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Research paper

Inter- and intra-subtype variation of *Blastocystis* subtypes isolated from diarrheic and non-diarrheic patients in Iran

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ABSTRACT

Blastocystis is a common intestinal parasitic protist infecting birds and mammals. *Blastocystis* comprises at least 17 subtypes (ST), of which ST1–ST9 have been detected in humans. Significant correlation between certain subtypes and pathogenicity remains to be established. Nevertheless, some studies suggest a potential linkage between subtypes (inter- and intra-subtype variation) and clinical manifestations. The aim of this study was to identify intra-subtype genetic variation of subtypes of *Blastocystis* in stools samples submitted by diarrheic and non-diarrheic patients. A 550-bp fragment of the nuclear small subunit ribosomal rRNA gene was amplified from 58 culture-positive samples isolated from diarrheic and non-diarrheic Iranian patients. PCR products were sequenced and sequences subjected to phylogenetic analysis. Intra-and inter-subtype variation was calculated. Based on comparison with reference sequences in GenBank, ST1, ST2 and ST3 were found in 18 (31.03%), 21 (36.22%), and 19 (32.75%) of the samples, respectively. Diarrheic stools were observed in eight (44.44%), 10 (47.61%), and nine (47.36%) patients with ST1, ST2, and ST3, respectively. No statistically significant correlation was found between subtypes and diarrhea ($P = 1.000$). Multiple sequence alignment exhibited a within-subtype similarity of 98.76%, 97.17%, and 99.78% in ST1, ST2, and ST3, respectively. Highest similarity was seen among ST3 isolates, while lowest similarity was seen among ST2 isolates. Phylogenetic analysis did not suggest any correlation between diarrhea and intra-subtype variation. Inter- and intra-subtype variation in SSU rRNA gene appears not to reflect differences in the clinical outcome of *Blastocystis* carriage.

1. Introduction

Blastocystis is an intestinal single-celled parasite infecting both human and a broad range of non-human hosts except insects and reptiles (Abe et al., 2003; Clark et al., 2013; Stensvold et al., 2009a; Tan, 2004; Thathaisong et al., 2003). It is one of the most common intestinal parasites and has been reported from many countries with low to high prevalence rates, depending on the level of hygiene, method of detection, geographical area, as well as study population (Belleza et al., 2015; Dogruman-Al et al., 2010; Graczyk et al., 2005). Although the parasite is common in both developing and developed countries, the colonization rate of *Blastocystis* appears significantly higher in developing countries, with reported prevalence figures of up to 100% in some African populations (El Safadi et al., 2014; Poulsen et al., 2016). Several studies have linked *Blastocystis* carriage with gastrointestinal symptoms, including diarrhea, abdominal pain, bloating and flatulence (Tan et al., 2010), irritable bowel syndrome (Dogruman-Al et al., 2009; Nagel et al., 2015; Stensvold et al., 2009b; Surangsrirat et al., 2010; Yakoob et al., 2010) as well as extra-intestinal disorders such as urticaria (Gupta and Parsi, 2006; Verma and Delfanian, 2013), palmoplantar pruritus (Kick et al., 2002), iron deficiency anemia (El Deeb et al., 2012; Yavasoglu et al., 2008), and chronic angioedema (Micheloud et al., 2007).

Based on diversity within the nuclear small subunit (SSU) ribosomal RNA gene, at least 17 different lineages (the so-called subtypes [ST]) (Alfellani et al., 2013c) have been identified; so far, ST1–ST9 have been found in humans. The four subtypes ST1–ST4 are the most common subtypes in humans, accounting for at least 90% of human *Blastocystis* carriage (Alfellani et al., 2013b; Malheiros et al., 2011). A role for *Blastocystis* in disease development in humans is yet to be demonstrated, and most subtypes have been found in both symptomatic and asymptomatic patients (Bohm-Gloning et al., 1997; Elwakil and Talaat, 2009; Moosavi et al., 2012). Even though ST3 was identified as the most common subtype in symptomatic patients (Scanlan, 2012),

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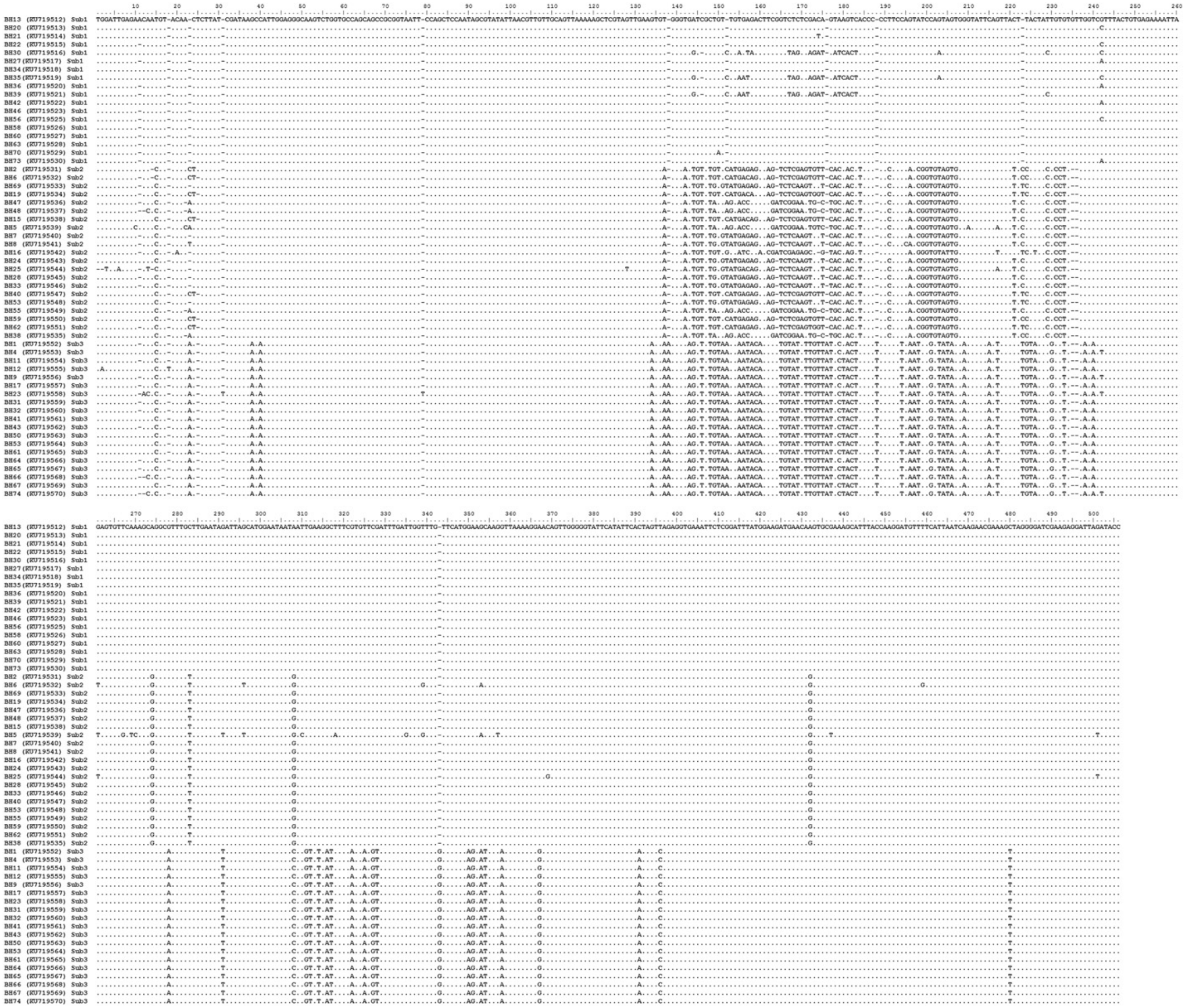

Fig. 1. Alignment of SSU rDNA nucleotide positions of ST1–ST3 obtained from the current study. The sequences were aligned using the Clustal W and Bioedit Software.

this fact might simply reflect the higher prevalence of this subtype in infected subjects. Meanwhile, several studies have suggested a potential correlation between specific subtypes with clinical symptoms (Dominguez-Marquez et al., 2009; Jones et al., 2009; Stensvold et al., 2008; Stensvold et al., 2011).

It has been suggested that intra-subtype variation reflected at the SSU rRNA gene level might be related to different symptoms presenting in patients with identical subtypes (Tan, 2004, 2008; Tan et al., 2010). However, while correlation between intra-subtype variations across SSU rRNA genes with clinical manifestations remains to be proven, differences in the ability to induce epithelial cell permeability was observed among intra-subtype variants of ST7 *in vitro* (Wu et al., 2014).

The objective of the current study was to identify subtypes of *Blastocystis* including intra-subtype genetic variation in stools samples submitted by diarrheic and non-diarrheic patients.

2. Materials and methods

2.1. Sample population

A total of 58 positive samples were collected from 400 diarrheic and non-diarrheic patients tested because of gastrointestinal disorders or in relation to general checkups, respectively, at the Enteric Parasitic Protozoan Laboratory in Dept. of Medical Parasitology and Mycology, Tehran University of Medical Sciences, during the period ranging from April 2015 to April 2016 (Jalallou et al., in press). Based on stool appearance, all stool samples had been categorized as 'formed' (completely formed or soft), 'diarrhea' and 'watery diarrhea'. All stool samples had been cultivated in 1 mL of autoclaved Locke's solution added to a 2-mL slant of clotted fetal bovine serum in a screw-cap tube. Next, 200 mg of stool was added together with starch and 4 mg/mL streptomycin to each

Fig. 2. Phylogenetic analysis of SSU rDNA nucleotide sequences of three subtypes of *Blastocystis* sp. isolates recovered from diarrheic and non-diarrheic patients in Iran. The tree was constructed using the Maximum-Likelihood test and the Tamura 3-parameter model as implemented in the MEGA6 software. The numbers above the branches indicate the percentage of bootstrap samplings supporting the interior branches. Branches without numbers have bootstrap values <75%. Diarrheic isolates are indicated with "D". THA: Thailand; MYS: Malaysia.