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Short note

Blastocystis isolates from a dog and their owners presenting with chronic diarrhoea. Dogs as reservoirs of *Blastocystis*: research in Poland and worldwide.

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ABSTRACT. *Blastocystis* cf. *hominis* is an unicellular protozoan parasite commonly found in the gastrointestinal tract of humans and animals. *Blastocystis* is characterized by high morphological and genetic diversity. Studies based on the analysis of *Blastocystis* spp. small subunit ribosomal RNA genes (SSU rDNA) have identified 26 subtypes (ST) so far, including at least 10 isolated from humans (STs 1–9 and ST12). In 2017, stool samples from a dog and its two owners living in Gdynia, Poland were examined; all three were suffering from chronic diarrhoea. In addition, 30 faecal samples were also examined from 30 dogs kept in one of Warsaw's hotels for animals. Stool specimens were analyzed using anaerobic cultivation at 37°C with a modified Jones' medium and molecular methods (PCR). Phylogenetic analysis using Bayesian inference was performed. Vacuolar forms of *Blastocystis* were identified in the stool samples of the dog and its owners; *Blastocystis* were not detected in any sample from the dogs living in the animal hotel. Based on the phylogenetic analysis, the obtained isolates were classified as subtype ST3 (for Owner 1) and subtype ST7 (for Owner 2 and the dog). To the best of our knowledge, the present study is the first to evaluate the presence of *Blastocystis* in canines in Poland, including domestic dogs.

Keywords: *Blastocystis* cf. *hominis*, dog, zoonosis, PCR

Introduction

Blastocystis cf. *hominis* is an unicellular protozoan parasite commonly found in the gastrointestinal tract of humans and animals [1,2]. Its worldwide distribution means it is one of the most frequently-detected protozoans in humans [3]; however, its occurrence depends on the geographic region and various socioeconomic and environmental factors. Epidemiological data demonstrates that prevalence of *Blastocystis* infection varies from 20% in developed countries [4] up to 100% in developing countries [5].

Blastocystis is characterized by high morphological and genetic diversity. Studies based on the analysis of *Blastocystis* spp. small subunit ribosomal RNA genes (SSU rDNA) have identified 26 subtypes (ST) so far, including at least 10 isolated from humans (STs 1–9 and ST12). Most of the STs isolated from humans are ST1 through ST4; subtypes ST5 through ST9, and ST12, considered animal, are less common in humans [4,6,7].

Zoonotic transmission of *Blastocystis* (from animals to humans) can occur through several routes: direct transmission (poor hand hygiene) or

via consumption of contaminated water or food. Research carried out in zoos in the United Kingdom, Philippines and Australia have confirmed the presence of the same genotypes of *Blastocystis* in animals and their carers [7–10]. In addition, Lee et al. [11] identified the same *Blastocystis* subtype in humans and their animals which draw water from a river.

The impact of *Blastocystis* on human health is still unclear, but many reports suggest it acts as a diarrheagenic agent [12].

In this paper we describe the first case of *Blastocystis* cf. *hominis* ST7 in a dog in Poland and the results of a study of 30 dogs kept in a hotel for animals.

Materials and Methods

In 2017, stool samples from a dog and its two owners living in Gdynia were examined; all three were suffering from chronic diarrhoea. In addition, 30 faecal samples were also examined from 30 dogs kept in one of Warsaw's hotels for animals.

Identification was performed using the microscopic method and by anaerobic cultivation at 37°C using modified Jones' medium supplemented with 10% horse serum [13]. The cultures were examined for the vacuolar form after 48 hours using light microscopy [14].

Any positive cultures were sub-cultured onto new medium and incubated for 48 hours, after which the cell pellet was separated by centrifugation at 70×g for one minute. DNA isolation from the cell pellet was performed using the Genomic Mini kit (A&A Biotechnology, Gdynia, Poland). A small subunit ribosomal RNA gene (SSU rDNA) fragment of about 560 bp was amplified by PCR method, with forward primer RD5 (5′-ATCTGGTTGATC CTGCCAGT-3′) [15] and reverse primer BhRDr (5′-GAGCTTTTTAACTGCAACAACG-3′) [16].

All PCR products were sequenced and the obtained sequences were compared to *Blastocystis* sequences deposited in GenBank. Phylogenetic analysis using Bayesian inference was performed using MrBayes 3.2.7a [17,18] including 24 reference sequences representing *Blastocystis* cf. *hominis* ST1–ST9 subtypes [8,14,19]. *Proteromonas lacertae* (GenBank: U37108) was used as an outgroup. *Blastocystis* subtype nomenclature follows that of Stensvold et al. [20]. The sequences reported in this paper were deposited in the GenBank database with the accession numbers MW346667–MW346669.

Figure 1. Vacuolar form of *Blastocystis* cf. *hominis* from culture (Owner 1 sample). Wet mount, Lugol's iodine. Scale bar 100 µm.

Results and Discussion

Vacuolar forms of *Blastocystis* were identified in the stool samples of the dog and its owners from Gdynia, Poland (Fig. 1). However, *Blastocystis* were not detected in any sample from the dogs living in the animal hotel. Based on the phylogenetic analysis, the obtained isolates were classified as subtype ST3 (for Owner 1) and subtype ST7 (for Owner 2 and the dog) (Figs. 2–3).

Both subtypes have previously been reported in Poland. *Blastocystis* cf. *hominis* ST3 is considered to be typically found in humans. In Poland, subtype

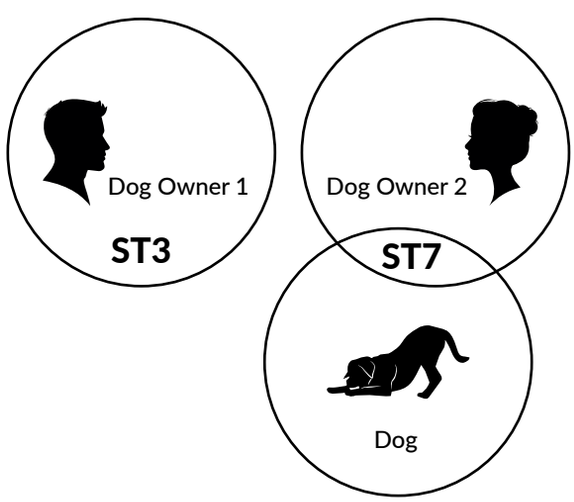

Figure 2. *Blastocystis* subtypes confirmed in the tested samples in this study

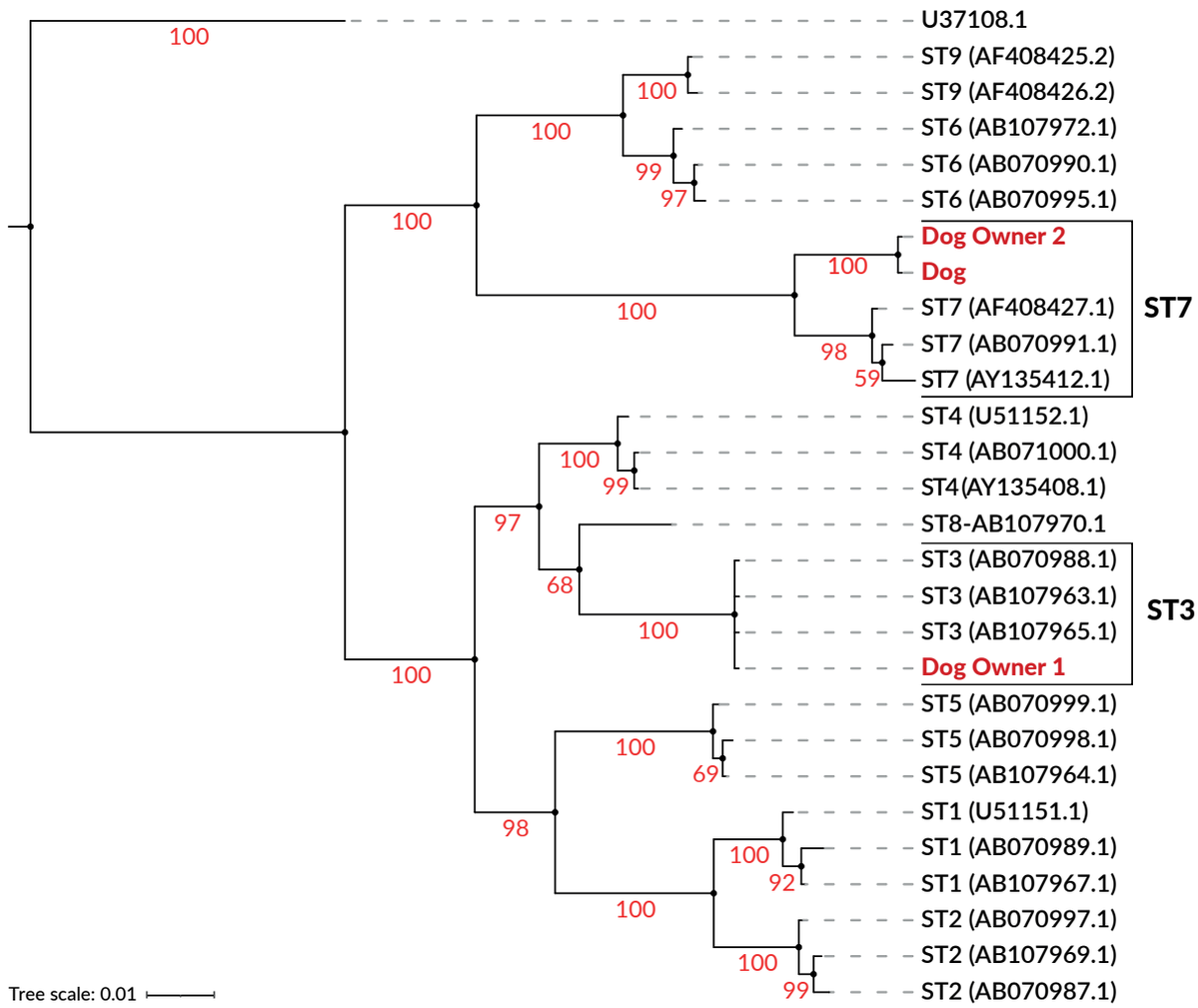

Figure 3. Bayesian inference tree based on fragment of sequences (536 bp and 548 bp) obtained at the small-subunit rRNA gene (SSU rDNA) of *Blastocystis* isolates of the present study, performed using MrBayes 3.2.7a [17,18]. The Bayesian posterior probabilities are shown adjacent to branch nodes.

ST3 was first detected in humans by Kotłowski [21] and later by Kaczmarek et al. [14,22]; Sałamatin et al. [23,24]; Wesołowska et al. [25,26]; its presence has also been noted by other researchers [27–32].

Blastocystis cf. *hominis* ST7 is considered typical for birds. While it was detected for the first time in Poland in chickens [33,34], it was later identified in humans [14,22,25,29–31].

The first animal detection of *Blastocystis* in Poland was made in 2014 in chickens [33–35]. Since then, *Blastocystis* has been detected in mammals at the Wrocław Zoo [36] and in pigs [32].

To the best of our knowledge, the present study is the first to evaluate the presence of *Blastocystis* in canines in Poland, including domestic dogs.

However, the presence of *Blastocystis* cf. *hominis* ST3 has been reported in dogs in Denmark [9] and Italy [37], and subtypes ST2 and ST10 in France [38]. *Blastocystis* has also been observed in dogs in several countries in Asia, South America USA and in Australia.

In contrast, no *Blastocystis* infection has been reported in dogs in Spain or Greece or in Japan [39–42] and no reports on *Blastocystis* infection in dogs from Africa have been published (Fig. 4, Table 1).

The results of our present study are consistent with those of Wang et al. [43] and Ruaux et al. [44], who propose that dogs cannot act as natural hosts for *Blastocystis* due to there being such a low percentage of infection.

In conclusion, animals, that live in close contact with humans, such as dogs, should be considered as potential reservoirs of *Blastocystis*, however, due to their low prevalence of *Blastocystis*, dogs do appear to not pose such an important threat. Nevertheless,

Table 1. Summary of published studies/reports on *Blastocystis* spp. in dog

Location	Subtypes (STs) identified	References
Argentina	Unknown	[45]
Australia	Unknown	[46,47]
Australia	ST1, ST3, ST4	[43,48]
Brazil	Unknown	[49–52]
Cambodia	ST2	[43]
Chile	Unknown	[53,54]
Colombia	ST2	[6,52]
Colombia	Unknown	[55]
Denmark	ST3	[2]
France	ST2, ST10	[38]
India	ST1, ST4, ST5, ST6	[43]
Iran	ST2, ST3, ST4, ST7, ST8, ST10	[58]
Iran	Unknown	[59–61]
Italy	ST3	[37]
Pakistan	Unknown	[62]
Philippines	Unknown	[56]
Philippines	ST1, ST2, ST3, ST4, ST5	[57]
Poland	ST7	This study
Thailand	ST5	[47]

larger studies are needed to more accurately evaluate the problem presented by the zoonotic reservoir of *Blastocystis* in animals living close to humans.

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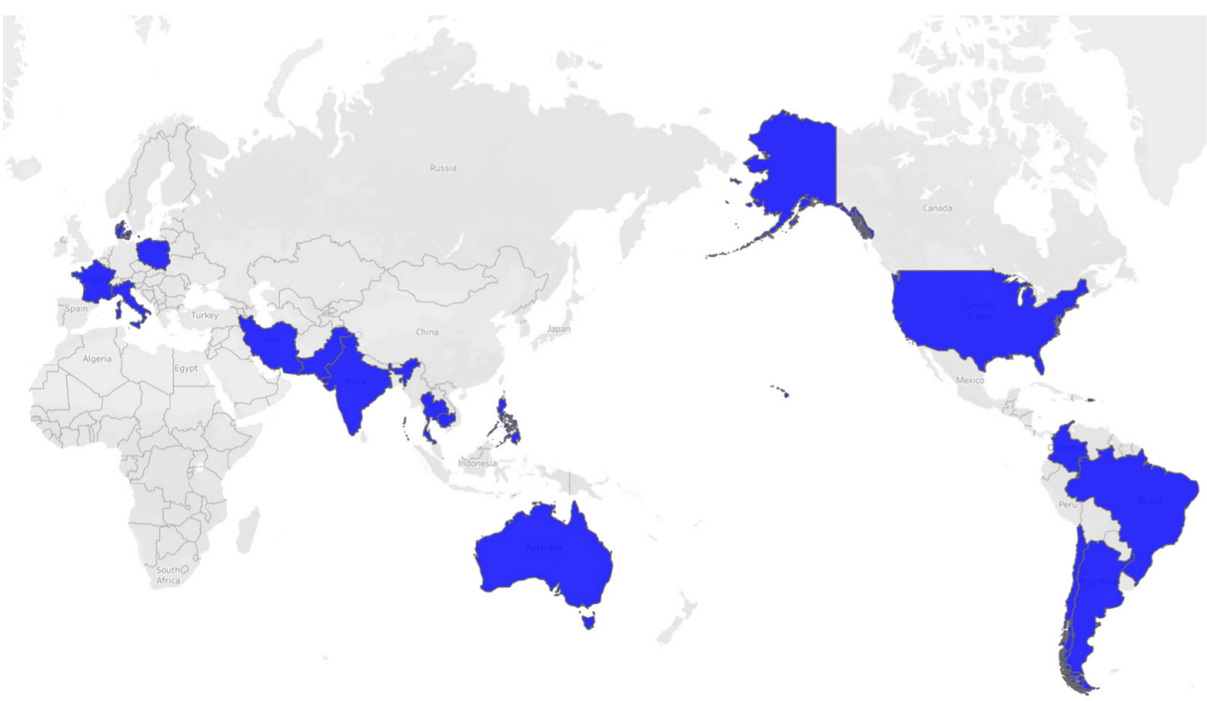

Figure 4. Geographical distribution of *Blastocystis* infection identified in dogs worldwide

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