

# Molecular characterization of *Blastocystis hominis* in irritable bowel syndrome patients and nursing staff in public and private clinic in Iraq

Mohammad Ismail ALBERFKANI

Medical Laboratory Technology, College of Health and Medical Techniques, Duhok Polytechnic University, Duhok, Zakho, Iraq

E-mail: Mohammad.said@dpu.edu.krd

**ABSTRACT.** Irritable bowel syndrome (IBS) is common gastrointestinal disorder with prevalence globally. Parasitic infection is one of the major risk factors for developing irritable bowel syndrome. We aim to estimate the incidence of *Blastocystis* sp. among IBS patients and nurse staff so as to assess the association between IBS and the *Blastocystis* infection by using microscopic and molecular techniques. Stool samples were collected from 136 people in the IBS group, 84 participates in the control group and 30 in nurse staff. The parasitic infection was recognized via a microscopic examination and confirmed by using PCR targeting SSU rRNA gene. The overall prevalence of the parasite through microscopic examination was 42.8%, including rates of 79.4%, 18.6% and 1.8% in the IBS, nurse staff and control groups, respectively. While the prevalence estimates for parasitic infections based on PCR was 46%, including rates of 74.7%, 21.7% and 3.4% in the IBS, nurse staff and control groups, respectively. The *Blastocystis* isolates of this study are *Blastocystis hominis* belong to subtype ST3 which was the predominant subtype isolated. All microscopically positive samples were also found positive by PCR, in addition eight microscopically negative samples were found positive by PCR. Hence PCR analysis was more sensitive than microscopic analyses. The rate of *Blastocystis* sp. using both methods was different significantly ( $P \leq 0.05$ ). Concerning the incidence of *Blastocystis* sp., statistically significant association were found between nurse staff and IBS patients by using the diagnostic technique performed.

**Keywords:** *Blastocystis* sp., irritable bowel syndrome, nursing staff, SSU rRNA gene, Iraq

## Introduction

Irritable bowel syndrome (IBS) is a common chronic gastrointestinal syndrome of large intestine characterized by abdominal discomfort with bowel movement and changes in the stool form, which may cause by several factors such as psychosocial factor, altered the hypersensitivity and the motility of the gastrointestinal tract, visceral hyperalgesia, dietary intakes, food sensitivity and intestinal inflammation caused by parasitic infection [1,2]. Post-infection is another common onset of IBS which following gastroenteritis infectious [3]. The average prevalence of postinfectious IBS following infectious gastroenteritis is 10% [4]. It has been mostly described as gastroenteritis outbreaks and traveler's diarrhea and several pathogens have been concerned in the development of IBS including

bacteria such as *Escherichia coli*, *Campylobacter jejuni*, and *Shigella sonnei* [5,6]. Besides that, several intestinal parasites such as *Entamoeba histolytica*, *Trichinella* spp. and *Blastocystis hominis* have been found as causative factor in the development of IBS symptoms [7,8]. Although, several factors may attribute in the development of IBS syndrome, post infections gastroenteritis caused by parasitic infection is considered as the main causes of developing IBS [9,10]. Among intestinal parasites, *Blastocystis hominis* is one the most common protozoan observed in the gastrointestinal tract of humans as well as livestock [11]. The parasite is spread directly by faecal-oral route from person to person or from animal [12]. *Blastocystis* has be present in several morphological forms (amoeboid, cyst, granular, vacuolar, avacuolar and multi-vacuolar forms) and is spread

from person to another by the faecal-oral route [13]. There are common symptoms among IBS and *Blastocystis* infected patients represented by abdominal pain associated with constipation diarrhea, nausea, fatigue and cramps [14]. Several molecular reports have been recorded to estimate the pathogenic potential of the parasite that detected in asymptomatic individuals as well as in symptomatic patients [15,16]. However, detection of parasite in stool by using microscopic examination and culturing are time consumed and required a skilled technologist, hence in the present study, we attempted to use 18S rRNA gene as DNA marker for detection the parasites among IBS patients visiting hospital and clinical care center. Therefore, this study provides the genetic diversity of *Blastocystis hominis* amongst asymptomatic individuals and IBS patients by using PCR based methods.

## Materials and Methods

### Sample collection

The study performed at private and public clinic (Duhok, Iraq) from April 2021 to April 2022. A total of 250 stool samples were collected from 250 individuals of both gender and different ages (18–70 years): 136 patients had irritable bowel syndrome (IBS) confirmed by Rome IV Criteria [17], 30 from nurse staff and 84 control group. During the interviews, demographic data was collected from all participants. Double faecal samples were screen by using microscopy and PCR methods for detection *Blastocystis* sp. Microscopic examination and molecular detection performed at the Research Laboratory, Duhok Polytechnic University (Duhok, Iraq). Stool samples were refrigerating at 4°C until sent to the Research Laboratory for DNA extraction.

### Microbiological and molecular techniques

For diagnosis of oocysts of *Blastocystis* sp., all stool samples were directly examined under the microscope using direct stool wet smears at the same time, one with Lugol's iodine and the second with normal saline. For molecular detection, the DNA extracted from 200 mg stool samples using the Presto Stool DNA Extraction Kit (Geneaid, Taiwan) according to the manufacturer's protocol. Detection of *Blastocystis* was accomplished by amplified conserved region of 18S small subunit ribosomal RNA (ssuRNA) gene [18]. Two pairs of universal primers; forward primer SR1 (5-GCTTAT

CTGGTTGATCCTGCCAGT-3) and reversed primer SR2 (5-TGATCCCGCAGGTTCA CCA-3) were used for amplify a PCR product of bp 1800 bps. The amplification conditions using a Gene Amp Thermocycler (Applied, Germany) under the following PCR settings: 94°C for 3 minutes, 35 cycles of 30 second at 94°C, 1 minute at 57°C, 2 minutes at 72°C and a final extension step of 7 minutes at 72°C. Obtained amplicons were separated by electrophoresis on 1.5% agarose gels stained with Safe Red DNA Dye. Then, PCR products were sent to Macrogen company (South Korean).

All sequences were compared with nine reference sequences in GenBank (<http://www.ncbi.nlm.nih.gov/BLAST>) [15]. The BLAST Basic Local Alignment Search Tool (<http://www.ncbi.nlm.nih.gov/BLAST>) were used for analysis DNA sequences. Trimming and clean-up of aligned sequences was done with BioEdit software and the obtained nucleotide sequences were recorded in the GenBank to obtain specific accession number. MEGA10 was used for conducting the phylogenetic tree by using Maximum Likelihood modes; Hasegawa-Kishino-Yano.

### Statistical analysis

Results were considered statistically significant if the  $P < 0.05$  and more than that was considered non-significant. SPSS software (Version 25) was used for statistical analyses.

## Results

The overall prevalence of *B. hominis* by using direct microscopy and PCR was 42.8% (107/250) and 46% (115/250), respectively. Based on microscopic examination, *B. hominis* was positive in 79.4% (85/107) among irritable bowel syndrome patients compared to 1.8% (2/107) in control and 18.6% (20/107) in nurse staff ( $P \leq 0.05$ ; Tab. 1). PCR for *B. hominis* was positive in 74.7% (86/115) in IBS patients compared to 3.4% (4/115) in control and 21.7% (25/115) in nurse staff ( $P \leq 0.05$ ; Tab. 1). All microscopically positive samples were also found positive by PCR, in addition eight microscopically negative samples (1 sample from IBS, 5 samples from nurse staff and 2 samples from control) were found positive by PCR. Hence PCR analysis was more sensitive than microscopic analyses. The difference between the prevalence of *Blastocystis* spp. using both methods was significant ( $P \leq 0.05$ ).

Table 1. Comparison of *Blastocystis* spp. in different groups by using microscopic and PCR techniques

Techniques		Patients IBS n=136	Nurse staff n=30	Control n=84	Total n=250	P-value $\chi^2$
Microscopy	Positive	85 (62.5%)	20 (66.6%)	2 (2.3%)	107 (42.8%)	$P<0.05$
	Negative	51 (37.5%)	10 (73.3%)	82 (83.3%)	143 (57.2%)	$\chi^2=84.594$
PCR	Positive	86 (63.2%)	25 (83.3%)	4 (4.7%)	115 (46%)	$P<0.05$
	Negative	50 (36.7%)	5 (16.6%)	80 (95.2%)	135 (54%)	$\chi^2=90.605$

Our study reported that *B. hominis* was positive in collected samples. In both IBS patients and nurse staff, *B. hominis* diagnosis by PCR was significantly positive compared to microscopic examination. Conventional PCR technique was used to target small subunit RNA gene sequence of *Blastocystis* in 250 samples. 115 samples exhibited distinct bands of 1800 bps on agarose gel which confirm the

presence of *Blastocystis* spp. Out of 115 samples that were positive by PCR, only 50 readable sequences were obtained and were search in NCBI using n-BLAST algorithm. The BLAST analyses revealed the presence of subtypes 3 (ST3) of *Blastocystis hominis* among the isolated samples. Our results confirmed that ST3 was the most prevalent subtype was detected among collected

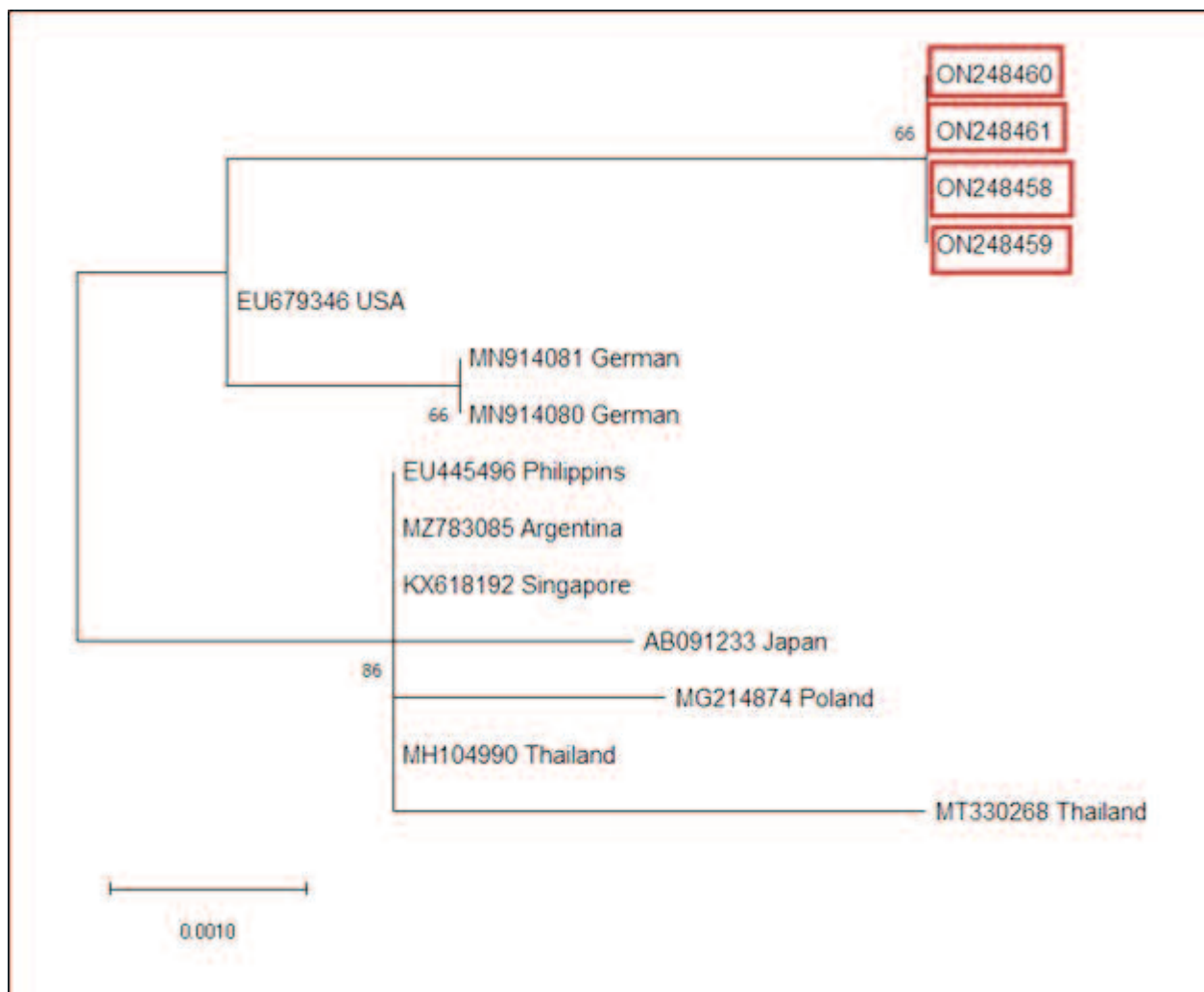


Figure 1. Maximum Likelihood tree displaying relationship amongst SSU rRNA gene sequences of *Blastocystis* from this study (red rectangle) with reference sequences from GenBank

samples. Four readable sequences were recorded in the GenBank under the following accession number; ON248458-ON248461. The present study showed that *Blastocystis hominis* is the causative agent of irritable bowel syndrome. According to the constructed phylogenetic tree (Fig. 1). All isolates were clustered together with other identified strains of the same species with 99.4–100% genotypes. All sequence of the *Blastocystis* with accession number ON248458-ON248461 were clustered together in one clade with high bootstrap support from Maximum Likelihood (66%). The sequence of EU679346 from USA joins our sequence in a well-supported clade. Other sequences showed closely related with different bootstrap as presented in figure 1. In To our knowledge, this is the first report revealing the presence of *Blastocystis hominis* belonging to the ST3 in IBS patients and nurse staff from Iraq.

## Discussion

The consequences of our study are that *B. hominis* was seen with a higher frequency in patients suffering from IBS followed by nurse staff and healthy individuals. Our finding is agreeing with several studies that have suggested a possible association between *Blastocystis hominis* and IBS [7,19–21]. However, the study shows a positive association between subtype of *Blastocystis* and IBS patients, which suggesting that *Blastocystis* ST3 can be regarded as a pathogenic subtype and probable associated with clinical manifestation diarrhoea. Domination of ST3 *Blastocystis* in collected samples from IBS patients is in line with previous studies [22–24]. It is possible that we are able to confirmed the infections with *B. hominis* in fixed stool samples by using microscopy with normal saline and Lugol's iodine staining and PCR examination of unfixed stool specimens. Stool examination under microscope is the standard diagnostic method for detecting protozoa and helminths due to easy access to the resources and less time required to conduct the methods [25]. However, in fresh samples, fat globules and other contaminants may microscopically diagnosis and reported as vegetative stages of *Blastocystis* sp. as well as the protozoan may rapidly deteriorate and become microscopically unrecognizable [26]. Besides that, the microscopic based technique was significantly affected by the number of parasite presence in the stool samples and false negative

result may associated with ST3 of *Blastocystis* which might encyst before shedding and become hardly recognized by microscopy techniques [27].

Hence, molecular methods using PCR assay for detection of protozoan has become the most favorable diagnostic method and used as reference technique for analysis the genetic information due to its highly sensitivity and specificity over other technique methods [28,29]. Our results concurred with other report suggestions that PCR analysis of *B. hominis* has a better result than that of microscopy [12,23,30,31]. In the current study PCR assay revealed the predominant of ST3 among the collected samples which is concordance with other studies conducted worldwide [32,33]. Previous reports have suggested that the possibility of subtypes of *B. hominis* may be linked with symptomatic and asymptomatic infections [12,23,30]. Studies among rates shown the different level of pathological changes in based on the subtypes. The mild degree was observed in asymptomatic subtypes (ST2, ST3 and ST4) infected rats, whereas moderate and severe degree was observed in symptomatic subtypes (ST1, ST3 and ST4) infected rats. The permeability of the intestinal cell was improved in symptomatic ST1 comparing with symptomatic ST3 and ST4, infected rats which shown minimum effects [23]. Hence, ST1 was considered as highly related to the pathogenicity of *B. hominis* [23,30,34].

## References

- [1] Quigley E.M., Fried M., Gwee K.A., Khalif I., Hungin A.P.S., Lindberg G., Abbas Z., Fernandez L.B., Bhatia S.J., Schmulson M., Olano C. 2016. World Gastroenterology Organisation global guidelines irritable bowel syndrome: a global perspective update September 2015. *Journal of Clinical Gastroenterology* 50(9): 704–713. doi:10.1097/MCG.0000000000000653
- [2] Lacy B.E., Mearin F., Chang L., Chey W.D., Lembo A.J., Simren M., Spiller R. 2016. Bowel disorders. *Gastroenterology* 150(6): 1393–1407. doi:10.1053/j.gastro.2016.02.031
- [3] Ibarra C., Herrera V., Perez de Arce E., Gil L.C., Madrid A.M., Valenzuela L., Beltran C.J. 2016. [Parasitosis and irritable bowel syndrome]. *Revista Chilena de Infectologia* 33(3): 268–274 (in Spanish with summary in English). doi:10.4067/S0716-10182016000300003
- [4] Downs I.A., Aroniadis O.C., Kelly L., Brandt L.J. 2017. Postinfection irritable bowel syndrome. *Journal of Clinical Gastroenterology* 51(10):

- 869–877. doi:10.1097/mcg.0000000000000924
- [5] Ragavan N.D., Kumar S., Chye T.T., Mahadeva S., Shiaw-Hooi H. 2015. *Blastocystis* sp. in irritable bowel syndrome (IBS)-detection in stool aspirates during colonoscopy. *PLoS One* 10(9): e0121173. doi:10.1371/journal.pone.0121173
- [6] Youn Y.H., Kim H.C., Lim H.C., Park J.J., Kim J.H., Park H. 2016. Long-term clinical course of post-infectious irritable bowel syndrome after shigellosis: a 10-year follow-up study. *Journal of Neurogastroenterology and Motility* 22(3): 490–496. doi:10.5056/jnm15157
- [7] Rajilić-Stojanović M., Biagi E., Heilig H.G., Kajander K., Kekkonen R.A., Tims S., de Vos W.M. 2011. Global and deep molecular analysis of microbiota signatures in fecal samples from patients with irritable bowel syndrome. *Gastroenterology* 141(5): 1792–1801. doi:10.1053/j.gastro.2011.07.043
- [8] Surangsrirat S., Thamrongwittawatpong L., Piyaniran W., Naaglor T., Khoprasert C., Taamasri P., Leelayoova S. 2010. Assessment of the association between *Blastocystis* infection and irritable bowel syndrome. *Journal of the Medical Association of Thailand* 93(11): 119–124.
- [9] Botschuijver S., Roeselers G., Levin E., Jonkers D.M., Welting O., Heinsbroek S.E., de Weerd H.H., Boekhout T., Fornai M., Masclee A.A., Schuren F.H. 2017. Intestinal fungal dysbiosis is associated with visceral hypersensitivity in patients with irritable bowel syndrome and rats. *Gastroenterology* 153(4): 1026–1039. doi:10.1053/j.gastro.2017.06.004
- [10] Klem F., Wadhwa A., Prokop L.J., Sundt W.J., Farrugia G., Camilleri M., Singh S., Grover M. 2017. Prevalence, risk factors, and outcomes of irritable bowel syndrome after infectious enteritis: a systematic review and meta-analysis. *Gastroenterology* 152(5): 1042–1054. doi:10.1053/j.gastro.2016.12.039
- [11] Haider S.S., Baqai R., Qureshi F.M., Boorom K. 2012. *Blastocystis* spp., *Cryptosporidium* spp., and *Entamoeba histolytica* exhibit similar symptomatic and epidemiological patterns in healthcare-seeking patients in Karachi. *Parasitology Research* 111(3): 1357–1368. doi:10.1007/s00436-012-2972-0
- [12] Kumarasamy V., Anbazhagan D., Subramaniyan V., Vellasamy S. 2018. *Blastocystis* sp., parasite associated with gastrointestinal disorders: an overview of its pathogenesis, immune modulation and therapeutic strategies. *Current Pharmaceutical Design* 24(27): 3172–3175. doi:10.2174/1381612824666180807101536
- [13] Prodeus T.V., Fedianina L.V., Frolova A.A. 2014. [Morphological identification of *Blastocystis*]. *Meditinskaja Parazitologija i Parazitarnye Bolezni* (1): 9–13 (in Russian with summary in English).
- [14] Beyhan Y.E., Yilmaz H., Cengiz Z.T., Ekici A. 2015. Clinical significance and prevalence of *Blastocystis hominis* in Van, Turkey. *Saudi Medical Journal* 36(9): 1118–1121. doi:10.15537/smj.2015.9.12444
- [15] Maloney J.G., Lombard J.E., Urie N.J., Shivley C.B., Santin M. 2019. Zoonotic and genetically diverse subtypes of *Blastocystis* in US pre-weaned dairy heifer calves. *Parasitology Research* 118(2): 575–582. doi:10.1007/s00436-018-6149-3
- [16] Stensvold C.R., Clark C.G. 2020. Pre-empting Pandora's box: *Blastocystis* subtypes revisited. *Trends in Parasitology* 36(3): 229–232. doi:10.1016/j.pt.2019.12.009
- [17] Mearin F., Rey E., Balboa A. 2016. Functional and motor gastrointestinal disorders. *Gastroenterologia y Hepatologia* 39(1): 3–13. doi:10.1016/S0210-5705(16)30169-8
- [18] Scicluna S.M., Tawari B., Clark C.G. 2006. DNA barcoding of *Blastocystis*. *Protist* 157(1): 77–85. doi:10.1016/j.protis.2005.12.001
- [19] Jimenez-Gonzalez D.E., Martinez-Flores W.A., Reyes-Gordillo J., Ramirez-Miranda M.E., Arroyo-Escalante S., Romero-Valdovinos M., Stark D., Souza-Saldivar V., Martinez-Hernandez F., Flisser A., Olivo-Diaz A. 2012. *Blastocystis* infection is associated with irritable bowel syndrome in a Mexican patient population. *Parasitology Research* 110(3): 1269–1275. doi:10.1007/s00436-011-2626-7
- [20] Krogsgaard L.R., Engsbro A.L., Stensvold C.R., Nielsen H.V., Bytzer P. 2015. The prevalence of intestinal parasites is not greater among individuals with irritable bowel syndrome: a population-based case-control study. *Clinical Gastroenterology and Hepatology* 13(3): 507–513. doi:10.1016/j.cgh.2014.07.065
- [21] Kesuma Y., Firmansyah A., Bardosono S., Sari I.P., Kurniawan A. 2019. *Blastocystis* ST-1 is associated with irritable bowel syndrome-diarrhoea (IBS-D) in Indonesian adolescences. *Parasite Epidemiology and Control* 6: e00112. doi:10.1016/j.parepi.2019.e00112
- [22] El-Badry A. A., Abd El Wahab W.M., Hamdy D.A., Aboud A. 2018. *Blastocystis* subtypes isolated from irritable bowel syndrome patients and co-infection with *Helicobacter pylori*. *Parasitology Research* 117(1): 127–137. doi:10.1007/s00436-017-5679-4
- [23] Salehi R., Haghighi A., Stensvold C. R., Kheirandish F., Azargashb E., Raeghi S., Bahrami F. 2017. Prevalence and subtype identification of *Blastocystis* isolated from humans in Ahvaz, Southwestern Iran. *Gastroenterology and Hepatology from Bed to Bench* 10(3): 235–241.
- [24] Alfellani M.A., Stensvold C.R., Vidal-Lapiedra A., Onuoha E.S.U., Fagbenro-Beyioku A.F., Clark, C.G. 2013. Variable geographic distribution of *Blastocystis* subtypes and its potential implications. *Acta Tropica* 126(1): 11–18. doi:10.1016/j.actatropica.2012.12.011
- [25] Anuar T.S., Al-Mekhlafi H.M., Ghani M.K.A., Bakar E.A., Azreen S.N., Salleh F.M., Mokhtar N. 2013. Evaluation of formalin-ether sedimentation and

- trichrome staining techniques: its effectiveness in detecting *Entamoeba histolytica/dispar/moshkovskii* in stool samples. *Journal of Microbiological Methods* 92(3): 344–348. doi:10.1016/j.mimet.2013.01.010
- [26] Bart A., Wentink-Bonnema E., Gilis H., Verhaar N., Wassenaar C. J., van Vugt M., Van Gool T. 2013. Diagnosis and subtype analysis of *Blastocystis* sp. in 442 patients in a hospital setting in the Netherlands. *BMC Infectious Diseases* 13(1): article number 389. doi:10.1186/1471-2334-13-389
- [27] Ragavan N.D., Govind S.K., Chye T.T., Mahadeva S. 2014. Phenotypic variation in *Blastocystis* sp. ST3. *Parasites and Vectors* 7: article number 404. doi:10.1186/1756-3305-7-404
- [28] Fidalgo B., Rubio E., Pastor V., Parera M., Ballesté-Delpierre C., Fernández M. J., Casals-Pascual C. 2021. Improved diagnosis of gastrointestinal infections using a semi-automated multiplex real-time PCR for detection of enteropathogens. *Journal of Medical Microbiology* 70(9): article number 001367. doi:10.1099/jmm.0.001367
- [29] Lepczyńska M., Dzika E., Chen W. 2021. Prevalence of *Blastocystis* subtypes in healthy volunteers in northeastern Poland. *Journal of Parasitology* 107(5): 684–688. doi:10.1645/20-170
- [30] 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(1): 43–47.
- [31] Adiyaman Korkmaz G., Doğruman A.F., Mumcuoğlu İ. 2015. Dışkı örneklerinde. *Blastocystis* spp. varlığının mikroskopik, kültür ve moleküler yöntemlerle araştırılması [Investigation of the presence of *Blastocystis* spp. in stool samples with microscopic, culture and molecular methods]. *Mikrobiyoloji Bülteni* 49(1): 85–97 (in Turkish with summary in English). doi:10.5578/mb.8439
- [32] Abu-Madi M., Aly M., Behnke J.M., Clark C.G., Balkhy H. 2015. The distribution of *Blastocystis* subtypes in isolates from Qatar. *Parasites and Vectors* 8(1): 1–7. doi:10.1186/s13071-015-1071-3
- [33] Popruk S., Udonsom R., Koompapong K., Mahittikorn A., Kusolsuk T., Ruangsittichai J., Palasuwan A. 2015. Subtype distribution of *Blastocystis* in Thai-Myanmar border, Thailand. *Korean Journal of Parasitology* 53(1): 13–19. doi:10.3347/kjp.2015.53.1.13
- [34] Yakoob J., Jafri W., Beg M.A., Abbas Z., Naz S., Islam M., Khan R. 2010. *Blastocystis hominis* and *Dientamoeba fragilis* in patients fulfilling irritable bowel syndrome criteria. *Parasitology Research* 107(3): 679–684. doi:10.1007/s00436-010-1918-7

Received 14 May 2022

Accepted 03 August 2022