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Short Communication

Occurrence of *Blastocystis* spp. in domestic animals in Triângulo Mineiro area of Brazil

***Renata Gregório Franco Moura*[1], *Márcia Benedita de Oliveira-Silva*[2], *André Luiz Pedrosa*[2], *Gabriel Antônio Nogueira Nascentes*[3] *and Marlene Cabrine-Santos*[4]**

[1]. Programa de Pós-Graduação Strictu Sensu em Medicina Tropical e Infectologia, Universidade Federal do Triângulo Mineiro, Uberaba, MG, Brasil.
[2]. Instituto de Ciências Biológicas e Naturais, Universidade Federal do Triângulo Mineiro, Uberaba, MG, Brasil.
[3]. Programa de Microbiologia e Imunologia, Instituto Federal de Educação, Ciência e Tecnologia do Triângulo Mineiro, Uberaba, MG, Brasil.
[4]. Instituto de Ciências da Saúde, Universidade Federal do Triângulo Mineiro, Uberaba, MG, Brasil.

Abstract

Introduction: Currently, there are few studies regarding *Blastocystis* epidemiology. This study aimed to evaluate the occurrence of *Blastocystis* in animals in Uberaba, Brazil. **Methods:** Fecal samples were examined by parasitological methods and screened for *Blastocystis* by polymerase chain reaction. **Results:** *Blastocystis* spp. were observed in pigs, sheep, cattle, and dogs. *Blastocystis* polymerase chain reaction was positive in 14/22 samples positive by parasitological methods. **Conclusions:** The occurrence of *Blastocystis* in animals is high, with a predominance of subtype 1 in the region. This is the first study conducted in Brazil showing the genetic profile of *Blastocystis* isolated from animals.

Keywords: *Blastocystis* spp. Domestic animals, Ritchie's method.

Blastocystis spp. are one of the most commonly found microorganisms infecting the intestine of humans and animals. In humans, this parasite is found in various age groups, with or without the occurrence of diarrhea[1]. Previous studies conducted in Uberaba, MG, Cabrine-Santos et al, in 2015, demonstrated that 17.8% (235/1,323) of the individuals analyzed were positive for *Blastocystis*. Although the pathogenicity of *Blastocystis* spp. is controversial, the parasite is found in stools of both healthy individuals and those with gastrointestinal symptoms[2] *Blastocystis* spp. have also been isolated from a wide range of animals, such as nonhuman primates, birds, amphibians, cattle, pigs, and less frequently rodents, reptiles, and insects[3].

Blastocystis spp. can be transmitted by the fecal-oral route via water and food contaminated with human or animal feces[2]. *Blastocystis* spp. have low host specificity and are considered potential zoonotic pathogens, since infections in humans have been associated with contact with primates, pigs, and birds[4,5]. Currently, an increasing interest in *Blastocystis* research reinforces the need for investigation of this parasite in areas where it has not yet been evaluated. In Brazil, there are no studies regarding occurrence of *Blastocystis* in animals, especially dogs, cats, and livestock. Thus, studies regarding *Blastocysts* are needed to determine the prevalence of this protozoan in animals, and its importance as a carrier for human infections, via direct contact and/or contamination of water and food supplies. Therefore, the aim of this study was to verify the occurrence of *Blastocystis* spp. in animals by parasitological and molecular methods in Uberaba, MG, a Brazilian city with a large population of cattle, raised for dairy and meat. This is the first study involving analysis for *Blastocystis* in animal samples in Brazil.

Between December 2013 and December 2014, fecal samples of cattle, pigs, and sheep were obtained from the *Instituto Federal de Educação, Ciência e Tecnologia do Triângulo Mineiro*, and those of dogs and cats from the Zoonoses Control Center in Uberaba, MG, Brazil. The dogs and cats used in the study were strays of the city, and information concerning where they were captured was not available. The stool specimens of adult and young animals were collected immediately after defecation. The specimens were examined using light microscopy, by direct observation of fresh fecal suspensions and after sedimentation by Ritchie's method[6]. *Blastocystis* spp. positive samples were washed three times with saline (0.9% NaCl), at 1,006×g/5min, and the pellet obtained was stored at -20ºC until deoxyribonucleic acid (DNA) extraction. Fecal DNA extraction was performed using the Magnex® DNA kit (Labtest Diagnóstica SA, MG, Brazil), according to the manufacturer's recommendations.

***Corresponding author*:** Profª. Marlene Cabrine dos Santos.
e-mail: marlenecabrine@yahoo.com.br; marlene.silva@uftm.edu.br
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Samples were also screened for *Blastocystis* by polymerase chain reaction (PCR) at the Parasitology Laboratory of the *Universidade Federal do Triângulo Mineiro* (UFTM) as previously described[7], using the primers 1FB and 1RB, which amplified the 1.1-kb small subunit ribosomal DNA (SSU rDNA) gene fragments. Furthermore, the *Blastocystis* isolates were classified into subtypes by PCR-Restriction Fragment Length Polymorphism (PCR-RFLP), using the *Spe*I endonuclease, and PCR-Sequence-Tagged Site (PCR-STS), as previously described[7].

Stool specimens from 143 animals, including dogs (n=78), cats (n=16), cattle (n=28), pigs (n=18), and sheep (n=3), were analyzed. The microscopic analysis showed the presence of hookworms and *Toxocara* sp. (4.2%), *Balantidium* sp. (1.4%), *Isospora* sp. (2.8%), *Entamoeba* sp. (23.8%), and *Giardia* sp. (31.5%) in the samples. The presence of *Giardia* in dogs and cats were 44.8% and 37.5%, respectively. *Blastocystis* spp. were detected in pigs (72.2%), sheep (33.3%), cattle (21.4%), and dogs (2.6%), in 15.4% of the investigated animals (**Table 1**). The occurrence of *Blastocystis* spp. in pigs was higher than that in cattle and dogs (*p-value*<0.01).

In the analysis of fecal samples for the identification of *Blastocystis*, direct microscopy of fresh fecal suspensions was more sensitive than Ritchie's method, with 90.9% (20/22 positive samples) and 40.9% (9/22 positive samples), respectively (*p-value*<0.01). The percentage agreement between the methods was 89.5% (**Table 2**).

Blastocystis-PCR was positive in 14/22 (63.6%) samples in which *Blastocystis* spp. were identified on parasitological methods, consisting of 5/6 (83.3%) cattle, 8/13 (61.5%) pigs, and 1/1 (100%) sheep (**Table 3**). In dogs, the positivity of *Blastocystis* was observed only by parasitological methods.

PCR products showed distinct sizes between 0.9-1.0 kb in one pig (12.5%) and two cattle (40%), 1.0-1.1kb in six pigs (75%) and three cattle (60%), and 1.3 kb in one pig. These products were sequenced and showed similarity with the sequences of GenBank *Blastocystis* (AF408407) of approximately 98%.

The classification of *Blastocystis* isolates showed the presence of sub-type 1 (ST1) in the three swine isolates (**Table 3**). The other isolates were negative by PCR-STS for ST1-ST7 genotypes, despite being classified by PCR-RFLP as possible genotypes ST3/ST4/ST8 or ST5/ST7.

The evaluation of *Blastocystis* spp. in Brazil is still in the early stages. Data concerning their occurrence in humans and animals are limited, as are data on their genotypes. This study demonstrated the occurrence of *Blastocystis* in dogs (2.6%), cattle (21.4%), pigs (72.2%), and sheep (33.3%) of the Triângulo Mineiro area. The occurrence of *Blastocystis* was the highest in pigs, and was demonstrated in dogs only by parasitological methods. These data are

TABLE 1: Occurrence of *Blastocystis* spp. in stool samples from domestic animals collected in Uberaba, MG, between Dec 2013 and Dec 2014.

Host	Occurrence of *Blastocystis spp.* n (%)	PR (CI95%)*	p-value**
Pigs	13/18 (72.2)	1.00	< 0.01
Sheep	1/3 (33.3)	0.46 (0.09-2.35)	
Cattle	6/28 (21.4)	0.30 (0.14-0.64)	
Dogs	2/78 (2.6)	0.04 (0.01-0.14)	
Cats	0/16 (0.0)	NA	
Total	**22/143 (15.4)**		

PR: prevalence ratio; **CI95%:** 95% confidence interval; **NA:** not applicable. *PR (CI95%) using the positivity of *Blastocystis spp.* in pigs as reference. **The p-value refers to the result of the chi-square test.

TABLE 2: Positivity of *Blastocystis* spp. in fecal samples of animals by direct microscopy and Ritchie's method.

Ritchie	Direct microscopy		
	positive n (%)	negative n (%)	total n (%)
Positive	7 (4.9)	2 (1.4)	9 (6.3)
Negative	13 (9.1)	121 (84.6)	134 (93.7)
Total	**20 (14.0)**	**123 (86.0)**	**143 (100.0)**

Percent agreement = 128/143 (89.5%). Percent disagreement = 15/143 (10.5%). Kappa coefficient = 0.43 (0.20-0.66).

TABLE 3: Positivity of *Blastocystis* SSU rDNA gene-PCR relative to parasitological methods (direct microscopy and Ritchie's method) in stool samples and genotyping by PCR-RFLP (*Spe*I) and PCR-STS.

Host	Positivity by parasitological methods (n_p)	PCR [n_{PCR+}/n_p]* (%)	ST1 genotypes (%)**
Dogs	2	0/2 (0.0)	ND
Cattle	6	5/6 (83.3)	0/5
Pigs	13	8/13 (61.5)	3/8 (37.5)
Sheep	1	1/1 (100.0)	0/1
Total	**22**	**14/22 (63.6)**	**3/14 (21.4%)**

SSU rDNA: small subunit ribossomal DNA; **PCR:** polymerase chain reaction; **RFLP**: retriction fragment length polymorphism; **STS**: *Sequence-Tagged* Site; **ST:** sub-type; n_p: number of positive samples by parasitological methods**; ND:** not done. *n_{PCR+}/n_p: number of positive samples by PCR/ number of positive samples by parasitological methods. **PCR-RFLP results were compatible with groups corresponding to ST1/ST2/ST6. PCR-STS confirmed ST1 for the three swine samples. All other samples were negative by PCR-STS.

consistent with the literature showing a prevalence of 71% in pigs, 9.6%-19.2% in cattle, and 3.4%-14.5% in dogs, by parasitological methods[8-10]. None of the cats in this study showed *Blastocystis* in their stool samples, although this parasite has been noted in cats by others authors, with a prevalence between 11.4% and 37.4%[9,11].

The analysis of feces from the different species also showed the presence of multiple intestinal parasites. The most important of which was the high prevalence of *Giardia* in dogs and cats, with percentages of 44.8% and 37.5%, respectively, corroborating literature data showing that these animals are a source of infection for humans[4,12].

The high prevalence of *Blastocystis* spp. in the cattle and pigs in this study suggests that these animals could serve as a source of infection for their handlers. In fact, there are reports of animal handlers having the same genotype of *Blastocystis* found in animals[13,14]. Moreover, these animals are a source of contamination of water used by water treatment plants for human use. The environmental contamination level, size (5-10μm), resistance of cysts to water treatment methods, such as chlorination, and poor removal of cysts during the filtration process allow for the zoonotic transmission of *Blastocystis*[15].

This study also evaluated the methods for parasitological diagnosis of *Blastocystis,* and direct microscopy showed higher sensitivity than Ritchie's method. However, Ritchie's method performed better than did direct microscopy in previous studies. According to our experience and that of other researchers, this discrepancy may be due to the instability of vacuolar forms of *Blastocystis* that rupture in the presence of the water or 10% formaldehyde used in the preparation method. Therefore, we suggest that the use of both methods would ensure better results.

The PCR method can also be used to diagnose *Blastocystis*. However, in this study, only 63.6% of samples positive by parasitological methods were positive by PCR. This may have occurred due to the low concentration of DNA, or its degradation.

In contrast to other studies, PCR products amplified with 1FB and 1RB primers showed sizes of 1.1kb, which was expected. Sequencing of these products confirmed a similarity with the sequences of GenBank *Blastocystis* of 98%. This demonstrates that the SSU rDNA gene is polymorphic. PCR-STS showed that pigs presented the ST1. However, the genotypes of the other isolates could not be determined.

In conclusion, the occurrence of *Blastocystis* spp. in domestic animals in the Triângulo Mineiro area is high, and pigs presented the ST1. Such animals can be a source of infection for human *Blastocystis* via direct contact (animal handlers) or contamination of the water supply. This is the first study conducted in Brazil to show the genetic profile of the *Blastocystis* isolates obtained from animals. Genotyping studies regarding *Blastocystis* in Brazil are still scarce and should be conducted to better understand this parasite and its epidemiology.

Ethical considerations

Positivity for *Blastocystis* and the sensitivity of the parasitological methods were evaluated using the chi-square test. Kappa coefficients were applied to evaluate the agreement among parasitological exams. The MedCalc 12.2 software (MedCalc Software, Ostend, Belgium) was used to perform the statistical analysis, with a significance level of 0.05. This study was approved by the Ethics Committee in Research at the UFTM under number 1,804.

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Conflict of interest

The authors declare that there is no conflict of interest.

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