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Blastocystis subtypes detected in humans and animals from Colombia

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ABSTRACT

Blastocystis is a common enteric protist colonizing probably more than 1 billion people along with a large variety of non-human hosts. This protist has been linked to symptoms and diseases such as abdominal pain, constipation, diarrhea, flatulence and irritable bowel syndrome (IBS). Remarkable genetic diversity has been observed, leading to the subdivision of the genus into multiple subtypes (ST), some of which are exclusively found in non-human hosts. The aim of this study was to determine the distribution of *Blastocystis* STs in different Colombian hosts. We obtained fecal samples positive for *Blastocystis* by microscopy from 277 humans, 52 birds, and 117 mammals (25 cattle, 40 opossums, 40 dogs, 10 rats and 2 howler monkeys). The samples were submitted to DNA extraction, PCR and sequencing using primers targeting the small subunit rRNA gene, and ST identification was performed according to DNA barcoding. We observed the occurrence of ST1 (34%) and ST2 (23%) and lower proportions of STs 3 (11.4%), 4 (0.8%), 6 (19.8%) and 8 (10.5%). Domesticated mammals shared the same STs as those usually seen in humans (ST1, ST2, ST3), while birds and marsupials had STs, which are usually rare in humans (ST6, ST8). Further studies implementing high-resolution molecular markers are necessary to understand the phylodynamics of *Blastocystis* transmission and the role of this stramenopile in health and disease in Colombian populations, and to expand on the phylogeographic differences observed so far with a view to exploring and understanding host–parasite co-evolution.

1. Introduction

Blastocystis comprises a genus of single-celled parasites belonging to the Stramenopiles. This protist presents a number of subtypes living in the gastrointestinal tract of humans, farm animals, birds, rodents, reptiles and others (Yoshikawa et al., 2004a,b; Clark et al., 2013). The transmission is thought to occur via the fecal-oral route and the cyst is probably the only form involved; the extent to which human–human, human-animal and animal-human transmission occurs remain under debate (Yoshikawa et al., 2000, 2004b; Parkar et al., 2007, 2010; Stensvold et al., 2012). *Blastocystis* is considered a cosmopolitan enteric protist with a worldwide distribution and the most frequently detected micro-eukaryote in epidemiological surveys. Many carriers probably suffer no or little abdominal or intestinal discomfort. Some authors report that symptoms caused by this protist comprise abdominal pain, constipation, diarrhea, flatulence and irritable bowel syndrome (IBS) (Dogruman-Al et al., 2009; Stensvold et al., 2009a). The prevalences reported vary according to geographical regions but are generally higher in developing countries than in developed countries due to differences in the standards of hygiene, waste disposal, exposure to animals, and consumption of contaminated food or water (Clark et al., 2013; Tan, 2008).

Remarkable intra-genetic diversity exists, and several distinct ribosomal lineages, the so-called subtypes (ST), have been reported. In 2007, a consensus of *Blastocystis* nomenclature was proposed revealing nine distinct STs colonizing humans, other mammals and birds, based on polymorphic regions across the small subunit (SSU) rRNA gene (Stensvold et al., 2007). Additionally, the discrimination of the distinct STs can be performed using allelic profiling of the SSU rRNA region using the *Blastocystis* 18S and Sequence Typing (MLST) databases (http://pubmlst.org/blastocystis/); to date, a total of at least 135 different 18S alleles exists (Stensvold et al., 2012). So far, no strict associations between the STs and the hosts have been reported, although moderate host specificity is seen (Stensvold et al., 2009b). Today, at least 17 genetically distinct SSU rRNA clusters are known, 8 of which have only been found in non-human hosts (Alfellani et al., 2013a). Additional molecular epidemiology studies using Multilocus

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Sequence Typing strategies have shown that intra-genetic diversity varies dramatically among the STs surveyed (Stensvold et al., 2012). In humans, subtypes 1 through 9 occur with varying frequency. 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/or irritable bowel syndrome (IBS) in Europe (Dominguez-Márquez et al., 2009; Stensvold et al., 2011; Alfellani et al., 2013a).

There is only limited data on *Blastocystis* STs in South America. Human colonization with STs 1, 2 and 3 appears common (Santín et al., 2011; Malheiros et al., 2011), while ST4 has not been detected in humans so far. The finding of ST4 in non-human primates in Colombia suggests that ST4 exists in South America, at least in sylvatic cycles. Therefore, the aim of the present study was to sample and DNA barcode *Blastocystis* from human and non-human hosts in Colombia to obtain an indication of subtype distribution and host specificity.

2. Materials and methods

2.1. Specimens and identification of Blastocystis

This is a descriptive study of *Blastocystis* STs in fecal samples collected across Colombia. We obtained samples from humans and animals: For the animals, fresh fecal samples were preserved in ethanol from: birds (52 samples) (*Passer domesticus*, *Thraupis episcopus, Oryzoborus maximiliani, Sicalis flaveola Petrochelidon pyrrhonota*), cattle (25 samples) (*Bos primigenius*), dogs (40 samples) (*Canis lupus familiaris*), opossums (40 samples) (*Didelphis marsupialis*), rodents (10 samples) (*Rattus rattus*) and howler monkeys (2 samples) (*Alouatta* spp.). Regarding the human samples, fecal samples from symptomatic and asymptomatic individuals were obtained during a previous study of intestinal parasites in Colombia. These samples were obtained from seven different regions of Colombia (Cundinamarca, Bogotá, Boyacá, Casanare, Huila, Santander and Vaupés) (Fig. 1). In total, we obtained 277 samples from humans that accepted to provide fresh fecal samples upon informed consent. Fecal specimens were evaluated with Lugol's stain and trichrome staining to detect *Blastocystis*. Samples positive for *Blastocystis* were submitted to DNA extraction using 250 mg of fecal sample. In total, we obtained 237 samples positive for *Blastocystis*: 125 samples from humans (70 without intestinal symptoms as part of previous study of intestinal disorders, 40 with diarrhea, and 15 with IBS that were diagnosed by a gastroenterologist based on physical examination using Rome III criteria; Drossman, 2006), 47 samples from birds and 65 samples from mammals (20 cows (cattle), 25 *D. marsupialis*, 15 dogs, 3 *R. rattus* and 2 *Alouatta* spp.).

2.2. Discrimination of Blastocystis STs

From each sample, 250 mg was submitted to DNA extraction using the QIAamp DNA Stool Mini Kit (Qiagen, Hilden, Germany) according to manufacturer's instructions. The samples were verified for the presence of *Blastocystis* by PCR amplification of *Blastocystis*-specific SSU rDNA using the primers RD5 (5′ATCTGGTTG ATCCTGCCAGT3′) and BhRDr (5′GAGCTTTTTAACTGCAACAACG3′) (Scicluna et al., 2006) as recently recommended (Stensvold, 2013). PCR products were purified and sequenced by both strands using dedideoxy-sequencing (Macrogen, Korea). Sequences were edited in MEGA 4.0 and compared with retrieved reference sequences from each ST in GenBank by BLAST queries. Additionally, sequences were submitted to sequence queries in the *Blastocystis* 18S database for allele calling. To test the reliability of ST discrim-

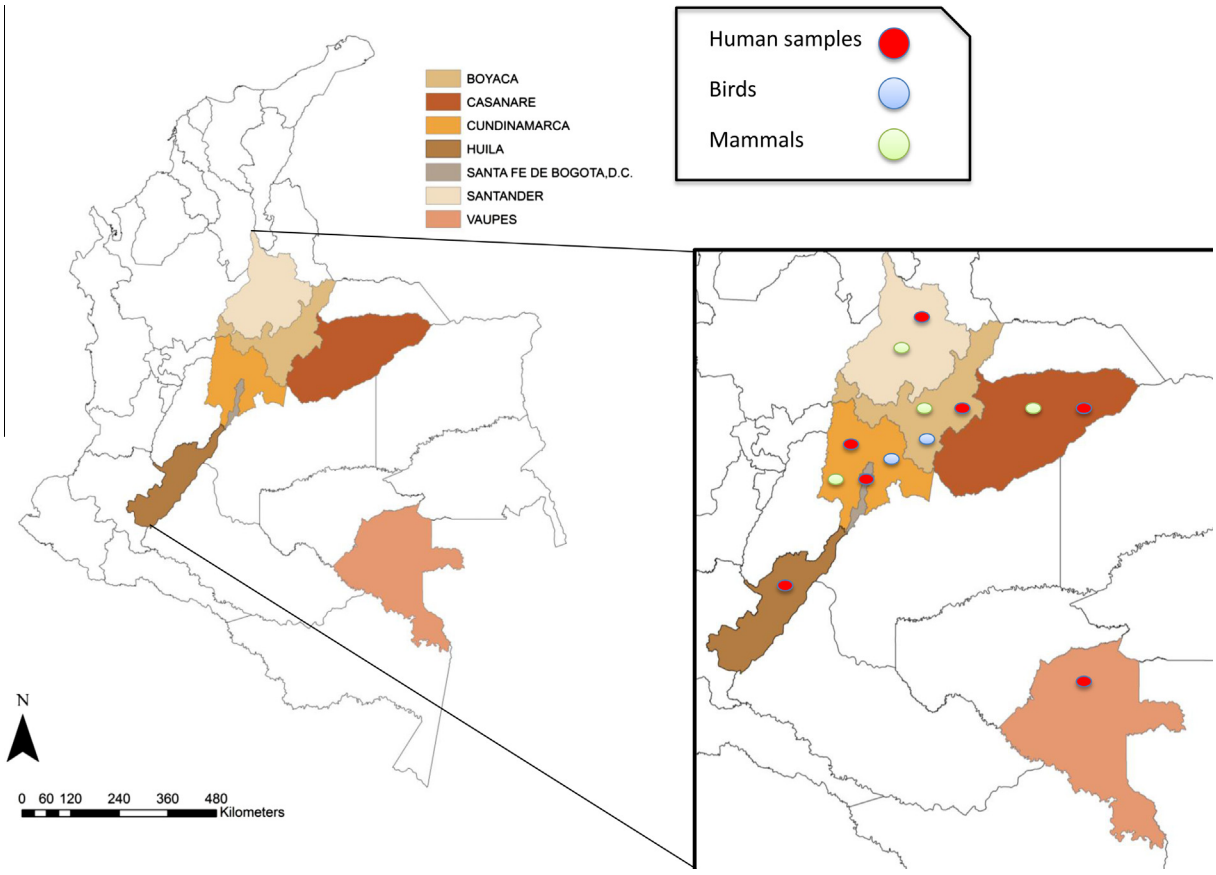

Fig. 1. Geographical distribution of positive-*Blastocystis* fecal samples analyzed.