Parasite Epidemiology and Control 15 (2021) e00227

Contents lists available at ScienceDirect

Parasite Epidemiology and Control

journal homepage: www.elsevier.com/locate/parepi

Frequency and distribution of *Blastocystis* sp. subtypes in patients with spondyloarthritis in Bogotá, Colombia

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ARTICLE INFO

Keywords:
Spondyloarthritis
Blastocystis
Intestinal parasites
Subtypes
Colombia

ABSTRACT

Although *Blastocystis* sp. is one of the most prevalent intestinal parasites worldwide, its role as a pathogen remains unclear. The use of molecular techniques to assess the genetic heterogeneity of *Blastocystis* sp. has become important to understand its function in some intestinal pathologies and if it is a key component of intestinal microbiota. Spondyloarthritis is a group of immune-mediated autoinflammatory diseases in which microbial dysbiosis in the gut (including parasites, bacteria and fungi) and intestinal inflammation are common features apparently associated with the pathophysiology of these disorders. This study included 74 patients diagnosed with spondyloarthritis and 57 systemically healthy individuals (included as controls), who were screened for intestinal parasites. *Blastocystis* sp. was detected in 68% and 73% of the patients with spondyloarthritis and controls, respectively. In faecal samples positive for *Blastocystis* sp., an 18S rRNA gene fragment of *Blastocystis* sp. was amplified and sequenced to identify their genetic subtypes. Patients with spondyloarthritis showed similar frequencies of ST1, ST2 and ST3 subtypes of *Blastocystis* sp. (30% each). The same subtypes were observed in controls, wherein almost 60% of the samples showed ST3. In addition, ST6 was found only in one sample from each group. ST1 subtype showed the greatest genetic variability. Although the same subtypes were detected in both patients with spondyloarthritis and controls, subtype prevalence studies conducted in Colombia indicate an association between ST3 and individuals with irritable bowel syndrome. This opens an interesting research avenue to further study of the epidemiology of *Blastocystis* sp. and its possible relationship with intestinal conditions in immunocompromised patients.

1. Introduction

Blastocystis sp. is an intestinal protozoan parasite that has long been identified as a commensal organism in the gut flora of humans and animals (Denoeud et al., 2011). Over the last few years, research on *Blastocystis* sp. has gained relevance as several studies have

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https://doi.org/10.1016/j.parepi.2021.e00227
Received 28 July 2021; Received in revised form 26 August 2021; Accepted 30 September 2021
Available online 6 October 2021

claimed that *Blastocystis* sp. infection leads to gastrointestinal symptoms, inflammatory bowel disease, ulcerative colitis and extra-intestinal manifestations such as urticaria and iron deficiency anaemia (Yavasoglu et al., 2008; Deeb et al., 2012; Jiménez-Gonzalez et al., 2012; Verma and Delfanian, 2013; Casero et al., 2015). To date, the pathogenicity of *Blastocystis* sp. has not been categorically demonstrated, since studies have reported inconclusive findings (Lukeš et al., 2015). Other studies on *Blastocystis* sp. have focused on assessing its genetic clusters or subtypes because there could be a potential relationship between the genetic diversity of populations and the wide range of responses to infection, including asymptomatic hosts (Clark, 1997; Stensvold et al., 2011). Molecular research has involved the analysis of the small-subunit ribosomal RNA gene (18S rRNA) and has led to the identification of 17 different subtypes of *Blastocystis* sp. (Alfellani et al., 2013). Recently, five new subtypes have been proposed, namely ST21 and ST23–ST26, which have been identified in wild and domestic animals (Maloney et al., 2019; Stensvold and Clark, 2020).

Blastocystis sp. infection can be regarded as a zoonotic disease because ST1–ST8 and ST12 subtypes have been isolated from humans and animals (Ramírez et al., 2016). Although ST9 has been exclusively isolated from humans (Yoshikawa and Iwamasa, 2016), 95% of infections in humans have been associated with ST1–ST4 subtypes (Stensvold and Clark, 2016). ST3, ST1 and ST2 are the most frequent subtypes identified in South America, whereas ST4 is the most frequent one in Europe and Australia and ST7 prevails in Africa, Nepal, Pakistan, Malaysia and Singapore (Ajjampur and Tan, 2016). A study conducted in 2013 on animal and human samples from different regions of Colombia reported an association between clinical symptoms and the infecting subtypes. Individuals colonised by ST1 were asymptomatic, those infected with ST2 had diarrhea and those infected with ST3 had irritable bowel syndrome (IBS) (Ramirez et al., 2014). Although several studies have been conducted worldwide to differentiate pathogenic subtypes from non-pathogenic subtypes, results have been contradictory even in studies performed in the same country (Stensvold et al., 2007; Mattiucci et al., 2015).

Studies on chronic diseases have suggested that *Blastocystis* sp. acts as an opportunistic pathogen in immunocompromised patients with HIV/AIDS and cancer, causing diarrhea (Tan et al., 2009). However, few studies have attempted to determine the distribution of *Blastocystis* subtypes and their relationship with these pathologies (Tan et al., 2009; Yersal et al., 2016; Mülayim et al., 2021) or in patients who have undergone organ transplants (Silva et al., 2019) or haemodialysis (Gulhan et al., 2020). In general, no relationship has been found in these studies.

Spondyloarthritis (SpA) is a group of autoinflammatory rheumatic diseases that include ankylosing spondylitis (AS), psoriatic arthritis (PsA), reactive arthritis (ReA), inflammatory bowel disease (IBD)-associated arthritis and undifferentiated spondyloarthritis (uSpA). All these conditions share genetic, pathophysiological, clinical and radiological features that suggest a common origin (Dougados and Baeten, 2011). Some of these features include arthritis affecting the axial skeleton with inflammatory low back pain, uveitis, dermatological and gastrointestinal involvement and genetic association with HLA-27 (Stolwijk et al., 2015).

Gastrointestinal symptoms (Romero-Sánchez et al., 2017) associated with increased permeability of the intestinal barrier (Gill et al., 2015), microbial dysbiosis (including bacteria and fungi) (Longman and Littman, 2015; Wheeler et al., 2016) and inflammation are frequently observed in patients with SpA and are common features associated with the pathophysiology of these disorders. In other models of infectious diseases associated with SpA, results have not been conclusive in prove this relationship. There are no known reports about the involvement of parasite-induced intestinal dysbiosis in patients with SpA. Similarly, there are very few publications on the occurrence of intestinal parasites in patients with SpA (Aikawa et al., 2011; Jiménez-Balderas et al., 2012), including one case report of ReA caused by *Blastocystis* (Tejera et al., 2012).

The aim of this study was to assess the intestinal parasite populations and to determine the distribution of *Blastocystis* sp. subtypes in a group of patients with spondyloarthritis in Colombia.

2. Materials and methods

2.1. Study population

Two study groups were included: 74 patients diagnosed with SpA and 57 systemically healthy individuals (controls). Patients aged 18–65 years selected by a team of rheumatologists using the criteria of the Assessment of SpondyloArthritis International Society (ASAS) were included (Rudwaleit et al., 2011). They were treated in the Rheumatology Service of Hospital Militar Central and Clinicos IPS and assessed for gastrointestinal symptoms (diarrhea, mucus in stool, haematochezia, increased frequency of daily bowel movements, abdominal pain and abdominal distension). Pregnant women and patients with a history of infection in the previous month, autoimmune disease, neoplasia, immunodeficiency, chronic pancreatitis, liver disease or diabetes were excluded. Similarly, candidates who had been administered antibiotic or antiparasitic medication within the previous 3 months or were on any dose of systemic steroids at the time of recruitment were not included. The controls comprised age-matched healthy individuals, and the same exclusion criteria were used in addition to absence of gastrointestinal symptoms.

2.2. Ethical approval and questionnaire

This study involved minimal risk and was conducted in accordance with the principles outlined in the Declaration of Helsinki and guidelines established by the Ministerio de Salud de Colombia for research involving human subjects. Faecal samples were collected from each subject after they signed the informed consent form. This research was approved by the Ethics Committee of Hospital Militar Central in Bogotá, Colombia (records number 09 of May 5, 2017, and 09 of June 1, 2018).

Information on demographic and clinical characteristics and disease progression was obtained from each subject via a questionnaire by direct interview.

2.3. Faecal samples and microscopic examination

Each subject (patients and controls) was provided with a plastic container for the collection of a faecal sample along with detailed instructions for a correct collection procedure. One sample per subject was collected and stored at 4 °C until arrival at the Molecular Parasitology Laboratory of Universidad El Bosque. The samples were immediately analysed using microscopy; three types of mounting techniques were used: 1) direct examination with 0.9% saline solution and 4% Lugol, 2) examination of concentrated samples using the Mini-Parasep SF faecal parasite concentrator (DiaSys Ltd., Berkshire, England) and 3) mounting with the Kato–Katz system (Sterlitech Corp., USA) for helminth detection. Each mount preparation was examined by certified microscopists. From each stool sample collected, an aliquot was taken for the measurement of calprotectin. Quantification was done with an ELISA assay using the KAPEPKT849 kit (DIAsource ImmunoAssays SA) according to the manufacturers' instructions. The remaining concentrated samples were stored at −20 °C for subsequent DNA extraction.

2.4. Molecular analysis

DNA was extracted from approximately 200 μL of concentrated faecal sample (Mini-Parasep SF, DiaSys Ltd., Berkshire, England) using the QIAamp Stool extraction mini-kit (Qiagen) according to manufacturer's instructions with some modifications, as previously described by Hernández et al., 2019 (Hernández et al., 2019). The presence of DNA was confirmed via quantitative polymerase chain reaction (qPCR) using specific primers to amplify a fragment of the 16S rRNA gene of Enterobacteriaceae and a TaqMan probe, as described by Menu et al., 2018 (Menu et al., 2018). If any sample was negative for this test, it was re-extracted. The primers used were TTB_F: AGAGTTTGATCMTGGCTCAG (forward) and TTB_R: TTACCGCGGCKGCTGGCAC (reverse), and the probe used was S_TTB38K: FAM-CCAKACTCCTACGGGAGGCAGCAG-BHQ1. The qPCR mixture contained 0.75 μM of each primer, 0.15 μM of probe, 1 μL of extracted DNA as template and TaqMan® Fast Advanced Master Mix (Biorad). The PCR was run on a CFX96™ Touch system (BioRad), and amplification programme consisted of initial denaturation at 95 °C for 5 min, 45 cycles of denaturation at 95 °C for 30 s and 60 °C for 1 min. DNA from *Enterococcus faecalis* ATCC 29212 was used as a positive control for the reaction. A molecular analysis complementary to the microscopic analysis was performed to identify *Blastocystis* sp. and *Giardia intestinalis*, as described by Stensvold et al., 2012 (Stensvold et al., 2012a) and Mejía et al., 2013 (Mejía et al., 2013), respectively. Additionally, conventional PCR was performed for *Entamoeba histolytica* identification with the primers reported by Mejía et al., 2013 (Mejía et al., 2013) and Hamzah et al., 2006 (Hamzah et al., 2006).

Blastocystis sp. typing was performed by amplification of the 18S rRNA gene using semi-nested PCR and a combination of primers previously reported by Scicluna in 2006 (Scicluna et al., 2006) and Stensvold in 2012 (Stensvold et al., 2012a). A 1722 bp fragment was amplified in the first reaction with primers RD5 Fwd (ATCTGGTTGATCCTGCCAGT) (forward) and Blasto18SR Rv (CCTACGGAAACCTTGTTACGACTTCA) (reverse). Universal PCR reaction mixture contained 1× Buffer GoFlexiTaq, 2 mM $MgCl_2$, 0.2 mM dNTPs, 0.4 μM oligonucleotides, 1 U DNA GoTaq® Flexi DNA Polymerase (Promega) enzyme and 2 μL of total DNA, resulting in a 15 μL final reaction volume. The following amplification programme was used for universal PCR and semi-nested PCR: 94 °C for 3 min; 37 cycles at 94 °C for 30 s, at 59 °C for 30 s and at 72 °C for 1 min and a final extension at 72 °C for 5 min. DNA from *Blastocystis* sp. strain BT1 (ATCC 50608D) was used as positive control. Nested PCR yielded a 611-bp fragment using the primers RD5 Fwd and BhRDr Rv (GAGCTTTTTAACTGCAACAACG). 1 μL of the universal reaction was used as a template at 25 μL reaction volumes under the above described conditions. PCR products were visualised in ethidium bromide-stained agarose gels and subsequently sequenced at Macrogen Inc. (Korea). Chromatograms obtained for each DNA sample of both strands were edited and analysed using MEGA X software (Kumar et al., 2018). Sequencing data were analysed using the PubMLST database (https://pubmlst.org/organisms/blastocystis-spp) to differentiate subtypes and alleles of *Blastocystis* sp. The obtained sequences were deposited in the GenBank database under accession numbers MW683249–MW683288.

For phylogenetic analysis, sequences obtained in the present study were compared with sequences of some reference subtypes (ST1: DQ232777, ST2: DQ232805, ST3: DQ232803 and ST6: AB070990) retrieved from GenBank. Using MEGA X software, multiple alignments were performed with Clustal W and a phylogenetic tree was constructed using the maximum likelihood algorithm and the Tamura Nei Parameter model. Cluster confidence values were assessed using a bootstrap of 1000 replicates.

2.5. Statistical analysis

Descriptive analyses, frequency distributions and analyses of central tendency and dispersion were performed for clinical, laboratory and demographic data. Associations between clinical variables and parasite frequencies were evaluated using the chi square/Fisher's exact tests. Comparisons were performed using the Mann–Whitney *U* test; *p*-values of <0.05 were considered statistically significant. All analyses were performed using SPSS version 26 for Windows.

3. Results

Seventy-four patients diagnosed with SpA were included. Among them, 39% were females, frequency of ankylosing spondylitis was 82% and the age median was 43 years. All patients were receiving treatment: 36% with conventional modifier treatment and 64% with biological treatment. Age median of healthy control group was 38 years and 54% belonged to male genre.

Table 1 shows general characteristics of the study subjects (patients with SpA and healthy controls).

According to parasitological analysis of samples using microscopy and molecular techniques, *Endolimax nana* was identified in

98.6% of patients with SpA and 98.2% of controls while *Blastocystis* sp. was identified in 67.6% of patients and 73.4% of controls (Table 2). Specifically, results of positives samples for *Blastocystis* found by microscopy were confirmed by qPCR.

DNA was extracted from all samples positive for *Blastocystis* sp. to characterise this parasite (42 control samples and 50 patient samples). Amplicons were obtained for 28 control samples and 18 patient samples. Chromatograms well defined without mixed peaks were obtained for 23 control samples and 17 patient samples. The 40 sequences were analysed and compared to the *Blastocystis* sequences recorded in the PubMLST database (https://pubmlst.org/bigsdb?db=pubmlst_blastocystis_seqdef&page=sequenceQuery) to assign the corresponding subtypes and alleles. Table 3 and Fig. 1 show these results. ST3 was the most frequent subtype in controls (56%, $p = 0.065$), whereas ST1 was the most frequent subtype among SpA patients (35%, $p = 0.270$).

Following the editing and analysis of the chromatograms, a phylogenetic analysis of sequences obtained for each sample was performed. Reference sequences for ST1, ST2, ST3 and ST6 subtypes were retrieved from GenBank database to build a radial phylogenetic tree, as shown in Fig. 2. The tree specifies distribution and clustering of samples with corresponding reference subtype.

4. Discussion

Blastocystis sp. is one of the most common enteric human protozoa in the world (Stensvold et al., 2020), with a prevalence of 0.5%–24% in developed countries and 63%–100% in developing countries (Stensvold et al., 2012b; El Safadi et al., 2014; Tito et al., 2019). *Blastocystis* sp. infection may be asymptomatic or cause non-specific symptoms such as flatulence, vomiting, abdominal pain and diarrhea (Wawrzyniak et al., 2013). In Colombia, estimated prevalence of *Blastocystis* sp. is >50% according to several studies of different regions of the country (Ramirez et al., 2014; Hernández et al., 2019; Ministerio de Salud y Protección Social, Universidad de Antioquia, 2015; Higuera et al., 2020a). In the present study, frequency of *Blastocystis* sp. infection was approximately 70% in controls and SpA patients, with no statistically significant differences between the groups ($p = 0.209$). High prevalence of this infection is generally associated with demographic, socio-economic and epidemiological factors (Wawrzyniak et al., 2013; Ostan et al., 2007), which in developing countries can be translated as inadequate sanitary conditions, unsafe water sources and contact with animals (Tan, 2008; Lee et al., 2012).

Our results demonstrate a particular state of high infection by intestinal protozoa (*E. nana* and *Blastocystis* sp.) in sampled population, which corresponds to adults living in urban areas with lifestyles similar to those in developed countries. We could not account for the high rates of infection observed because the scope of this study did not include a specific epidemiological focus on associated causes or factors. Nevertheless, it is known that 72% of SpA patients presented gastrointestinal symptoms, unlike controls, as this was an exclusion criterion. In light of these findings, it would be very interesting to study water and food samples and even parasites in pets to determine the origin of these infections.

Table 1
Demographic characteristics of the study population.

Variable	SpA patients ($n = 74$)	Healthy controls ($n = 57$)	p value
Age (years)			
Median (IQR)	43 (36–51)	38 (29–53)	0.117[b]
Sex (n - %)			
Female	29–39.20	26–45.60	0.271[a]
Male	45–60.80	31–54.40	
Calprotectin (ng/ml)			
Median (IQR)	62.3 (43.4–110.0)[+]	45.5 (42.0–68.9)[+]	0.029[b]
Type of SpA (n - %)			
Ankylosing spondylitis (AS)	61–82.43		
Female	24–39.30		0.939[a]
Male	37–60.70		
Undifferentiated spondyloarthritis (uSpA)	7–9.45		
Female	3–42.90		0.939[a]
Male	4–57.10		
Psoriatic arthritis (PsA)	6–8.11		
Female	2–33.30		0.939[a]
Male	4–66.70		
Gastrointestinal symptoms (n - %)	53–71.62		
Treatment (n - %)			
Biological[*]	48–64.86		0.342 [a]
Conventional[**]	26–36.13		
Disease activity (n - %)			
Clinically active	46–63.90		0.903[a]
Clinical remission	26–36.10		

IQR: interquartile range.
[a] Chi square/Fisher's exact test.
[b] Mann-Whitney U test.
[+] Shapiro-Wilk normality test $p = 0.001$.
[*] TNFa inhibitor and IL17 inhibitor.
[**] Methotrexate and sulfasalazine.

Table 2
Parasites identified in patients with SpA and controls.

Parasites	Total	SpA patients (n = 74)		Healthy controls (n = 57)		*p* value*
	n	n	%	n	%	
Endolimax nana	129	73	98.6	56	98.2	0.701
Blastocystis spp	92	50	67.6	42	73.4	0.209
Entamoeba coli	6	5	6.7	1	1.7	0.344
Entamoeba histolytica	6	4	5.4	2	3.5	0.607
Chilomastix mesnili	4	2	2.7	2	3.5	0.614
Entamoeba dispar/moshkosvki	4	1	1.3	3	5.3	0.241
Giardia intestinalis	3	1	1.3	2	3.5	0.241
Iodamoeba bütschlii	1	0	0	1	1.7	0.452

* Fisher's exact test and Mann-Whitney U test.

Table 3
Subtypes of *Blastocystis* sp. in SpA patients and healthy controls.

Subtype	SpA patients (n)	Healthy controls (n)	*p* value*
ST1	6	4	0.270
ST2	5	5	0.714
ST3	5	13	0.065
ST6	1	1	0.802

* Chi squared test and Fisher's exact test.

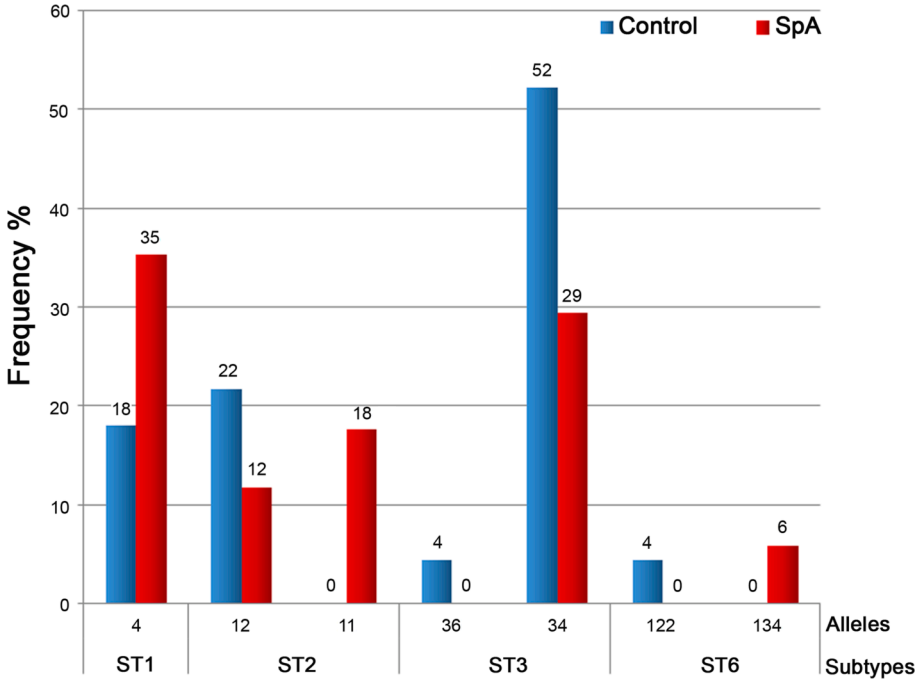

Fig. 1. Frequency of *Blastocystis* sp. subtypes and alleles in spondyloarthritis (SpA) patients and controls. Frequency percentage values are relative to the total for each group, 23 sequences from controls and 17 sequences from SpA patients.

Regarding subtypes of *Blastocystis* sp., our results are consistent with previous reports for Colombia and the Americas (Jiménez et al., 2019). Few studies have described subtypes circulating in humans and animals in Colombia; however, they agree that ST1, ST2 and ST3 subtypes are the most frequent (Ramírez et al., 2016; Ramirez et al., 2014; Higuera et al., 2020a; Sánchez et al., 2017; Villamizar et al., 2019; Potes-Morales et al., 2020). In this study, one sample from a control and one sample from a SpA patient were clustered with ST6, which is not frequent in the Americas. Some studies have reported ST6 in human and avian samples (Ramírez et al., 2016; Oliveira-Arbex et al., 2018; Greige et al., 2018), and it has been described as zoonotic.

Alleles associated with each subtype were also identified. In agreement with previous reports by Ramírez et al., 2014 (Ramirez et al., 2014), Sánchez et al., 2017 (Sánchez et al., 2017) and Higuera et al., 2020 (Higuera et al., 2020a), allele 4 was identified in ST1

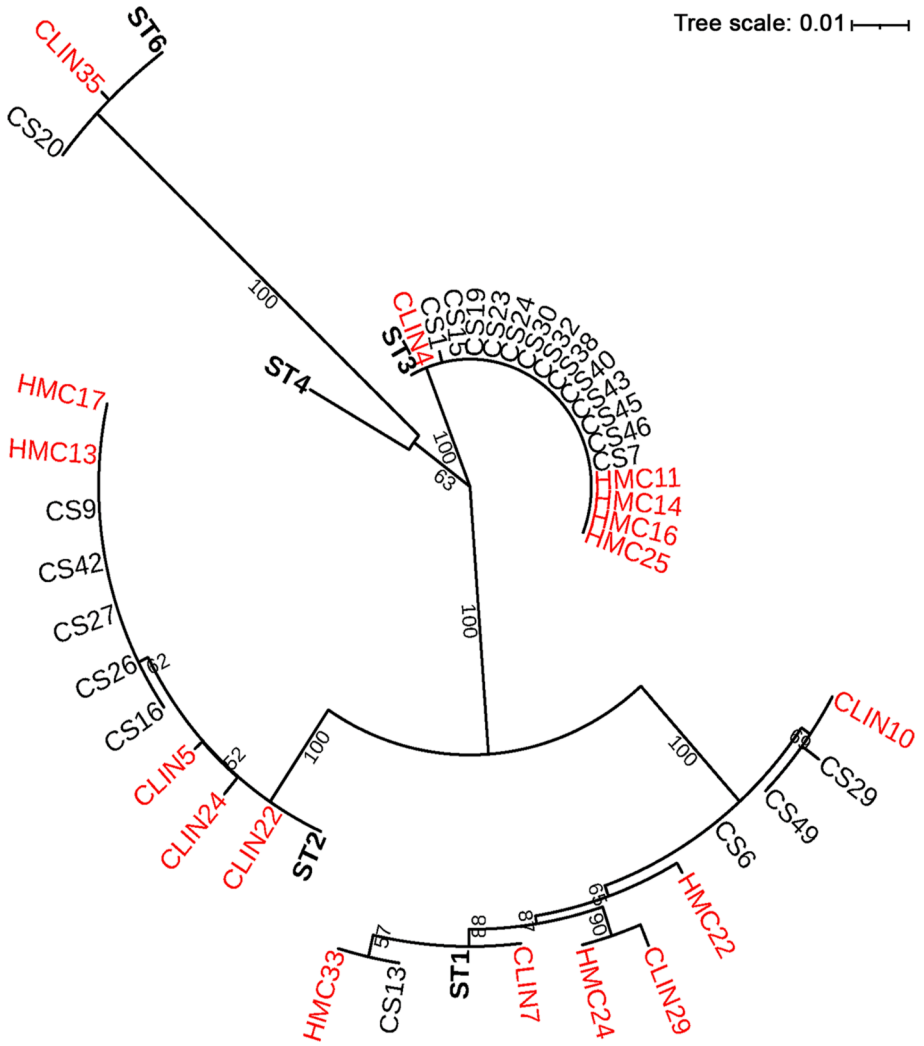

Fig. 2. Phylogenetic tree of 18S rRNA gene fragment of *Blastocystis* sp. samples. Constructed with the maximum likelihood method and the Tamura Nei model. Bootstrap values of >50% are shown for the nodes. Tree was constructed in MEGA X and edited using iTOL v5 (https://itol.embl.de/). *Blastocystis* sp. sequences of spondyloarthritis patients are shown in red and those of healthy controls in black. Reference subtype sequences retrieved from GenBank and ST4 sequence from DNA ATCC used as control are shown in bold type. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

subtype. Similarly, alleles 12 and 11 were found in ST2, alleles 36 and 34 in ST3 and alleles 122 and 134 in ST6. All these alleles have been previously reported in Colombia (Ramirez et al., 2014; Higuera et al., 2020a; Sánchez et al., 2017).

Regarding sequence polymorphisms, ST3 was the most homogeneous subtype as no variations or changes were observed in the sequence compared with that of the reference (DQ232803); this was also observed for ST2 (DQ232805). ST1 subtype showed a high degree of polymorphisms; since the phylogenetic tree (Fig. 2) shows that this branch was divided into sub-nodes, indicating more changes compared with reference sequence. These results support other studies that have shown high intra-ST diversity, which is apparently associated with the ssu-rRNA genetic marker and the geographical region to which the samples belong (Higuera et al., 2020a; Higuera et al., 2020b).

An association between intestinal and systemic immune disorders and altered composition of gut microbiota has been suggested for diseases such as SpA (Mauro and Ciccia, 2019). Gut microbiota consists of all microorganisms that colonise the intestine, such as bacteria, viruses, parasites and fungi. Hence, the purpose of the present study was to investigate intestinal parasites infecting SpA patients. Its been suggested that *Blastocystis* sp. may have a modulatory effect on some populations of gut bacterial microbiota (Deng et al., 2021); however, no definitive conclusion has been reached due to varying results. Some studies have reported that *Blastocystis* sp. is associated with a healthy microbiota (Tito et al., 2019; Castañeda et al., 2020), whereas others have linked it to dysbiosis and gastrointestinal diseases (Nourrisson et al., 2014). In this study, faecal calprotectin was measured in both patients and controls (Table 1), as intestinal inflammation biomarker. Although faecal calprotectin levels were significantly higher in patients compared to controls ($p = 0.029$), it is not related to the presence of *Blastocystis*, since the prevalence were similar in the two groups.

There are some limitations in the present study: Although the control group consisted of people without SpA or gastrointestinal symptoms, presence of other underlying and unknown diseases may have affected the study results. Also, other limitation is not having included patients without treatment as a group to compare, given the difficulty of finding well-diagnosed patients who do not receive treatment. Moreover, *Blastocystis* infection could be underestimated because in vitro culture of samples to promote growth of parasite was no used. Thus, there may be positive samples that did not undergo PCR and genotyping. Finally, mixed subtypes infections of *Blastocystis* cannot be solved because Sanger sequencing of 18S rRNA gene (used in this study) does not allow that discrimination.

The main objective of this study was to assess whether any *Blastocystis* sp. subtype was particularly associated with patients with SpA. Although ST1 was the most frequent subtype in this group, no significant results were obtained ($p = 0.270$).

Our study makes an important contribution to the field of molecular epidemiology, genetic diversity and possible *Blastocystis* sp. host–parasite interactions in populations with rheumatic diseases such as SpA, which greatly affect quality of life of general population. To the best of our knowledge, no previous studies have analysed *Blastocystis* sp. subtypes in these pathologies.

Funding statement

This work was funded by the Departamento Administrativo de Ciencia, Tecnología e Innovación (COLCIENCIAS) (Grant No. 130877757442), Universidad El Bosque (PCI2018–10091), Hospital Militar Central (Grant No. 2017–023), Clinicos IPS, Gastro-advanced, Fundación Instituto de Reumatología Fernando Chalem in Bogotá, Colombia and Biomedicina de Chihuahua, México.

Declaration of Competing Interest

The authors declare that they have no potential conflicts of interest to disclose.

References

Aikawa, N.E., Twardowsky, O., Carvalho, J.F., Silva, C.A., Silva, I.L., Ribeiro, A.C., Saad, C.G., Moraes, J.C., Toledo, R.A., Bonfá, E., 2011. Intestinal microsporidiosis: a hidden risk in rheumatic disease patients undergoing anti-tumor necrosis factor therapy combined with disease-modifying anti-rheumatic drugs? Clinics (Sao Paulo) 66 (7), 1171–1175. https://doi.org/10.1590/s1807-59322011000700008.

Ajjampur, S.S., Tan, K.S., 2016. Pathogenic mechanisms in Blastocystis spp. - Interpreting results from in vitro and in vivo studies. Parasitol. Int. 65, 772–779. https://doi.org/10.1016/j.parint.2016.05.007.

Alfellani, M.A., Taner-Mulla, D., Jacob, A.S., Imeede, C.A., Yoshikawa, H., Stensvold, C.R., Clark, C.G., 2013. Genetic diversity of blastocystis in livestock and zoo animals. Protist. 164 (4), 497–509. https://doi.org/10.1016/j.protis.2013.05.003.

Casero, R., Mongi, F., Sánchez, A., Ramírez, J.D., 2015. Blastocystis and urticaria: examination of subtypes and morphotypes in an unusual clinical manifestation. Acta Trop. 126, 12–16. https://doi.org/10.1016/j.actatropica.2015.05.004.

Castañeda, S., Muñoz, M., Villamizar, X., Hernández, P.C., Vásquez, L.R., Tito, R.Y., Ramírez, J.D., 2020. Microbiota characterization in Blastocystis-colonized and Blastocystis-free school-age children from Colombia. Parasit. Vectors 13 (1), 521. https://doi.org/10.1186/s13071-020-04392-9.

Clark, C.G., 1997. Extensive genetic diversity in *Blastocystis hominis*. Mol. Biochem. Parasitol. 87, 79–83. https://doi.org/10.1016/s0166-6851(97)00046-7.

Deeb, H.K.E., Salah-Eldin, H., Khodeer, S., 2012. Blastocystis hominis as a contributing risk factor for development of iron deficiency anemia in pregnant women. Parasitol. Res. 110 (6), 2167–2174. https://doi.org/10.1007/s00436-011-2743-3.

Deng, L., Wojciech, L., Gascoigne, N.R.J., Peng, G., Tan, K.S.W., 2021. New insights into the interactions between Blastocystis, the gut microbiota, and host immunity. PLoS Pathog. 17 (2), e1009253 https://doi.org/10.1371/journal.ppat.1009253.

Denoeud, F., Roussel, M., Noel, B., Wawrzyniak, I., Da Silva, C., Diogon, M., Viscogliosi, E., Brochier-Armanet, C., Couloux, A., Poulain, J., 2011. Genome sequence of the stramenopile Blastocystis, a human anaerobic parasite. Genome Biol. 12 (3), R29. https://doi.org/10.1186/gb-2011-12-3-r29.

Dougados, M., Baeten, D., 2011. Spondyloarthritis. Lancet 377 (9783), 2127–2137. https://doi.org/10.1016/S0140-6736(11)60071-8.

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 Infect. Dis. 14, 164. https://doi.org/10.1186/1471-2334-14-164.

Gill, T., Asquith, M., Rosenbaum, J.T., Colbert, R.A., 2015. The intestinal microbiome in spondyloarthritis. Curr. Opin. Rheumatol. 27 (4), 319–325. https://doi.org/10.1097/BOR.0000000000000187.

Greige, S., El Safadi, D., Bécu, N., Gantois, N., Pereira, B., Chabé, M., Benamrouz-Vanneste, S., Certad, G., El Hage, R., Chemaly, M., Hamze, M., Viscogliosi, E., 2018. Prevalence and subtype distribution of Blastocystis sp. isolates from poultry in Lebanon and evidence of zoonotic potential. Parasit. Vectors 11 (1), 389. https://doi.org/10.1186/s13071-018-2975-5.

Gulhan, B., Aydin, M., Demirkazik, M., Koltas, I.S., Cikman, A., Turkmen, K., Duran, T., 2020. Subtype distribution and molecular characterization of Blastocystis from hemodialysis patients in Turkey. J. Infect. Dev. Ctries. 14 (12), 1448–1454. https://doi.org/10.3855/jidc.12650.

Hamzah, Z., Petmitr, S., Mungthin, M., Leelayoova, S., Chavalitshewinkoon-Petmitr, P., 2006. Differential detection of Entamoeba histolytica, Entamoeba dispar, and Entamoeba moshkovskii by a single-round PCR assay. J. Clin. Microbiol. 44 (9), 3196–3200. https://doi.org/10.1128/JCM.00778-06.

Hernández, P.C., Morales, L., Chaparro-Olaya, J., Sarmiento, D., Jaramillo, J.F., Ordoñez, G.A., Cortés, F., Sánchez, L.K., 2019. Intestinal parasitic infections and associated factors in children of three rural schools in Colombia. A cross-sectional study. PLoS One 14 (7), e0218681. https://doi.org/10.1371/journal.pone.0218681.

Higuera, A., Villamizar, X., Herrera, G., Giraldo, J.C., Vasquez-A, L.R., Urbano, P., Villalobos, O., Tovar, C., Ramírez, J.D., 2020a. Molecular detection and genotyping of intestinal protozoa from different biogeographical regions of Colombia. PeerJ. 8, e8554 https://doi.org/10.7717/peerj.8554.

Higuera, A., Muñoz, M., López, M.C., Reyes, P., Urbano, P., Villalobos, O., Ramírez, J.D., 2020b. Succinate dehydrogenase gene as a marker for studying Blastocystis genetic diversity. Heliyon. 6 (11), e05387 https://doi.org/10.1016/j.heliyon.2020.e05387.

Jiménez, P.A., Jaimes, J.E., Ramírez, J.D., 2019. A summary of Blastocystis subtypes in north and South America. Parasit. Vectors 12 (1), 376. https://doi.org/10.1186/s13071-019-3641-2.

Jiménez-Balderas, F.J., Camargo-Coronel, A., Gargia-Jaimes, J., Zonana-Nacach, A., Alcantara-Anguianoi, I., Carrillo-Becerril, L., Tapia-Romero, R., Gonzalez, T.S., Villalobos-Gomez, F., Martinez-Hernandez, S., Ventura-Juarez, J., Jimenez-Balderas, E.A., Méndez-Samperio, P., De-La-Rosa-Arana, J.L., 2012. A study on parasites in Mexican rheumatic disease patients. J. Egypt. Soc. Parasitol. 42 (2), 271–280.

Jiménez-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., Maravilla, P., 2012. Blastocystis infection is associated with irritable bowel syndrome in a Mexican patient population. Parasitol. Res. 110 (3), 1269–1275. https://doi.org/10.1007/s00436-011-2626-7.

Kumar, S., Stecher, G., Li, M., Knyaz, C., Tamura, K., 2018. MEGAX: molecular evolutionary genetics analysis across computing platforms. Mol. Biol. Evol. 35, 1547–1549. https://doi.org/10.1093/molbev/msy096.

Lee, L., Chye, T.T., Karmacharya, B.M., Govind, S.K., 2012. Blastocystis sp.: waterborne zoonotic organism, a possibility? Parasit. Vectors 5, 130. https://doi.org/10.1186/1756-3305-5-130.

Longman, R.S., Littman, D.R., 2015. The functional impact of the intestinal microbiome on mucosal immunity and systemic autoimmunity. Curr. Opin. Rheumatol. 27 (4), 381–387. https://doi.org/10.1097/BOR.0000000000000190.

Lukeš, J., Stensvold, C.R., Jirků-Pomajbíková, K., Wegener Parfrey, L., 2015. Are human intestinal eukaryotes beneficial or commensals? PLoS Pathog. 11 (8), e1005039 https://doi.org/10.1371/journal.ppat.1005039.

Maloney, J.G., Molokin, A., Santin, M., 2019. Next generation amplicon sequencing improves detection of Blastocystis mixed subtype infections. Infect. Genet. Evol. 73, 119–125. https://doi.org/10.1016/j.meegid.2019.04.013.

Mattiucci, S., Crisafi, B., Gabrielli, S., Paoletti, M., Cancrini, G., 2015. Molecular epidemiology and genetic diversity of Blastocystis infection in humans in Italy. Epidemiol. Infect. 21, 1–12. https://doi.org/10.1017/S0950268815001697.

Mauro, D., Ciccia, F., 2019. Gut dysbiosis in Spondyloarthritis: cause or effect? Best Pract. Res. Clin. Rheumatol. 33 (6), 101493. https://doi.org/10.1016/j.berh.2020.101493.

Mejía, R., Vicuña, Y., Broncano, N., Sandoval, C., Vaca, M., Chico, M., Cooper, P.J., Nutman, T.B., 2013. A novel, multi-parallel, real-time polymerase chain reaction approach for eight gastrointestinal parasites provides improved diagnostic capabilities to resource-limited at-risk populations. Am. J. Trop. Med. Hyg. 88 (6), 1041–1047. https://doi.org/10.4269/ajtmh.12-0726.

Menu, E., Mary, C., Toga, I., Raoult, D., Ranque, S., Bittar, F., 2018. Evaluation of two DNA extraction methods for the PCR-based detection of eukaryotic enteric pathogens in fecal samples. BMC Res. Notes. 11 (1), 206. https://doi.org/10.1186/s13104-018-3300-2.

Ministerio de Salud y Protección Social, Universidad de Antioquia, 2015. Encuesta nacional de parasitismo intestinal en población escolar 2012–2014. Facultad Nacional Salud Pública, Universidad de Antioquia, El Ministerio, Medellín. https://www.minsalud.gov.co/sites/rid/Lists/BibliotecaDigital/RIDE/VS/PP/ET/encuesta-nacional-de-parasitismo-2012-2014.pdf.

Mülayim, S., Aykur, M., Dağcı, H., Dalkılıç, S., Aksoy, A., Kaplan, M., 2021. Investigation of isolated Blastocystis subtypes from Cancer patients in Turkey. Acta Parasitol. 66 (2), 584–592. https://doi.org/10.1007/s11686-020-00322-y.

Nourrisson, C., Scanzi, J., Pereira, B., NkoudMongo, C., Wawrzyniak, I., Cian, A., Viscogliosi, E., Livrelli, V., Delbac, F., Dapoigny, M., Poirier, P., 2014. Blastocystis is associated with decrease of fecal microbiota protective bacteria: comparative analysis between patients with irritable bowel syndrome and control subjects. PLoS One 9 (11), e111868. https://doi.org/10.1371/journal.pone.0111868.

Oliveira-Arbex, A.P., David, E.B., Guimarães, S., 2018. Blastocystis genetic diversity among children of low-income daycare center in southeastern Brazil. Infect. Genet. Evol. 57, 59–63. https://doi.org/10.1016/j.meegid.2017.11.005.

Ostan, I., Kilimcioğlu, A.A., Girginkardeşler, N., Ozyurt, B.C., Limoncu, M.E., Ok, U.Z., 2007. Health inequities: lower socio-economic conditions and higher incidences of intestinal parasites. BMC Public Health 7, 342. https://doi.org/10.1186/1471-2458-7-342.

Potes-Morales, C., Osorio-Delgado, L.A., Carranza, J.C., Vallejo, G.A., 2020. The first molecular detection of Blastocystis subtypes in human faecal samples from Ibague, Colombia. Parasite Epidemiol. Control. 9, e00132 https://doi.org/10.1016/j.parepi.2020.e00132.

Ramirez, J.D., Sanchez, L.V., Bautista, D.C., Corredor, A.F., Florez, A.C., Stensvold, C.R., 2014. Blastocystis subtypes detected in humans and animals from Colombia. Infect. Genet. Evol. 22, 223–228. https://doi.org/10.1016/j.meegid.2013.07.020.

Ramírez, J.D., Sánchez, A., Hernández, C., Flórez, C., Bernal, M.C., Giraldo, J.C., Reyes, P., López, M.C., García, L., Cooper, P.J., 2016. Geographic distribution of human Blastocystis subtypes in South America. Infect. Genet. Evol. 41, 32–35. https://doi.org/10.1016/j.meegid.2016.03.017.

Romero-Sánchez, C., Bautista-Molano, W., Parra, V., De Avila, J., Rueda, J.C., Bello-Gualtero, J.M., Londoño, J., Valle-Oñate, R., 2017. Gastrointestinal symptoms and elevated levels of anti-Saccharomyces cerevisiae antibodies are associated with higher disease activity in Colombian patients with Spondyloarthritis. Int. J. Rheumatol. 2017, 4029584. https://doi.org/10.1155/2017/4029584.

Rudwaleit, M., van der Heijde, D., Landewé, R., Akkoc, N., Brandt, J., Chou, C.T., Dougados, M., Huang, F., Gu, J., Kirazli, Y., Van den Bosch, F., Olivieri, I., Roussou, E., Scarpato, S., Sørensen, I.J., Valle-Oñate, R., Weber, U., Wei, J., Sieper, J., 2011. The assessment of SpondyloArthritis international society classification criteria for peripheral spondyloarthritis and for spondyloarthritis in general. Ann. Rheum. Dis. 70 (1), 25–31. https://doi.org/10.1136/ard.2010.133645.

Sánchez, A., Munoz, M., Gómez, N., Tabares, J., Segura, L., Salazar, Á., Restrepo, C., Ruíz, M., Reyes, P., Qian, Y., Xiao, L., López, M.C., Ramírez, J.D., 2017. Molecular epidemiology of Giardia, Blastocystis and Cryptosporidium among indigenous children from the Colombian Amazon Basin. Front. Microbiol. 8, 248. https://doi.org/10.3389/fmicb.2017.00248.

Scicluna, S.M., Tawari, B., Clark, C.G., 2006. DNA barcoding of Blastocystis. Protist. 157, 77–85. https://doi.org/10.1016/j.protis.2005.12.001.

Silva, M.D.R.A., Melo, G.B., Malta, F.M., Abdala, E., Costa, S.F., Pierrotti, L.C., Gonçalves, E.M.N., Castilho, V.L.P., Chieffi, P.P., Gryschek, R.C.B., Paula, F.M., 2019. Subtypes of Blastocystis sp. isolated in fecal samples from transplant candidates in Sao Paulo, Brazil. Parasite Epidemiol. Control. 8, e00128. https://doi.org/10.1016/j.parepi.2019.e00128.

Stensvold, C.R., Clark, C.G., 2016. Current status of Blastocystis: a personal view. Parasitol. Int. 65, 763–771. https://doi.org/10.1016/j.parint.2016.05.015.

Stensvold, C.R., Clark, C.G., 2020. Pre-empting pandora's box: Blastocystis subtypes revisited. Trends Parasitol. 36 (3), 229–232. https://doi.org/10.1016/j.pt.2019.12.009.

Stensvold, C.R., Suresh, G.K., Tan, K.S., Thompson, R.C., Traub, R.J., Viscogliosi, E., Yoshikawa, H., Clark, C.G., 2007. Terminology for Blastocystis subtypes–a consensus. Trends Parasitol. 23 (3), 93–96. https://doi.org/10.1016/j.pt.2007.01.004.

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. https://doi.org/10.4269/ajtmh.2011.11-0005.

Stensvold, C.R., Ahmed, U.N., Andersen, L.O., Nielsen, H.V., 2012a. Development and evaluation of a genus-specific, probe-based, internal-process-controlled real-time PCR assay for sensitive and specific detection of Blastocystis spp. J. Clin. Microbiol. 50 (6), 1847–1851. https://doi.org/10.1128/JCM.00007-12.

Stensvold, C.R., Alfellani, M., Clark, C.G., 2012b. Levels of genetic diversity vary dramatically between Blastocystis subtypes. Infect. Genet. Evol. 12 (2), 263–273. https://doi.org/10.1016/j.meegid.2011.11.002.

Stensvold, C.R., Tan, K.S.W., Clark, C.G., 2020. Blastocystis. Trends Parasitol. 36 (3), 315–316. https://doi.org/10.1016/j.pt.2019.12.008.

Stolwijk, C., van Tubergen, A., Castillo-Ortiz, J.D., Boonen, A., 2015. Prevalence of extra-articular manifestations in patients with ankylosing spondylitis: a systematic review and meta-analysis. Ann. Rheum. Dis. 74 (1), 65–73. https://doi.org/10.1136/annrheumdis-2013-203582.

Tan, K.S., 2008. New insights on classification, identification, and clinical relevance of Blastocystis spp. Clin. Microbiol. Rev. 21 (4), 639–665. https://doi.org/10.1128/CMR.00022-08.

Tan, T., Ong, S., Suresh, K., 2009. Genetic variability of Blastocystis sp. isolates obtained from cancer and HIV/AIDS patients. Parasitol. Res. 105 (5), 1283. https://doi.org/10.1007/s00436-009-1551-5.

Tejera, B., Grados, D., Martinez-Morillo, M., Roure, S., 2012. Reactive arthritis caused by Blastocystis hominis. Reumatol. Clin. 8 (1), 50–51. https://doi.org/10.1016/j.reuma.2011.07.008.

Tito, R.Y., Chaffron, S., Caenepeel, C., Lima-Mendez, G., Wang, J., Vieira-Silva, S., Falony, G., Hildebrand, F., Darzi, Y., Rymenans, L., Verspecht, C., Bork, P., Vermeire, S., Joossens, M., Raes, J., 2019. Population-level analysis of Blastocystis subtype prevalence and variation in the human gut microbiota. Gut. 68 (7), 1180–1189. https://doi.org/10.1136/gutjnl-2018-316106.

Verma, R., Delfanian, K., 2013. Blastocystis hominis associated acute urticaria. Am J Med Sci 346 (1), 80–81. https://doi.org/10.1097/MAJ.0b013e3182801478.

Villamizar, X., Higuera, A., Herrera, G., Vasquez-A, L.R., Buitron, L., Muñoz, L.M., Gonzalez-C, F.E., Lopez, M.C., Giraldo, J.C., Ramírez, J.D., 2019. Molecular and descriptive epidemiology of intestinal protozoan parasites of children and their pets in Cauca, Colombia: a cross-sectional study. BMC Infect. Dis. 19 (1), 190. https://doi.org/10.1186/s12879-019-3810-0.

Wawrzyniak, I., Poirier, P., Viscogliosi, E., Dionigia, M., Texier, C., Delbac, F., Alaoui, H.E., 2013. Blastocystis, an unrecognized parasite: an overview of pathogenesis and diagnosis. Ther. Adv. Infect. Dis. 1 (5), 167–178. https://doi.org/10.1177/2049936113504754.

Wheeler, M.L., Limon, J.J., Bar, A.S., Leal, C.A., Gargus, M., Tang, J., Brown, J., Funari, V.A., Wang, H.L., Crother, T.R., Arditi, M., Underhill, D.M., Iliev, I.D., 2016. Immunological consequences of intestinal fungal Dysbiosis. Cell Host Microbe 19 (6), 865–873. https://doi.org/10.1016/j.chom.2016.05.003.

Yavasoglu, I., Kadikoylu, G., Uysal, H., Ertug, S., Bolaman, Z., 2008. Is Blastocystis hominis a new etiologic factor or a coincidence in iron deficiency anemia? Eur. J. Haematol. 81 (1), 47–50. https://doi.org/10.1111/j.1600-0609.2008.01080.x.

Yersal, O., Malatyali, E., Ertabaklar, H., Oktay, E., Barutca, S., Ertug, S., 2016. Blastocystis subtypes in cancer patients: analysis of possible risk factors and clinical characteristics. Parasitol. Int. 65, 792–796. https://doi.org/10.1016/j.parint.2016.02.010.

Yoshikawa, H., Iwamasa, A., 2016. Human Blastocystis subtyping with subtype-specific primers developed from unique sequences of the SSU rRNA gene. Parasitol. Int. 65 (6), 785–791. https://doi.org/10.1016/j.parint.2016.03.002.