Dicle Üniversitesi Veteriner Fakültesi Dergisi
https://dergipark.org.tr/tr/pub/duvetfd
Araştırma Makalesi/Research Article
ISSN:1307-9972 Dicle Üniv Vet Fak Derg 2022;15(1):41-44 e-ISSN:1308-0679
DOI: 10.47027/duvetfd.1098763

The Role of Inhabited Animals With People in Human Transmission of *Blastocystis sp.*

Fatih ÇAKIR[1,a,✉], Muttalip ÇİÇEK[2,b], Hasan İÇEN[,c], İbrahim Halil YILDIRIM[4,d], Akın KOÇHAN[3,e]

[1]Department of Microbiology, Faculty of Medicine, Dicle University, Diyarbakır, TÜRKİYE.
[2]Department of Microbiology, Faculty of Medicine, Ahi Evran University, Kırşehir, TÜRKİYE.
[3]Department of İnternal Disease, Faculty of Veterinary, Dicle University, Diyarbakır, TÜRKİYE.
[4]Dicle University, Faculty of Veterinary, Department of Genetics, TÜRKİYE.

[a]ORCID: 0000-0002-9808-4366; [b]ORCID: 0000-0003-4807-4482; [c]ORCID: 0000-0002-6034-3203
[d]ORCID: 0000-0001-5518-5004; [e]ORCID: 0000-0003-0199-4539

Geliş Tarihi/Received	Kabul Tarihi/Accepted	Yayın Tarihi/Published
09.04.2022	21.06.2022	30.06.2022

Abstract

Blastocystis sp. is a common intestinal parasite worldwide that can cause infection in humans and animals. *Blastocystis* sp. has a high genetic diversity with 17 different subtypes (ST) identified to date. Since nine of these subtypes are common in both humans and animals, it has been proposed that animals may have a role in the transmission of *Blastocystis sp*. to humans. The aim of this study is to investigate the epidemiological effect of animals on the human transmission of *Blastocystis* species by molecular methods in our country. A total of 420 faecal samples were collected from cattle, sheep, dogs, horses and chickens. Samples were stained with trichrome staining and cultivated by Jones's medium culture method. *Blastocystis* was detected in 53 (12.6%) of 420 samples. The samples were examined by Polymerase Chain Reaction (PCR) to identify nine common human subtypes. Subtypes were not detected in 33 (62.3%) of the 53 *Blastocystis* positive samples. Subtypes were detected in 20 (37.7%) samples. The detected subtypes were as follows: ST5 in four (7.5%) sheep, ST6 in six (11.3%) chickens, ST7 in 11 (20.7%) chickens, both ST6-ST7 were detected in one chicken. ST1, ST2, ST3 and ST4 - common subtypes in our country- were not detected in any animal. Sheep and chickens may be the source of human transmission of ST5, ST6 and ST7, the rare subtypes in Turkey. As a result, humans rather than animals, seem to be the source of the human transmission of *Blastocystis sp.* in Turkey.

Key Words: *Blastocystis sp.*, molecular epidemiology, polymerase chain reaction.

Blastocystis sp.'nin İnsanlara Bulaşında İnsanlarla Yakın İlişkili Hayvanların Rolü

Öz

Blastocystis sp. Dünyada yaygın görülen, insanlarda ve hayvanlarda hastalıklara sebep olabilen enterik bir parazittir. *Blastocystis sp.*'nin genetik çeşitliliği çok fazladır ve günümüzde 17 farklı subtipi (ST) tespit edilmiştir. Bu subtiplerden dokuz tanesi insanlarda ve hayvanlarda ortak olduğu için *Blastocystis sp.*'nin insana bulaşında hayvanların etken olabileceği belirtilmiştir. Bu çalışmanın amacı, ülkemizde *Blastocystis* sp.'nin insanlara bulaşında hayvanların etkisini moleküler yöntemlerle epidemiyolojik olarak araştırmaktır. Çalışmamızda insanlarla yakın ilişkili çeşitli hayvanlardan 420 dışkı örneği toplandı. Bu örnekler trikrom boyama, Jones' medium kültür yöntemi ve Polimeraz Zincir Reaksiyonu (PZR) yöntemi ile çalışıldı. 420 örnekten 53 (%12.6)'ünde *Blastocystis* tespit edildi. *Blastocystis sp.* pozitif örneklerde insana ait subtiplerin tespiti için PZR yöntemi ile çalışıldı. *Blastocystis* pozitif 53 örnekten 33 (%62.3)'ünde (15 Sığır, 14 koyun, 2 tavuk ve 2 at dışkı örneği) dokuz *Blastocystis* subtipinden hiçbiri tespit edilmedi. 20 (%37.7) örnekte ise insana ait subtipler tespit edildi. Bu subtiplerin dağılımı şu şekildedir: 4 (%7.5) koyunda ST5, 6 (%11.3) tavukta ST6, 11 (%20.7) tavukta ST7, 1 tavukta ST6-ST7 beraber tespit edildi. Çalışma sonucunda, ülkemizde sık görülen subtiplerden ST1, ST2, ST3 ve ST4 hiçbir hayvanda tespit edilemedi. Ülkemizde nadir görülen subtiplerden ST5, ST6 ve ST7'nin insana bulaşında koyun ve tavukların kaynak olabileceği değerlendirildi. Sonuç olarak, ülkemizde *Blastocystis sp.*'nin insana bulaşında zoonotik kaynaklardan çok insanlar sorumlu gözükmektedir.

Anahtar Kelimeler: *Blastocystis sp.*, moleküler epidemiyoloji, polimeraz zincir reaksiyonu.

INTRODUCTION

Blastocystis sp. is a common parasite that can infect humans and many different animal species. *Blastocystis sp.* has been taxonomically classified in recent years and has been placed into the *Stramenopile* line as a result of molecular studies investigating the 18s rRNA region (1). *Blastocystis sp.* can cause a variety of diseases such as gastrointestinal symptoms (GIS), urticaria, and irritable bowel syndrome besides existing in humans without any symptoms (2-6).

To date, a total of 17 *Blastocystis sp.*- 10 of which infect humans- were found (7, 8). ST1-ST9 were reported as the most widespread subtypes common in humans and animals; ST1, ST3, and ST5 in cattle and even-toed ungulate, ST4 in

rodents, and ST6 and ST7 in poultry (2, 9-17). *Blastocystis sp.* ST10-17, except ST12, is not seen in humans and is present in primates, odd-toed ungulate, even-toed ungulate, carnivorous animals, birds, and rodents (18, 19).

One of the most discussed aspects of *Blastocystis sp.* is transmission sources. Transmission to humans is thought to be caused by oral-faecal ingestion of the cyst form of the parasite (20). *Blastocystis sp.* can infect many different mammalian and poultry species as well as humans. In recent years, it was stated that animals associated with humans can play a role in infection (21). Therefore, it is important to know the *Blastocystis* transmission routes in the prevention of *Blastocystis* related diseases (2).

The aim of this study is to investigate whether animals or humans are the main origins of *Blastocystis* transmission to humans in Turkey.

MATERIAL AND METHODS

The study was conducted under the responsibility of veterinarians from an animal shelter, hippodrome and pasture areas. In this study, animal faecal samples collected after spontaneous defecation were analyzed. Therefore, this study did not require Animal Ethics Committee approval in accordance with Turkish Law.

In this study, stool samples were collected from cattle, sheep, and chickens grazing in pasture areas. Dog stool samples were obtained from animal shelters and horse samples from farm and hippodrome, as no outdoor dog and horses were found.

All stool samples were screened with the trichrome staining method. In addition, the samples were cultivated to the modified jones medium. Trichrome and culture-positive samples were preserved at -20°C until the genomic investigation.

DNA Extraction

Total genomic DNA was extracted from 250 mg faeces using a DNA isolation kit (Gene MATRIX Stool DNA Purification Kit, Poland). The isolation process was according to the manufacturer's recommended procedures.

Detection of Molecular Subtypes by PCR Method

Trichrome and jones medium culture-positive samples were studied with the general primer for *Blastocystis sp.*. General primer for *Blastocystis sp.* utilized previously published by Bohm-Gloning et al. (22) primers. F: 5'-GGA GGT AGT GAC AAT AAA TC-3' R: 5'-ACT AGG AAT TCC TCG TTC ATG-3'

The samples detected positive with *Blastocystis* general primers were included in the study to determine subtypes. For the detection nine subtypes of *Blastocystis sp.*, which cause human infection, ST-specific primers were used, which were developed by Yoshikawa et al. (23)

All PCR amplifications with ST-specific primers were accomplished in a 10-µl volume including 2 µl of the template DNA (5 µg/ml), 1× Ex Taq buffer, 0.2 U of TaKaRa Ex Taq® (Takara Bio Inc., Japan), 0.5 pM primers, and 0.2 mM dNTP mixture. The cycles for PCR used were firstly 94 °C for 5 min, followed by 38 cycles at 94 °C for 1 min, 55 °C for 45 s, 72 °C for 1 min and the final step at 72 °C for 10 min.

PCR products were visualised in 1.5% agarose gels stained with ethidium bromide with a 98 50-bp ladder marker (50-bp to 1.5-kbp; GeneDirex Taiwan). DNAs processed according to agarose gel weights were transferred to a UV gel imaging system (Quantum ST4). The bands visualised according to the weights of the DNAs were analyzed under UV light. The detected bands are shown in (Figure 1).

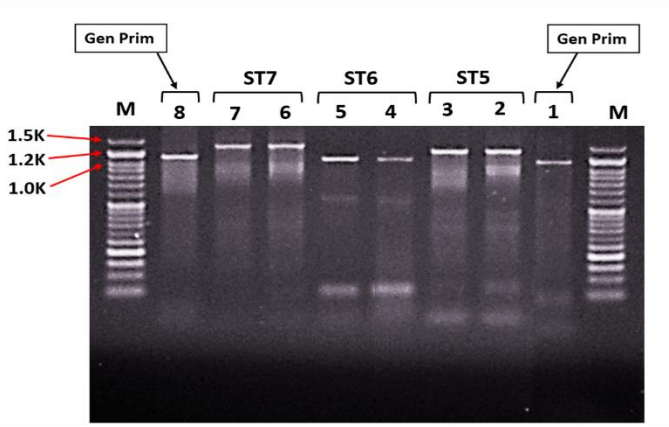

Figure 1. Agarose gel images of DNA fragments. Lane M DNA Ladder (50 bp): DNA fragments in Lanes 1 and 8 generated by general primers (1100 bp). PCR products were monitorized in other Lanes generated by subtype-specific primer pairs: Lane 2 and 3, subtype 5 (1200 bp). Lane 4 and 5, subtype 6 (1050 bp). Lane 6 and 7, subtype 7 (1340 bp).

Statistical evaluation was not performed because the numerical values of the subtypes obtained were insufficient for statistical evaluation. The results were considered fold values when compared to each other.

RESULTS

In the present study, a total of 420 animal samples were collected from cattle, sheep, chickens, horses and dogs.

Blastocystis was detected in 53 (12.6%) of 420 stool samples by trichrome staining and culture method. The distribution of *Blastocystis* positive stool samples was as follows: 15 (28.3%) cattle, 18 (33.9%) sheep, 18 (33.9%) chickens, 2 (3.7%) horses. None of the 89 dogs stool samples tested positive for *Blastocystis*.

A total of 53 samples were studied by the polymerase chain reaction (PCR) method to identify *Blastocystis* subtypes. First of all, using *Blastocystis sp.* general primers, 53 isolates were confirmed to be *Blastocystis*.

In the PCR performed with nine subtype-specific primers of *Blastocystis sp.*,33 (62.2%) of 53 samples were not found to have human subtypes. The distribution of human subtype negative 33 samples was as follows: 15 (28.3%) cattle samples, 14 (26.4%) sheep samples, 2 (3.7%) chicken and horse samples (Table 1).

20 (37.7%) of 53 samples were determined to have human subtypes, ST5 was detected in four sheep stool samples, ST6 in six chicken samples, ST7 in 11 chicken samples. ST6 and ST7 were seen together in one of the chicken stool samples (Table 1).

Table 1. Distribution of *Blastocystis sp.* subtypes in animals

	ST5	ST6	ST7	Nonsubtype	Total
Cattle	0	0	0	15	15
Sheep	4	0	0	14	18
Chickena	0	6	11	2	18
Horse	0	0	0	2	2
Dog	0	0	0	0	0
Total	4	6	11	33	53

[a] ST6 and ST7 were seen together in one of the chicken stool samples.

DISCUSSION AND CONCLUSION

In the present study, samples identified as positive with the trichrome staining and culture methods were detected as positive by PCR as well. Studies on this subject show that molecular tests are as reliable as the culture method (24-26). In a study by Santos et al. (25), comparing the detection methods of *B.* hominis, the culture method was found to be the most reliable one, followed by PCR performed with culture samples, and PCR from the direct stool. Roberts et al. (24) in a study comparing PCR and culture methods in the detection of *B. hominis*, claimed that the PCR method was more sensitive than the culture method. Furthermore, in another study conducted by Parkar et al. (26), molecular methods were found to be superior to the culture method. When the results of the current study are evaluated in terms of other studies, it can be said that the trichrome staining method, culture method, and the PCR method are reliable methods for identification of *Blastocystis sp*.. These results show that although there are many steps in the isolation process of the stool and it is labor-consuming, the PCR method is as reliable as trichrome and culture methods in the detection of *Blastocystis sp.*

On the other hand, only 2 out of 98 horse stool samples collected from the hippodrome were positive, while none of the 73 dog stool samples collected from the dog shelters were positive. The reason for this situation might be the antiprotozoal drugs which were given to these animals parenterally and added to their feed in hippodrome or in dog shelters.

Furthermore, ST5 was detected in 4 (7.5%) sheep samples (Table 1). Studies conducted around the world indicate that ST5 is an animal-sourced subtype and that poor hygiene plays a very important role in the transmission of the agent to humans (11, 12).

Studies in the world have identified ST5 in cattle and pigs (11, 12). Interestingly in this study, ST5 was detected in sheep. Alfellani et al. (27) in a study investigating the prevalence of *Blastocystis sp.* in livestock and zoo animals, reported that ST5 was generally present in even-toed ungulates. In this respect, it is possible to find ST5 in sheep. However, this data should be supported by molecular sequence analyzes and different studies using more samples to be made on this subject.

In addition, ST6 was detected in six (11.3%) chicken stool samples in this study (Table 1). There is no study investigating the prevalence of ST6 among chickens in Turkey. In this respect, this data is the first relevant data obtained in Turkey. In 2008, a review of the prevalence of *Blastocystis sp.* species prepared by Tan stated that ST6 was seen especially in poultry and that they are responsible for human infections (2). In this study, ST6 *Blastocystis sp.* was detected positive at a high rate of six (35.3%) in chickens. This suggests that chickens are a potential source of *Blastocystis* infections of the ST6 subtype in humans.

ST7 was detected in 11 (20.7%) of the stool samples collected from the chickens (Table 1). Tan et al. (10) reported in their review that ST7 was seen in chickens and the host distribution was very limited as well as this subtype showed protease activity and contained serious virulence factors. In a study investigating the prevalence of *Blastocystis sp.* in humans and animals, researchers detected the ST7 in eight chickens and in eight pigs while none in humans (11). In the present study, ST7, *Blastocystis sp.* positive found with a high rate of 64.7% of the chicken group.

Subtype distribution in animal samples was observed as follows. In the samples positive with general primer, nine subtypes of *Blastocystis sp.* causing disease in humans were not detected in 15 cattle stool samples, in 14 of the 18 sheep samples, 2 of the 18 chicken samples and 2 horse stool samples.

Studies conducted in this field presented that ST1, ST3, and ST5 were common subtypes in even-toed ungulate (13-15, 28). The findings in this study are not compatible with these studies. In some studies, investigating subtypes in cattle and sheep, it was reported that ST10 and ST14 were the highest in even-toed ungulates (16, 17). Regarding these studies, since only 9 subtypes of *Blastocystis sp.* were investigated in this study, there might be subtypes like ST10 or ST14 existing in these animal samples. Cian et al., in a study about the relationship between subtypes in even-toed ungulates and humans, indicated that ST10, ST14, and ST1 were the most common in even-toed ungulates and ST10 and ST14 were not seen in humans. In a study about the subtype relationships in even-toed ungulates and humans, Cian et al. indicated that ST10, ST14, and ST1 were the most common subtypes in even-toed ungulates while ST10 and ST14 were not present in humans.The study, also stated that the studies claiming ST1 transmission to humans via animals were very limited (17). The findings of the current study as well as Cian and colleagues' work show that cattle and sheep are not very effective in human infection of *Blastocystis sp.*. The fact that the most common subtypes in humans are ST3, ST1, ST2 and ST4 in Turkey, are not seen in sheep and that none of the human subtypes detected in cattle support this claim.

Among the *Blastocystis sp.* subtypes, ST3, ST1, ST2, and ST4 are the most commonly observed subtypes in humans. ST5, ST6, ST7, ST8 and ST9 are very rare in our country. In this study, the most common subtypes in humans, i.e., ST1, ST2, ST3, and ST4, were not detected in any of the animals. Among the subtypes that infect humans, sheep in the transmission of ST5 and chickens in the transmission of ST6-ST7 may be sources.

According to this study, it was observed that ST5 could cause infection in sheep. On the other side, cattle have not been identified as a serious source of infection for humans.

In conclusion, animals do not seem to be an important source of infection of *Blastocystis* subtypes, which are common in humans in our country.

CONFLICT OF INTEREST

The authors declared that there is no conflict of interest.

REFERENCES

1. Arisue N, Hashimoto T, Yoshikawa H, et al. (2002). Phylogenetic Position of Blastocystis Hominis and of Stramenopiles Inferred from Multiple Molecular Sequence Data. J Eukaryot Microbiol. 49: 42-53.
2. Tan KS. (2008). New Insights on Classification, Identification, and Clinical Relevance of Blastocystis spp. Clinical Microbiology Reviews. 21: 639-65.
3. Yakoob J, Jafri W, Beg MA, et al. (2010). Irritable Bowel Syndrome: Is it Associated with Genotypes of *Blastocystis hominis.* Parasitol Res. 106: 1033-8.
4. Balint A, Doczi I, Bereczki L, et al. (2014). Do not Forget the Stool Examination-Cutaneous and Gastrointestinal Manifestations of Blastocystis sp Infection. Parasitol Res. 113: 1585-90.
5. Lepczynska M, Chen WC, Dzika E. (2016). Mysterious Chronic Urticaria Caused by Blastocystis spp. Int J Dermatol. 55: 259-66.
6. AL FD, Hökelek M. (2007). *Blastocystis hominis* Fırsatçı Bir Patojen mi. Türkiye Parazitol Derg. 31: 28-36.
7. Ramirez JD, Sanchez A, Hernandez C, et al. (2016). Geographic Distribution of Human Blastocystis Subtypes in South America. Infect Genet Evol. 41: 32-5.
8. Navarro C, Domínguez-Márquez M, Garijo-Toledo M, et al. (2008). High Prevalence of Blastocystis *sp.* in Pigs Reared under Intensive Growing Systems: Frequency of Ribotypes and Associated Risk Factors. Veterinary Parasitology. 153: 347-58.
9. Greige S, El Safadi D, Becu N, et al. (2018). Prevalence and Subtype Distribution of Blastocystis *sp.* Isolates from Poultry in Lebanon and Evidence of Zoonotic Potential. Parasites and Vectors. 11: 389.
10. Tan KS, Mirza H, Teo JD, et al. (2010). Current Views on the Clinical Relevance of Blastocystis spp. Current Infectious Disease Reports. 12: 28-35.
11. Yoshikawa H, Tokoro M, Nagamoto T, et al. (2016). Molecular Survey of Blastocystis *sp.* from Humans and Associated Animals in an Indonesian Community with Poor Hygiene. Parasitology International. 65: 780-4.
12. Yan Y, Su SL, Ye JH, et al. (2007). Blastocystis sp Subtype 5: A possibly Zoonotic Genotype. Parasitol Res. 101: 1527-32.
13. Stensvold CR, Alfellani MA, Norskov-Lauritsen S, et al. (2009). Subtype Distribution of Blastocystis Isolates from Synanthropic and Zoo Animals and Identification of A New Subtype. Int J Parasitol. 39: 473-9.
14. Noel C, Dufernez F, Gerbod D, et al. (2005). Molecular Phylogenies of Blastocystis Isolates from Different Hosts: Implications for Genetic Diversity, Identification of Species, and Zoonosis. Journal of Clinical Microbiology. 43: 348-55.
15. Ramirez JD, Sanchez LV, Bautista DC, et al. (2016). Blastocystis Subtypes Detected in Humans and Animals from Colombia. Infect Genet Evol. 22: 223-8.
16. Fayer R, Santin M, Macarisin D. (2012). Detection of Concurrent Infection of Dairy Cattle with Blastocystis, Cryptosporidium, Giardia, and Enterocytozoon by Molecular and Microscopic Methods. Parasitol Res. 111: 1349-55.
17. Cian A, El Safadi D, Osman M, Moriniere R, et al. (2017). Molecular Epidemiology of Blastocystis *sp.* in Various Animal Groups from Two French Zoos and Evaluation of Potential Zoonotic Risk. PloS one. 12: e0169659.
18. Stensvold CR, Suresh GK, Tan KS, et al. (2007). Terminology for Blastocystis Subtypes–A Consensus. Trends in Parasitology. 23: 93-6.
19. Clark CG (1997): Extensive Genetic Diversity in *Blastocystis hominis.* Molecular and Biochemical Parasitology. 87: 79-83.
20. Lee H, Lee S-H, Seo MG, et al. (2018). Occurrence and Genetic Diversity of Blastocystis in Korean Cattle. Veterinary Parasitology. 258: 70-3.
21. Zhu W, Tao W, Gong B, et al. (2017). First Report of Blastocystis Infections in Cattle in China. Veterinary Parasitology. 246: 38-42.
22. Bohm-Gloning B, Knobloch J, Walderich B. (1997). Five subgroups of *Blastocystis hominis* from Symptomatic and Asymptomatic Patients Revealed by Restriction Site Analysis of PCR-Amplified 16S-like rDNA. Trop Med Int Health. 2(8): 771–778.
23. Yoshikawa H, Iwamasa A. (2016). Human Blastocystis Subtyping with Subtype-Specific Primers Developed from Unique Sequences of The SSU rRNA Gene. Parasitology International. 65: 785-791.
24. Roberts T, Barratt J, Harkness J, et al. (2011). Comparison of Microscopy, Culture, and Conventional Polymerase Chain Reaction for Detection of Blastocystis sp in Clinical Stool Samples. Am J Trop Med Hyg. 84: 308-12.
25. Santos HJ, Rivera WL. (2011). Comparison of Direct Fecal Smear Microscopy, Culture, and Polymerase Chain Reaction for The Detection of Blastocystis sp in Human Stool samples. Asian Pac J Trop Med. 6: 780-4.
26. Parkar U, Traub RJ, Kumar S, et al. (2007). Direct characterization of Blastocystis from Faeces by PCR and Evidence of Zoonotic Potential. Parasitology. 134: 359-67.
27. Alfellani MA, Taner-Mulla D, Jacob AS, et al. (2013). Genetic Diversity of Blastocystis in Livestock and Zoo Animals. Protist. 164: 497-509.
28. Thathaisong U, Worapong J, Mungthin M, et al. (2003). Blastocystis Isolates from A Pig and A Horse Are Closely Related to *Blastocystis hominis.* Journal of Clinical Microbiology. 41: 967-75.

✉ **Corresponding Author:**
Fatih ÇAKIR
Dicle University, Faculty of Medicine, Department of Microbiology, Diyarbakır, TÜRKİYE
E-mail: sfcakir@gmail.com