

OCCURRENCE OF *Blastocystis* spp. IN UBERABA, MINAS GERAIS, BRAZIL

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SUMMARY

Intestinal parasites are a problem for public health all over the world. The infection with *Blastocystis*, a protozoan of controversial pathogenicity, is one of the most common among them all. In this study, the occurrence of intestinal parasites, with emphasis on *Blastocystis*, in patients at the Universidade Federal do Triângulo Mineiro was investigated in Uberaba (MG) through microscopy of direct smears and fecal concentrates using Ritchie's method. Feces of 1,323 patients were examined from April 2011 to May 2012. In 28.7% of them at least one intestinal parasite was identified, and the most frequent organisms were *Blastocystis* spp. (17.8%) and *Giardia intestinalis* (7.4%). The occurrence of parasitism was higher in children aged 6 -10 years old, and the infection with *Blastocystis* spp. was higher above the age of six ($p < 0.001$). The exclusive presence of *G. intestinalis* and of *Blastocystis* spp. was observed in 5.4% and 12.2% of the patients, respectively. Regarding patients with diarrheic feces, 8% revealed unique parasitism of *Blastocystis* spp. Other intestinal parasites observed in children were *Ascaris lumbricoides* (0.3%) and *Entamoeba histolytica/dispar/moshkovskii* (1.4%). The Ritchie's method was more sensitive (92.8%) when compared to direct microscopy (89.8%), with high agreement between them (97.7%, kappa = 0.92). In conclusion, the occurrence of *Blastocystis* spp. in Uberaba is high and the presence of diarrheic feces with exclusive presence of the parasite of *Blastocystis* spp. was observed.

KEYWORDS: *Blastocystis* spp.; Intestinal parasites; Stools; Uberaba (MG).

INTRODUCTION

Intestinal infections by protozoa are frequent all over the world, being most prominent in developing countries, since the majority of the infections are generally acquired by the ingestion of foods or water contaminated by human and/or animal feces, generally caused by the lack of basic sanitation and conditions of hygiene^{4,6,12}. In this context the infection with *Blastocystis* spp., an anaerobic intestinal protozoan is one of the most prevalent^{6,9,12}, occurring in approximately 1.5% to 10% of the population in developed countries and 30% to 60% in developing countries¹⁹. However, these data are underestimated, since laboratory technicians are generally not sufficiently trained to detect it or simply do not report their findings. Moreover, routine techniques for stool analysis such as the water spontaneous sedimentation (HOFFMAN-PONS-JANER)⁸ which leads to the breakage of the vacuolar stage of the parasite, is one of the mostly detected stages in the stool examination, leading to the false negative results¹⁴.

Although the infection with *Blastocystis* spp. is one of the most prevalent amongst the intestinal parasites, its impact on public health is not known, since its pathogenicity has been noted as controversial by several authors^{6,9,10,21}. However, in spite of the controversial issue that *Blastocystis* pathogenesis represents, there are no explanations for

patients who present symptoms like diarrhea, fever, vomit, abdominal pain, and nausea in the absence of any other parasite but *Blastocystis*^{6,9,10}. In addition to this, studies have shown that stress conditions can lead to increased susceptibility and pathogenicity of *Blastocystis*, it is also an opportunistic parasite in immunocompromised patients^{5,16}. There is a huge lack of information regarding the pathogenesis, the diagnosis and the epidemiology of this protozoan²⁰. In this study, it is shown that the occurrence of *Blastocystis* in Uberaba is high, followed by the infection of *Giardia intestinalis*, and that direct methods, especially Ritchie's, are suitable for the diagnosis of the parasite. Moreover, the presence of diarrheal stools with unique parasitism by *Blastocystis* spp. was observed.

MATERIAL AND METHODS

The present paper is a cross-sectional study with a non-probability sample of patients who were treated at the Universidade Federal do Triângulo Mineiro Hospital, between April 2011 and May 2012. All patients referred to carry out a stool test suffered from acute or chronic diarrhea or complaints of constant abdominal pain and/or weakness was included. Age, presence of underlying diseases, HIV/AIDS or gastrointestinal symptoms were not considered as exclusion criteria. The specimens were examined by the microscopy direct of smears and fecal concentrates by Ritchie's method¹⁸. Briefly, the examination by direct

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microscopy was conducted with an amount of stool placed in a drop of Lugol solution in a slide/coverlip and observed by optical microscopy with a 400× objective lense. Ritchie's method was performed by keeping the feces in 3.7% formaldehyde, adding ethyl ether and then centrifuging the mixture at 1,200×g/5min. The sediment was observed with the 400× objective. The statistical software Statistica 10.0 (Statsoft, Tulsa, OK, 2011) was used to perform the statistical analysis.

The association between risk factors and presence of *Blastocystis* spp. was verified by the chi-squared classic or, whenever necessary by the chi-square test with Yates correction and Fisher's exact test. Moreover, the association force was measured by the calculation of the Odds ratio with confidence intervals of 95%. The agreement between the microscopy direct and the Ritchie's method was evaluated by means of Kappa coefficient. Results which demonstrate a level of significance lower than 0.05 ($p < 0.05$) were considered significant. This study was approved by the ethics committee in research at the UFTM under number 1804.

RESULTS

Stool specimens from 1,323 patients were examined, with 44.1% male and 55.9% female. From the analyzed samples, 28.7% presented an intestinal pathogenic parasite or not, being *Blastocystis* spp. (17.8%) and *G. intestinalis* (7.4%) the most observed (Table 1). The known pathogenic parasites analysis showed a positivity of 10.4% (138/1323), with the highest occurrence detected by *G. intestinalis*. The presence of non-pathogenic parasites occurred in 7.3% of the samples (Table 1).

Table 1

Occurrence of intestinal parasites in stool samples of patients from the Clinical Hospital at the Universidade Federal do Triângulo Mineiro, Uberaba, MG

Positivity of the diagnostic tests	n	%
Intestinal parasites	379	28.65
<i>Blastocystis</i> sp.	235	17.76
<i>Giardia intestinalis</i>	98	7.41
<i>Entamoeba coli</i>	59	4.46
<i>Endolimax nana</i>	36	2.72
<i>Entamoeba histolytica/dispar/moshkovskii</i>	26	1.97
<i>Taenia</i> sp.	6	0.45
<i>Ascaris lumbricoides</i>	2	0.15
<i>Isospora belli</i>	1	0.08
<i>Strongyloides stercoralis</i>	2	0.15
<i>Chilomastix mesnili</i>	1	0.08
Hookworms	1	0.08
<i>Enterobius vermicularis</i>	1	0.08
<i>Hymenolepis nana</i>	1	0.08
Exclusive presence of <i>Blastocystis</i>	161	12.17
Exclusive presence of <i>Giardia</i>	71	5.37

From the evaluated samples, 33.47% (442) were from children aged 0-5 years and 11.04% (146) were from children aged between 6-10 years

(Table 2), and in 27.8% and 44.1 % of them, respectively, the presence of parasitism by at least one organism was observed, whether pathogenic or not. *G. intestinalis* infection occurred in 86.7% of cases (85/98) in patients aged between 0-10 years (Table 2). *Ascaris lumbricoides* (3, 0.3%) and *Entamoeba histolytica/dispar/moshkovskii* (13, 0.7%) were also observed in children.

Table 2

Parasitism by *Giardia intestinalis* and *Blastocystis* spp. according to the age group of patients treated at the Clinical Hospital, Universidade Federal do Triângulo Mineiro, Uberaba (MG)

Age (years)	No. of patients	<i>Giardia intes- tinalis</i> (n/%)	<i>Blastocystis</i> spp. (n/%)
0-5	442	70 (15.8)	51 (11.5)
6-10	146	15 (10.2)	44 (30.1)
11-20	103	6 (5.8)	25 (24.1)
21-50	407	5 (1.2)	70 (17.2)
> 50	225	2 (0.8)	45 (20.0)
Total	1,323	98	235

Overall, parasitism was higher in male patients (32.6%) than in females (25.5%) (95% CI = 1.1 to 1.79, $p < 0.005$) and higher in the range age of 6-10 years (95% IC = 1.48-3.60; $p < 0.001$). Interestingly, parasitism of *Blastocystis* spp. was significantly higher in patients presenting over six years of age ($p < 0.001$, Table 2). The analysis of parasitism by *G. intestinalis* or *Blastocystis* spp. by age in relation to gender showed no significant difference.

The unique presence of *Blastocystis* in feces occurred in 161/1,323 samples (12.1%). The analysis of the consistency of feces at the moment of the examination showed that, among the solid samples, softened and liquid, 12.0% (107/892), 15.8% (34/215) and 8.0% (6/75), respectively, were positive exclusively for *Blastocystis* spp., showing no statistical difference ($p = 0.152$). The information of the consistency of 14 stool samples with exclusively positivites for *Blastocystis* sp was not taken.

The analysis by Ritchie's method was more sensitive for the diagnosis of *Blastocystis* (92.8%) than that by direct microscopy (89.8%), with a ratio of 97.7% agreement (Kappa = 0.92).

DISCUSSION AND CONCLUSIONS

In this study the occurrence of intestinal infections by protozoa and/ or helminths in Uberaba (MG) was of 28.7%. However, only 10.4% of the stool samples presented some pathogenic parasite, in which 7.4% corresponded to the infection by *Giardia* that occurred mainly in children between 0-10 years of age. These data are in accordance with other studies carried out in several regions of Brazil^{11,14,17}. Regarding age and gender, the presence of intestinal parasites was higher in male children aged between the ages of six and 10 years. In relation to parasitism by *Blastocystis* (17.8%) it was higher in patients over six years of age and had no direct relation to gender. *G. intestinalis* infection presented no correlation with gender either. Regarding age, the data agrees with other

studies¹⁹ and differs from some authors which showed that *Blastocystis* infection was higher in children than in adults^{9,14,15}. In relation to gender, there is no agreement which indicates that gender shows the highest occurrence of *Blastocystis* spp.^{14,19}.

The occurrence of *Blastocystis* spp. infection was higher when compared with all other parasites, an observation that corroborates other studies^{4,6,9,12,16}. The exclusive occurrence of *Blastocystis* in 8% of diarrheal stools suggests that it may have a pathogenic character, as some authors agree^{19,21}. Some authors observed the presence of *Blastocystis* in stool samples from HIV-infected, homosexuals, travelers, day care children, animal handlers, and mentally handicapped individuals^{2,16}. Besides, in immunocompromised patients, the parasite must be considered pathogenic and patients should be treated accordingly for *Blastocystis* if no other pathogens are detected^{2,16}. According to them, the pathogenicity of *Blastocystis* is possibly associated with low host immunity, modified intestinal microbiota, and concomitant presence of irritable bowel syndrome and the virulence of the parasite strain. According to CHANDRAMATHI *et al.* (2014), pathogenicity may also be host stress dependent, which would lead to a suppression of both immune responses and to the oxidant-antioxidant regulatory system. However, more studies are needed to exclude other possible causes of diarrhea, such as rotavirus infection or metabolic disorders.

In the literature, several authors suggest that the search of this protozoan via the direct method^{6,9,12}, trichrome staining and cultivation^{1,10,21}, states that the concentration methods should not be employed for observation of *B. hominis* as they destroy cell morphology. In this study, both methods, direct and Ritchie's showed higher sensitivity to 89.8%, with a high agreement percentage (97.7%, kappa = 0.92), being appropriate to the diagnosis of *Blastocystis*. Although the culture is efficient, its cost is higher than the direct method, which has good sensitivity for detecting *Blastocystis*, since the vacuolar shapes of this parasite are usually released in large amounts in feces. In the authors' experience and unlike that of other authors¹, staining of fecal smears for direct identification of *Blastocystis* from feces is not easy to analyze, as the microscopist needs experience to obtain a good result. Thus, the Ritchie's method is a good concentration method, as it is fast and effective, as demonstrated by other authors¹⁴. The HPJ method is also effective if used to dilute 3.7% of formaldehyde stools, since the water breaks the vacuolar, granular and amoeboid shapes of the parasite.

Infection with non-pathogenic parasites (*Endolimax nana*, *Entamoeba coli*, *Chilomastix mesnilli*) occurred in 7.3% of the samples (Table 1). Human infection by non-pathogenic protozoa has been reported by several authors in Brazil^{7,13,14,22} and it highlights the need of their own reports in laboratory reports, therefore it should be considered as an indicator of fecal contamination of food and water consumed by the population.

In conclusion, the occurrence of *Blastocystis* spp. in Uberaba (MG) was high, this scenario indicates the importance of investigating the main route of parasite transmission and their association with the clinical symptoms manifestation. Furthermore, this study showed that the direct and Ritchie's method were effective for the diagnosis of *Blastocystis* spp. and that there is a need for the description of commensal protozoa in laboratory reports and for the training of laboratory technicians to improve in order to detect it.

RESUMO

Ocorrência de *Blastocystis* spp. em Uberaba, Minas Gerais, Brasil

Parasitas intestinais são um problema de saúde pública no mundo e a infecção por *Blastocystis*, protozoário de patogenicidade controversa, é uma das mais frequentes. Nesse estudo foi investigada a ocorrência de parasitos intestinais em pacientes atendidos na Universidade Federal do Triângulo Mineiro, em Uberaba (MG), com ênfase em *Blastocystis*, pelos métodos parasitológicos direto e de Ritchie. Foram examinadas fezes de 1.323 pacientes de abril/2011 a maio/2012. Em 28,7% deles foi identificado um parasito intestinal, sendo *Blastocystis* spp. (17,8%) e *Giardia intestinalis* (7,4%) os mais frequentes. A ocorrência de parasitismo foi maior em crianças de 6-10 anos e a infecção por *Blastocystis* spp. foi maior acima de seis anos ($p < 0,001$). Presença exclusiva de *G. intestinalis* e de *Blastocystis* spp. foi observada em 5,4% e 12,2% dos pacientes, respectivamente, sendo que dos pacientes com fezes diarreicas, 8% apresentavam parasitismo exclusivo por *Blastocystis* spp. Outros parasitos intestinais observados em crianças foram *Ascaris lumbricoides* (0,3%) e *Entamoeba histolytica/dispar/moshkovskii* (1,4%). O método de Ritchie foi mais sensível (92,8%) que o direto (89,8%), com alta concordância entre eles (97,7%, kappa = 0,92). Em conclusão, a ocorrência de *Blastocystis* spp. em Uberaba é elevada e foi observada a presença de fezes diarreicas com parasitismo exclusivo por *Blastocystis* spp.

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AUTHOR'S CONTRIBUTIONS

MCS and ALP were responsible for the experimental design of the study; ENC and RAC were responsible for the execution techniques and parasitological examination of stools along with MCS and MBOs. GANN was responsible for the statistical analysis and DC for the attending and for the referral of the patients. All authors reviewed and contributed to the writing of this manuscript. MCS is responsible for the manuscript.

CONFLICT OF INTERESTS

No conflict of interests was declared.

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