

## Intestinal symptoms and *Blastocystis* load in schoolchildren of Paranaguá Bay, Paraná, Brazil

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### ABSTRACT

The symptomatology of *Blastocystis* cannot be attributed to any particular subtype, although can be related to a high *Blastocystis* infection load. One stool sample from each of 217 schoolchildren of Vale de Sol Paranaguá Bay (Paraná, Brazil) was collected. Three milliliters of each fixed stool sample were processed applying the formalin-ether concentration technique. After obtaining the overall prevalence of intestinal parasites, quantification was carried out in *Blastocystis* positive samples. A total of 75/217 (34.6%) children suffered from intestinal symptoms (abdominal pain and/or persistent diarrhea), of whom 41.3% (31/75) presented moderate/heavy *Blastocystis* load with a statistically significant risk to present intestinal symptoms (OR 0.039 [0.006-0.15];  $p < 0.001$ ). Moreover, those symptomatic schoolchildren monoparasitized only by *Blastocystis* (10/75, 13.3%), and those polyparasitized by *Blastocystis* with other non-pathogenic species (15/75, 20%) with moderate/heavy loads, also entail a statistically significant risk of intestinal symptomatology, both in monoparasitism (12%, OR 0.10 [0.004-0.63];  $p = 0.021$ ) and in polyparasitism with a non-pathogenic parasite (18.6%, OR 0.059 [0.002-0.35];  $p = 0.001$ ). For the first time in Brazil, using data from schoolchildren of Paranaguá Bay, we demonstrated that moderate/heavy loads of *Blastocystis* could be related to intestinal symptoms.

**KEYWORDS:** *Blastocystis* spp. Intestinal symptoms. Load. Schoolchildren intestinal parasitism. Parasite load. Parasite burden.

Intestinal parasite infection decreases quality of life and increases susceptibility to other infections<sup>1</sup>. *Blastocystis* infects between 1-2 billion people on a global scale<sup>2</sup>. *Blastocystis* prevalence in humans varies from 30-76% in developing countries<sup>3</sup>. The role of *Blastocystis* in human health and disease remains unclear. Although commonly represented by asymptomatic infections, it does not mean that *Blastocystis* is not pathogenic, which is a similar situation found in *Giardia intestinalis* infections<sup>4</sup>.

In patients with negative results for other potentially pathogenic microorganisms, the symptomatology of *Blastocystis* could not be attributed to any particular subtype<sup>5</sup>. However, it can be related to hosts' related factors such as host genetics, immune status, host response/stress, concomitant infections, dysbiosis of gut microbiota, etc. A high *Blastocystis* load could also be considered the cause of the patient's intestinal symptomatology<sup>2,6</sup>. Herein, for the first time in Brazil, the relationship between intestinal symptoms and the *Blastocystis* load in schoolchildren of Paranaguá Bay (Paraná, Brazil) is examined.

Each of the 217 recruited schoolchildren, aged 1-15 years, from Vale de Sol, a coastal city of Paranaguá (Brazil), provided a stool sample, which was collected

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in a plastic container. Stool samples were fixed in 10% formalin using a 1/3 proportion. Informed consent was obtained from each participant's legal guardian before the recruitment following the instructions of *Comité Ético de la Universitat València* (H1431958278890).

Three milliliters of each fixed stool sample were processed using the formalin-ether concentration technique<sup>7</sup>. The entire sediment was examined to establish the intestinal parasite prevalence. Samples were considered negative when no parasite structures were observed in the entire sediment. In addition, in positive *Blastocystis* samples, quantification was carried out in the entire sediment based on the number of parasite structures observed at a magnification of 400x following Speich *et al.*<sup>8</sup> with some modifications. Briefly, a total of five fields per slide were observed and an absolute number was assigned for categorizing *Blastocystis* loads as follows: a) one to four *Blastocystis* structures per slide were observed (5 fields); b) one parasite structure per field was observed; c) > one parasite structure per field was observed. The mean number after all observations was calculated, considering: a) low infection load; b) moderate infection load; and c) heavy infection load. Percentages of each infection load were calculated.

Statistical analysis was performed using the Open Source Epidemiologic Statistics for Public Health, version 3.03a. The odds ratios (OR) and 95% confidence interval of those having symptoms and being infected with low loads of *Blastocystis* were compared to the ones from patients with moderate and/or heavy loads, were calculated using a logistic regression. The level of statistical significance was  $p < 0.05$ .

In the entire study, an overall prevalence of intestinal infection of 43.8% (95/217) was found. No statistical differences were noticed regarding sex, but the group composed of 5-9 year old children was the most infected  $p < 0.001$ . A total of eight protozoan and three helminth species were detected (Table 1), with *Blastocystis* being the most prevalent (31.8%). The low prevalence of soil transmitted helminth species was noteworthy.

From the total of 217 schoolchildren, 34.6% (75/217) suffered from intestinal symptoms (abdominal pain and/or persistent diarrhea). Among them, 44% (33/75) were parasitized by *Blastocystis*, and only 2.7% (2/75) presented a low parasite load (Table 2), which suggests that the parasitism caused by *Blastocystis* by means of a moderate to heavy load (41.3%, 31/75) represent a statistically significant risk of the onset of symptoms (OR 0.039 [0.006-0.15];  $p < 0.001$ ).

Due to the detection of other pathogenic intestinal parasites (Table 1), in order to rule out their possible involvement with intestinal symptoms, symptomatic

**Table 1** - Prevalence of intestinal parasite species in the 217 schoolchildren studied in Paranaguá Bay (Paraná, Brazil)

Parasites	N=217 % (95% C.I.)*
PROTOZOA	43,3(36,8-50)
<i>Blastocystis</i> spp.	31,8 (25,9-38,2)
<i>Endolimax nana</i>	13,4 (9,3-18,4)
<i>Giardia intestinalis</i>	12,9 (8,9-17,9)
<i>Entamoeba hartmanni</i>	5,1 (2,7-8,6)
<i>Entamoeba</i> complex#	2,8 (1,1-5,7)
<i>Entamoeba coli</i>	2,3 (0,8-5)
<i>Iodamoeba butschlii</i>	2,3 (0,8-5)
<i>Chilomastix mesnili</i>	0,9 (0,2-3)
HELMINTHS	7,8 (4,8-12)
<i>Ascaris lumbricoides</i>	6,5 (3,7-10,3)
<i>Trichuris trichiura</i>	0,9 (0,2-3)
Ancylostomatidae	0,5 (0-2,3)
TOTAL	43,8 (37,3-50,4)

\* %= percentage; 95% C.I.= 95% confidence interval. #*Entamoeba* complex= *Entamoeba histolytica*/E. *dispar*/ E. *moshkovskii*

**Table 2** - *Blastocystis* loads in symptomatic schoolchildren studied in Paranaguá Bay (Paraná, Brazil)

	Intestinal Symptoms N=75	
	Low (%)	Moderate-Heavy (%)
<i>Blastocystis</i>	2(2.7)	31(41.3)
Monoparasitism	1(1.3)	9(12)
Polyparasitism (non-pathogen)	1(1.3)	14(18.6)

schoolchildren monoparasitized only by *Blastocystis* (13.3%, 10/75), and those polyparasitized by *Blastocystis* and other non-pathogenic species (20%, 15/75) were selected.

A moderate to heavy *Blastocystis* load (Table 2) entails a statistically significant risk of intestinal symptomatology, both in monoparasitism (12%, OR 0.10 [0.004-0.63];  $p = 0.021$ ), as well as in polyparasitism with a non-pathogenic parasite (18.6%, OR 0.059 [0.002-0.35];  $p = 0.001$ ).

Classifying an organism as pathogenic is not always straightforward<sup>2</sup>. The pathogenic potential of *Blastocystis* is associated with non-specific gastrointestinal symptoms, such as diarrhea, nausea, vomiting, abdominal pain and irritable bowel syndrome<sup>9,10</sup>, having direct and indirect effects on the human gut. The direct effects, triggered by the adherence to the gut epithelium and mediated by cysteine proteases and secretion of the diarrheagenic toxins

as virulence factors<sup>11</sup>, include apoptosis, degradation of tight-junction proteins, increased intestinal permeability, induction of pro-inflammatory cytokines and down-regulation of iNOS. The indirect pathogenic effects are immunomodulatory properties that facilitate other enteric pathogens and produce alterations in the microbiota<sup>9</sup>.

Not all humans are susceptible to infections caused by *Blastocystis*, and this parasite can be detected in healthy asymptomatic hosts. However, data from schoolchildren of *Paranaguá Bay* demonstrate that moderate/heavy *Blastocystis* loads could be related to intestinal symptoms. *Blastocystis* does not only colonize, but also multiply in the intestine. There seems to be a need for certain intestinal conditions for this replication to take place without braking and/or control. Thus, in a favorable environment, the *Blastocystis* load can increase and reach levels that may lead to harmful effects, with appearance of symptoms.

This study was affected by three limitations: first, multiple stool samples should be examined per child; second, stool samples were not tested for bacteria, viruses or fungus and, therefore, such etiologies cannot be ruled out; and third, more sensitive molecular approaches should be added to the research so as to improve diagnostic accuracy and allow determination of the subtype of *Blastocystis* involved. In this sense, this study represents the first step towards a more comprehensive study to clarify the clinical significance of *Blastocystis* in *Paranaguá, Brazil*.

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## AUTHORS' CONTRIBUTIONS

CMA created and designed the study protocol; RS, DK and CYO carried out the sampling collection; RS and CMA carried out the data analysis and interpretation; CMA and RS drafted the manuscript; RT and JGE critically revised the manuscript. All authors read and approved the final manuscript.

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