
USA	ST10 (13), ST14 (1), mixed type (2)	16/84 (19%)	Fayer et al. 2012; Santin et al. 2011
USA	ST3 (4), ST4 (18), ST5 (27), ST10 (5), ST14 (8), ST17 (3), ST21 (2), ST23 (1), ST24 (2), ST25 (1), ST26 (5)	73/2539 (2.9%)	Maloney et al. 2018
China	ST 4 (2), ST 5 (1), ST10 (41), ST14 (10)	54/526 (10.3%)	Zhu et al. 2017
China	ST3 (2), ST10 (10), ST14 (2)	14/147(9.5%)	Wang et al. 2018
Turkey	ST14 (7), ST10 (2)	9/80 (11.3%)	This study

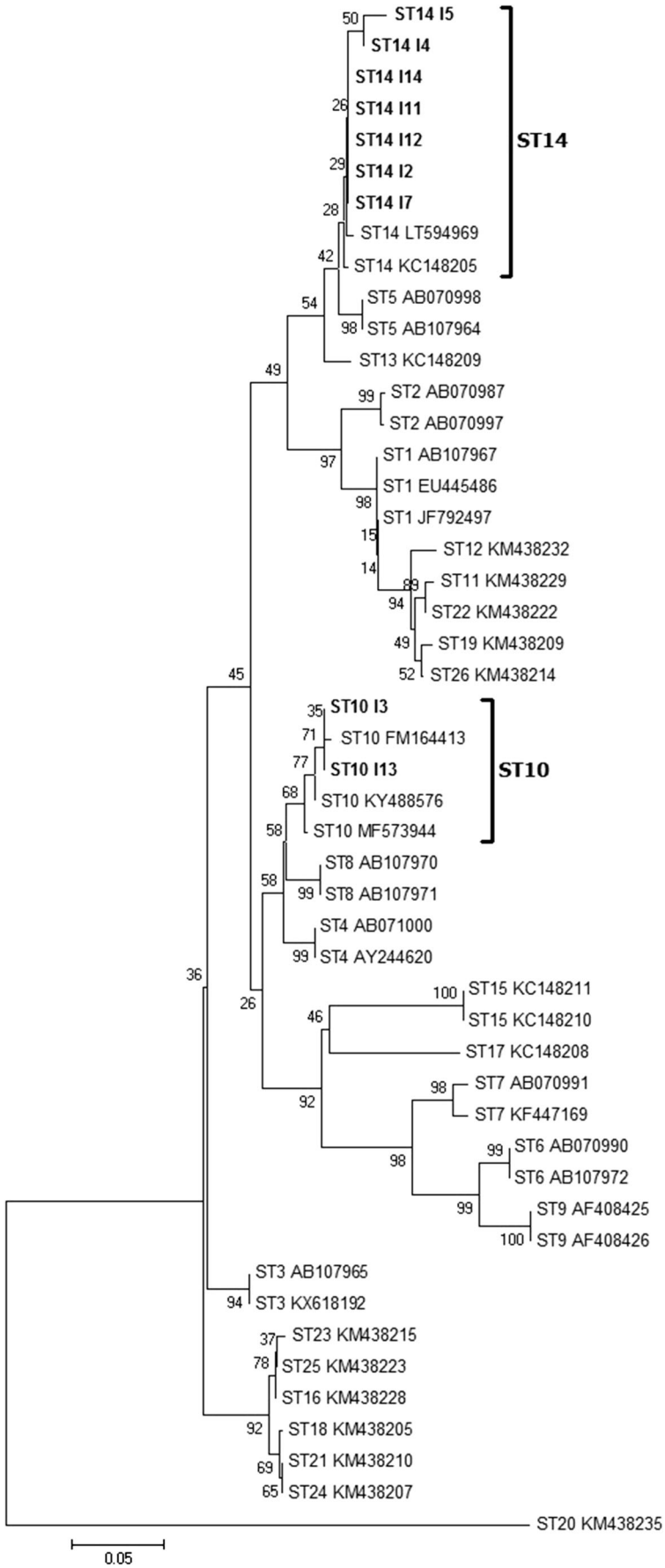

Fig 1 SSU rRNA gene sequences based neighbor-joining tree on maximum likelihood pairwise distance. Each subtype is named along with the Genbank Accession number. Numbers indicate bootstrap values of bootstrap replicates (Kimura-2 parameter, 1000 replications). Branch lengths indicate percent difference among haplotypes

evolved neutrally, thereby validating the use of phylogenetic analyses (Tajima 1989). In the present study, the *Blastocystis* subtypes were found in the same clade with ST10 and ST14 (Fig. 1).

Some of the *Blastocystis* subtypes have zoonotic potential. The probability of zoonotic potential of *Blastocystis* species isolated in Japan, Nepal, Iran, and Colombia is up to 100% (Yoshikawa et al. 2003, 2004; Abe et al. 2003; Lee et al. 2012; Badparva et al. 2015; Ramirez et al. 2014). Recent study in cattle reported other subtypes ST4 and ST5 as the most common subtypes in pre-weaned calves in the USA. However non-zoonotic subtypes are more frequent in USA (Santin et al. 2011; Fayer et al. 2012). The results of our study indicated the presence of two non-zoonotic subtypes (ST10 and ST14) among cattle in Turkey. Most of the studies investigated the *Blastocystis* subtypes in human fecal samples and environmental samples. Presence of ST1 and ST3 was shown from environmental samples taken from rivers in Black sea region in Turkey (Koloren et al. 2018). Dogan et al. 2017 reported that the most frequent *Blastocystis* subtype was ST3 (43.4%) followed by ST1 (26.1%), ST4 (10.9%), and ST2 (8.7%). They also found mixed subtypes in five samples: ST1 + ST3 ($n = 3$), ST1 + ST2 ($n = 1$), and ST2 + ST3 ($n = 1$). In addition, Sankur et al. (2017) identified a total of 33 *Blastocystis* isolates from children and found ST3 as the predominant subtype (34.2%), 11 isolates were ST1 (31.4%), nine isolates were (25.7%) were ST2, and one was (2.8%) ST7. In reviewing the literature so far, the *Blastocystis* subtypes reported from our country were ST1-4, 6, 7 (Dagcı et al. 2014; Ozyurt et al. 2008).

Our study is the first study that genotyped *Blastocystis* isolates from cattle and no previous study have reported the presence of *Blastocystis* ST10 and ST14 in Turkey. Further studies are required to follow up variations in subtypes present in cattle and zoonotic potential of the subtypes identified.

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