Annals of Parasitology 2020, 66(1), 19–25
doi: 10.17420/ap6601.234


Original papers

# Molecular evidence for zoonotic transmission of *Blastocystis* subtypes in Kurdistan province, West of Iran

**Fares Bahrami[1,2], Ali Haghighi[2], Ghasem Zamini[3], Mohammadbagher Khademerfan[3,4]**

[1]Zoonoses Research Center, Research Institute for Health Development, Kurdistan University of Medical Sciences, Sanandaj, Iran
[2]Department of Medical Parasitology and Mycology, School of Medicine, Shahid Beheshti University of Medical Sciences, Tehran, Iran
[3]Department of Parasitology and Mycology, Faculty of Medicine, Kurdistan University of Medical Sciences, Sanandaj, Iran
[4]Cellular and Molecular Research Center, Research Institute for Health Development, Kurdistan University of Medical Sciences, Sanandaj, Iran

Corresponding Author: Ghasem Zamini; e-mail: Kurdistan_parasitologist@yahoo.com

**ABSTRACT.** *Blastocystis* sp. is one of the most prevalent human parasites with a vast variety of non-human hosts. The aim of the present study was to determine the subtype distribution of *Blastocystis* in humans and trace the route of transmission by molecular data and phylogenetic analysis. Stool samples were collected from patients who referred to 14 medical laboratories in Kurdistan, Iran. All the samples were examined using the direct wet mount and formalin-ether concentration techniques. DNA extraction was carried out for 30 microscopically positive isolates and 33 negative samples. DNA amplification and subtype identification were also performed using the barcoding method and sequencing techniques. Of 1383 stool samples, 239 (17.3%) were infected with *Blastocystis* sp. Out of the 24 sequenced isolates, two (8.3%), six (25%), and 16 (66.6 %) belonged to the ST1, ST2, and ST3 subtypes, respectively. Bioinformatics analysis indicated that all the isolates were genetically similar to animal isolates. *Blastocystis* sp. was very common and ST1, ST2, and ST3 subtypes were prevalent in the study population. Bioinformatics analysis suggests that zoonotic transmission plays an important role in *Blastocystis* sp. distribution in Kurdistan province.

**Keywords:** *Blastocystis*, prevalence, subtypes, zoonoses, Kurdistan, Iran

## Introduction

*Blastocystis* sp. is one of the most common intestinal anaerobic unicellular eukaryotic parasites infecting humans with a large variety of animal hosts [1]. This parasite is found in normal and diarrheic fecal samples [2]. Although *Blastocystis* organisms have been isolated from a wide range of non-human hosts including mammals, birds, reptiles, amphibians, and insects [3,4], all isolates are morphologically indiscernible [3]. Due to the morphological diversity, it is difficult to assign a standard morphology for the diagnosis of clinical samples [2].

The prevalence of human *Blastocystis* infection differs from region to region because of several environmental and socioeconomic factors [5]. In general, the prevalence of *Blastocystis* is higher in developing countries than in developed ones due to the poor sanitary and hygienic conditions [2,5].

The transmission of *Blastocystis* possibly occurs through animal-to-animal, animal-to-human, human-to-human, and human-to-animal routes [6]. However, the transmission of the parasite via the fecal-oral route from person-to-person or indirectly by eating food or drinking water has been demonstrated among individuals in closed human communities [2,7], as well as among experimental rats in the same cage [8]. The cystic form is also known to be responsible for transmission [7].

*Blastocystis* sp. isolated from humans and animals have been reported to be morphologically identical. However, an extensive genetic variation was observed among numerous *Blastocystis* sp. in human and animal isolates by sequencing PCR products [5]. Furthermore, molecular genetic studies have demonstrated that the genus *Blastocystis* comprises at least 17 subtypes, nine of which, ST1-ST9, have been isolated from both humans and animals worldwide, while the others have been reported only in animals [9,10]. The recent molecular data indicate that the majority of human isolates are ST1 to ST4 in most countries, with a predominance of ST3 [11,12]. Conversely, only a few isolates from animals are found to belong to ST3. Interestingly, all these animal ST3 isolates are found to be from domestic animals living in direct contact with humans. These lines of evidence suggest that only ST3 can easily infect humans, and other animals could be possible reservoirs for it, whereas subtypes 5–9 only sporadically cause infection in humans and are more suitable for animal hosts [2].

Accordingly, molecular epidemiological surveys have been conducted to investigate the genetic diversity of *Blastocystis* in different geographical settings and host assemblages in order to identify host specificity and investigate the possibility of zoonotic transmission routes [6] and the possible pathogenicity of some subtypes [13]. Thus, the molecular studies of *Blastocystis* isolates and the discrimination of subtypes are useful to identify the transmission routes and effective prevention strategies.

The aim of the present study was to identify the subtypes of *Blastocystis* isolates and investigate the possibility of zoonotic transmission routes of *Blastocystis* in Kurdistan province, north-western Iran.

## Materials and Methods

**Parasitological study.** A total number of 1383 stool samples were collected from individuals who referred to 14 medical laboratories in Sanandaj city, the capital of Kurdistan province, from June 2015 to February 2017. All the fecal samples were examined by direct smear (wet mount with normal saline and Lugol's iodine) and formalin-ether concentration techniques for the detection of *Blastocystis* sp. In addition, some positive samples were cultivated in the xenic HSr + S and LIT medium.

Table 1. Characterization of *Blastocystis* subtypes detected in humans in Kurdistan province based on sequence analysis

| | Sample ID | Subtype | Accession No. |
|---|---|---|---|
| 1 | FB(B.ST3)41-IR | ST3 | KY359227 |
| 2 | FB(B.ST3)87-IR | ST3 | KY359228 |
| 3 | FB(B.ST2)99-IR | ST2 | KY359229 |
| 4 | FB(B.ST2)120-IR | ST2 | KY359230 |
| 5 | FB(B.ST3)179-IR | ST3 | KY359231 |
| 6 | FB(B.ST3)282-IR | ST3 | KY359232 |
| 7 | FB(B.ST3)404-IR | ST3 | KY359233 |
| 8 | FB(B.ST1)444-IR | ST1 | KY359234 |
| 9 | FB(B.ST3)513-IR | ST3 | KY359235 |
| 10 | FB(B.ST3)592-IR | ST3 | KY359236 |
| 11 | FB(B.ST3)641-IR | ST3 | KY359237 |
| 12 | FB(B.ST3)669-IR | ST3 | KY359238 |
| 13 | FB(B.ST3)704-IR | ST3 | KY359239 |
| 14 | FB(B.ST3)741-IR | ST3 | KY359240 |
| 15 | FB(B.ST2)773-IR | ST2 | KY359241 |
| 16 | FB(B.ST2)783-IR | ST2 | KY359242 |
| 17 | FB(B.ST2)827-IR | ST2 | KY359243 |
| 18 | FB(B.ST1)912-IR | ST1 | KY359244 |
| 19 | FB(B.ST3)985-IR | ST3 | KY359245 |
| 20 | FB(B.ST3)1039-IR | ST3 | KY359246 |
| 21 | FB(B.ST2)1120-IR | ST2 | KY359247 |
| 22 | FB(B.ST3)1129-IR | ST3 | KY359248 |
| 23 | FB(B.ST3)1369-IR | ST3 | KY359249 |
| 24 | FB(B.ST3)1370-IR | ST3 | KY359250 |

**DNA extraction and PCR amplification.** The DNA of 30 positive samples with morphological diversity was extracted using a commercial DNA extraction kit (YTA, FavorGen, Cat. No YT9032, Taiwan) according to the manufacturer's instructions. DNA was also extracted from 33 samples negative for *Blastocystis* by microscopy.

**PCR amplification.** A 620-bp fragment from the 18S rRNA gene was amplified using the DNA barcoding method and RD5 and BhRDr primers as previously described [14]. PCR was performed using the Taq DNA Polymerase Master Mix Red (Amplicon, Denmark). PCR products were electrophoresed and visualized on 1.5% agarose gel stained with ethidium bromide.

**Sequence analysis and accessions.** The sequence chromatograms were observed using Chromas Version 1.0 (Technelysium Pty. Ltd.,

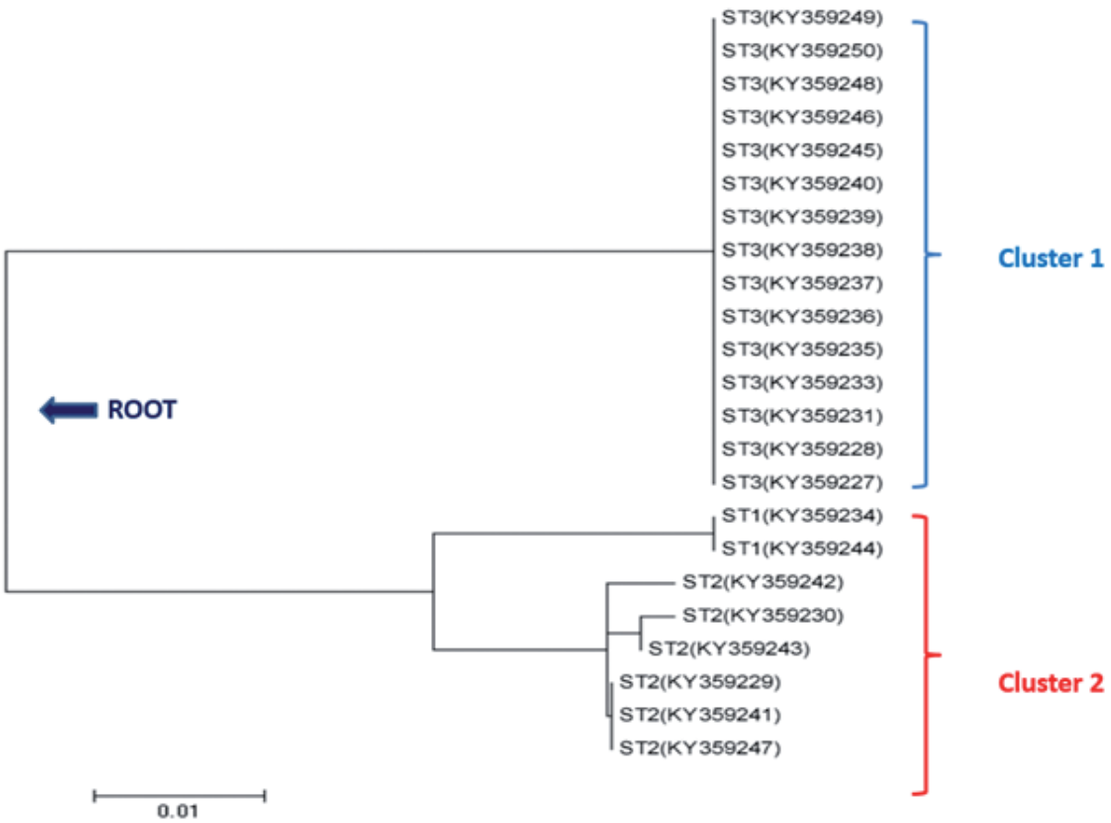


Figure 1. Phylogenetic relationships of *Blastocystis* subtypes in our study

Helensvale, Queensland, Australia). The nucleotide sequences were manually edited. The nucleotide sequences of 24 isolates in the present study were submitted to the GenBank/EMBL/DDBJ database under the accession numbers KY359227 to KY359250 (Table 1).

**Ethical clearance.** All the procedures were approved by the Ethics Committee of Shahid Beheshti University of Medical Sciences (SBMU), Iran (Grant No. 6598) with respect to the human rights. The authors asserted that all the procedures of this work complied with the ethical standards of the Declaration of Helsinki as revised in 2008.

## Results

The microscopy results showed that 239 (17.3%) individuals were positive for *Blastocystis* sp. Based on PCR, all the 30 selective microscopy-positive samples showed a positive band. In addition, out of the 33 randomly selected negative samples, five isolates showed a 620-bp fragment band on the agarose gel.

Thirty positive samples by PCR method were sent to Macrogen® Corp for sequencing. Six samples failed to generate a sequence. The sequence analysis revealed that 2 (8.3%), 6 (25%), and 16 (66.6 %) isolates belonged to subtypes 1, 2, and 3, respectively. All the subtypes showed 99-100 homology with their own kind and sequences in the GenBank.

According to the phylogenetic tree (Fig. 1), all the sequences generated in the current study were placed in two branches. Subtypes I and II were placed in the same branch and subtype III in a different one. The results of the phylogenetic analysis also represented that *Blastocystis* subtypes isolated in our study were placed near to the subtypes isolated from animals (Fig. 2).

In order to investigate the relationship between *Blastocystis* subtypes and morphological diversity, 12 out of 30 selected samples for sequencing with different morphological forms, including avacuolar, granular, cyst, and vacuolar forms in the microscopic method, and three cases in the growth medium (amoeboid form) were selected to examine the relationship between the morphological form and *Blastocystis* subtypes. All morphological forms were observed in the three subtypes.

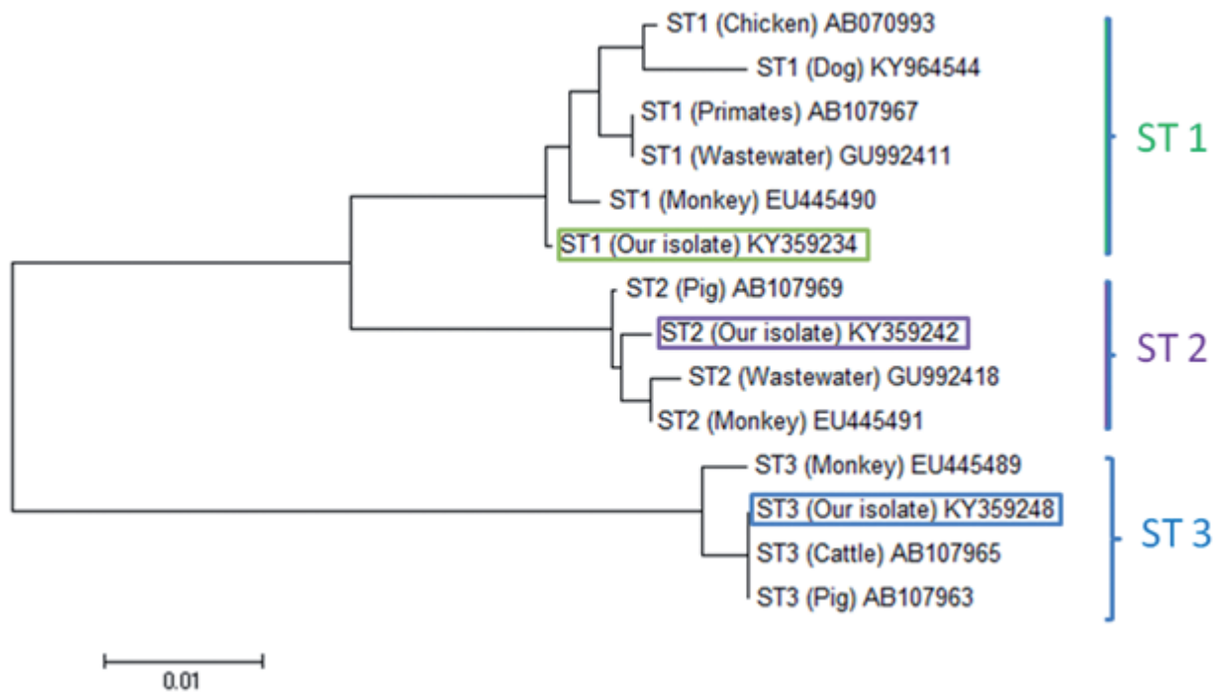


Figure 2. Phylogenetic relationships between *Blastocystis* subtypes (KY isolate) in this study and animal isolates of *Blastocystis* subtypes previously published in GenBank

## Discussion

To date, there have been a few studies concerning the molecular epidemiology of *Blastocystis* infections in Iran, mostly describing the distribution of *Blastocystis* subtypes. Most of them proposed the investigation of the association between *Blastocystis* subtypes and clinical symptoms. However, the phylogenetic analysis and transmission route of *Blastocystis* have not yet been investigated. Therefore, this study aimed to improve our understanding of the aspects of transmission routes of human *Blastocystis* in Kurdistan province, the northwest of Iran.

In the current study, the results obtained from sequencing revealed that ST1, ST2, and ST3 were the subtypes obtained from human fecal samples. In similar studies, these subtypes were isolated from humans in different parts of Iran [15–19], Lebanon [20], Senegal [21], Turkey [22], Libya [23], Makkah [24], Egypt [13], China [25], Poland [26], and France [11]. The predominance of subtype 3 in this study is consistent with the studies from Iran [15,18], Turkey [22], Saudi Arabia [24], China [25], Singapore [27], Colombia [28], and France [11]. However, in another study from Iran [17], subtype 2, and in studies from Libya [23] and China [29], subtype 1 was predominant. Furthermore, in Iran, subtype 3 was identified from the cattle [30]. These three subtypes were also common in water and wastewater [31,32]. Additionally, these subtypes have been introduced as the most common *Blastocystis* subtypes in cancer and HIV+/AIDS patients [33]. Subtypes 4 [18,34,35], 5 [15,18,34, 35], and 7 [35] have also been reported in humans in Iran.

The results of the phylogenetic analysis also represented that *Blastocystis* subtypes isolated from both human and animal subjects were placed beside each other, indicating the high possibility of zoonotic transmission of this microorganism (Fig. 2). In that way, phylogenetic analysis indicated ST1 isolates in our study were 99-100% homologous with ST1 sequences obtained from chicken (AB070993), monkey (EU445490), primates (AB107967), dog (KY964544), and wastewater (GU992411). In addition, this homology was observed between ST2 isolates in our study and ST2 sequenced from pig (AB107969), monkey (EU445491), and wastewater (GU992418). Furthermore, ST3 isolates in our study had 95-100% homology with sequences from the cattle (AB107965), monkey (EU445489), and pig (AB107963). Accordingly, the potential zoonotic transmission of *Blastocystis* is tracked in the region.

Therefore, we conclude that animals could play

an important role in the epidemiology of *Blastocystis* infection. It is noteworthy to indicate that *Blastocystis* is considered as the most common water-borne intestinal infection. *Blastocystis* commonly exists in water contaminated with faeces of infected humans or animals [31,32]. Thus, it has been suggested that people can be infected with *Blastocystis* of zoonotic origin without direct contact with animals. Therefore, zoonotic *Blastocystis* can be transmitted to humans by ingesting *Blastocystis*-contaminated food and water.

*Blastocystis* is one of the most common intestinal parasites in Kurdistan province and it specifically belongs to the ST1, ST2, and ST3 subtypes. Bioinformatics analysis to compare the phylogenetic patterns of *Blastocystis* subtypes sequenced in this study and the isolates from animals in GenBank showed that zoonotic transmission plays a crucial role in *Blastocystis* transmission. However, further studies should be performed on the distribution of *Blastocystis* subtypes in animals and different water sources in Iran, where *Blastocystis* is reported in humans.

## Acknowledgements

We thank all technicians and personnel working in the medical laboratories, School of Medicine, Kurdistan University of Medical Sciences and also those who work at the Department of Parasitology and Mycology, School of Medicine, Shahid Beheshti University of Medical Sciences who contributed to the study.

This work was a part of the Ph.D thesis of Fares Bahrami under supervision of Prof. Ali Haghighi and Dr. Ghasem Zamini, which was supported financially by Kurdistan University of Medical Sciences (Grant No. 335) and Shahid Beheshti University of Medical Sciences (Grant No. 6598).

## References

[1] Denoeud F., Roussel M., Noel B., Wawrzyniak I., Da Silva C., Diogon M., Viscogliosi E., Brochier-Armanet C., Couloux A., Poulain J., Segurens B., Anthouard V., Texier C., Blot N., Poirier P., Ng G.C., Tan K.S.W., Artiguenave F., Jaillon O., Aury J.-M., Delbac F., Wincker P., Vivarès C.P., El Alaoui H. 2011. Genome sequence of the stramenopile *Blastocystis*, a human anaerobic parasite. *Genome Biology* 12: R29. doi:10.1186/gb-2011-12-3-r29

[2] Mehlhorn H., Tan K.S.W., Yoshikawa H. 2012. *Blastocystis*: pathogen or passenger? An evaluation of 101 years of research. Parasitology Research Monographs. Vol. 4. Springer-Verlag, Berlin-Heidelberg, Germany.

[3] Poirier P., Wawrzyniak I., Vivarès C.P., Delbac F., El Alaoui H. 2012. New insights into *Blastocystis* spp.: a potential link with irritable bowel syndrome. *PLoS Pathogens* 8: e1002545. doi:10.1371/journal.ppat.1002545

[4] Zierdt C.H. 1991. *Blastocystis hominis* – past and future. *Clinical Microbiology Reviews* 4: 61-79. doi:10.1128/CMR.4.1.61

[5] Wawrzyniak I., Poirier P., Viscogliosi E., Dionigia M., Texier C., Delbac F., El Alaoui H. 2013. *Blastocystis*, an unrecognized parasite: an overview of pathogenesis and diagnosis. *Therapeutic Advances in Infectious Disease* 1: 167-178. doi:10.1177/2049936113504754

[6] Noël C., Dufernez F., Gerbod D., Edgcomb V.P., Delgado-Viscogliosi P., Ho L.-C., Singh M., Wintjens R., Sogin ML., Capron M., Pierce R., Zenner L., Viscogliosi E. 2005. Molecular phylogenies of *Blastocystis* isolates from different hosts: implications for genetic diversity, identification of species, and zoonosis. *Journal of Clinical Microbiology* 43: 348-355. doi:10.1128/JCM.43.1.348-355.2005

[7] Parija S.C., Jeremiah S.S. 2013. *Blastocystis*: taxonomy, biology and virulence. *Tropical Parasitology* 3: 17-25. doi:10.4103/2229-5070.113894

[8] Yoshikawa H., Yoshida K., Nakajima A., Yamanari K., Iwatani S., Kimata I. 2004. Fecal-oral transmission of the cyst form of *Blastocystis hominis* in rats. *Parasitology Research* 94: 391-396. doi:10.1007/s00436-004-1230-5

[9] Stensvold C.R., Suresh G.K., Tan K.S.W., Thompson R.C.A., Traub R.J., Viscogliosi E., Yoshikawa H., Clark C.G. 2007. Terminology for *Blastocystis* subtypes – a consensus. *Trends in Parasitology* 23: 93-96. doi:10.1016/j.pt.2007.01.004

[10] Santín M., Gómez-Muńoz M.T., Solano-Aguilar G., Fayer R. 2011. Development of a new PCR protocol to detect and subtype *Blastocystis* spp. from humans and animals. *Parasitology Research* 109: 205-212. doi:10.1007/s00436-010-2244-9

[11] Souppart L., Sanciu G., Cian A., Wawrzyniak I., Delbac F., Capron M., Dei-Cas E., Boorom K., Delhaes L., Viscogliosi E. 2009. Molecular epidemiology of human *Blastocystis* isolates in France. *Parasitology Research* 105: 413. doi:10.1007/s00436-009-1398-9

[12] Cian A., El Safadi D., Osman M., Moriniere R., Gantois N., Benamrouz-Vanneste S., Delgado-Viscogliosi P., Guyot K., Li L.-L., Monchy S., Noël C., Poirier P., Nourrisson C., Wawrzyniak I., Delbac F., Bosc S., Chabé M., Petit T., Certad G., Viscogliosi E. 2017. Molecular epidemiology of *Blastocystis* sp. in various animal groups from two French zoos and evaluation of potential zoonotic risk. *PloS One* 12:

e0169659. doi:10.1371/journal.pone.0169659
[13] Souppart L., Moussa H., Cian A., Sanciu G., Poirier P., El Alaoui H., Delbac F., Boorom K., Delhaes L., Dei-Cas E., Viscogliosi E. 2010. Subtype analysis of *Blastocystis* isolates from symptomatic patients in Egypt. *Parasitology Research* 106: 505-511. doi:10.1007/s00436-009-1693-5
[14] Scicluna S.M., Tawari B., Clark C.G. 2006. DNA barcoding of *Blastocystis*. *Protist* 157: 77-85. doi:10.1016/j.protis.2005.12.001
[15] Moosavi A., Haghighi A., Mojarad E.N., Zayeri F., Alebouyeh M., Khazan H., Kazemi B., Zali M.R. 2012. Genetic variability of *Blastocystis* sp. isolated from symptomatic and asymptomatic individuals in Iran. *Parasitology Research* 111: 2311-2315. doi:10.1007/s00436-012-3085-5
[16] Alinaghizade A., Mirjalali H., Mohebali M., Stensvold C.R., Rezaeian M. 2017. Inter-and intra-subtype variation of *Blastocystis* subtypes isolated from diarrheic and non-diarrheic patients in Iran. *Infection, Genetics and Evolution* 50: 77-82. doi:10.1016/j.meegid.2017.02.016
[17] Jalallou N., Iravani S., Rezaeian M., Alinaghizade A., Mirjalali H. 2017. Subtypes distribution and frequency of *Blastocystis* sp. isolated from diarrheic and non-diarrheic patients. *Iranian Journal of Parasitology* 12: 63-68.
[18] Khademvatan S., Masjedizadeh R., Yousefi-Razin E., Mahbodfar H., Rahim F., Yousefi E., Foroutan M. 2018. PCR-based molecular characterization of *Blastocystis hominis* subtypes in southwest of Iran. *Journal of Infection and Public Health* 11: 43-47. doi:10.1016/j.jiph.2017.03.009
[19] Mirjalali H., Abbasi M.R., Naderi N., Hasani .Z, Mirsamadi E.S., Stensvold C.R., Balaii H., Aghdaei HA., Zali M.R. 2017. Distribution and phylogenetic analysis of Blastocystis sp. subtypes isolated from IBD patients and healthy individuals in Iran. *European Journal of Clinical Microbiology and Infectious Diseases* 36: 2335-2342. doi:10.1007/s10096-017-3065-x
[20] El Safadi D., Meloni D., Poirier P., Osman M., Cian A., Gaayeb L., Wawrzyniak I., Delbac F., El Alaoui H., Delhaes L., Dei-Cas E., Mallat H., Dabboussi F., Hamze M., Viscogliosi E. 2013. Molecular epidemiology of *Blastocystis* in Lebanon and correlation between subtype 1 and gastrointestinal symptoms. *The American Journal of Tropical Medicine and Hygiene* 88: 1203-1206. doi:10.4269/ajtmh.12-0777
[21] El Safadi D., Gaayeb L., Meloni D., Cian A., Poirier P., Wawrzyniak I., Delbac F., Dabboussi F., Delhaes L., Seck M., Hamze M., Riveau G., Viscogliosi E. 2014. Children of Senegal River Basin show the highest prevalence of *Blastocystis* sp. ever observed worldwide. *BMC Infectious Diseases* 14: 164. doi:10.1186/1471-2334-14-164
[22] Dogruman-Al F., Yoshikawa H., Kustimur S., Balaban N. 2009. PCR-based subtyping of *Blastocystis* isolates from symptomatic and asymptomatic individuals in a major hospital in Ankara, Turkey. *Parasitology Research* 106: 263-268. doi:10.1007/s00436-009-1658-8
[23] Abdulsalam A.M., Ithoi I., Al-Mekhlafi H.M., Al-Mekhlafi A.M., Ahmed A., Surin J. 2013. Subtype distribution of *Blastocystis* isolates in Sebha, Libya. *PLoS One* 8: e84372. doi:10.1371/journal.pone.0084372
[24] Mohamed R.T., El-Bali M.A., Mohamed A.A., Abdel-Fatah M.A., El-Malky M.A., Mowafy N.M., Zaghlool D.A., Bakri R.A., Al-Harthi S.A. 2017. Subtyping of *Blastocystis* sp. isolated from symptomatic and asymptomatic individuals in Makkah, Saudi Arabia. *Parasites and Vectors* 10: 174. doi:10.1186/s13071-017-2114-8
[25] Li L.-H., Zhou X.-N., Du Z.-W., Wang X.-Z., Wang L.-B., Jiang J.-Y., Yoshikawa H., Steinmann P., Utzinger J., Wu Z., Chen J.-X., Chen S.-H., Zhang L. 2007. Molecular epidemiology of human *Blastocystis* in a village in Yunnan province, China. *Parasitology International* 56: 281-286. doi:10.1016/j.parint.2007.06.001
[26] Kaczmarek A., Gołąb E., Żarnowska-Prymek H., Rawska A., Jańczak D., Lewicki A., Wesołowska M., Rożej-Bielicka W., Cielecka D., Sałamatin R. 2017. Genetic diversity of *Blastocystis hominis* sensu lato isolated from humans in Poland. *Epidemiological Review* 71: 539-546.
[27] Wong K.H.S., Ng G.C., Lin R.T.P., Yoshikawa H., Taylor M.B., Tan K.S.W. 2008. Predominance of subtype 3 among *Blastocystis* isolates from a major hospital in Singapore. *Parasitology Research* 102: 663-670. doi:10.1007/s00436-007-0808-0
[28] Ramírez J.D., Sánchez LV., Bautista D.C., Corredor A.F., Flórez A.C., Stensvold C.R. 2014. *Blastocystis* subtypes detected in humans and animals from Colombia. *Infection, Genetics and Evolution* 22: 223-228. doi:10.1016/j.meegid.2013.07.020
[29] Yan Y., Su S, Lai R., Liao H., Ye J., Li X., Luo X., Chen G. 2006. Genetic variability of *Blastocystis hominis* isolates in China. *Parasitology Research* 99: 597-601. doi:10.1007/s00436-006-0186-z
[30] Badparva E., Sadraee J., Kheirandish F. 2015. Genetic diversity of *Blastocystis* isolated from cattle in Khorramabad, Iran. *Jundishapur Journal of Microbiology* 8: e14810. doi:10.5812/jjm.14810
[31] Banaticla J.E.G., Rivera W.L. 2011. Detection and subtype identification of *Blastocystis* isolates from wastewater samples in the Philippines. *Journal of Water and Health* 9: 128-137. doi:10.2166/wh.2010.127
[32] Noradilah S.A., Lee I.L., Anuar T.S., Salleh F.M., Abdul Manap S.N.A., Mohd Mohtar N.S.H., Azrul S.M., Abdullah W.O., Moktar N. 2016. Occurrence of *Blastocystis* sp. in water catchments at Malay villages

and Aboriginal settlement during wet and dry seasons in Peninsular Malaysia. *PeerJ* 4: e2541. doi:10.7717/peerj.2541

[33] Tan T.C., Ong S.C., Suresh K.G. 2009. Genetic variability of *Blastocystis* sp. isolates obtained from cancer and HIV/AIDS patients. *Parasitology Research* 105: 1283. doi:10.1007/s00436-009-1551-5

[34] Khademvatan S., Masjedizadeh R., Rahim F., Mahbodfar H., Salehi R., Yousefi-Razin E., Foroutan M. 2017. *Blastocystis* and irritable bowel syndrome: frequency and subtypes from Iranian patients. *Parasitology International* 66: 142-145. doi:10.1016/j.parint.2017.01.005

[35] Khoshnood S., Rafiei A., Saki J., Alizadeh K. 2015. Prevalence and genotype characterization of *Blastocystis hominis* among the baghmalek people in southwestern Iran in 2013-2014. *Jundishapur Journal of Microbiology* 8: e23930. doi:10.5812/jjm.23930

*Received 16 April 2019*
*Accepted 23 October 2019*