Blastocystis hominis transmission by non-potable water: a case report in Italy

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SUMMARY

In the reported case, a 41-year-old Italian man came to the clinician's observation reporting cramps, bloating and watery diarrhoea a few days after drinking water indicated as unpotable from a fountain in a farm area. The medical suspicion was directed at both gluten intolerance and enteric infection, eventually of waterborne origin. Gluten intolerance was investigated by intestinal biopsy and excluded, while stool analyses ruled out infective bacteriological or viral agents and parasites. Subsequently, a persistent eosinophilia was revealed and a parasitological analysis was again suggested, planning for a more sensitive molecular method. Therefore, a multiplex-PCR of enteric protozoa species DNA was performed on an intestinal biopsy and faecal samples revealing only *Blastocystis hominis* protozoa, subsequently typed as subtype 1 by RFLP-PCR method. *B. hominis* is an anaerobic protozoa found in the human and animal intestinal tract, recently associated with a pathogenic role characterized by chronic development. Since blastocystosis has been demonstrated as a waterborne infection, a sample of water matrix was analysed, revealing the *B. hominis* subtype 1 DNA inside. A probable water transmission of *Blastocystis* infection has been demonstrated in this case report. Only a probiotic treatment based on *Saccharomyces boulardii* was administered to the patient and this apparently resolved the infection. In summary, the case described here is a chronic blastocystosis of possible waterborne origin, controlled by assuming a yeast treatment.

INTRODUCTION

B. hominis is a eukaryotic microbe frequently found in the human and animal intestinal tract. It can be part of the gut microbiota generally resulting harmless to the subject (Scanlan *et al.*, 2014; Audebert *et al.*, 2016). In some cases, it is a pathogen causing gastrointestinal symptoms such as diarrhoea, nausea, abdominal pain, vomiting and bloating (Wawrzyniak *et al.*, 2013). *B. hominis* has a global distribution and a faecal-oral transmission route has been suggested. From the taxonomic point of view it was previously considered a yeast, or fungus or ameboid protozoa since in 1991 it was classified it as a protozoon by morphology (Zierdt, 1991). Afterwards small-subunit ribosomal DNAsequencing definitively assigned this protist to the *Phylum Heterokonta* (*Stramenopiles*), including also water molds, diatoms, brown algae and other photosynthetic protozoa (Cavalier-Smith, 1999; Arisue *et al.*, 2002). Four major cellular forms of this species have been described in stools samples and *in vitro* cultures: vacuolar, granular, amoeboid and cystic. Upon ingestion of cysts by the host, this protozoon undergoes excystation in the large intestine and develops into vacuolar forms. These vacuolar forms divide developing amoeboid and granular forms. Then, encystation may occur along the colon, followed by cysts excretion in the faeces (Suresh *et al.*, 2009). *B. hominis* is the only *Stramenopile* known to colonize humans and animals. From the epidemiological point of view it is considered the most common protist inhabitant of the human gut and has been also associated with inflammatory bowel disease and irritable bowel syndrome (Nagler *et al.*, 1993; Poirier *et al.*, 2012; Azizian *et al.*, 2016). The different behaviour shown by *B. hominis* could be associated with the high genetic diversity found in this species (Arisue *et al.*, 2003). In fact, using isoenzymes and karyotyping techniques, these species have been divided into subgroups showing different pathogenic skills (Stenzel and Boreham, 1996; Clark, 1997; Bohm-Gloning *et al.*, 1997). In particular, the mammalian and avian *Blastocystis* spp. groups were subdivided into seventeen subtypes (STs), nine of which, referred to as ST1 to ST9, have also been found in humans (Stensvold and Clark, 2016). It is a rising idea that some subtypes of *Blastocystis* may be more likely associated with symptoms and it has been demonstrated that several of them show different degrees of pathogenicity in experimentally inoculated rats, subtype 1 being the most virulent (Hussein *et al.*, 2008). A very exhaustive study, published in the current year, described the very high biodiversity of *Blastocystis* genotypes, differing especially in the coding sequences for proteins implicated in the pathogenic molecular machine (Gentekaki *et al.*, 2017). According to different studies, *B. hominis* is also associated with cutaneous manifestations and chronic urticaria (Zuel-Fakkar *et al.*, 2011; Verma and Delfagnan, 2013; Haamed *et al.*, 2011) also in Italy (Pasqui *et al*, 2004). A recent study showed a strong association between an allele of ST3 *B. hominis* and urticaria recurrence in patients (Casero *et al.*, 2015) and ST4 has been highly associated with acute gas-

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troenteric symptoms (Stensvold *et al.*, 2011). Additionally, it was observed that *B. hominis* can frequently be found in mixed infections together with *Dientamoeba fragilis* (Stensvold *et al.*, 2009), another intestinal protozoan able to cause urticaria (Windsor and Johnson, 1999) and intestinal disorders (Stensvold *et al.*, 2007). Moreover, substantial molecular evidence for zoonotic transmission of *Blastocystis* was provided (Yoshikawa *et al.*, 2000) and its transmission route being oro-faecal, environmental faecal contamination plays a key role. Effectively, *Blastocystis* spp. was detected in sewage in Malaysia and Scotland, and other studies provided evidence for waterborne transmission of the parasite (Lee *et al.*, 2012; Leelayoova *et al.*, 2002; Baldursson and Karanis, 2011). Thick-walled cysts have been correlated with environmental transmission, while thin-walled cysts might be responsible for internal reinfection within the host gut (Stenzel *et al.*, 1991; Singh *et al.*, 1995). Finally, *Blastocystis* has been correlated with outbreaks due to the use of unfiltered drinking water (Tan, 2008). In conclusion, many factosrs such as the asserted pathogenicity of most *Blastocystis* spp. strains, a possible association with inflammatory gut diseases, environmental transmission and zoonotic potential, and contribute to currently considering this protist an emergent parasite.

CASE REPORT

A 41-year-old Italian man came to a doctor's observation reporting symptoms of acute gastroenteritis, considered a self-limiting viral affection. After several weeks, cramps, bloating and watery diarrhoea were persistent and associated with weight loss, general malaise and anorexia, so clinicians suggested an investigation for celiac disease. Therefore, the patient underwent a gastro-duodenoscopy with an intestinal biopsy, which showed atrophy of the intestinal villi, a mild-chronic nonspecific inflammation and a duodenal lymphocytic infiltrate (intraepithelial lymphocytes/enterocytes 23/100). However, he resulted negative for celiac disease after serological tests and gluten-free diet. Further clinical investigations revealed deficiency of D vitamin assimilation and increased duodenal lymphocytic infiltrate CD3+ (intraepithelial lymphocytes/enterocytes 30/100), but gastric ulcer, colon cancer and inflammatory bowel disease were excluded. Therefore, the patient's history highlighted an intestinal malabsorption and chronic intestinal inflammation of possible infective origin.

Since intestinal symptoms persisted and the patient reported drinking unpotable water from a rural fountain a few days before the acute symptomatology, clinicians performed a microbiological stool analysis. Stool analysis by microscope for bacteria and parasites and by serological diagnosis for virus excluded enteric bacterial/parasitological as well as viral infections (*Table 1*). In particular, infections by *Giardia* spp. and *Cryptosporidium* spp. waterborne parasites were ruled out. In the following months, the patient complained of persistent muscle-tendon pain, tremors, and intermittent gastro-enteric disturbance; blood-analyses were again performed and a peripheral persistent eosinophilia was highlighted. Therefore, a parasitic infection was again strongly suspected and he arrived at our laboratory for a molecular parasitological analysis more sensitive than the microbiological analysis. Firstly, to investigate the patient's infective initial stage and to increase the diagnostic sensitivity/specificity degree

Table 1 - *Enteric microorganisms researched in the stool sample. Diagnoses by and serological microscopy analyses. All the diagnosis results were negative.*

Bacteria	E. coli O157, Salmonella spp., Brucella spp., Helicobacter pilori, Borrelia spp., Clostridium difficile and C. difficile toxin A and B, Yersinia spp., Campilobacter jejuni, Proteus vulgaris, Mycobacterium spp., Rickettsia prowazekii.
Viruses	HIV, HCV, HBV, HHV-6, Adenovirus.
Parasites	Giardia intestinalis, Cryptosporidium spp., Entamoeba histolytica, Toxoplasma gondii, Leishmania donovani, Balantidium coli, Cyclospora cayetanensis, Cystoisospora belli, Microsporidia spp., Taenia saginata, Enterobius vermicularis, Echinococcus granulosus, Strongyloides stercoralis, Trichinella spiralis, Filariidae.

by molecular technique, it was decided to analyse a paraffin-fixed slide specimen of the gut biopsy, already performed several months earlier. A slide scraping was then carried out to extract tissue material and total DNA production was performed according to the Promega Wizard Genomic DNA Purification Kit. A multiplex PCR amplification, previously standardized, was applied to search for different parasitic species DNA (*Giardia, Cryptosporidium, Cyclospora, Dientamoeba, Blastocystis*) using a standardized primer panel shown in *Table 2*. Among the target sequences, only *B. hominis* SSU rRNA gene target (Scicluna *et al.*, 2006) was amplified, showing the specific DNA in the sample. The molecular diagnosis on the bioptic material confirmed the previously demonstrated negativity for other enteric parasites (*Table 1*), showing only a high gut colonization by *Blastocysts* protozoa, not searched for before. Since the patient reported drinking unpotable water from a rural fountain, and *Blastocystis* has been isolated from surface water also in Italy (Angelici *et al.*, 2013; Ade *et al.*, 2011), this event was assumed to be the putative cause of infection. The indicted fountain is normally intended for watering livestock and provides canalized water from the nearby river contaminated by animal faeces. The fountain was designated as NOT DRINKABLE water. Due to the faecal contamination of river water by livestock, a risk of zoonotic waterborne infections appeared consistent. Therefore, the fountain water was monitored a few months after the patient's infection occurred. The same environmental conditions and livestock presence in the fountain area were, indeed, confirmed. Five litres of water were collected and concentrated by an initial filtration and then serial centrifugations to obtain a final pellet. Cell fraction in the pellet was lysed by Promega Genomic DNA Purification Kit while PCR and DNA amplification was performed by the same primer panel shown in Table 2. The presence of *Giardia duodenalis* and *B. hominis* was revealed. A genotyping protocol of the *Blastocystis* isolates by restriction enzymes analysis of SSU rRNA, 1800bp amplicon (RFLP-PCR analysis) from both human and environmental specimens was then performed, as suggested by Clark (Clark, 1997). Concerning the amplified gene target of *B. hominis*, and the Rsa1-RFLP electrophoretic pattern, results are reported in *Figure 1* and *Figure 2*, respectively. As the electrophoretic Rsa1 restriction patter corresponds to *B. hominis* Subtype 1, in agreement with Clark (Clark, 1997) in both the specimens, we can assert they belong to the same genotype 1. This datum supports the hypothesis

Table 2 - *Target genes used for diagnosis of enteric protozoa in the intestinal biopsies.*

Protozoan Species	Target Gene / Amplicon bp	Reference
Cyclospora cayetanensis	ITS 2 / 116 bp	(Lalonde and Gajadhar, 2008)
Cryptosporidium parvum	COWP nested / 311 bp	(Yu *et al.*, 2009)
Giardia duodenalis	16S rRNA nested / 292 bp	(Appelbee *et al.*, 2003)
Dientamoeba fragilis	5.8S rRNA / 98bp	(Verweij *et al.*, 2007)
Blastocystis hominis	SSU rRNA / 600 bp	(Scicluna *et al.*, 2006)
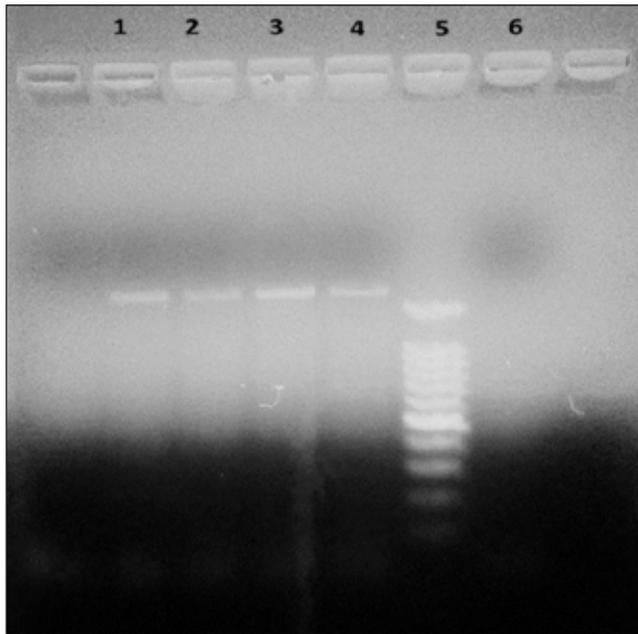
Figure 1
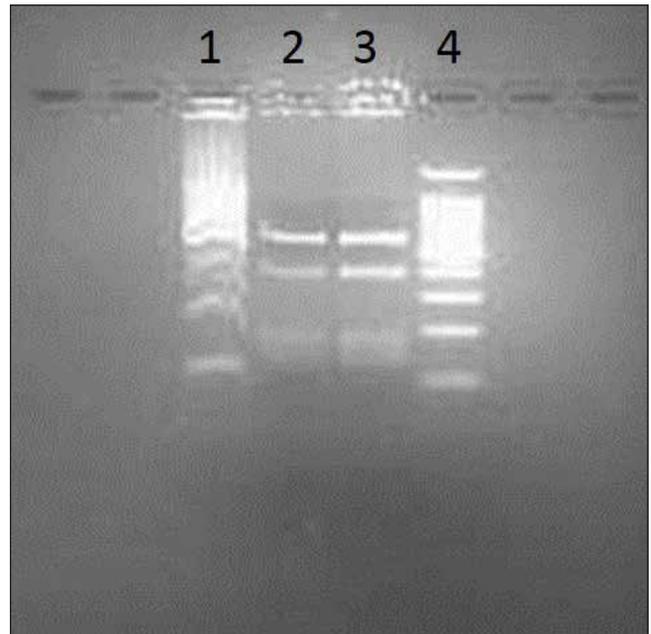
Figure 2

that the patient was infected by drinking the contaminated water from the fountain some days before the onset of enteric symptomatology.

The patient was recommended for metronidazole treatment, but he declined, reporting his intolerance of nitroimidazole derivatives, previously tested (genetic polymorphism of the coding gene for CYP2C19 substrate). Consequently, he was recommended to assume a probiotic treatment, containing *Lactobacillus* and *Bifidobactrium*, to control the intestinal dysbiosis and a month after he returned to our observation he had no more intestinal symptoms, but claimed chronic fatigue, muscle cramps, pallor, chronic urticaria and psychological malaise. A faecal analysis by PCR on three serial specimens was then performed, with multiplex PCR (*Table 2*), again detecting *B. hominis* and suggesting that the parasite had chronically colonized the intestinal tract of this subject. Furthermore, faecal analysis revealed the presence of *Dientamoeba fragilis*, showing a co-infection between these two parasites, often described during the chronic stage of a blastocystosis (Stensvold *et al.*, 2007; Windsor and Johnson, 1999). He was also diagnosed with a high level of faecal lactoferrin, a sensitive and specific marker identifying intestinal inflammation (Kane *et al.*, 2003), suggesting that, almost certainly, the situation had not yet resolved. He continues to be affected by general malaise, muscle-tendon pain, pinprick sensation in his legs, chronic itching especially at calf level, and persistent peripheral eosinophilia. Clinicians suggested the patient

take a different kind of probiotic, containing *Saccharomyces boulardii*. Recently, 18 months after the last positivity for blastocystosis, the patient twice repeated the molecular search for *B. hominis* in faecal samples and resulted negative showing parasite eradication. He resulted negative for *B. hominis* and *D. fragilis* parasites also by microscope assay and, currently, specific symptoms like eosinophilia, enteric disturbance and urticaria are no longer claimed.

DISCUSSION AND CONCLUSION

B. hominis has been definitively demonstrated as a human gut pathogen, mostly characterized by a high genetic variability with subtypes showing different degrees of pathogenicity (Stensvold and Clark, 2016). It has also been implicated as cause of long-term chronic inflammation and difficult to eradicate (Vitetta *et al.*, 2016; Roberts *et al.*, 2014). The case reported here showed a symptomatic *Blastocystis* infection with a chronic course because of the lack of pharmacological treatment and a possible waterborne origin of this infection was demonstrated. Indeed, the patient's symptoms appeared immediately after drinking from a contaminated fountain. A molecular analysis of both the patient's biological samples and the watery matrix of the fountain specimens indicated the subtype 1 *B. hominis* DNA, suggesting possible infection by the contaminated water. Subtype 1 has been very frequently isolated from human cases also in Italy (Mattiucci *et al.*,

2016) and often isolated in animals, showing a certain zoonotic potential (Wang *et al.*, 2013; Wang *et al.*, 2014). The high prevalence of *Blastocystis* as a parasite in the Italian population has been previously demonstrated by clinical studies (Calderaro *et al.*, 2010), but it is not yet correlated to specific routes of transmission and risk factors. Usually, an enteric microorganism transmitted by the faecal-oral route with a zoonotic cycle should be easily transmitted by contact with contaminated surface water. Indeed, *B. hominis* has been isolated in surface water (Elshazly *et al.*, 2007) and in different animal species (Stensvold and Clark, 2016), proving both a waterborne and zoonotic parasite. Therefore, a possible waterborne origin of the infection was proposed. This is the first case in Italy in which the diagnostic approach indicates a water-related transmission risk factor. Few data are available on the waterborne origin of *B. hominis* infections in Italy, neglecting a very important transmission risk to humans and animals (Angelici *et al.*, 2013; Ade *et al.*, 2011).

The case presented here shows the chronic appearance of intestinal symptoms, eosinophilia, diffuse pain at muscle level and positivity for faecal lactoferrin, strongly suggesting a chronic infection by *Blastocystis* as the only etiological agent isolated by repeated diagnosis in the patient's intestinal tract. Specific symptoms like chronic fatigue (Tan, 2008), persisting urticaria (Windsor and Johnson, 1999) and general discomfort support this diagnosis. In addition, *D. fragilis* co-infection was also revealed confirming data from other authors that these infections can occasionally occur simultaneously (Stensvold *et al.*, 2007; Windsor and Johnson, 1999). The long-term chronic symptoms associated with the infection claimed by the subject could be caused by the lack of therapy giving rise to the chronic pathology. In any case, a specific treatment by only long-term probiotic intake finally resolved the infection especially when *Saccharomyces* was supplied. This yeast is a eukaryotic microorganism like the protozoon *Blastocystis* and a possible trophic competition between them could be the reason inhibiting parasite survival. Likely, in this clinical case *S. boulardii* administration was sufficient to control the *Blastocystis* infection, as reported by several authors (Sekar and Shanthi, 2013; Dinleyici *et al.*, 2011). In summary, this case report highlights for the first time a possible transmission of *B. hominis* through contaminated drinking water in Italy, the relevance of considering *Blastocystis* a pathogen able to support a chronic infection and the possibility to control balstocistosis by yests administration. This enteric protozoon is very common, but highly neglected in the clinical diagnostic panel and its chronicity is often not revealed. The role of *B. hominis* as a potential pathogenic agent responsible for intestinal and extra-intestinal disorders is still underestimated at clinical level, although scientific knowledge exists at a basic research level (Wawrzyniak *et al.*, 2012; Basak *et al.*, 2014). For this reason, this parasite is often not investigated and can occasionally produce an alteration of the intestinal flora (Vitetta *et al.*, 2016), exposing the subject to other co-infections. Since *B. hominis* infection is associated with chronic intestinal diseases or other gut disturbances, we strongly recommend considering the risk of transmission through surface water contaminated by faeces.

Conflict of interest

The authors declare that there is no conflict of interest regarding the publication of this paper.

Authors' Contribution

All authors read and agreed the paper.

References

Ade P., Funari E., Angelici M.C. Approccio metodologico per la ricerca di protozoi parassiti in un bacino eutrofico. In: Caciolli S, Giovannangeli R, Musmeci L, ed. Convegno nazionale Salute ed ambiente in Italia. Istituto Superiore di Sanità. Roma, 5-6 dicembre 2011. Riassunti (ISTISAN Congressi 11/C9) p.47i, Roma: Istituto Superiore di Sanità; 2011.

Angelici M.C, Ade P., Funari E. Molecular Monitoring For Pathogenic Eukaryotic Microbes In Lake Water. First EMBO Conference on Aquatic Microbial Ecology - SAME13, Stresa 8-13 September 2013. Abstract book: PS-S1.01, 361.

Appelbee A.J., Frederick L.M., Heitman T.L., Olson M.E. (2003). Prevalence and genotyping of *Giardia duodenalis* from beef calves in Alberta, Canada. *Vet Parasitol*. **112**, 289-294.

Arisue N., Hashimoto T., Yoshikawa H., Nakamura Y., Nakamura G., et al. (2002). Phylogenetic position of *Blastocystis hominis* and of *stramenopiles* inferred from multiple molecular sequence data. *J Eukaryot Microbiol*. **49**, 42-53.

Arisue N., Hashimoto T., Yoshikawa H. (2003). Sequence heterogeneity of the small subunit ribosomal RNA genes among *Blastocystis* isolates. *Parassitol*. **126**, 1-9.

Audebert C., Even G., Cian A.; Blastocystis Investigation Group, Loywick A., Merlin S., Viscogliosi E., Chabé M. (2016). Colonization with the enteric protozoa *Blastocystis* is associated with increased diversity of human gut bacterial microbiota. *Sci Rep*. **5**, 25255.

Azizian M., Basati G., Abangah G., Mahmoudi M.R., Mirzaei A. (2016). Contribution of *Blastocystis hominis* subtypes and associated inflammatory factors in development of irritable bowel syndrome. *Parasitol Res*. **115**, 2003-2009.

Baldursson S., Karanis P. (2011). Waterborne transmission of protozoan parasites: review of worldwide outbreaks - an update 2004-2010. *Water Res*. **45**, 6603-6614.

Basak S., Rajurkar M.N., Mallick S.K. (2014). Detection of *Blastocystis hominis*: a controversial human pathogen. *Parasitol Res*. **113**, 261-265.

Böhm-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**, 771-778.

Calderaro A., Montecchini S., Rossi S., Gorrini C., De Conto F., et al. (2010). Intestinal parasitoses in a tertiary-care hospital located in a non-endemic setting during 2006-2010. *BMC Infect Dis*. **14**, 264.

Casero RD, Mongi F, Sánchez A, Ramírez JD. (2015). *Blastocystis* and urticaria: Examination of subtypes and morphotypes in an unusual clinical manifestation. *Acta Trop*. **148**, 156-161.

Cavalier-Smith, T. (1999). "The kingdom *Chromista*, origin and systematics". In Round, F.E.; Chapman, D.J. Progress in Phycological Research. 4. Elsevier. 309-347.

Clark C.G. (1997). Extensive genetic diversity in *Blastocystis hominis*. *Mol Biochem Parasitol*. **87**, 79-83.

Dinleyici E.C., Eren M., Dogan N., Reyhanioglu S., Yargic Z.A., et al. (2011). Clinical efficacy of *Saccharomyces boulardii* or metronidazole in symptomatic children with *Blastocystis hominis* infection. *Parasitol Res*. **108**, 541-545.

Elshazly A.M., Elsheikha H.M., Soltan D.M., Mohammad K.A., Morsy T.A. (2007). Protozoal pollution of surface water sources in Dakahlia Governorate, Egypt. *J Egypt Soc Parasitol*. **37**, 51-64.

Gentekaki E., Curtis B.A., Stairs C.W., Klimeš V., Eliáš M., et al. (2017). Extreme genome diversity in the hyperprevalent parasitic eukaryote *Blastocystis*. *PLoS Biol*. **15**, e2003769.

Haamed D.M.A., Hassanin O.M., Zuel-Fakkar N.M. (2011). Association of *Blastocystis hominis* genetic subtypes with urticarial. *Parasitol Res*. **108**, 553-560.

Hussein E.M., Hussein A.M., Eida M.M., Atwa M.M. (2008). Pathophysiological variability of different genotypes of human *Blastocystis hominis* Egyptian isolates in experimentally infected rats. *Parasitol Res*. **102**, 853-860.

Kane S.V., Sandborn W.J., Rufo P.A., Zholudev A., Boone J., et al. (2003). Fecal lactoferrin is a sensitive and specific marker in identifying intestinal inflammation. *Am J Gastroenterol*. **98**, 1309-1314.

Lalonde L.F., Gajadhar A.A. (2008). Highly sensitive and specific PCR assay for reliable detection of *Cyclospora cayetanensis* oocysts. *Appl Environ Microbiol*. **74**, 4354-4358.

Lee L.I., Chye T.T., Karmacharya B.M., Govind S.K. (2012). *Blastocystis* sp.: waterborne zoonotic organism, a possibility? *Parasit Vectors*. **28**, 130.

Leelayoova S., Rangsin R., Taamasri P., Naaglor T., Thathaisong U., et al. (2004). Evidence of waterborne transmission of *Blastocystis hominis*. *Am J Trop Med Hyg*. **70**, 658-662.

Mattiucci S., Crisafi B., Gabrielli S., Paoletti M., Cancrini G. (2016). Molecular epidemiology and genetic diversity of *Blastocystis* infection in humans in Italy. *Epidemiol Infect*. **144**, 635-646.

Nagler J., Brown M., Soave R. (1993). *Blastocystis hominis* in inflammatory bowel disease. *J Clin Gastroenterol*. **16**, 109-112.

Pasqui A.L., Savini E., Saletti M., Guzzo C., Puccetti L., et al. (2004). Chronic urticaria and *Blastocystis hominis* infection: a case report. *Eur Rev Med Pharmacol Sci*. **8**, 117-120.

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 Pathog*. **8**, e1002545.

Roberts T., Ellis J., Harkness J., Marriott D., Stark D. (2014) Treatment failure in patients with chronic *Blastocystis* infection. *J. Med. Microbiol*. **63**, 252-257.

Scanlan P.D., Stensvold C.R., Rajilic-Stojanovic M., Heilig H.G.H.J., De Vos W..M, et al. (2014). The microbial eukaryote *Blastocystis* is a prevalent and diverse member of the healthy human gut microbiota. *FEMS Microbiol Ecol*. **90**, 326-330.

Scicluna S.M., Tawari B., Clark C.G. (2006). DNA barcoding of *Blastocystis*. *Protist*. **157**, 77-85.

Sekar U., Shanthi M. (2013). *Blastocystis*: Consensus of treatment and controversies. Trop Parasitol. **3**, 35-39.

Singh M., Suresh K., Ho L.C., Ng G.C., Yap E.H. (1995). Elucidation of the life cycle of the intestinal protozoan *Blastocystis hominis*. *Parasitol Res*. **81**, 446-450.

Stensvold C.R., Arendrup M.C., Mølbak K., Nielsen H.V. (2007). The prevalence of *Dientamoeba fragilis* in patients with suspected enteroparasitic disease in a metropolitan area in Denmark. *Clin Microbiol Infect*. **13**, 839-842.

Stensvold C.R., Lewis H.C., Hammerum A.M., Porsbo L.J., Nielsen S.S., et al. (2009). *Blastocystis*: unravelling potential risk factors and clinical significance of a common but neglected parasite. *Epidemiol Infect*. **137**, 1655-1663.

Stensvold C.R., Christiansen D.B., Olsen K.E., Nielsen H.V. (2011). *Blastocystis* sp. subtype 4 is common in Danish *Blastocystis*-positive patients presenting with acute diarrhea. *Am. J. Trop. Med. Hyg*. **84**, 883-885.

Stensvold C.R., Alfellani M., Clark C.G. (2012). Levels of genetic diversity vary dramatically between *Blastocystis* subtypes. *Infect Genet Evol*. **12**, 263-273.

Stensvold C.R., Clark C.G. (2016). Current status of *Blastocystis*: A personal view. *Parasitol Int*. **65**, 763-771.

Stenzel D.J., Boreham P.F., McDougall R. (1991). Ultrastructure of *Blastocystis hominis* in human stool samples. *Int J Parasitol*. **21**, 807-812.

Stenzel D.J., Boreham P.F. (1996). *Blastocystis hominis* revisited. *Clin Microbiol Rev*. **9**, 563-584.

Suresh K., Venilla G., Tan T., Rohela M. (2009). In vivo encystation of *Blastocystis hominis*. *Parasitol Res*. **104**, 1373-1380.

Tan K.S. (2008). New insights on classification, identification, and clinical relevance of *Blastocystis* spp. *Clin Microbiol Rev*. **21**, 639-665.

Verweij J.J., Mulder B., Poell B., van Middelkoop D., Brienen EA., van Lieshout L. (2007). Real-time PCR for the detection of *Dientamoeba fragilis* in fecal samples. *Mol Cell Probes*. **21**, 400-404.

Verma R., Delfanian K. (2013). *Blastocystis hominis* associated acute urticaria. *Am J Med Sci*. **346**, 80-81.

Vitetta L., Saltzman E.T., Nikov T., Ibrahim I., Hall S. (2016). Modulating the Gut Micro-Environment in the Treatment of Intestinal Parasites. *J Clin Med*. **5**, 11.

Wang W., Cuttell L., Bielefeldt-Ohmann H., Inpankaew T., Owen H., et al. (2013). Diversity of *Blastocystis* subtypes in dogs in different geographical settings. Parasites & Vectors. **6**, 215.

Wang W., Bielefeldt-Ohmann H., Traub R.J., Cuttell L., Owen H. (2014). Location and Pathogenic Potential of *Blastocystis* in the Porcine Intestine. *PLoSONE*. **9**, e103962.

Wawrzyniak I., Poirier P., Viscogliosi E., Dionigia M., Texier C., et al. (2013). *Blastocystis*, an unrecognized parasite: an overview of pathogenesis and diagnosis. *Ther Adv Infect Dis*. **1**, 167-178.

Wawrzyniak I., Texier C., Poirier P., Viscogliosi E., Tan K.S., et al. (2012). Characterization of two cysteine proteases secreted by *Blastocystis* ST7, a human intestinal parasite. *Parasitol Int*. **61**, 437-442.

Windsor J.J., Johnson E.H. (1999). *Dientamoeba fragilis*: the unflagellated human flagellate. *Br J Biomed Sci*. **56**, 293-306.

Yoshikawa H., Abe N., Iwasawa M., Kitano S., Nagano I. (2000). Genomic analysis of *Blastocystis hominis* strains isolated from two long-term health care facilities. *J Clin Microbiol*. **38**, 1324-1330.

Yu J.R., Lee S.U., Park W.Y. (2009). Comparative sensitivity of PCR primer sets for detection of *Cryptosporidium parvum*. *Korean J Parasitol*. **47**, 293-297.

Zierdt CH. (1991). *Blastocystis hominis* - past and future. *Clin Microbiol Rev*. **4**, 61-79.

Zuel-Fakkar N.M., Abdel Hameed D.M., Hassanin O.M. (2013). Study of *Blastocystis hominis* isolates in urticaria: a case-control study. *Clin Exp Dermatol*. **36**, 908-910.