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Short Report: Molecular Characterization of *Blastocystis* Obtained from Members of the Indigenous Tapirapé Ethnic Group from the Brazilian Amazon Region, Brazil

Antonio F. Malheiros,* C. Rune Stensvold, C. Graham Clark, Guilherme B. Braga, and Jeffrey J. Shaw

Department of Biology, Department of Nursing, Mato Grosso State University, Cáceres, Mato Grosso, Brazil;
Department of Microbiological Diagnostics, Statens Serum Institut, Copenhagen S, Denmark; Faculty of Infectious and Tropical Diseases,
London School of Hygiene and Tropical Medicine, London, United Kingdom; Department of Medicine Veterinary Preventive and Animal Health,
São Paulo University, São Paulo, Brazil; Department of Parasitology, University of São Paulo, São Paulo, Brazil

Abstract.   A total of 382 stool samples were examined during a survey of intestinal parasites in members of the Tapirapé ethnic group, who live in the Brazilian Amazon region of Mato Grosso. Fecal DNAs from *Blastocystis*-positive samples were extracted, polymerase chain reaction amplified using *Blastocystis*-specific primers targeting the small subunit rRNA gene, and sequenced. Three subtypes (STs) were identified: ST1 (41%), ST2 (32%), and ST3 (17%). Seven mixed infections were found (11%). The subtype distribution was markedly different from that reported in Europe in that ST4 was not detected and ST3 was not the most common subtype. This study is the first to include molecular characterization of *Blastocystis* in Brazil and in indigenous communities from Latin America.

Blastocystis is a very common parasitic protist found in humans and a large variety of other animals.[1–3] Numerous epidemiological surveys carried out in different countries report *Blastocystis* as the most common eukaryotic organism in human fecal samples,[4] but the prevalence of *Blastocystis* infection in humans is still unknown in many parts of the world. The role of *Blastocystis* as a cause of diarrhea or other symptoms is controversial because it can be found in both symptomatic and asymptomatic individuals.[2]

Morphological identification of *Blastocystis* is challenging[5] and traditional diagnostic methods differ significantly in their diagnostic sensitivity.[6] Polymerase chain reaction (PCR) amplification of *Blastocystis* DNA from cultures or feces is probably the most sensitive detection method and also enables molecular characterization.[7–10]

Blastocystis exhibits extensive genetic diversity and comprises at least 13 subtypes (STs), nine of which have been found in humans.[9,11–13] On a global scale, ST3 appears to be the most common subtype found in humans, followed in prevalence by ST1, ST2, and ST4.[2] ST5–ST9 have a more sporadic occurrence and may be of zoonotic origin[12,14]; human infection with ST6 and ST7 appears to be more common in certain countries, such as Japan.[15,16]

The background prevalence of *Blastocystis* in Brazilian indigenous populations has been determined by direct examination of fecal samples on only two previous occasions,[17,18] and nothing is known about the subtype distribution of *Blastocystis* in these cohorts. The only published study on *Blastocystis* subtypes in Latin America, a region inhabited by > 500 million people, was performed on only 12 isolates from Colombia.[19] The aim of this study was to genetically characterize *Blastocystis* from inhabitants of six indigenous Indian communities in the Brazilian Amazonia, and to compare the subtypes present with those found in other countries.

The study was carried out in the Tapirapé community, situated 30 km from the Confresa municipality, state of Mato Grosso, Brazil (Figure 1). The indigenous reserve is located in Legal Amazonia and inhabited by 542 members of the Tapirapé ethnic group. The members of this tribe have many free roaming animals including dogs, cats, chickens, and pigs. The principal source of protein for this population is derived from fishing and hunting animals such as the paca, agouti, anteater, tortoise, capuchin monkey, deer, armadillo, tapir, mallard, and curassow. Piped water is drawn from nearby rivers and is delivered to every household, but it is neither filtered nor chlorinated. There is a general lack of adequate sanitation; only one public latrine is available for the entire village; however, this latrine is not used by all the villagers.

A total of 382 fecal specimens (210 from females and 172 from males) were collected during January and February 2010 and examined by light microscopy (×400) of concentrates obtained by the techniques of Hoffmann and Ritchie.[20–23] The consistency (formed, soft, loose, and watery) of all fecal samples was noted on collection. Demographic data and clinical information were recorded for all members of the tribe. The research was approved by the Ethics Committee of the Institute of Biomedical Sciences of the University of São Paulo, and consent was obtained from all human adult participants and from parents or legal guardians of minors according to National Committee for Ethics in Research (CONEP-120/2008).

Part of each microscopy-positive stool samples was preserved in 70% ethanol for subsequent DNA extraction and molecular analysis. Samples were washed three times in phosphate-buffered saline before extraction using the QIAamp DNA Stool Mini Kit (Qiagen, Hilden, Germany) according to the recommendations of the manufacturer, except that DNAs were eluted in 100 μL AE buffer.

The PCR and sequencing were performed as previously described,[7] and sequences edited with Chromas version 2.33 (Technelysium Pty. Ltd., Queensland, Australia) were individually compared with *Blastocystis* SSU-rRNA gene sequences available in GenBank using the basic local alignment search tool (BLAST) algorithm. Subtypes were identified by determining a match or closest similarity to all known *Blastocystis* subtypes.[9] Mixed subtype infections were identified by the presence of double peaks in semi-conserved gene areas.[6]

Demographic, such as age and gender, clinical, such as stool consistency and abdominal pain, and parasitological data

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*Address correspondence to Antonio F. Malheiros, Department of Biology, Department of Nursing, Mato Grosso State University, Av. São João, s/n, Cáceres, Mato Grosso, Brazil 78200-000. E-mail: malheiros@unemat.br and afmalheiros@usp.br
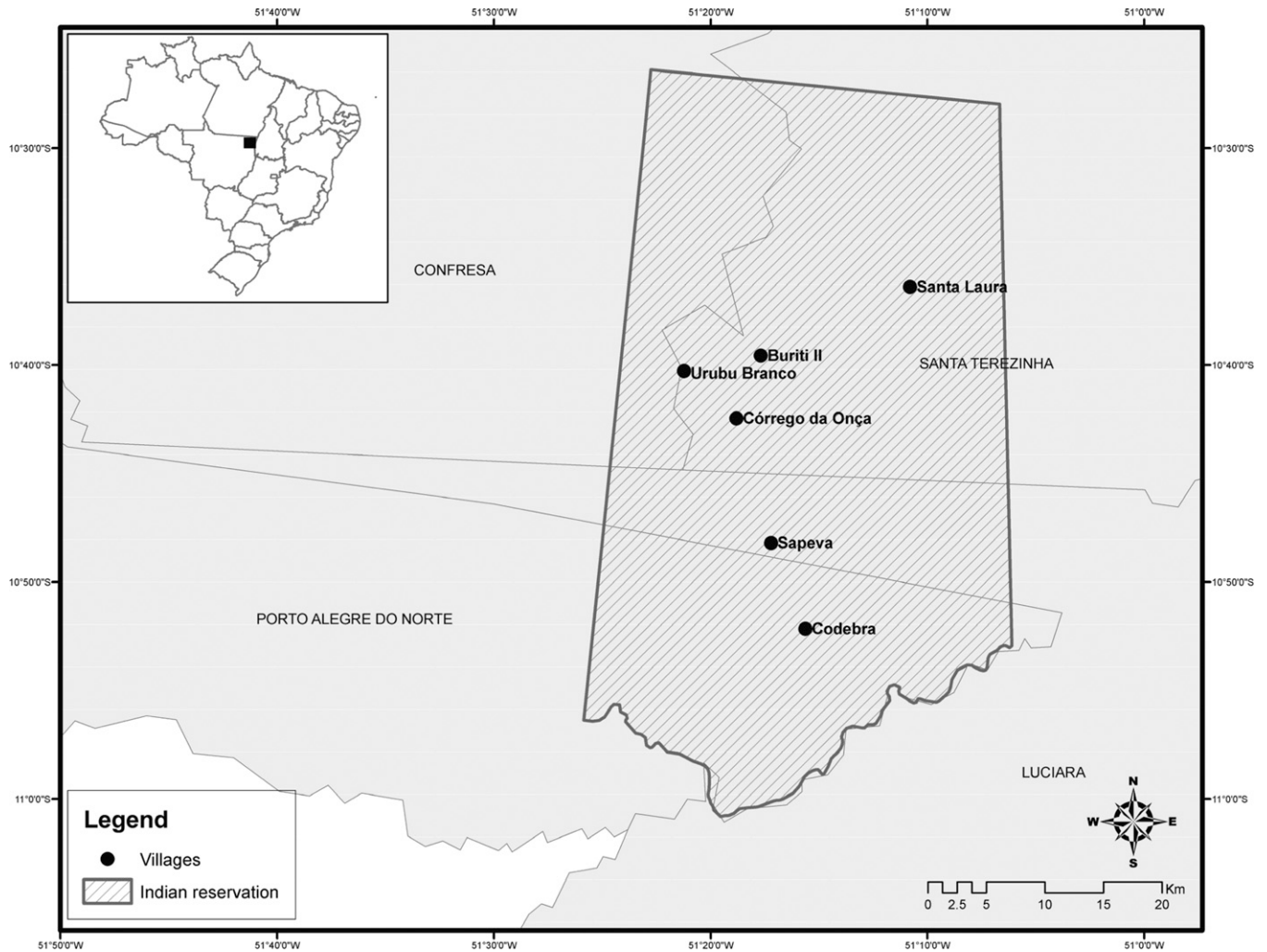
FIGURE 1.   Localization of the six indigenous villages, Confresa Mato Grosso, Brazilian Amazon, Brazil.

were analyzed statistically using the Pearson χ^2 test, in the cases with an expected count of < 20, and Fisher Exact test, when the expected count was > 20 (two-sided). We considered P values lower than 0.05 to be statistically significant.

Overall, 382 individuals from six indigenous villages were enrolled of whom 80 (21%) were positive for *Blastocystis* based on microscopy of fecal concentrates, 66 of which were confirmed by PCR. *Blastocystis* was more common in males than females ($P < 0.05$), and being < 15 years of age was associated with positivity for *Blastocystis* ($P < 0.03$).

Of 66 *Blastocystis* infections confirmed by PCR, sequences revealed single subtype infections in 59 cases and mixed

subtype infections in seven cases (Table 1). ST1 was the predominant subtype, colonizing 27 individuals (41%), followed by ST2 in 21 individuals (32%), and ST3 in 11 (17%). Mixed infections consisting of ST1 and ST2 were seen in five individuals, ST1 and ST3 in one individual, and ST2 and ST3 in another individual.

Among the 66 cases, 28 individuals had abdominal pain. Eleven of the latter had ST1, 10 had ST2, five had ST3, and two had a mixture of ST1 and ST2; there was no statistically significant association between *Blastocystis* STs and abdominal pain ($P > 0.05$). Likewise, no significant associations were found between infection with any of the observed *Blastocystis*

TABLE 1

Number of infections with *Blastocystis* confirmed by polymerase chain reaction (PCR) and *Blastocystis* subtype distribution among 382 indigenous people living in Tapirapé villages, Brazilian Amazon, Brazil

Gender and age group	Microscopy-positive				*Blastocystis* sp. subtype						
	PCR-positive	PCR-negative	Microscopy-negative	Total	ST1	ST2	ST3	ST1/ST2	ST1/ST3	ST2/ST3	Total
Females (< 15 y old)	24	3	99	126	12	8	1	2	0	1	24
Males (< 15 y old)	22	7	73	102	7	8	6	1	0	0	22
Females (> 15 y old)	7	2	74	83	3	1	3	0	0	0	7
Males (> 15 y old)	13	2	56	71	5	4	1	2	1	0	13
Total	66	14	302	382	27	21	11	5	1	1	66

subtypes and stool consistency, between gender and subtypes, or between age and subtypes (all $P > 0.05$).

An important step in understanding the geographical distribution of genetically distinct variants of *Blastocystis* was the adoption in 2007 of a standard nomenclature.[9] Since then the most common subtype has proven to be ST3, followed by ST1, ST2, and ST4.[4,12] Unlike almost all previously reported studies, the dominant subtype in this study was ST1 (41%), followed by ST2 (32%) and ST3 (17%). However, ST4, which has been recorded in studies from Europe and North America and some regions of Asia, was not detected in the Tapirapé Indian samples or, incidentally, in the small study of Colombian samples.[19] Assuming the subtype distribution identified in this study and the Colombian samples[19] is representative for Latin American *Blastocystis*, why is ST4 relatively prevalent throughout Europe and North America but absent in this and most other parts of the world? Further studies are needed to answer this and other questions involving the epidemiology of *Blastocystis* in Latin America, but the distribution of subtypes could be linked to the ethnic origin of the infected population, because there is little contact between indigenous groups and people in other communities.

Our subtyping results corroborate other studies in tropical developing countries. In Thai school children, 77.9% were infected with ST1 and 22.1% with ST2.[24] ST1 was found in the school's water supply. In water treatment plants in the Philippines *Blastocystis* was found in 23% of the influent water and 7% of the effluent water, showing that treatment was not eliminating *Blastocystis*.[25] Typing revealed ST1 and ST2 in both influent and effluent waters. As in these regions, one of the most likely sources of infection in our Brazilian study area is the water, because there is no water treatment in the Tapirapé community, and it raises the question as to whether certain subtypes are more resistant to water. More data from other ethnic groups in Brazil living in different biomes are needed to investigate whether the dominance of ST1 is a regional phenomenon or is linked to populations with a particular ethnic/ecological background.

In our study, 11% of the infections were subtype mixtures, which suggests either multiple sources of infection or one source containing multiple subtypes. This prevalence is similar to studies in China,[26] Turkey,[27,28] France,[4] Denmark,[2] and Egypt,[29] and mixed infections were also seen in the Colombian samples.[19] The true prevalence of mixed infections is difficult to ascertain as detection is likely to depend on the method used for subtyping.[10]

Understanding the prevalence and genetic structure of intestinal parasites, such as *Blastocystis*, in endemic areas may contribute to a better understanding of the risk factors for infection in different ecological situations. Because *Blastocystis* is apparently transmitted fecal-orally and is very common, this parasite appears to be an appropriate indicator for the overall level of intestinal parasitism in different populations. Therefore, data on *Blastocystis* could be helpful in elaborating and evaluating intervention methods to reduce the burden of intestinal parasites.

This study of *Blastocystis* subtypes is only the second in Latin America but represents the first in Brazil and the first involving an indigenous community of Latin America.

Authors' addresses: Antonio F. Malheiros, Department of Biology, Department of Nursing, Mato Grosso State University, Cáceres, Mato Grosso, Brazil; and Department of Parasitology, São Paulo University, São Paulo, Brazil, E-mails: malheiros@unemat.br and afmalheiros@usp.br. C. Rune Stensvold, Department of Microbiological Diagnostics, Statens Serum Institut, Copenhagen S, Denmark, E-mail: run@ssi.dk. C. Graham Clark, Faculty of Infectious and Tropical Diseases, London School of Hygiene and Tropical Medicine, London, UK, E-mail: graham.clark@lshtm.ac.uk. Guilherme B. Braga, Department Medicine Veterinary Preventive and Animal Health, São Paulo University, São Paulo, Brazil, E-mail: guibraga@vps.fmvz.usp.br. Jeffrey J. Shaw, Department of Parasitology, São Paulo University, São Paulo, Brazil, E-mail: jayusp@hotmail.com.

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