Prevalence and Genotype Characterization of *Blastocystis hominis* Among the Baghmalek People in Southwestern Iran in 2013-2014

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Background: *Blastocystis hominis* is a common globally distributed parasite. The prevalence of this parasite has been shown to vary among different countries. Molecular studies have also shown that there is a high level of genetic diversity among *Blastocystis* spp. isolated from humans and animals. Extensive information on parasitic genotypes will aid in devising more effective strategies for the identification and potential control of these pathogenic parasites.

Objectives: This study aimed to gain information on the prevalence and abundance of *Blastocystis* subtypes in Iran.

Materials and Methods: Over a period of 3 months, 1,410 stool samples were collected and examined by microscopy. Samples found to be positive for *B. hominis* were concentrated and phylogenetic analysis was subsequently performed. A questionnaire was completed by all study participants.

Results: *Blastocystis hominis* was found to have a prevalence of 3.33% in the study population. There was no significant association of Blastocystis infection with age (P = 0.3) or gender (P = 0.57). The *Blastocystis* subtypes (ST) identified in this study were ST3, ST4, ST5, and ST7 with the most prevalent being ST4 (40.9%).

Conclusions: The prevalence of *B. hominis* in the study area was lower than that reported for most developed countries, and unlike in other countries in the Middle East, ST4 was the most prevalent subtype.

Keywords: Subtypes, Phylogenetic Analysis, ST4, *Blastocystis hominis*

1. Background

*Blastocystis hominis* is a common intestinal protozoan parasite of humans and many animals (1, 2). This parasite is perhaps the most common eukaryotic organism in the human digestive system (3), with a prevalence of up to 60% reported in some developing countries (4). The parasite’s prevalence in developed countries, on the other hand, is reportedly 5% - 20% (1). Standards of health care, garbage disposal, as well as food and water contamination must be improved in developing countries to lower the parasite prevalence (5). *Blastocystis* spp. are zoonotic parasites and do not have a specific host (2, 6). *Blastocystis* transmission occurs primarily by fecal-oral means, which is exacerbated in poor sanitary conditions (7). Contaminated water is the most important issue in spread of the parasite (8). Despite extensive research, there is no consensus regarding the pathogenesis of *Blastocystis* spp. Some of the symptoms that have been attributed to *Blastocystis* are nausea, anorexia, abdominal pain, bloating, and acute or chronic diarrhea (9-12).

*Blastocystis* spp. have great genetic diversity (13, 14). Based on molecular analysis, *Blastocystis* isolates from humans, mammals, and birds can be assigned to nine subtypes (15). Differentiation of *Blastocystis* species by routine methods is not possible, and DNA-based methods are therefore essential for the detection of genetic variation in these organisms. Molecular epidemiological studies are particularly useful in ascertaining transmission patterns, host specificity, and chemotherapeutic drug resistance. The prevalence and genotype diversity of *Blastocystis* spp. in Iran have not been studied extensively. Previously, PCR-RFLP and PCR with seven pairs of sequence-tagged site (STS) primers were used to assess the genetic diversity of *Blastocystis* spp. in Iran (16, 17), and in this study, specific PCR products were sequenced for further assessment of genetic diversity among *Blastocystis* spp. in Iran.

2. Objectives

The aim of this study was to investigate the prevalence and genotype distribution of *Blastocystis* spp. among the Baghmalek people in southwestern Iran.
3. Materials and Methods

3.1. Study Area
Baghmalek city is located 120 km from the Ahvaz metropolitan in southwestern Iran. The city has a population of 110,000 of which more than 80% live in rural areas.

3.2. Sample Collection and Microscopic Diagnostics
Over a period of 3 months, after study subjects completed questionnaires, 1,410 stool samples were collected from patients from five districts of Baghmalek city: north, south, east, west, and central. Patients completed questionnaires and provided written consent. Stool samples were used for two fecal smear tests: normal saline and Lugol's iodine smears. Formalin ethyl acetate concentration and trichrome staining methods were also performed (1). Approximately 200 mg of each positive sample was stored at -20°C until subsequent molecular analysis.

3.3. PCR Amplification and Sequencing
DNA was extracted from fecal specimens by using an AccuPrep® Stool DNA extraction kit (Bioneer, Daejeon, South Korea) according to the manufacturer's instructions. The specific primers used to amplify the SSU rDNA gene from Blastocystis-positive specimens were Blast 505 - 532 (5’ GGA GGT AGT GAT GAC AAT AATC 3’; forward) and Blast 998 - 1017 (5’TGC TTT CGC ACT TGT TCATC 3’; reverse) (18). PCR amplifications were performed using AccuPower® PCR PreMix, a ready-to-use PCR reagent (Bioneer), where each reaction contained 7 µL distilled water, 2 µL of each primer, and 4 µL extracted DNA. The PCR conditions were as follows: an initial pre-heating step at 95°C for 4 minutes; cycles of 95°C for 30 seconds, 54°C for 30 seconds, and 72°C for 30 seconds; and a final extension step at 72°C for 5 minutes. Amplicons were visualized with agarose gel electrophoresis (1.5% agarose), and samples yielding products of 500 (479) bp were considered positive for Blastocystis. Positive and negative controls were included and PCR products were sent to South Korea for sequencing.

3.4. Sequence Analysis
DNA sequence analysis was performed on 22 PCR-positive samples. The SSU rDNA sequences obtained were compared with those available in GenBank by using the BLASTN program (http://www.ncbi.nlm.nih.gov/BLAST/). ST-1|HQ641595, ST-2|HQ641605, ST-3|HQ641611, ST-4|HQ641621, ST-5|HQ641630, ST-6|HQ641658, ST-7|HQ641661. Distance-based analysis was conducted on the sequencing data by using molecular evolutionary genetic analysis version 4 (MEGA 4) (19) and a phylogenetic tree was constructed using the neighbor-joining method (20) with the Kimura 2-parameter model (21). The support of monophyletic groups was assessed by the bootstrap method with 1,000 replicates (22).

3.5. Statistical Analysis
Frequency distributions of strains and demographic information were analyzed by descriptive statistical methods, and qualitative variables were analyzed by the chi-square test.

4. Results
The prevalence of Blastocystis spp. based on microscopic analysis was found to be 3.33% among people referred to Baghmalek medical centers. Of 1,410 patients whose stool samples were examined by microscopy, 602 (42.7%) were males, 22 of whom were infected with Blastocystis spp., and 808 (57.3%) were females, 25 of whom were infected (age range: 6 - 60 years). Although the infection rate was greater in males than in females, there was no significant statistical association between Blastocystis infection and gender (P = 0.57; Table 1). The highest positivity rate (4.51%) was observed in the group of subjects less than 15 years old, but statistical analysis indicated no significant association between Blastocystis infection and age (P = 0.3; Table 2). Of 47 samples characterized as Blastocystis-positive by direct microscopy methods, 25 did not yield Blastocystis-specific PCR products.

Seventeen samples found to be Blastocystis-positive by PCR were sequenced and Blastocystis subtype analysis yielded the following results: subtype 4 was the most predominant subtype, infecting nine (40.9%) individuals, while subtypes 3, 5, and 7 were isolated from only three (13.6%), two (9.1%), and three (13.6%) cases, respectively (Table 3). It should be noted that, despite specific PCR products being obtained, the subtype(s) of five sequenced samples could not be determined.

A phylogenetic tree of the Blastocystis isolates is displayed in Figure 2. The nucleotide sequences of the 21 sequenced and subtyped samples from this study have been deposited in GenBank under accession numbers AB891594 - AB891524.

### Table 1. Prevalence of Blastocystis hominis Among the Baghmalek People in Southwestern Iran, Grouped by Gender a

| Gender | No. Examined | Positive Cases |
|--------|-------------|----------------|
| Male   | 602         | 22 (3.65)      |
| Female | 808         | 25 (3.09)      |
| Total  | 1,410       | 47 (3.33)      |

a The values are presented as No. (%).

### Table 2. Prevalence of Blastocystis hominis among the Baghmalek people of southwestern Iran Grouped by Age a

| Age, y | No. Examined | Positive Cases |
|--------|-------------|----------------|
| 0 - 15 | 487         | 22 (4.51)      |
| 16 - 30| 544         | 10 (1.83)      |
| > 30   | 379         | 15 (3.95)      |
| Total  | 1,410       | 47 (3.33)      |

a The values are presented as No. (%).
In this study, Blastocystis was detected in 3.33% of stool specimens examined by microscopy, which is in agreement with the prevalence (2.2% - 28.2%) of Blastocystis previously reported in microscopy-based studies in Iran (23, 24). The prevalence of this parasite determined by traditional parasitological techniques is lower than that determined by PCR- and cultivation-based studies (25, 26). This discrepancy can be attributed to the resemblance of Blastocystis with yeast, Cyclospora spp., and fat globules. The prevalence rate of infection in the current study was comparable to that reported in developed countries, which may be because of acceptable primary health and environmental conditions in the restricted area.

In this study, the highest prevalence rate of infection was observed in the ≤ 15 year age group. This may be because of lack of parental supervision or lack of personal hygiene in this age group, increasing the susceptibility of these subjects to infection. Higher parasite prevalence was observed among males in this study compared with females, which is consistent with the findings of similar studies in Libya (27) and Turkey (28), which also reported higher rates of infection in males than in females. The differences in the prevalence rates of Blastocystis infection with age (P = 0.3) and gender (P = 0.57) in this study were not statistically significant, and thus, it seems that age and sex are not risk factors for Blastocystis infection.

This is one of few studies to date investigating the molecular epidemiology of Blastocystis infections in Iran. Subtype 4 (ST4) of Blastocystis was identified in the current study, and in two studies on Blastocystis previously conducted in Iran, ST4 was not reported (15, 16). The subtyping in these previous studies was conducted by PCR-RFLP, which is not considered an accurate approach for Blastocystis subtype identification, because the same RFLP profile does not always represent the same sequence (29-31) and sequence-tagged site primers of some genotypes cannot be detected by this method (32). In the present study, specific PCR product sequencing was used instead, yielding results similar to those reported by Poirier et al. (33) in France and Stensvold et al. (34) in Denmark. Sequencing was used for subtyping in both these studies. Noel et al. (35) and Silberman et al. (36) believe that rodents are reservoirs host of ST4, and high prevalence of ST4 in the study area may therefore be associated with rodent infection. This potential relationship, however, requires further investigation for confirmation.

The results of this and other studies demonstrated significant variation in Blastocystis strain prevalence within populations: ST3 has been reported to be predominant in several studies (37-39), while it was not very prevalent (13.6%) among the positive isolates in the current study. ST5, which was previously reported at a low rate in humans (5), was found to have a prevalence of 13.6% among positive samples in this study. Although there is no clear explanation for these differences, they may be a

Table 3. Blastocystis Subtypes (ST) Identified in Baghmalek in Southwestern Iran

| Type  | Number | Percent |
|-------|--------|---------|
| ST3   | 3      | 13.6    |
| ST4   | 9      | 40.0    |
| ST5   | 2      | 9.1     |
| ST7   | 3      | 13.6    |
| Unknown | 5    | 22.8    |
| Total | 22     | 100     |

5. Discussion

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result of differences in lifestyles as well as differences in the animal reservoirs in different study areas. In this current study, ST7 was identified, like in other studies in the Middle East (40, 41). Other known Blastocystis subtypes including ST1, ST2, ST6, ST8, and ST9 were not identified in our study. Larger sampling in further studies may help to explain this finding. As also observed in other studies (16, 38, 41), some samples (n = 25) found to be Blastocystis-positive by direct microscopy in this study did not yield Blastocystis-specific PCR products. This may be due to a number of factors including instability in sampling, improper storage and transportation, feces inhibitors, and low concentration of parasites (42-44).

This study was an assessment of the prevalence and subtypes of Blastocystis in Baghmalek in southwestern Iran. Unlike many studies conducted in the Middle East and Far East, ST4 was found in this study to be the most common subtype a discrepancy that requires further investigation. As previously reported in other studies, ST3, ST5, and ST7 were also identified in our study. Further investigation into the prevalence and subtypes of Blastocystis in this and other study areas should include investigation into routes of transmission and identification of zoonotic subtypes.

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Authors’ Contributions
Saleh Khoshnood: collection of samples, detection of parasites, molecular analysis, and writing. Abdollah Rafiei and Jasem Saki: research design, scientific advice, writing, and molecular analysis. Kobra Alizadeh: collection of samples and writing.

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References
1. Stenzel DJ, Boreham PF. Blastocystis hominis revisited. Clin Microbiol Rev. 1996;9(4):563-84.
2. Tan KS. Blastocystis in humans and animals: new insights using modern methodologies. Vet Parasitol. 2004;126(1-2):131-44.
3. Clark CG. Ctgic genetic variation in parasitic protozoa. J Med Microbiol. 2000;49(6):489-91.
4. Pegelow K, Gross R, Pietrzk K, Lukito W, Richards AL, Fryauf DF. Parasitological and nutritional situation of school children in the Sukara district, West Java, Indonesia. Southeast Asian J Trop Med Public Health. 1997;28(1):173-90.
5. Tan KS. New insights on classification, identification, and clinical relevance of Blastocystis spp. Clin Microbiol Rev. 2008;21(4):639-65.
6. Santin M, Gomez-Munoz MT, Solano-Aguilar G, Fayer R. Development of a new PCR protocol to detect and subtype Blastocystis spp. from humans and animals. Parasitol Res. 2018;117(9):2005-12.
7. Ustun S, Turgay N. Blastocystis hominis and bowel diseases. Turk J Parasitol Derg. 2006;30(1):272-6.
8. Kuo HY, Chiang DH, Wang CC, Chen TL, Fung CP, Lin CP, et al. Clinical significance of Blastocystis hominis: experience from a medical center in northern Taiwan. J Microbiol Immunol Infect. 2008;41(3):222-6.
9. El-Shazly AM, Abdel-Magied AA, El-Beshbishi SN, El-Nahas HA, Fouad MA, Monib MS. Blastocystis hominis among symptomatic and asymptomatic individuals in Tảkha Center, Dakahila Governorate, Egypt. J Egypt Soc Parasitol. 2005;35(2):553-66.
10. Kaya S, Cetin ES, Aridogan BC, Arikan S, Demirci M. Pathogenicity of Blastocystis hominis, a clinical reevaluation. Turk J Parasitol Derg. 2007;31:245-7.
11. Suresh K, Smith H. Comparison of methods for detecting Blastocystis hominis. Eur J Clin Microbiol Infect Dis. 2004;23(6):509-11.
12. Tasova X, Sahin B, Koltas S, Paydas S. Clinical significance and frequency of Blastocystis hominis in Turkish patients with hematological malignancy. Acta Med Okayama. 2000;54(3):131-6.
13. Clark CG. Extensive genetic diversity in Blastocystis hominis. Mol Biochem Parasitol. 1997;87(1):79-83.
14. Sardarian K, Hajiloo M, Maghsoudi A, Mohgimbeigi A, Alkikhi M. WITHDRAWN: A Study of the Genetic Variability of Blastocystis hominis Isolates in Hamadan, West of Iran. Jundishapur J Microbiol. 2012;6(1):31-5.
15. Stensvold CR, Suresh GK, Tan KS, Thompson RC, Traub RJ, Visco-gliosi E, et al. Terminology for Blastocystis subtypes—a consensus. Trends Parasitol. 2007;23(1):3-6.
16. Motazedian H, Ghasemi H, Sadjadi SM. Genomic diversity of Blastocystis hominis from patients in southern Iran. Ann Trop Med Parasitol. 2008;102(2):185-8.
17. Moosavi A, Haghighi A, Mojarad EN, Zayeri F, Alebouyeh M, Khasan H, et al. Genetic variability of Blastocystis sp isolated from asymptomatic and asymptomatic individuals in Iran. Parasitol Res. 2012;111(6):2311-5.
18. Bohm-Gloning B, Knobloch J, Walderich B. 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. 1997;2(8):773-8.
19. Tamura K, Dudley J, Nei M, Kumar S. MEGA4: Molecular Evolutionary Genetics Analysis (MEGA) software version 4.0. Mol Biol Evol. 2007;24(1):1596-9.
20. Saitou N, Nei M. The neighbor-joining method: a new method for reconstructing phylogenetic trees. Mol Biol Evol. 1987;4(4):406-25.
21. Kimura M. A simple method for estimating evolutionary rates of base substitutions through comparative studies of nucleotide sequences. J Mol Evol. 1980;16(2):111-20.
22. Felsenstein J. Phylogenies and the comparative method. Am Nat. 1985;125:15.
23. Haghighi A, Khorashad AS, Nazemalhosseini M, Ojarud E, Kazemi B, Rostami Nejad M, Rasti S. Frequency of enteric protozoan parasites among patients with gastrointestinal complaints in medical centers of Zahedan, Iran. Trans R Soc Trop Med Hyg. 2007;101(3-4):542-4.
24. Daryani N, Barmaki GH, Ettehad H, Sharif MH, Dehghan A. A cross-sectional study of Blastocystis hominis in primary school children, Northwest Iran. Int J Trop Med. 2006;53:5-57.
25. Saksirisampant W, Nuchprayoon S, Wiwanitkit V, Yenthakam S, Tasova Y, Sahin B, Koltas S, Paydas S. Clinical significance of Blastocystis hominis. Parasitol Res. 2003;96(6-Suppl):S263-70.
26. Saksirisampant W, Prownebon J, Kulkumthorn M, Yenthakam S. Some characteristics of Blastocystis sp. isolated from humans and animals of Blastocystis hominis, a clinical reevaluation. J Med Assoc Thai. 2003;86(3):563-70.
27. Saksirisampant W, Nuchprayoon S, Wisanuttik Y, Yenthakam S, Amapasiri A. Inestinal parasitic infestations in children in an orphanage in Pathum Than province. J Med Assoc Thai. 2003;86 Suppl 2:5263-70.
28. Saksirisampant W, Prownebon J, Kulkumthorn M, Yenthakam S, Janpla S, Nuchprayoon S. Prevalence of intestinal parasitic infestations among school children in the central region of Thailand. J Med Assoc Thai. 2006;89(11):2928-33.
29. Al-Fellani MA, Khan AH, Al-Gazouli RM, Zaid MK, Al-Ferjani MA. Prevalence and Clinical Features of Blastocystis hominis Infection among Patients in Sebha, Libya. Sultan Qaboos Univ Med J. 2007;7(1):35-40.
30. Ozyurt M, Kurt O, Molbak K, Nielsen HV, Nazendaroglu T, Stensvold CR. Molecular epidemiology of Blastocystis infections in Turkey. Parasitol Int. 2008;57(3):300-6.
29. Arisue N, Hashimoto T, Yoshikawa H. Sequence heterogeneity of the small subunit ribosomal RNA genes among Blastocystis isolates. *Parasitology*. 2003;126(Pt 1):3–9.

30. Yoshikawa H, Wu Z, Nagano I, Takahashi Y. Molecular comparative studies among Blastocystis isolates obtained from humans and animals. *J Parasitol*. 2003;89(3):585–94.

31. Yoshikawa H, Abe N, Wu Z. PCR-based identification of zoonotic isolates of Blastocystis from mammals and birds. *Microbiology*. 2004;150(Pt 5):1477–51.

32. Yano Y, Su S, Lai R, Liao H, Ye J, Li X, et al. Genetic variability of *Blastocystis hominis* isolates in China. *Parasitol Res*. 2006;99(5):597–601.

33. Poirier P, Warzywinski I, Albert A, El Alaoui H, Delbac F, Livrelli V. Development and evaluation of a real-time PCR assay for detection and quantification of Blastocystis parasites in human stool samples: prospective study of patients with hematological malignancies. *J Clin Microbiol*. 2011;49(3):975–83.

34. Stensvold CR, Christiansen DB, Olsen KE, Nielsen KE. Blastocystis sp. subtype 4 is common in Danish Blastocystis-positive patients presenting with acute diarrhea. *Am J Trop Med Hyg*. 2011;84(6):881–5.

35. Noel C, Dufey F, Gerbod D, Edgcomb VP, Delgado-Viscogliosi P, Ho LC, et al. Molecular phylogenies of Blastocystis isolates from different host: implications for genetic diversity, identification of species, and zoonosis. *J Clin Microbiol*. 2005;43(1):348–55.

36. Silberman JD, Sogin ML, Leipe DD, Clark CG. Human parasite finds taxonomic home. *Nature*. 1996;380(6573):398.

37. Yoshikawa H, Wu Z, Kimata I, Iseki M, Ali IK, Hossain MB, et al. Polymerase chain reaction-based genotype classification among human Blastocystis hominis populations isolated from different countries. *Parasitol Res*. 2004;92(1):22–9.

38. Tan TC, Ong SC, Suresh KG. Genetic variability of Blastocystis sp. isolates obtained from cancer and HIV/AIDS patients. *Parasitol Res*. 2009;105(5):821–6.

39. Hussein EM, Hussein AM, Eida MM, Atwa MM. Pathophysiologic variability of different genotypes of human Blastocystis hominis Egyptian isolates in experimentally infected rats. *Parasitol Res*. 2008;102(5):853–60.

40. Meloni D, Sanciu G, Poirier P, El Alaoui H, Chabe M, Delhaes L, et al. Molecular subtyping of Blastocystis sp. isolates from symptomatic patients in Italy. *Parasitol Res*. 2011;109(3):613–9.

41. Yakoob J, Jafri W, Beg MA, Abbas Z, Naz S, Islam M, et al. Irritable bowel syndrome: is it associated with genotypes of Blastocystis hominis. *Parasitol Res*. 2009;106(5):1033–8.

42. Jacobson M, Englund S, Ballagi-Pordany A. The use of a mimic to detect polymerase chain reaction-inhibitory factors in feces examined for the presence of Lawsonia intracellularis. *J Vet Diagn Invest*. 2003;15(3):268–73.

43. Lantz PG, Mattsson M, Wadström T, Rådström P. Removal of PCR inhibitors from human faecal samples through the use of an aqueous two-phase system for sample preparation prior to PCR. *J Microbiol Methods*. 1997;31(3):159–67.

44. Monteiro L, Bonnemaison D, Vekris A, Petry KG, Bonnet J, Vidal R, et al. Complex polysaccharides as PCR inhibitors in feces: Helicobacter pylori model. *J Clin Microbiol*. 1997;33(4):995–8.