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Non-Infection to *Blastocystis* in Cats of Lorestan Province, Iran

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ABSTRACT

Blastocystis spp. is a globally distributed zoonotic protozoan parasite with various subspecies found in the gastrointestinal tract in many types of vertebrates such as carnivorous and herbivorous mammals? This study aimed to evaluate the Blastocystis spp. parasite among the cats in Lorestan province. In this study, 120 stool samples of a cat were collected in the sterile plastic disposable cans and transferred to the laboratory and directly investigated. The DNA of the samples was extracted and Specific Primer-PCR was performed. To control and ensure the results of PCR, a mixed sample containing a Blastocystis-positive stool sample with cat samples was used, the DNA of which was extracted and examined similar to the study on cat samples. The results of this study showed that the studied cats were not infected with Blastocystis spp. parasite. Also, the PCR result of the mixed sample was positive. The prevalence of the parasite in various hosts and geographic areas is different. The results showed that cats of Lorestan province did not infect Blastocystis spp., similar to some other countries, so they are not considered as a source or host for humans and other animals. Since the infection is reported in other animals in the region, further studies on the physiology and nutrition of cats are suggested.

Keywords: *Blastocystis, Prevalence, Cat, Lorestan, Infection, Protozoa.*

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INTRODUCTION

Infection with the protozoan, anaerobic and zoonotic parasite of *Blastocystis* spp. is common in the tropical, subtropical, and temperate areas of the world, especially in the communities with economic and health poverty [1, 2]. This is a globally distributed parasite, but its prevalence is higher in developing countries [1], and this is the most common gastrointestinal parasite in Iran with a prevalence of 14.6% [3]. It is estimated that more than one billion people in the world are infected with this parasite [4]. *Blastocystis* spp. is a polymorphic parasite with various forms of the vacuole, granulate, amoeboid, multi vacuole, and cyst [5, 6]. *Blastocystis* spp. belongs to a separated eukaryotic kingdom known as Chromista and the only infectious form of this kingdom [7].

The studies have shown that this gastrointestinal parasite has various hosts of invertebrates and vertebrates including reptiles, amphibians, birds, and a wide variety of mammals, such as humans and many herbivores and carnivores [8, 9]. It has two types of cysts, one with a thin wall causing internal infection and the other one with a thick wall that is a parasite transferable form, which is directly transferred in contact with humans and other infected animals or through the water and food [10-12]. WHO considers it among the water health criteria and health programs, and there is a relationship between the quality of drinking water and infection to this parasite [13, 14]. After entering the gastrointestinal system and large intestine, the cysts are immediately opened and turned into the form of vacuoles, which are multiplied by bipolar division and transformed into forms of amoeboid

and granulate, and finally, the cystic stage is completed in the colon region before the departure [5, 12].

Formerly, the diagnosis of parasites in different hosts was based on the morphological forms, which does not only have high sensitivity for being a polymorphic parasite but also could not distinguish biological details [15, 16]. But the recent molecular studies have shown that *Blastocystis* spp. is not only polymorphic but also heterogeneous with numerous subspecies that seven cases of which (ST_1-ST_7) as the standard subspecies were considered as the basis for many epidemiological studies [15, 17].

For example, ST3 is the most prevalent subspecies in humans, which is known as human subspecies. ST2 is reported in monkeys, ST4 in rodents, ST5 in cattle and pigs, and ST6 and ST7 are both reported in birds [12]. However, none of these hosts are dedicated, and as a result, any of which can be transferred to other hosts, such as humans, and as a result, they can turn into zoonosis parasite, on the other hand, each of these hosts is also considered as the source of infection or storage host for humans [12, 15, 18].

Molecular studies of *Blastocystis* parasite have been conducted widely in some hosts, but it is rarely performed on cats worldwide, while never performed in Iran. According to studies carried out in Lorestan province on humans, cattle, and birds [18-20], to complete the epidemiological condition of the parasite in this province, a molecular study is essential to determine the prevalence of *Blastocystis* parasite and the relevant subspecies in cats.

MATERIALS AND METHODS

Study area

Lorestan Province is a region with temperate and mountainous weather located between 46 ° and 51′ to 50 °and 22 ′ north latitude in the middle Zagros Mountains, with an area of 28294 km2 and a population of about 1760000 people, which is located between provinces of Isfahan and Chaharmahal and Bakhtiari in the east, Markazi and Hamedan in the north, Kermanshah and Ilam in the west, and Khuzestan in the south [21, 22].

Sample collection

This study was carried out on the stray cats in Lorestan province in 2017. In the present research 120, cat stool samples were collected in the labeled plastic disposable cans and transferred to the Laboratory of Parasitology Department, Lorestan University of Medical Sciences.

Direct examination of samples

Immediately after receiving the samples and recording the characteristics, an expansion is prepared directly by gram staining of each sample and examined by optical microscopy, and the rest of the samples were stored -20 ° C for DNA extraction.

DNA extraction

Similar to the methods that were also used in earlier studies, the DNA of samples was extracted and stored at -20 ° C until PCR [15, 19, 20].

PCR reaction

PCR was performed to investigate the presence of *Blastocystis* spp. using the specific primer pair with similar sequences of previous studies [15, 18, 20].

B11400 for 5' – GGA ATC CTC TTA GAG GGA CAC TAT ACAT-3', B11710 Rev 5' – TTA CTA TCC AAA GTG TTC ATC GGA C-3'. In brief, PCR was initially performed with one cycle for 5′ at 94 ° C, and then 30 cycles including 1′ at 94 ° C, 1′ at 58 ° C, 1′ at 72 ° C, and finally, one cycle for 5′ at 72 ° C. The expected size of PCR product was 310 bp. The resulting PCR products were evaluated on 1.5% agarose gel. Positive and non-DNA samples were respectively used as positive and negative controls.

Controlling PCR Results

To control and ensure the results of PCR and investigating the presence of inhibitors in the testing samples, a mixed sample containing 100 mg of *Blastocystis* positive stool samples and 100 mg of cat samples was used, the DNA of which was extracted and investigated similar to the study on cat samples.

Ethical approval

The present study was approved by the Ethics Committee of Lorestan University of Medical Sciences.

RESULTS

The results of this study that was performed by direct and molecular PCR methods on 120 cat stool samples were negative for infection to *Blastocystis*. Therefore, the studied cats did not have *Blastocystis*, while the outcome of positive PCR reaction for the control sample and mixed samples was positive, indicating the presence of *Blastocystis* and accuracy of extraction and PCR reaction (Figure 1).

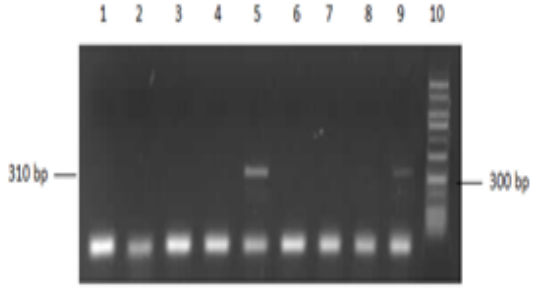

Figure 1. Electrophoresis of PCR product on 1.5% agarose gel

Lane 1-4, 6,7 Negative samples; Lane 5 Controlling PCR; Lanes 8 Negative control; Lanes 9 Positive control; Lanes10: 50 bp DNA ladder marker

DISCUSSION

The prevalence of *Blastocystis* in cats of Lorestan province was investigated using direct and molecular PCR methods, and the results showed the lack of parasite in the testing samples.

The studies have shown that the prevalence of *Blastocystis* is different in various geographic regions because each region has its own cultural, climatic, economic, health, nutritional and even genetic conditions affecting the prevalence of parasites [23, 24].

For example, the prevalence of this parasite in the cats in Australia was reported 63.7% (22), while it was zero in Iraq [25], China [26], and France [9].

Also in the microscopic examination, the prevalence of *Blastocystis* spp. is reported 14.3% in the stray cats in Khuzestan province which is located in southwestern Iran and south of Lorestan province [27].

However, according to the results of this research, *Blastocystis* infection was not reported in cats in Lorestan province that has been evaluated by the molecular method. This outcome can approve the suggestions of previous studies regarding the effect of various conditions on the prevalence of parasites.

The difference in the prevalence of this parasite in cats in different regions of the world is similar to the difference in its prevalence in other animals. For example, while 71% of dogs in Australia are infected with this parasite but in Japan was not reported [28].

In the study on the birds in Lorestan province that could be hunted by cats for feeding, the *Blastocystis* parasite was only reported in one case [19].

Since no study is reported on the prevalence of *Blastocystis* parasite by molecular PCR method in cats of Iran, hence, no criteria are available for comparing the results of this study.

It can be concluded from the results of this study that the stray cats of Lorestan province with a fairly large population that is dealing with people in different ways did not have the zoonotic parasite of *Blastocystis* spp. and are not probably considered as the source of infection for humans.

The further studies are suggested on the physiology and type of nutrition of cats in the region to find that why cats lack this parasite despite the presence of *Blastocystis* spp. in other hosts in the province [19, 20].

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Authors' contribution

Ebrahim Badparva designed the research. Ebrahim Badparva and Farnaz Kheirandish collaborated on the laboratory assays and manuscript writing.

Conflict of interest

The authors declare that there is no conflict of interest.

REFERENCES

1. Stenzel, D. J., Boreham, P. F. Blastocystis hominis revisited. Clinical Microbiology Reviews, 1996; 9(4), 563-584.

2. Tan, K. S., Singh, M., Yap, E. H. Recent advances in Blastocystis hominis research: hot spots in terra incognita. International journal for parasitology, 2002; 32(7), 789-804.
3. Badparva, E., Ezatpour, B., Mahmoudvand, H., Behzadifar, M., Behzadifar, M., Kheirandish, F. Prevalence and genotype analysis of blastocystis hominis in Iran: a systematic review and meta-analysis. Archives of Clinical Infectious Diseases, 2017; 12, 1-9.
4. Andersen, L. O. B., Stensvold, C. R. Blastocystis in health and disease: are we moving from a clinical to a public health perspective?. Journal of clinical microbiology, 2016; 54(3), 524-528.
5. Vdovenko, A. A. Blastocystis hominis: origin and significance of vacuolar and granular forms. Parasitology Research, 2000; 86(1), 8-10.
6. Singh, M., Suresh, K., Ho, L. C., Ng, G. C., Yap, E. H. Elucidation of the life cycle of the intestinal protozoan Blastocystis hominis. Parasitology Research, 1995; 81(5), 446-450.
7. Cavalier-Smith, T. A revised six-kingdom system of life. Biological Reviews, 1998; 73(3), 203-266.
8. Boreham, P. F., Stenzel, D. J. Blastocystis in humans and animals: morphology, biology, and epizootiology. In Advances in parasitology (Vol. 32, pp. 1-70). Academic Press., 1993
9. Cian, A., El Safadi, D., Osman, M., Moriniere, R., Gantois, N., Benamrouz-Vanneste, S., ... Noël, C. Molecular epidemiology of Blastocystis sp. in various animal groups from two French zoos and evaluation of17; potential zoonotic risk. PLoS One, 20712(1), e0169659.
10. Sienzel, D. J., Boreham, P. F. L., McDougall, R. Ultrastructure of Blastocystis hominis in human stool samples. International journal for parasitology, 1991; 21(7), 807-812.
11. Yoshikawa, H., Yoshida, K., Nakajima, A., Yamanari, K., Iwatani, S., Kimata, I. Fecal-oral transmission of the cyst form of Blastocystis hominis in rats. Parasitology Research, 2004; 94(6), 391-396.
12. Tan, K. S. New insights on classification, identification, and clinical relevance of Blastocystis spp. Clinical microbiology reviews, 2008; 21(4), 639-665.
13. World Health Organization, Guidelines for drinking–water Quality world health organization, Fourth edition, Geneva. 2011.
14. Leelayoova, S., Rangsin, R., Taamasri, P., Naaglor, T., Thathaisong, U., Mungthin, M. Evidence of waterborne transmission of Blastocystis hominis. The American journal of tropical medicine and hygiene, 2004; 70(6), 658-662.
15. Stensvold, R., Brillowska-Dabrowska, A., Nielsen, H. V., Arendrup, M. C. Detection of Blastocystis hominis in unpreserved stool specimens by using polymerase chain reaction. Journal of Parasitology, 2006; 92(5), 1081-1087.
16. Termmathurapoj, S., Leelayoova, S., Aimpun, P., Thathaisong, U., Nimmanon, T., Taamasri, P., Mungthin, M. The usefulness of short-term in vitro cultivation for the detection and molecular study of Blastocystis hominis in stool specimens. Parasitology Research, 2004; 93(6), 445-447.
17. Stensvold, C. R., Arendrup, M. C., Jespersgaard, C., Mølbak, K., Nielsen, H. V. Detecting Blastocystis using parasitologic and DNA-based methods: a comparative study. Diagnostic microbiology and infectious disease, 2007; 59(3), 303-307.
18. Badparva, E., Sadraee, J., Kheirandish, F., Frouzandeh, M. Genetic diversity of human blastocystis isolates in khorramabad, central Iran. Iranian Journal of parasitology, 2014; 9(1), 44.
19. Badparva, E., F. Kheirandish, J. Sadraee, Molecular study of zoonotic parasite of Blastocystis sp. in birds in Khorramabad city, Lorestan province. Pajouda. 2014. 9: p. 53-59 (In Persian).
20. Badparva, E., Sadraee, J., Kheirandish, F. Genetic diversity of Blastocystis isolated from cattle in Khorramabad, Iran. Jundishapur Journal of Microbiology, 2015; 8(3).
21. Azizi, K., Heidari, S. A comparative study on energy balance and economical indices in irrigated and dry land barley production systems. International Journal of Environmental Science and Technology, 2013; 10(5), 1019-1028.

22. Lorestan Governorate, Statistical yearbook. Khorram Abada, Iran: Lorestan Governorate; http://www.amar.org.ir/Default.aspx?tabid=1693. 2011.
23. Li, L. H., Zhang, X. P., Lv, S., Zhang, L., Yoshikawa, H., Wu, Z., ... Zhou, X. N.. Cross-sectional surveys and subtype classification of human Blastocystis isolates from four epidemiological settings in China. Parasitology Research, 2007; 102(1), 83-90.
24. Alfellani, M. A., Stensvold, C. R., Vidal-Lapiedra, A., Onuoha, E. S. U., Fagbenro-Beyioku, A. F., Clark, C. G. Variable geographic distribution of Blastocystis subtypes and its potential implications. Acta Tropica, 2013; 126(1), 11-18.
25. Al-Aredhi, H. S. Prevalence of gastrointestinal parasites in domestic cats (Felis catus) in Al-Diwaniya province/Iraq. Int. J. Curr. Microbiol. Appl. Sci, 2015; 4(5), 166-171.
26. Chen, J., Xu, M. J., Zhou, D. H., Song, H. Q., Wang, C. R., Zhu, X. Q. Canine and feline parasitic zoonoses in China. Parasites & Vectors, 2012; 5(1), 152.
27. Khademvatan, S., Rahman Abdizadeh, F. R., Hashemitabar, M., Ghasemi, M., Tavalla, M. Stray cats gastrointestinal parasites and its association with public health in Ahvaz City, South Western of Iran. Jundishapur Journal of Microbiology, 2014; 7(8).
28. Tan, K. S.. Blastocystis in humans and animals: new insights using modern methodologies. Veterinary parasitology, 2004; 126(1-2), 121-144.