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Molecular Prevalence of *Blastocystis* sp. in Anatolian Water Buffaloes in Diyarbakır, Turkey

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ABSTRACT

The water buffaloes raised in Turkey originate from the Mediterranean water buffalo, a subgroup of the river buffalo, and are called the Anatolian buffalo. *Blastocystis spp.* is an anaerobic enteric protozoan parasite that lives in the gastrointestinal tract of a wide variety of hosts, including humans. This study aims to determine the molecular prevalence of *Blastocystis* spp. in Anatolian buffaloes in Diyarbakır province. Fresh fecal samples from animals were placed in individual fecal sample containers. The sex and age of the animal were recorded for each sample collected. As a result of PCR analysis, specific bands of 500 bp were obtained in 34 (17%) of 200 samples. Prevalence by age groups was found 27.69% in the 0–1-year age group and 11.85% in those older than one year ($P<0.05$). The prevalence by sex was 17.46% in females and 16.22% in males ($P>0.05$). As a result of this study, the presence of *Blastocystis* spp. was revealed in Anatolian Water Buffaloes in Diyarbakır. It is thought that further studies are needed to determine the zoonotic subtype potential of the agent in the region.

1.INTRODUCTION

Blastocystis spp. is an anaerobic enteric protozoan parasite that lives in the gastrointestinal tract of a wide variety of hosts, including humans (AbuOdeh et al., 2019, Maloney et al., 2019, Moura et al., 2018, Sreekumar et al., 2014, Zhu et al., 2017). *Blastocystis spp.* is probably the most common human intestinal parasite in the world, with an estimated one billion infections worldwide (Andersen & Stensvold, 2016, Maloney et al., 2019).

Blastocystis is currently subdivided into at least 28 subtypes (ST1-ST17, ST21, ST23-ST32) that are probably separate species, all found on mammalian and avian hosts (Jinatham et al., 2021, Maloney et al., 2019). Several genetically distinct *Blastocystis* lineages have also been described in amphibian, insect, and reptile hosts, but they are not yet part of the subtyping nomenclature (Jinatham et al., 2021).

Blastocystis spp., a polymorphic organism, has four forms: vacuolar, granular, amoeboid, and cysts (Hemalatha et al., 2014, Jinatham et al., 2021, Kamaruddin et al., 2020). Among all forms, the fecal cyst is the only environment-resistant infectious form (Hemalatha et al., 2014). It has been suggested that *Blastocystis* sp. Is transmitted by the fecal-oral route through water and food contaminated with human and animal fecal wastes (Hemalatha et al., 2014, Lee et al., 2018, Maloney et al., 2019, Moura et al., 2018, Noradilah et al., 2017, Zhu et al., 2017). The pathogenicity of *Blastocystis* is associated with factors such as subtype variation and host immune status, but the exact mechanism is controversial (Elwakil & Hewedi, 2010, Zhu et al., 2017). Close contact with infected animals may pose risks of zoonotic transmission of Blastocystis (Zhu et al., 2017).

While the pathogenic potential of Blastocystis in humans remains unclear (AbuOdeh et al., 2019), one study reports that *Blastocystis* sp. is pathogenic (El

Safadi et al., 2014). It has been reported that the rate of infection is higher in zoos and slaughterhouse workers who are in contact with animals more often than in others (Abe et al., 2002, Lee et al., 2018). It has been reported that *Blastocystis* spp. is seen in both symptomatic and asymptomatic individuals (AbuOdeh et al., 2019, El Safadi et al., 2014), Recently, the organism has also been shown to be associated with human irritable bowel syndrome (Maloney et al., 2019, Sreekumar et al., 2014).

The water buffaloes raised in Turkey originate from the Mediterranean water buffalo, a subgroup of the river buffalo, and are called the Anatolian buffalo (Şahin et al., 2013). In Turkey, buffaloes are mostly found in the provinces of Samsun, Sinop, Çorum, Amasya, Afyon, Balıkesir, Sivas, Muş and Diyarbakır (Atasever & Erdem, 2008, Yılmaz & Kara, 2019). This study was carried out to determine the molecular prevalence of *Blastocystis* spp. in Anatolian buffaloes in Diyarbakır province.

2.MATERIAL AND METHODS

2.1. The Study Area

This study was carried out in Diyarbakır province located in the Southeastern Anatolia Region of Turkey (370.52' N, 400.13' E) (Figure 1).

2.2. Animal Material and Sample collection

The animal material of the study consisted of 200 Anatolian Water Buffaloes of different sexes and ages (Figure 2). Fresh fecal samples from animals were placed in individual fecal sample containers. The sex and age of the animal were recorded for each sample collected. The samples were brought to the laboratory in the cold chain and stored at +4°C until analyzed.

2.3. DNA extraction

DNA extraction was performed from all samples using the GeneMATRIX Stool DNA Purification Kit following the kit protocol. The obtained DNAs were stored at -20°C until further analysis.

2.4. PCR amplification

Forward Blast (5′- GGA GGT AGT GAC AAT AAA TC-3′) and Reverse Blast (5′- TGC TTT CGC ACT TGT TCA TC-3′) primers were used for the amplification of the SSU rDNA gene region of *Blastocystis* spp. (Santín et al., 2011). 8 pmol of forward and reverse primers, 4 µl of 5x FIREPol® Master Mix (7.5 mM MgCl2, Solis BioDyne, Estonia) 1.6 µl of DNA and 12.8 µl of Nuclease Free Water were used in 20 µl of mastermix. The reaction was followed by pre-denaturation for 5 minutes at 95°C, with each cycle consisting of denaturation (30 s at 95°C), bonding (30 s at 54°C), and elongation (30 s at 72°C) steps and 35 cycles and a final elongation of 5 minutes at 72°C. The resulting PCR products were stained with RedSafe™ Nucleic Acid Staining Solution and images were obtained on 1.5% agarose gel.

2.5. Ethical approval:

This study was approved by Dicle University Animal Experiments Local Ethics Committee (Document number: 30/05/2022-294534)

2.6. Statistical analysis

The relationship between grouped variables was analyzed using an SPSS V16.0 for the chi-square test. $P<0.05$ was considered statistically significant.

3.RESULTS

The prevalence of *Blastocystis* spp. in Buffaloes by sex and age is given in Table 1. As a result of PCR analysis, specific bands of 500 bp were obtained in 34 (17%) of 200 samples (Figure 3). Prevalence by age groups was found 27.69% in the 0-1year age group and 11.85% in those older than one year ($P<0.05$). The prevalence by sex was 17.46% in females and 16.22% in males ($P>0.05$).

Table 1. Sex and age-wise prevalence of *Blastocystis* spp. In Anatolian water buffalo

Variable	Examined (n)	Positive		P
		(n)	(%)	
Sex				
Female	126	22	17.46	0.821
Male	74	12	16.22	
Age (year)				
0-1	65	18	27.69	0.005
>1	135	16	11.85	
Total	200	34	17.00	

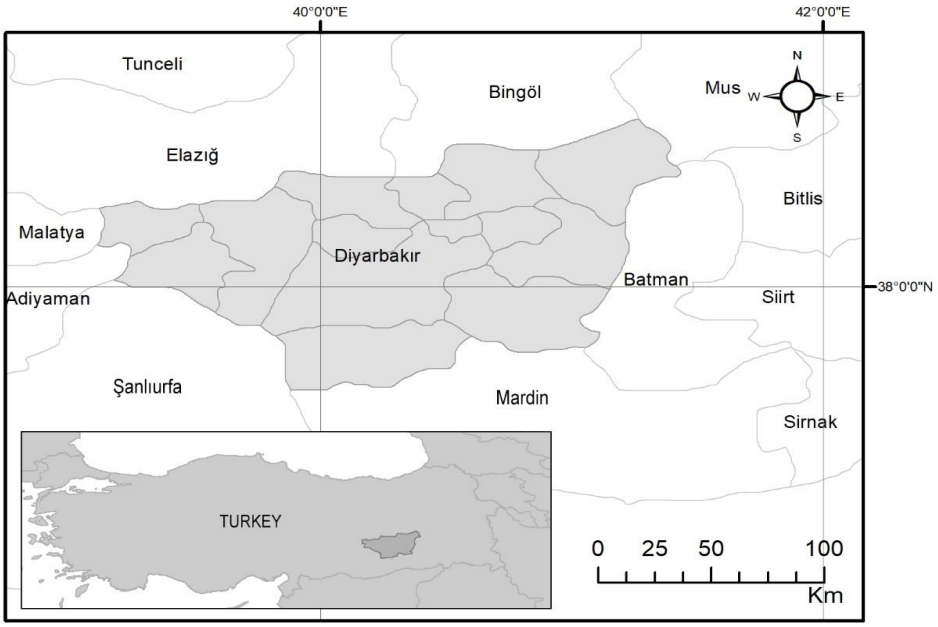

Figure 1. Maps of the study area

Figure 2. Anatolian Water Buffalo (*Bubalis bubalis*)

4.DISCUSSION

Blastocystis is a disease that has been found in both humans and animals (Maloney et al., 2019). Although the prevalence of the disease in humans varies according to geographical regions, it is reported that it is higher in developing countries than in developed countries, probably due to differences in hygiene standards, waste disposal, contact with infected animals, and consumption of contaminated food or water (AbuOdeh et al., 2019).

Many studies have been carried out to determine the prevalence of *Blastocystis* in cattle worldwide (Abarca et al., 2021, Abe et al., 2002, AbuOdeh et al., 2019, Aynur et al., 2019, Gabrielli et al., 2020, Hemalatha et al., 2014, Kamaruddin et al., 2020, Lee et al., 2018, Maloney et al., 2019, Mohammad Rahimi et al., 2021, Moura et al., 2018, Ramírez et al., 2014, Tavur & Önder, 2022, Zhu et al., 2017). However, studies investigating the prevalence of *Blastocystis* sp. in buffaloes are very limited. In studies on buffaloes, 17.82% in Italy (Gabrielli et al., 2021), 21.05% in Nepal (Lee et al., 2012), 35% in Turkey (Onder et al., 2021), in Thailand it was reported that *Blastocystis* sp. was detected in all four buffalo fecal samples (Jinatham et al., 2021), in one buffalo calf feces in India (Sreekumar et al., 2014).

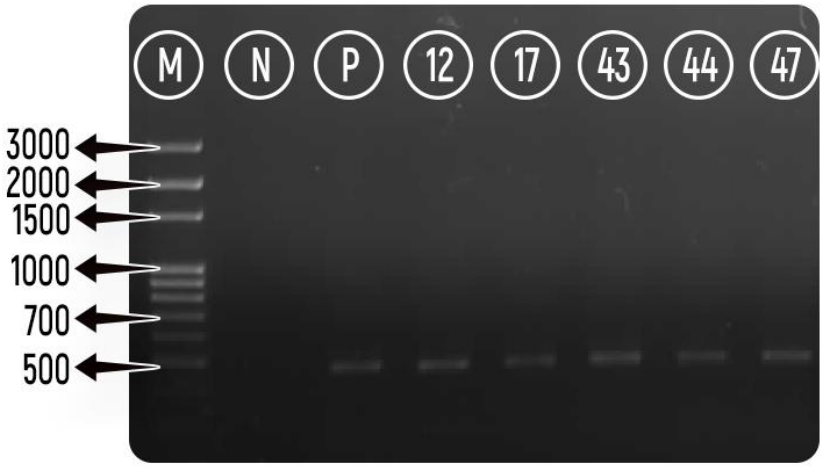

Figure 3. SSU rDNA amplification of *Blastocystis* spp. using nested-PCR. Lanes M: Marker, N: Negative control, P: positive control, Lanes 12, 17, 43, 44, and 47represent Blastocystis positive samples (500 bp).

Microscopic methods, culture, and molecular methods (PCR) are used in the diagnosis of *Blastocystis* (Ertuğ et al., 2015). It is reported that the PCR protocol is more sensitive and specific when compared to the microscopic examination and culture method (Malatyali & Özçelik, 2011, Popruk et al., 2013, Stensvold et al., 2009). As a result of this study, 34 (17%) of the 200 samples for which PCR analyzes were performed were found to be *Blastocystis* positive. These results are similar to the results reported by the researchers (Gabrielli et al., 2021, Lee et al., 2012).

Geographical location, sampling season, number, age and immune status of animals, care and feeding conditions, stress, and methods used can be counted among the reasons for the differences observed between studies (Lee et al., 2018, Onder et al., 2021, Zhu et al., 2017).

Researchers (Daryani et al., 2008, Duda et al., 1998, Kamaruddin et al., 2020) reported that the infection was more common in females than males. In this study, the higher prevalence in females (17.46%) than males (16.22%) support the researchers (P>0.05). While Tavur & Önder (2022) and Daryani et al. (2008) reported a higher prevalence in adults, other researchers (Kamaruddin et al., 2020, Lee et al., 2018) reported a higher prevalence in young ones. The fact that the highest prevalence was detected in the under 1-year-old group in this study supports the researchers (P<0.05) (Kamaruddin et al., 2020, Lee et al., 2018)

5.CONCLUSION

As a result of this study, the presence of *Blastocystis* spp. was revealed in Anatolian Water Buffaloes in Diyarbakır. ST4 and ST7 subtypes, which are also seen in humans, have been identified in studies carried out in Nepal and Thailand (Jinatham et al., 2021, Lee et al., 2018). Such animals can be a source of infection for human *Blastocystis* through direct contact or contamination of the water supply. Therefore, it is thought that further studies are needed to determine the zoonotic subtype potential of the agent in the region.

6.ACKNOWLEDGEMENT

Conflicts of interest

The authors declare that there is no conflict of interest.

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