Infection, Genetics and Evolution 41 (2016) 32–35

Contents lists available at ScienceDirect

Infection, Genetics and Evolution

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

Short communication

Geographic distribution of human *Blastocystis* subtypes in South America

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ARTICLE INFO

Article history:
Received 18 February 2016
Received in revised form 16 March 2016
Accepted 17 March 2016
Available online 24 March 2016

Keywords:
Blastocystis
18S alleles
Subtypes
Molecular epidemiology
Genotyping

ABSTRACT

Blastocystis is a cosmopolitan enteric protist colonizing probably more than 1 billion people. This protozoan exhibits genetic diversity and is subdivided into subtypes (STs). The aim of this study was to determine the distribution of *Blastocystis* STs in symptomatic and asymptomatic human samples from different countries of South America. A total of 346 fecal samples were genotyped by SSU rDNA showing ST1 (28.3%), ST2 (22.2%), ST3 (36.7%), ST4 (2%), ST5 (2.3%), ST6 (2%), ST7 (2.3%), ST8 (0.6%), ST12 (0.9%) and a novel ST (2.7%). These findings update the epidemiology of *Blastocystis* in South America and expand our knowledge of the phylogeographic differences exhibited by this stramenopile.

Blastocystis comprises a genus of single-celled parasites that present a cosmopolitan distribution and colonizes an estimated 1 billion people, many of whom are asymptomatic. Some authors report that symptoms caused by this protist comprise abdominal pain, constipation, diarrhea, flatulence and irritable bowel syndrome (IBS) (Dogruman et al., 2009). This parasite comprises several different species living in the gastrointestinal tract of humans, farm animals, birds, rodents, reptiles and other animals (Clark et al., 2013). Transmission of cysts is thought to occur via fecal–oral route and the cyst is probably the only form involved; the extent to which human–human, human–animal and animal–human transmissions occur remains unclear (Parkar et al., 2007, 2010; Stensvold et al., 2012).

Remarkable genetic diversity exists, and there are several distinct ribosomal lineages, the so-called subtypes (STs) based on polymorphic regions across the small subunit (SSU) rRNA gene (Stensvold et al., 2007). So far, moderate host-specificity for STs has been reported (Alfellani et al., 2013). Today, at least 17 genetically distinct SSU rRNA clusters are known (Alfellani et al., 2013). In humans, nine STs have been reported. STs 1, 2, 3 and 4 are all common in Europe. While ST1, ST2 and ST3 seem to be equally prevalent in patients with diarrhea and healthy individuals, ST4 appears to be linked to diarrhea and irritable bowel syndrome (IBS) in Europe (Stensvold et al., 2011; Alfellani et al., 2013). Molecular epidemiological studies have demonstrated that humans can be colonized by one or more of several STs, some of which are commonly found in non-human hosts.

Data are scarce from Latin America: in Colombia, ST1 (34%) and ST2 (23%) and lower proportions of ST3 (11.4%) were detected in humans (Ramírez et al., 2014). In Brazil, ST1 (41%), ST2 (32%) and ST3 (17%) were identified in an ethnic group (Tapirapé ethnic group) (Malheiros et al., 2011); in Mexico a local survey identified 51% of ST1, 23% of ST2, 19% of ST3 and 2% of ST7 (Villalobos et al., 2014). Lastly, in Argentina a survey on asymptomatic and symptomatic patients showed a predominance of ST3 (71.6%) followed by ST1 (14.9%), ST6 (7.5%) and ST2 (5.9%) (Casero et al., 2015). While these studies suggest the absence of ST4 in humans native to South America, other studies have demonstrated the presence of ST4 in non-human primates in Colombia at a low proportion (Ramírez et al., 2014). Apart from data from Brazil, Mexico, Argentina and Colombia (Malheiros et al., 2011; Villalobos et al., 2014; Ramírez et al., 2014), there are no comparative data of

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http://dx.doi.org/10.1016/j.meegid.2016.03.017
1567-1348/

Blastocystis STs from South America at a regional level. The aim of the present study was to do a regional survey in human samples from Argentina, Bolivia, Ecuador, Peru, Brazil and Colombia in order to develop SSU rRNA *Blastocystis* barcoding to establish STs and 18S allele frequency and their geographical distribution in South America.

We conducted a continental survey in six different countries of South America (Bolivia, Ecuador, Peru, Brazil, Colombia and Argentina) (Fig. 1). In this survey 346 stool specimens were collected from humans (40 samples from Bolivia, 25 samples from Ecuador, 13 samples from Peru, 22 samples from Brazil, 181 samples from Colombia and 65 samples from Argentina). The criteria to collect the samples were by convenience, there was nosample size calculation conducted and the samples included in this study were positive by microscopy for *Blastocystis* infection. Fresh samples were collected and stored in ethanol. Fecal specimens were evaluated with Lugol's stain and trichrome staining to detect *Blastocystis*. Samples from Bolivia, Ecuador, Peru and Brazil were obtained from asymptomatic patients while samples from Colombia were obtained from asymptomatic patients, patients with diarrhea or other gastrointestinal symptoms. Patients from Argentina presented intestinal symptoms like diarrhea, constipation, abdominal pain and bloating (Casero et al., 2015). From each sample, 250 mg was submitted to DNA extraction using the QIAmp DNA Stool Mini Kit (Qiagen, Hilden, Germany) according to the manufacturer's instructions. Genomic DNA was preserved at −20 °C until analysis. The samples were verified for the presence of *Blastocystis* via PCR amplification of *Blastocystis*-specific SSU rDNA using the primers RD5 (5′-ATC TGG TTG ATC CTG CCAG T-3′) and BhRDr (5′-GAG CTT TTT AAC TGC AAC AAC G-3′) (Scicluna et al., 2006), as recently recommended (Stensvold, 2013). PCR products were purified and sequenced by both strands using the dideoxy-terminal method. Sequences were edited in MEGA 5.0 (Tamura et al., 2010) and compared with reference sequences representing each ST in GenBank by BLAST queries. Additionally, sequences were submitted to sequence queries at the *Blastocystis* 18S database available at http://pubmlst.org/blastocystis/ for *Blastocystis* 18S allele calling and confirmation of ST. The final sequences were deposited on GenBank under the accession numbers KU928039–KU928128.

We detected *Blastocystis* cysts in all the samples analyzed, after performing barcoding we observed an overall distribution of ST1 98/346 (28.3%), ST2 77/346 (22.2%), ST3 127/346 (36.7%), ST4 7/346 (2%), ST5 8/346 (2.3%), ST6 7/346 (2%), ST7 8/346 (2.3%), ST8 2/346 (0.6%), ST12 3/346 (0.9%) and a novel ST 9/346 (2.7%). Discriminated by countries, the frequencies are shown in Table 1 and Fig. 1. Regarding the alleles retrieved from the 18S database; for ST1 five alleles were detected, for ST2 seven alleles, for ST3 nine alleles, for ST4 only one allele, for ST6 one allele, in the case of samples typed as ST5, ST6, ST7, ST8 and ST12 no match was found on the database (Fig. 2 and Table S1).

Blastocystis is a recurrent commensal and its pathogenic role is still controversial. Due to the epidemiological characteristics of South America the frequency of patients infected with this protist tends to be high (Ramírez et al., 2014). The information regarding the molecular epidemiology of *Blastocystis* subtypes in South America is scarce with limited information in Colombia, Brazil and Argentina. This is to our knowledge the first attempt to try to elucidate the circulating subtypes and ribosomal alleles in this region of the world. *Blastocystis* samples from Colombia constituted half of the total panel analyzed, whereas isolates from Peru, Brazil, and Ecuador were sub-represented. This fact strongly suggests that molecular epidemiological data from these three countries should be considered as preliminary. One of the most interesting findings was the vast diversity detected in humans from Bolivia, Ecuador, Peru, Brazil, Colombia and Argentina observing the

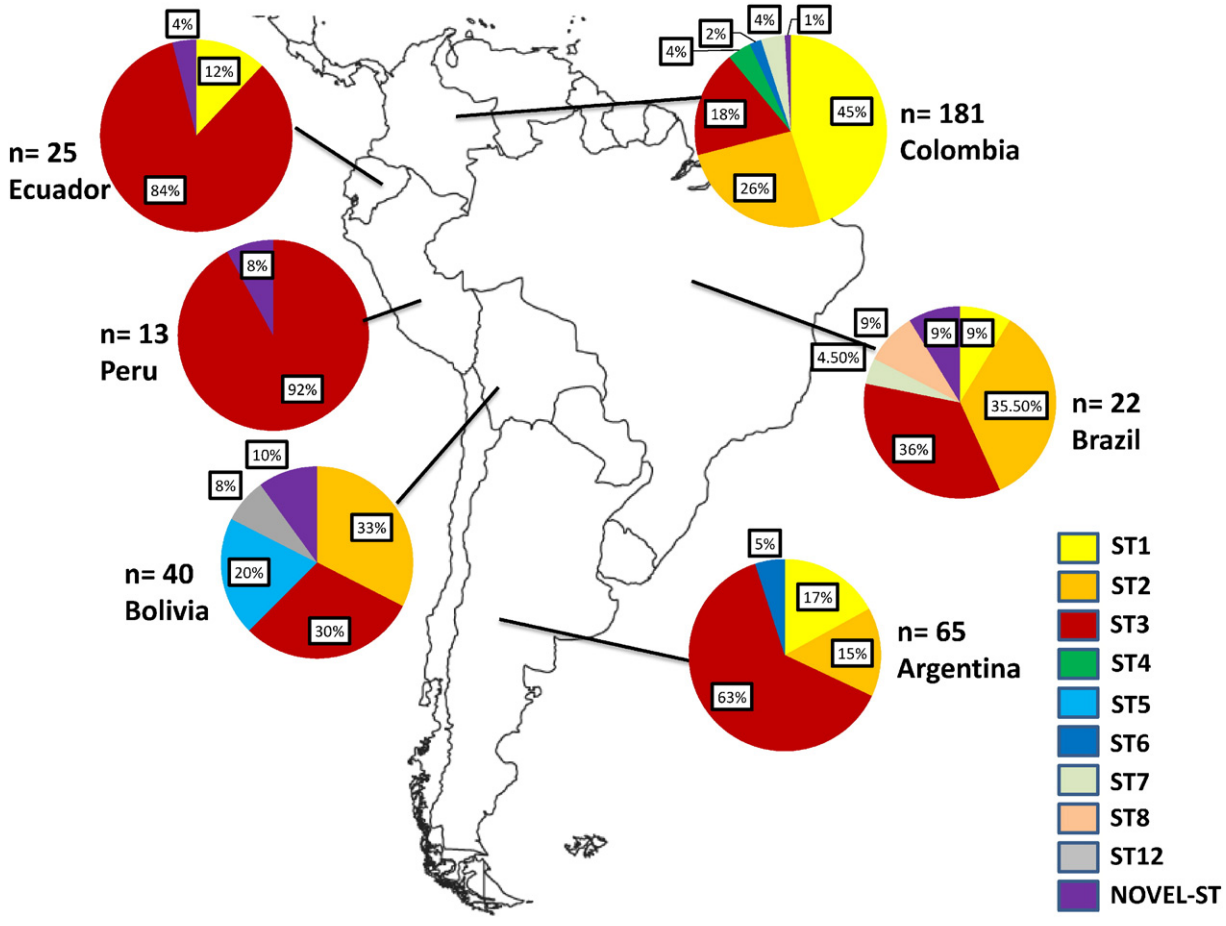

Fig 1. Geographical distribution of *Blastocystis* subtypes detected in 346 human fecal samples from South America.