



First report of *Blastocystis* spp. subtypes in ZOO animals in Slovakia, Central Europe

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A – Research concept and design, B – Collection and/or assembly of data, C – Data analysis and interpretation,
D – Writing the article, E – Critical revision of the article, F – Final approval of the article

Danišová O, Valenčáková A, Kandráčová P, Tomko M, Sučík M. First report of *Blastocystis* spp. subtypes in ZOO animals in Slovakia, Central Europe. Ann Agric Environ Med. 2022; 29(1): 149–151. doi: 10.26444/aaem/145826

Abstract

Blastocystis spp. has been reported in wildlife, domestic animals and animals housed in ZOO. To-date, 17 genetically diverse lines have been reported in mammals and birds (designated ST) based on differences in the SSU rRNA. In this study, faeces samples were collected from 24 ZOO animals with clinical signs suggestive of gastrointestinal disease in Košice ZOO, Slovakia. After DNA isolation, PCR was conducted to amplify the SSU region of DNA of *Blastocystis* species. Forward primer- Blast F and reverse primer- Blast R were used in the reaction. From 25 faeces samples, *Blastocystis* spp. was detected in 5 animals (3 mammals, 2 birds), with a prevalence of 20%. Subsequent molecular analyses identified the ST 5 (n = 3), ST 7 (n = 1), and ST 12 (n = 1) subtypes, where the ST 5 subtype was identified in the mammalian group and birds, and the ST 7 and ST 12 subtypes were identified only in mammals. Based on these findings, focusing on ZOO animals as a potential source of infection for humans is highly recommended.

Key words

PCR, Slovakia, *Blastocystis* spp., subtypes, ZOO animals

INTRODUCTION

Blastocystis spp. is an unicellular microorganism found in the gastrointestinal tract of humans and various animals, including primates, amphibians, reptiles, and even insects [1, 2, 3, 4, 5]. Although samples taken from infected humans and animals were morphologically indistinguishable from each other, a remarkable genetic diversity between samples from both humans and animals was demonstrated [2, 6]. To-date, 17 genetically diverse lines have been reported in mammals and birds (subtypes; designated ST) based on differences in the small subunit of ribosomal RNA (SSU rRNA) [7]. *Blastocystis* spp. has a wide range of hosts, with the same subtype found in several animal species. Ten of the 17 STs (ST1 – ST10) were detected in humans, while ST 9 was identified only in humans, which can be considered a human-specific subtype. *Blastocystis* spp. has been reported in wildlife, domestic animals, and ZOO animals [3, 8, 9, 10, 11, 12], and several authors have described the distribution of *Blastocystis* spp. among animals in ZOOs [2, 3, 13]. The diversity of subtypes of *Blastocystis* spp. in animals in such a small area as a ZOO is an alarming fact. Infected animals are a threat not only to other species but also to staff and visitors [13, 14]. Therefore, additional epizootiologic data need to be provided to identify potential sources of *Blastocystis* infection in animals, which was also the aim of this ZOO animal study.

MATERIALS AND METHOD

Study population – samples. Samples of faeces were collected from 24 animals (9 mammals, 15 birds, Tab.1) in the ZOO in Košice, Slovakia. Faecal samples were collected only from animals with clinical signs of gastrointestinal disease (anorexia, diarrhoea, abdominal pain, and weight loss to cachexia).

DNA isolation, PCR, electrophoresis, sequencing. The procedure for DNA isolation, subsequent PCR, electrophoresis and sequencing, followed the protocol according to Danišová et al. (2021) [15].

RESULTS

In 25 faeces samples, *Blastocystis* spp. was detected in 5 animals (3 mammals, 2 birds), with a prevalence of 20 %. Subsequent molecular analyses identified the ST 5 (n = 3), ST 7 (n = 1), and ST 12 (n = 1) subtypes. The ST 5 subtype was detected in the mammalian and bird groups, and the ST 7 and ST 12 subtypes only in mammals. Five sequences identified by the sequencing service and evaluated by BLAST as *Blastocystis* spp. were used to create a phylogenetic tree. There were also 29 sequences of 16 individual subtypes stored in GenBank, 2 sequences of the group *Katotomorpha* spp. DQ431243 and *Protoopalina intestinalis* AY576545. The comparison and placement of branches in the phylogenetic tree confirmed the identification of individual subtypes (Fig. 1). The sequences identified in this study were deposited in the GenBank database (Accession Nos. OK431085, OK431086, OK431087, OK431088, OK431089) (Tab. 1).

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Received: 09.06.2021; accepted: 13.01.2022; first published: 27.01.2022

DISCUSSION

In the authors' epizootiologic survey, mammalian and bird animal groups were represented. Primates were the most tested animals in the group of mammals due to their high incidence of this disease worldwide. In Italy, there was the highest prevalence of *Blastocystis* spp. in long-tailed macaques 87.6% [16], in Ecuador, the majority of this species reached 60.4% in a group of monkeys [17], and Tanzania reported a prevalence of 49% – 51.3% in wild chimpanzee communities and 71.4% to 92% in monkeys [18, 19, 20]. In the current study, *Blastocystis* spp. was found in 2 of the 5 examined primates, which represents a prevalence of 40%. In a group of other tested mammals (Tab. 1), blastocystosis was confirmed in a single wild boar, which suggests that the detection of blastocystosis in wild animals is difficult because of their free movement in nature. A study conducted by Danišová et al. in Slovakia (2021) [15], also focused on identifying this

Table 1. Species of tested animals and subtypes found.

Isolate	Mammals	<i>Blastocystis</i> sp./subtype	GenBank Accession Nos.
1 przewalski's horse	<i>Equus ferus przewalskii</i>		
2 bactrian camel	<i>Camelus bactrianus</i>		
3 wild boar	<i>Sus scrofa</i>		
4 wild boar	<i>Sus scrofa</i>	<i>Blastocystis</i> ST 12	OK431086
5 tufted capuchin	<i>Sapajus apella</i>		
6 patas monkey	<i>Erythrocebus patas</i>		
7 barbary macaque	<i>Macaca sylvanus</i>		
8 ring-tailed lemur	<i>Lemur catta</i>	<i>Blastocystis</i> ST 7	OK431089
9 black-and-white ruffed lemur	<i>Varecia variegata variegata</i>	<i>Blastocystis</i> ST5	OK431085
Birds			
10 spur-winged goose	<i>Plectropterus gambensis gambensis</i>		
11 spur-winged goose	<i>Plectropterus gambensis gambensis</i>		
12 southern ground hornbill	<i>Bucorvus leadbeateri</i>		
13 papuan hornbill	<i>Rhyticeros plicatus ruficollis</i>		
14 marabou stork (male)	<i>Leptoptilos crumeniferus</i>	<i>Blastocystis</i> ST5	OK431088
15 marabou stork (female)	<i>Leptoptilos crumeniferus</i>		
16 emu	<i>Dromaius novaehollandiae</i>		
17 emu	<i>Dromaius novaehollandiae</i>		
18 emu	<i>Dromaius novaehollandiae</i>		
19 mandarin duck	<i>Aix galericulata</i>		
20 bernier's teal	<i>Anas bernieri</i>	<i>Blastocystis</i> ST5	OK431087
21 white-winged duck	<i>Asarcornis scutulata</i>		
22 eurasian spoonbill	<i>Platalea leucorodia</i>		
23 little egret	<i>Egretta garzetta</i>		
24 greater rhea	<i>Rhea americana</i>		
Σ ZOO animals		5	

pathogen. The highest prevalence of *Blastocystis* spp. has been reported in birds (up to 82%), making them the highest risk for humans. ZOO birds, however, show a much lower prevalence, presumably due to more frequent cleaning their cages and runs than other animals, as confirmed by other surveys [13, 21, 22]. In the current study, *Blastocystis* spp. was identified only in 2 of the 15 samples collected from birds (13.3%), consistent with study results worldwide.

Out of the 17 *Blastocystis* ST, subtypes ST1-ST8, ST10, ST13- ST15, and ST17 have been identified worldwide, with varying prevalence among groups of animals. The huge genetic diversity of *Blastocystis* spp. was also pointed out among the animals in the ZOO [2, 4, 21, 23]. In this study, of the 25 analyzed samples, 3 subtypes were identified. In Košice ZOO, the waterfowl of the avian group is located at 2 different places (Fig. 2). The most commonly found subtypes of *Blastocystis* spp. in birds are ST6 and ST7, characterized as 'avian' [6, 24]. However, ST1, ST4, and ST5 have also been identified in ZOO animals; ST5 is the most often presented in pigs. ST 12 was found in a positive wild boar's sample, which is typical for ruminants [3].

The third subtype – ST7, was identified in Lemur catta and ST5 was also identified in black and white ruffed lemur (*Varecia variegata variegata*). These 2 primates are placed next to each other, although different subtypes of *Blastocystis* were identified (Fig 1, Fig 2). Worldwide, ST1-ST8 subtypes have been detected in primates, and interestingly, animal isolates of all the isolated subtypes have been matched to isolates from stool samples in members of staff [2, 14, 25].

CONCLUSION

This study is the first in Slovakia to deal with *Blastocystis* spp., in ZOO animals. The results indicate the need to focus on ZOO animals as a potential source of infection for humans [13, 23]. These are, in particular, the zoonotic subtypes ST5 and ST7, which have been identified in primates that are close to care keepers, or the subtype ST5 in waterfowl, where visitors can also be in the proximity of the animals. These data

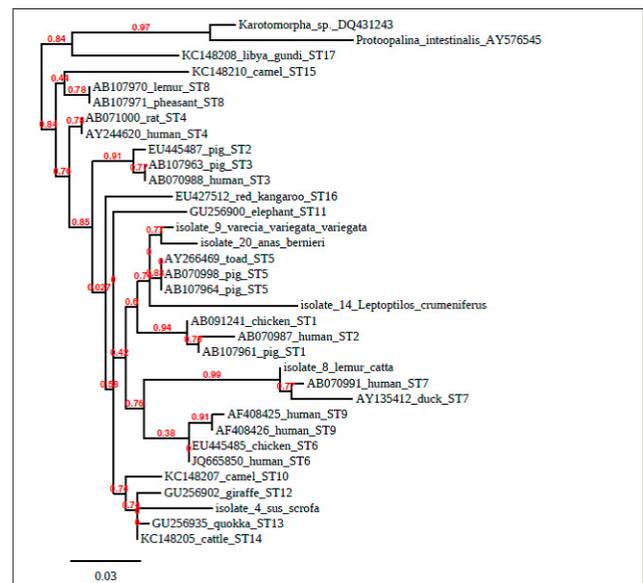


Figure 1. Branches in the phylogenetic tree confirming the identification of individual subtypes



Figure 2. Map of the ZOO Košice

also emphasize the importance of screening other *Blastocystis* spp. hosts to broaden epizootiological and epidemiological information on the occurrence of this parasite, and to take preventive and control measures to help reduce the incidence of *Blastocystis* spp. in ZOO animals, and thus minimize the risk of transmission of the zoonosis from animals to staff.

Funding Statement

This project was created with the support of the grant project of the Ministry of Education, Science, Research and Sport of the Slovak Republic VEGA no. 1/0113/20.

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