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*Original article*

# Molecular detection and subtype distribution of *Blastocystis* sp. from shelter dogs and cats in Van, Turkey: First report of ST10 in cats and ST1, ST10 and ST30 in dogs

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## Abstract

*Blastocystis* is an intestinal protist commonly found in humans and many different animal species. It is probably the most common enteric parasite with an estimated one billion infections worldwide. The fecal materials for this study were collected from 100 cats and 200 dogs different age and sex in shelter in Van, Turkey. DNA extraction, PCR amplification and sequence analysis were performed on the fecal samples. As a result, a prevalence of 1% (1/100) in cats and 1.5% (3/200) in dogs was detected. The prevalence was higher in both cats and dogs, in age groups younger than one year and in females according to gender. Sequence analysis revealed *Blastocystis* sp. ST10 in cats and *Blastocystis* sp. ST1, ST10 and ST30 in dogs. The sequences obtained were deposited in Genbank. In conclusion, stray cats and dogs may be a source of infection for other cats and dogs, and the detection of zoonotic ST1 in dogs suggests that dogs may be a reservoir for human infection.

**Keywords:** *Blastocystis* sp., phylogeny, cat, dog, zoonos, Turkey

## Introduction

*Blastocystis* sp. is an anaerobic protozoan found in the intestines of humans and various animals and in the environment (Daryani et al. 2008, Can et al. 2021, Tavur and Önder 2022, Çelik 2023). It is probably the most common enteric parasite with an estimated one billion infections worldwide (Maloney et al. 2019, Shams et al. 2022). *Blastocystis* sp. was first described in 1911 and named "*Blastocystis enterocola*" and then updated to *Blastocystis hominis* in 1912. Later, since genetic studies showed that there was only one species of *Blastocystis* sp. infecting humans and animals, it was decided to use the "*Blastocystis* sp. subtype nn" naming system for species isolated from humans and animals (Malatyali and Özçelik 2011, Coyle et al. 2012, Can et al. 2021, Çelik 2023)

To date, 32 subtypes have been identified based on sequence analysis of the small subunit (SSU) rRNA gene in humans and different animal species (Mahdavi et al. 2022, Shams et al. 2022). Among these, ST1-ST9 and ST12 are zoonotic (Can et al. 2021, Onder et al. 2021, Çelik et al. 2022), while the others (ST10, ST11, ST13-17, ST21 and ST23-32) are only seen in animals (Mahdavi et al. 2022, Shams et al. 2022). *Blastocystis* sp. is a polymorphic organism and has four forms: vacuolar, granular, amoeboid and cyst (Hemalatha et al. 2014, Can et al. 2021, Shams et al. 2022). Among these forms, fecal cyst is the only environmentally resistant infectious form (Hemalatha et al. 2014, Çelik et al. 2022).

*Blastocystis* sp. is reported to be transmitted via fecal-oral route and contaminated water or food (Moura et al. 2018, Gazzonis et al. 2019, Tavur and Önder 2022). However, this has not been experimentally confirmed (Hemalatha et al. 2014). Close contact between humans and animals may also play a role in zoonotic transmission (Osman et al. 2015, Onder et al. 2021). However, the extent and frequency of such events are largely unknown and require more in-depth research (Shams et al. 2022). *Blastocystis* sp. is seen in humans of various age groups (Moura et al. 2018). Although it is usually asymptomatic (Can et al. 2021), symptomatic people may show gastrointestinal symptoms such as abdominal pain, diarrhea, nausea, vomiting, bloating and loss of appetite (Hemalatha et al. 2014, Can et al. 2021, Shams et al. 2022). People in close contact with their animals may be more susceptible to this infection (Hemalatha et al. 2014). Microscopy, culture and molecular tests are the main methods used to detect infection. However, differentiation of subtypes is only possible using DNA-based methods and sequence analysis of the small subunit ribosomal RNA (SSU rRNA) gene (Maloney et al. 2019, Onder et al. 2021, Shams et al. 2022).

The number of studies on *Blastocystis* sp. in different animal species in Turkey is quite limited and studies on the determination of subtypes are very rare. In this study, it was aimed to determine the prevalence of *Blastocystis* sp. in shelter cats and dogs in Van province by molecular method and to identify zoonotic or animal-specific subtypes.

## Materials and Methods

### The study area and sample collection

This study was conducted between June and August 2023 in Van province, located in the Eastern Anatolia Region of Turkey (38°31′54″ N, 43°24′47″ E). The fecal materials for this study were collected from 100 cats and 200 dogs aged 0-5 years in the Animal Care Home of Van province. Fresh fecal samples were placed in individual sample containers and labeled. Age and sex information of each animal was recorded. The samples were then brought to the laboratory and stored at +4°C until analyzed.

### DNA extraction

DNA extraction was performed from all stool samples using GeneMATRIX Stool DNA Purification Kit (EURx, Gdańsk, Poland) according to the manufacturer's protocol. The DNAs obtained were stored at -20°C for further use.

### PCR amplification

For amplification of the SSU rDNA gene region of *Blastocystis* sp., Forward Blast (5′- GGA GGT AGT GAC AAT AAA TC-3′) and Reverse Blast (5′- TGC TTT CGC ACT TGT TCA TC-3′) primers previously reported by Santín et al. (2011) were used. The PCR products were stained with Safe-T-Stain™ (BioShop, Canada) and visualized on a 1.5% agarose gel.

### Sequence analysis and phylogeny

Bidirectional sequence analysis of positive samples was performed by a private company (BM Labosis, Ankara, Turkey). The DNA sequences obtained were checked and alligned in BioEdit program and were ready for analysis (Hall 1999). SSU rRNA sequences were aligned with the alignment algorithm using Neighbor Joining analysis (Tamura et al. 2021)in MEGA11 and then all variables in the original trace files were double-checked for confirmation. The phylogenetic relationship between species was established using the T92 and G models determined by Jmodeltest 2.0 for SSU rRNA alignments using 1000 bootstraps to build

Table 1. Distribution of *Blastocystis* sp. prevalence by sex and age in cats and dogs.

| Parameters | Examined | Positive | | *p* |
|---|---|---|---|---|
| | (n) | (n) | % | |
| Cat | | | | |
| Sex | | | | |
| Female | 68 | 1 | 1.47 | NS |
| Male | 32 | 0 | 0.00 | |
| Age (Year) | | | | |
| <1 | 18 | 1 | 5.56 | NS |
| 1-3 | 56 | 0 | 0.00 | |
| >3 | 26 | 0 | 0.00 | |
| Subtotal | 100 | 1 | 1.00 | |
| | | | | |
| Dog | | | | |
| Sex | | | | |
| Female | 127 | 2 | 1.57 | NS |
| Male | 73 | 1 | 1.37 | |
| Age (Year) | | | | |
| <1 | 46 | 2 | 4.35 | NS |
| 1-3 | 101 | 1 | 0.99 | |
| >3 | 53 | 0 | 0.00 | |
| Subtotal | 200 | 3 | 1.50 | |
| Total | 300 | 4 | 1.33 | |

NS – Non Significant

a phylogenetic tree. *Proteromonas lacertae* (U37108.1) was used as an outgroup.

### Statistical analysis

The data obtained in the study were analyzed using the SPSS V16.0 (IBM, Chicago, IL, USA) program. The relationship between grouped variables was calculated using chi-square test. The difference was considered statistically significant when $p<0.05$.

### Ethical approval

This study was approved by Van Yüzüncü Yıl University Animal Experiments Local Ethics Committee (grant number of 06062023-374731).

## Results

As a result of PCR analysis, the total prevalence was determined as 1.33% (4/300) (Table 1). As a result of the analysis, 500 bp bands specific for *Blastocystis* sp. were obtained in 1% (1/100) of cats and 1.5% (3/200) of dogs. In both cats and dogs, the prevalence was higher in youngers than one year according to age groups and in females according to gender ($p>0.05$).

Four sequences obtained from cats (OR398186.1) and dogs (OR398172.1, OR400936.1, OR398171.1) were deposited in GenBank. BLAST analysis showed that the *Blastocystis* species obtained in this study had high similarity compared to the data sets in GenBank (Table 2). Phylogenetic analysis of SSU rRNA gene sequences confirmed *Blastocystis* sp. STs in this study (Fig. 1).

## Discussion

*Blastocystis* sp. is an important pathogen that is widespread throughout the world (Maloney et al. 2019, Tavur and Önder 2022). Due to the zoonotic potential of this pathogen, animals can serve as a potential reservoir for human infection (Maloney et al. 2019, Mohammadpour et al. 2020, Onder et al. 2021). Dogs and cats can be potential reservoirs for humans by carrying these pathogens. Therefore, detection of *Blastocystis* sp. infection in animals is very important for human health (Shams et al. 2022).

Several studies have been conducted in different countries to determine the prevalence of *Blastocystis* sp. in dogs worldwide. A prevalence of 70.8% has been reported in Australia (Duda et al. 1998), 28% in Iran

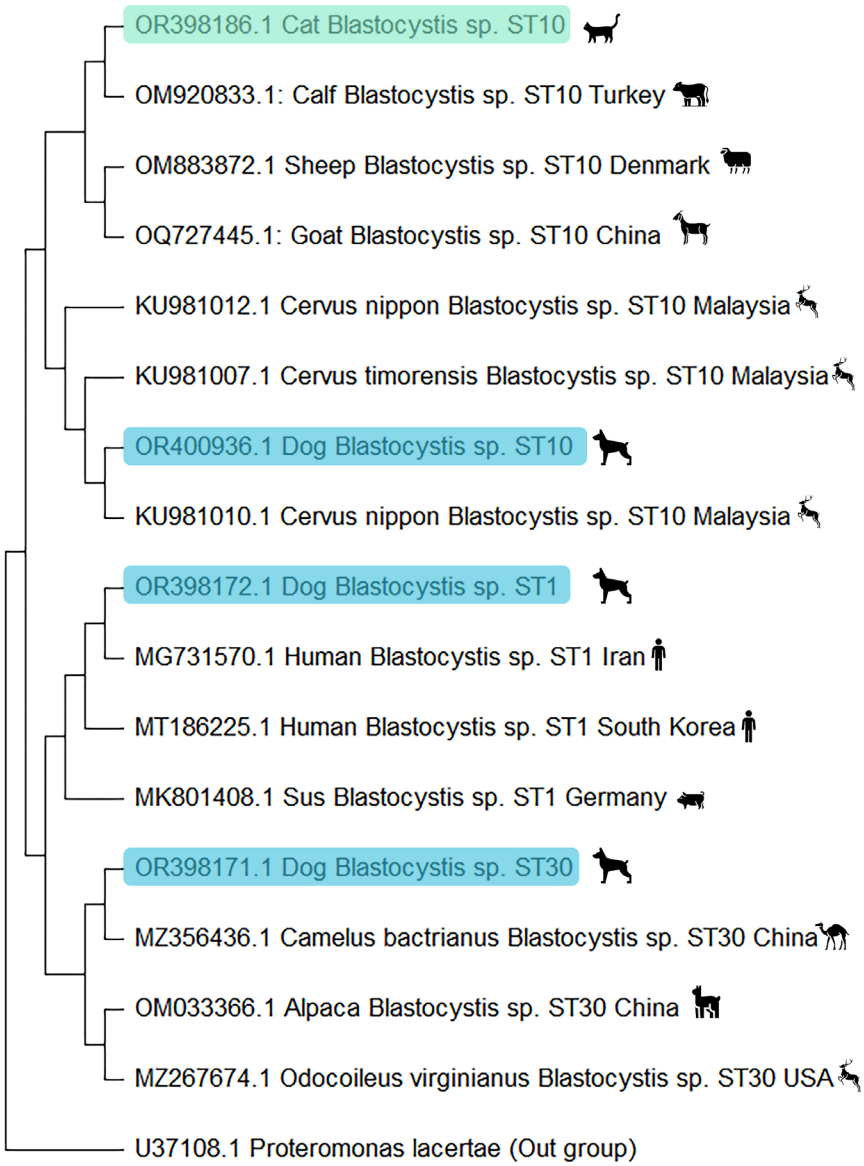


Fig. 1. Phylogenetic relationships of *Blastocystis* sp. isolates using the Neighbor Joining Method based on SSU rRNA gene region. Numbers in nodes represent Bootstrap values (1000 bootstrap). *Proteromonas lacertae* (U37108.1) was used as an outgroup.

Table 2. The DNA sequences deposited in GenBank as a result of this study.

| Obtained Sequences | | | | | Reference sequences from GenBank | |
|---|---|---|---|---|---|---|
| Pathogen | Host | Target Gene | Accession number | Length (bp) | Identity (%) | Accession number |
| *Blastocystis* sp. ST10 | Cat | SSU rDNA | OR398186.1 | 423 | 100 | OM920833 |
| *Blastocystis* sp. ST1 | Dog | SSU rDNA | OR398172.1 | 444 | 100 | MG731570.1 |
| *Blastocystis* sp. ST10 | Dog | SSU rDNA | OR400936.1 | 440 | 99.55 | KU981010.1 |
| *Blastocystis* sp. ST30 | Dog | SSU rDNA | OR398171.1 | 397 | 99.75 | MZ356436.1 |

(Daryani et al. 2008), 2.6% in Brazil (Moura et al. 2018), 24% in India (Wang et al. 2013), 1.3% in Cambodia (Wang et al. 2013), 3.4% in France (Osman et al. 2015), 14.5% in the Philippines (Belleza et al. 2016), 21.2% in Italy (Gazzonis et al. 2019), 5.4% in China (Liao et al. 2020), and 1.3% in Korea (Suh et al. 2022). In this study, 1.5% positivity was detected in dogs. This result was similar to the studies conducted by Wang et al. (2013), and Suh et al. (2022)

Several studies have been conducted in countries

such as the Philippines (ST1, ST2, ST3, ST4, ST5) (Belleza et al. 2016), Italy (ST3) (Gazzonis et al. 2019), Iran (ST1, ST2, ST3, ST4, ST7, ST8, ST10) (Mohammadpour et al. 2020, Mahdavi et al. 2022),, Korea (ST1, ST5, ST10, ST14) (Suh et al. 2022), and Poland (ST7) (Kaczmarek et al. 2020) to determine *Blastocystis* subtypes. In this study, as a result of sequence analysis of samples taken from dogs, ST1 and ST10 subtypes were detected, similar to studies in Iran (Mohammadpour et al. 2020, Mahdavi et al. 2022), and Korea (Suh et al. 2022), as well as ST30, a new subtype reported to be seen in white-tailed deer (Maloney et al. 2021) and sheep (Yang et al. 2023).

The ST1 sequence (OR398172.1) obtained from BLAST analysis in dogs showed 100% and 99.31% similarity with sequences obtained from humans in Iran (MG731570.1) and foxes in Spain (MK587500.1), respectively, while the ST10 sequence (OR400936.1) showed 99.55% similarity with the sequence obtained from sika deer in Malaysia (KU981010.1). The sequence ST30 (OR398171.1) is 99.75% and 99.50% similar to sequences obtained from camels (MZ356436.1) and alpacas (OM033366.1) in China, respectively. Blastocysts subtypes ST1-ST9 and ST12 are considered to be zoonotic since they occur in humans (Yan et al. 2007, Tan 2008, Can et al. 2021). The detection of ST1, which is reported to be zoonotic in this study, indicates that dogs may be a reservoir for humans.

In studies conducted in different parts of the world to determine the prevalence of *Blastocystis* sp. in cats; a prevalence of 67.3% % has been reported in Australia (Duda et al. 1998)1.7% in Nigeria (Okoye et al. 2014), 36% in Iraq (Albakri and Al-ani 2016), 0.6-0.82% in China (Li et al. 2019, Yongxia et al. 2023), 0.6% in Korea (Kwak and Seo 2020), 3.03%-17.7% in Iran (Mohammadpour et al. 2020, Karimi et al. 2023), 2.6% in Egypt (Naguib et al. 2022), and 3.65%-15.6% in Turkey (Can et al. 2021, Karakavuk et al. 2021). As a result of this study, 1% positivity was detected in cats. This result coincides with the findings of previous (Okoye et al. 2014, Li et al. 2019, Kwak and Seo 2020, Yongxia et al. 2023) research.

Studies have been conducted in various countries such as Korea (ST4) (Kwak and Seo 2020), Iran (ST1, ST3, ST4, ST10, ST14) (Mohammadpour et al. 2020, Karimi et al. 2023), Egypt (ST3, ST14) (Naguib et al. 2022), Algeria (ST2, ST3) (Boutellis et al. 2021), and Turkey (ST4) (Can et al. 2021) to determine *Blastocystis* sp. subtypes in cats.As a result of this study, the ST10 subtype was identified in agreement with the studies conducted by Mohammadpour et al. (2020) , and Karimi et al. (2023) in Iran. BLAST analysis shows that the sequence of ST10 (OR398186.1) is 100% similar to the sequence obtained from calves (OM920833) in Turkey.

In this study, the prevalence obtained from both dogs and cats was lower than the findings of some researchers (Duda et al. 1998, Daryani et al. 2008, Albakri and Al-ani 2016, Gazzonis et al. 2019, Mohammadpour et al. 2020). The reasons for the differences between the studies can be listed as hygienic conditions, immune status of animals, nutritional conditions, potential contamination of food and water, geographical variation, stress, sample sizes, sampling season and methods used (Onder et al. 2021).

In studies on *Blastocystis* sp. detection in animals, some researchers (Duda et al. 1998, Kwak and Seo 2020, Çelik et al. 2022, Suh et al. 2022) reported higher prevalence in females, while different studies (Daryani et al. 2008, Karimi et al. 2023) reported higher prevalence in males. The higher prevalence in females in both dogs and cats in this study is in line with the findings of researchers (Duda et al. 1998, Kwak and Seo 2020, Çelik et al. 2022, Suh et al. 2022) ($p>0.05$).

Studies by Daryani et al. (2008), and Tavur and Önder (2022)reported a higher prevalence in adults, while other (Osman et al. 2015, Çelik et al. 2022, Çelik 2023, Karimi et al. 2023) reported a higher prevalence in younger than one year. The higher prevalence in animals younger than one year of age in this study supports the findings of the researchers (Osman et al. 2015, Çelik et al. 2022, Çelik 2023, Karimi et al. 2023) ($p>0.05$). The reason for the higher prevalence in small animals may be due to the underdeveloped immune system of these animals.

## Conclusion

In this study, molecular characterization and subtype distribution of *Blastocystis* sp. were determined in shelter cats and dogs in Van province. As a result of this study, ST10 subtypes were detected in cats and ST1, ST10 and ST30 subtypes in dogs. This indicates that stray cats and dogs may be a source of infection for other cats and dogs. The fact that ST1, which was detected in dogs and suggested to be zoonotic, was also detected suggests that dogs may be a reservoir for human infection. We believe that it would be useful to conduct more large-scale molecular and genetic studies including different animal species in the region.

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