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Frequency of Blastocystosis and Its Association with Clinical Symptoms in 2 Years of Surveillance at "Pedro Kourí" Institute

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Abstract

Blastocystis sp. is one of the most common intestinal parasites diagnosed worldwide, but its pathogenic role is still regarded by some authors as controversial. The frequency of *Blastocystis sp.* was studied during a two year period of time and its association with some clinical symptoms. An observational descriptive study was carried out from January 2012 to December 2013. A total of 3140 stool samples were investigated for the presence of parasites by various parasitological techniques. Among 3140 analyzed stool samples the prevalence of *Blastocystis* infection observed was 3.54%, the fourth most prevalent. A total of 111 stool samples were positive for *Blastocystis sp.*, and 71 samples showed co-infection with one or more other intestinal parasites. The group of 5 to 14 years age group showed a higher percentage of infections in the group of symptomatic persons than in asymptomatic ones and it was statistically significant, while in persons with ages more than 40 years the frequency of infection with this parasite was higher in asymptomatic persons. *Blastocystis* infection is one of the most frequent parasitic infections diagnosed in our setting. The relationship with symptomatology found in individuals infected with *Blastocystis* was significant and this association was higher in younger children.

Introduction

The intestinal parasitic infections still remain as one of the most prevalent infections worldwide, especially in developing countries, where sanitary and socioeconomic conditions may be less developed. In these countries despite the fact that all age groups are susceptible to these infections, children and the elderly are the more vulnerable groups [1].

Blastocystis sp. belongs to the phylum Stramenopila, and is an unusual anaerobic, single-celled microorganism, considered as a remarkably successful intestinal protozoan of a vast array of host species including humans [2]. Interestingly, this is the only stramenopile living in the lower digestive tract of humans, and it also lives in other mammals, birds, reptiles, amphibians, and insects [2].

Nevertheless, *Blastocystis sp.* is an intestinal protozoan of controversial pathogenicity and has been raised during the last years as one of the most important intestinal protozoan identified worldwide in routine clinical parasitology [2]. Human infection is associated with poor personal hygiene, lack of sanitation, exposure to animals, and consumption of contaminated food or water, although prevalence can exceed 10% in developed countries [3].

Clinical manifestations described among the symptomatic individuals are mainly nonspecific symptoms and signs such as diarrhoea, abdominal pain, nausea, fatigue, vomiting, anorexia, and flatulence [4]. Cutaneous symptoms have also been described during blastocystosis [5], and several studies suggest an association with chronic colopathy such us irritable bowel syndrome [6].

In humans *Blastocystis* consists of at least 9 genetic subtypes, and some of these, may be associated with differences in pathogenicity and symptomatology [7,8]. Since it was first described more than a century ago, the question as to whether the protistan parasite *Blastocystis* causes disease or is a commensal of the human gut still remains unresolved. Several studies have explored the pathological basis of variability in clinical presentation of *Blastocystis* infection, including but not limited to subtype-dependent variability in parasite pathogenicity and drug resistance, variability in host response [9,10].

In Cuba, various national health programs have been instituted since 1959, and they have contributed to improving the quality of life of people and hygienic-sanitary conditions in almost all the country [11]. In the last National survey of intestinal parasitic infections (IPI) carried out in 2009, Enterobius vermicularis, Ascaris lumbricoides and Trichuris trichiura were the most prevalent infections among helminths, while Giardia intestinalis, Entamoeba histolytica/dispar and *Blastocystis sp.*, were the most frequently identified pathogenic protozoa [12].

Given the lack of epidemiological information of *Blastocystis* infection in Cuba, this study was aimed to determine the frequency of this protozoal infection and its correlation with some clinical symptoms among individuals attending the National Laboratory of parasitic infections at "Pedro Kouri" Institute.

Methods

Study population

An observational descriptive study was carried out between January 2012 and December 2013 in La Habana, capital of Cuba. A total of 3140 stool samples were collected from persons remitted at the National Reference Laboratory of Intestinal Parasitic Infections at "Pedro Kouri" Institute. These samples divided from patients admitting with gastrointestinal complaints (n=847) including acute diarrhoea, abdominal pain, vomiting, and flatulence; and. from population (n=2293) attending the outpatient department within the

similar time period on an outpatient basis as a part of a routine medical examination, without any accompanying gastrointestinal symptoms. In particular, each person were asked about demographic information and history of gastrointestinal symptoms were collected with a standardized questionnaire (face-to-face interviews). These patients had intact immunity.

Those who tested positive for *Blastocystis* infection were divided into two groups; one was composed of individuals who had digestive symptoms and the other one with individuals who did not. Individuals were also divided in group of ages (pre scholar, scholar, young adults, and older adults) in order to correlate them with symptomatology. For the analysis of association with clinical symptoms all co-infections with intestinal parasites of medical importance were not considered.

The design of this work has been approved by the ethical committee of our Institution, and the patient's written consent was obtained for every stool sample received in our Parasitology laboratory.

Stool examinations

Only one specimen per patient was examined for the presence of intestinal parasites. Every faecal sample was prepared with native–Lugol, formol ethyl acetate concentration method, and was examined under light microscope at X40, X10 or X100 magnification, respectively. Sediments were then examined as a wet mount in saline and iodine for detection of protozoa, eggs and larvae of intestinal helminthes. All samples were evaluated by experienced parasitology specialists and the results were recorded. A sample was accepted as positive for *Blastocystis sp.* if five more than one parasites were identified in every microscopic field at X40 magnification. In addition, permanent stained smears were also carried out by the modified Ziehl-Neelsen stain for intestinal coccidian parasites in cases of diarrheal samples [13].

No further information was available about potential viral or bacterial infections.

Statistical analysis

Statistical analysis was performed using the Statistical Package Epidat 3.1 and EpiInfo 6.02. Pearson's Chi Square tests were used to examine the associations of *Blastocystis* prevalence with clinical symptoms. Odds ratios (OR) and 95% confidence intervals (95% CI) were computed, as well. All $P \leq 0.05$ were considered statistically significant.

Results

Frequency of *Blastocystis sp.* infections in the study

A total of 3140 stool samples were processed during that period of time. From them, 1681 (53.54%) correspond to female individuals (median age, 37, range 0-82) and 1459 from males (46.46%) (median age 34, range 1-76). Overall, *Blastocystis sp.* was found in 111 cases, divided into 50 males (45.05%) and 61 females (54.95%). Forty samples were only positive for *Blastocystis* and 71 showed co-infection with one or more other parasites. Endolimax nana was the most common protozoan parasite found in conjunction with *Blastocystis* (32), followed by Giardia intestinalis and the Entamoeba histolytica/ dispar complex, with 8 cases, respectively.

Among intestinal parasites of medical importance, *Blastocystis sp.* is the fourth most prevalent organism with 3.54% (results are summarized in Table 1). Giardia intestinalis, Trichuris trichiura and Ascaris lumbricoides, were the intestinal parasites more prevalent in this study.

Species	No.	%	IC 95%
Giardia intestinalis	246	7.82	6.87-8.78
Trichuris trichiura	163	5.2	4.39-5.98
Ascaris lumbricoides	133	4.23	3.51-4.95
Blastocystis sp.	111	3.54	2.87-4.20
Entamoeba histolytica/ dispar	105	3.33	2.90-3.98
Enterobius vermicularis	40	1.27	0.87-1.68
Cyclospora cayetanensis	17	0.54	0.27-0.81
Taenia sp.	8	0.25	0.06-0.45
Inermicapsifer madagascariensis	8	0.25	0.06-0.45
Strongyloides stercoralis	7	0.2	0.04-0.40
Hymenolepis nana	4	0.13	0.04-0.33
Cryptosporidium spp.	2	0.06	0.008-0.23
Fasciola hepatica	1	0.03	0.001-0.18
Total	845	26.9	21.88-24.87

Table 1: Frequency of intestinal parasites of medical importance at the National Reference Laboratory of Intestinal Parasitic Infections in the period 2012-2013.

Consideration of *Blastocystis sp.*, frequency respect to gender revealed a 3.6% prevalence in females and a 3.4% in men (P=0.44). between the environment where the individuals live (urban or rural) with *Blastocystis* infection (P=0.06). Most gastrointestinal symptoms reported by symptomatic patients were nonspecific and included diarrhoea, abdominal pain, flatulence, nausea, and vomiting, as is exposed in Table 2.

From a total of 111 persons infected with *Blastocystis sp.*, 64 were symptomatic and 47 asymptomatic (Table 3). In patients infected with *Blastocystis sp.* who had gastrointestinal complaints, gender distribution was homogenous (n=64; 33 (52%) females and 31 (48%) males; P=0.09), as well as in asymptomatic individuals (n=47; 28 (60%) females and 19 (40%) males) (P=0.68).

	Blastocystis sp.		Total
	N	%	N
Demographic and clinical characteristics			
Gender			
Male %	50	3.4	1451
Female%	61	3.6	1689
Residing area			
Urban %	73	4.0	1821

Rural %	38	2.9	1319
Total symptomatic patients			
Diarrhoea	41	6.4	643
Abdominal pain	52	7.2	721
Nausea	34	6.2	545
Flatulence	21	5.5	379
Vomiting	13	2.9	415

Table 2: Clinical and demographic characteristics of *Blastocystis sp.* infections in the population studied.

Group of ages (years)	Total (%)	Infected with *Blastocystis*		P Value	Infected only with *Blastocystis*		P Value
		Symptomatic group* No. (%)	Asymptomatic group No. (%)	Symptomatic group* No. (%)	Asymptomatic group No. (%)		
<5	478 (15.2)	9 (1.88)	4 (0.84)	0,16	5 (1.05)	2 (0.42)	0.45
5-14	624 (19.9)	39 (6.25)	15 (2.40)	0,0008**	13 (2.08)	4 (0.64)	0.02**
15-40	1137 (36.2)	11 (0.97)	10 (0.88)	0,83	7 (0.62)	4 (0.35)	0.36
>40	901 (28.7)	5 (0.55)	18 (2.0)	0,006**	4 (0.44)	1 (0.11)	0.37
Total	3140	64 (2.04)	47 (1.5)	0,10	29 (0.92)	11 (0.35)	0.004**
*Diarrhoea or flatulence or abdominal pain or nausea ** Significant value P< 0.05							

Table 3: Distribution of symptomatic and asymptomatic individuals infected with *Blastocystis sp.* according to age group.

Clinical data	**Patterns of infection**		
	Individuals only infected with *Blastocystis sp.* (n= 40) n (%)	Individuals negative or infected with commensal protozoan. (n=2153) n (%)	Individuals co-infected with other intestinal pathogenic parasite (n=947) n (%)
digestive symptoms* Yes (n=847) No (n=2293)	29 (3.4) 11(0.5)	86 (10.2) 2067 (90.1)	732 (86.4) 215 (9.4)
OR (95 % IC) P Value	7.14 (3.55-14.35) P=0.000+ **	0.11 (0.09-0.14) P=0.000+ **	9.22 (7.77-109.94) P=0.000+ **
*Diarrhoea or flatulence or abdominal pain or nausea ** Significant value P< 0.05			

Table 4: Patterns of *Blastocystis* infection in the population studied according to the presence of some digestive symptoms.

Analysing the association between the infection of *Blastocystis* in the different age groups, a significant difference was found in the older children group (5-14 years old) infected by this intestinal protozoan and the symptoms that they developed (P<0.05). On the other hand, the group above 40 years old was more likely to have an asymptomatic infection (P<0.05), probably by the fact of When this analysis was done only with those individuals infected only with Blastocystis, the older children group remained statistically significant with the symptoms that they developed when compared with the asymptomatic ones (P=0.02). When considering all the individuals only infected with *Blastocystis*, there are significantly more subjects who have clinical manifestations (P < 0.05) as shown in Table 3.

Blastocystis sp. represented the only intestinal parasite in 29 of these symptomatic patients. The common symptoms among patients infected exclusively with *Blastocystis sp.* were abdominal pain (69%), diarrhoea (51.7%), and nausea (37.9%). Moreover, 17.2% and 13.8% of these patients had nausea/ vomiting and flatulence, respectively. Eighteen of the symptomatic patients (62.1%) had two or more gastrointestinal symptoms.

Regarding the infection pattern observed in the direct microscopic examination, we divided those who were infected only by *Blastocystis* and the others in which individuals were not parasitized or were infected with commensal protozoan, and those in which individuals

were co-infection with parasites of medical importance of intestinal tract. Individuals in which *Blastocystis* and other intestinal pathogens were observed had a odds ratio 9 times higher to develop gastrointestinal symptoms, whereas, those only infected with that intestinal protozoan had 7 times more probability to have intestinal disturbances (OR=7.14 % IC:3.55-14.35). Persons co infected with a commensal protozoa have not risk to present symptomatology (Table 4).

Discussion

In our frequency study we found a high number of multiple infections comparing with infections caused only by Blastocystis. Other intestinal protozoa found included Giardia intestinalis, Entamoeba histolytic/dispar. Moreover, helminth parasites, especially soil-transmitted helminths (*A. lumbricoides* and *T. trichiura*) had a higher prevalence indicating that the humidity of climate and the close contact with soil is favourable for the development of helminth parasites which need moist soil [1].

The prevalence of *Blastocystis sp.* infection in humans often exceeds 5% in developed countries and can reach as high as 76% in developing countries [2,4], and recently in a study carried out in a cohort of children living in a rural area from Senegal, the prevalence of *Blastocystosis sp.* reached a peak of 100%, a result never achieve before [14]. Having a cosmopolitan distribution, the parasite is a common laboratory finding in the stools of individuals with and without intestinal symptoms worldwide and remains extremely difficult to eradicate [3]. However, prevalence data are largely dependent on the methods used for detection, quantitative PCR being the most sensitive method, meaning that infections by *Blastocystis sp.* are likely underestimated [7,15].

Lately, *Blastocystis sp.* have been included in the water sanitation and health programs of the World Health Organization (WHO) [16]. Increasing interest of scientific and medical communities for *Blastocystis sp.* was coupled with new data about epidemiology, pathogenicity, and, more recently, the first whole genome of a human isolate [17]. Clinical studies also associate *Blastocystis* with other intestinal and dermatological inflammatory disorders, such as irritable bowel syndrome and urticarial, respectively [5]. Patients immunocompromised due to HIV or cancer are particularly susceptible to infections, suggesting that *Blastocystis* is also an opportunistic pathogen [18].

In the present study, the frequency of *Blastocystis* infection was 3.54%, which is low when compared with the range of the prevalence rate of previous studies in our country. For instance, Cañete et al., reported a prevalence above 38% among children who were attended to in a day care center in an urban area of Matanzas city [19]. In a cross-sectional study carried out by Escobedo et al., to determine the prevalence of intestinal parasitic infections among children at educational centers in a rural area of San Juan y Martinez, Pinar del Rio province, they found the same data of prevalence of *Blastocystis* [20] as were found by the researchers in the Matanzas study. On the other hand, in the second national survey of intestinal parasitic infections in Cuba, in which samples of all age groups were collected, the frequency of this protozoan was 8.89% [12]. In another study carried out in 456 children aged 1-5 years from 4 day-care centers located in San Miguel del Padrón municipality during November 1998, Mendoza et al., found a 29.6% of *Blastocystis* prevalence [21].

The higher frequency of *Blastocystis* found in those studies mentioned above could be explained by the fact that three stool samples were examined per individual. In our study only one stool sample remitted to our Laboratory was examined in the majority of individuals, as part of a national surveillance of Parasitic Infections. Previous reports from different countries have shown that *Blastocystis* infections are associated with several factors such as the consumption of contaminated food and water, close contact with animals, poor personal hygiene, inadequate sanitation, geographical distribution, agricultural activities and seasonal influences [22-24]. All samples in this study were remitted from La Habana, and the differences found in the prevalence rates between those studies, representing several provinces, could be related to different water sanitation and geographical distribution.

Previous studies have found a significantly higher infection rate in adults than in children with the highest prevalence rate among young adults aged between 18 and 30 years [25,26]. In contrast, other reports have found a higher prevalence rate in children and females as compared to adults and males [27,28]. Moreover, a recent study has reported a significant reduction in the *Blastocystis* infection prevalence rate in older children when compared with younger children [29]. In our study the group of schoolchildren (5-14 years old) was the most infected and a strong statistical association between the infection and the development of intestinal symptomatology was found. Our results show that *Blastocystis* in the juvenile population may be responsible for gastrointestinal symptoms. This result could be explained by the fact that bad hygienic practices (for instance, dirty hands) are more usual in this group of ages and reinfection with one or more subtypes of *Blastocystis* is more probable, carrying out the development of gastrointestinal disturbances due to *Blastocystis* infection

Today there is a concern that water may be a main source of infection by *Blastocystis* although large-scale waterborne outbreaks involving the parasite have not yet been documented. Ability of *Blastocystis* to survive for long periods of time in the environment together with their small size represent some factors that may favour waterborne transmission of the parasite, which is why in 2006, *Blastocystis* was added by the WHO to the list of waterborne parasites [2].

The fecal-oral route is the main mode of transmission of *Blastocystis sp.* like the other common gastrointestinal parasites [1]. Drinking contaminated water, especially surface water, was reported to be a significant risk factor for *Blastocystis* infection [22], although in a study made by Abdusalam et al. (2013) found no significant difference in the prevalence of *Blastocystis* infection between those who use treated water and those who use untreated water, indicating that the level of *Blastocystis* contamination in groundwater is low [4].

Our findings showed no significant difference in the prevalence of *Blastocystis* infection based on the age and gender of the participants, and this is consistent with the results of previous reports 4, 22, 24. The age and gender correlations reported in other studies may not represent physiological properties intrinsic to those hosts, but rather may be caused by the variation in environmental conditions associated with age and gender, and may indicate a higher exposure to the source of infection either at the work places including the food and environment and or exposure to animals. For that, further studies on animal and environmental isolates are required to identify different transmission routes and reservoirs of *Blastocystis sp.*

Numerous studies have focused on the pathogenic potential of *Blastocystis sp.* by investigating its prevalence in symptomatic and asymptomatic groups, and have thus either supported or denied pathogenic significance of this protozoan [2,29]. Wu et al. found that *Blastocystis* attaches to intestinal epithelium and leads to epithelial barrier dysfunction and that drug resistance might entail a fitness cost in parasite virulence by limiting entero-adhesiveness [10]. In another study made by Wu, they found that a *Blastocystis* subtype (ST-7) induced enterocyte-apoptosis by activating caspases 3 and 9, suggesting the involvement of the intrinsic apoptotic pathway in pathogenesis [30].

The intestinal inflammation induced by this parasite was reported to yield specific inflammatory changes in the gut wall. In this regard, in an experimental animal model study in mice, a vacuolar form of the parasite was reported to cause invasion of the lamina propia, submucosal and muscular layers of the large intestine leading mixed inflammatory cell infiltration and active colitis in infected mice [31].

Focus on symptomatic and asymptomatic individuals, regarding the infection pattern observed in the direct microscopic examination, we found that individuals in which *Blastocystis* and other intestinal pathogen was observed had a odds ratio 9 times higher to develop gastrointestinal symptoms, whereas, those only infected with that intestinal protozoan had 7 times more probability to have intestinal disturbances. Persons co infected with a commensal protozoa have not risk to present symptomatology (Table 4). However it is important to remember that this is based on only one sample, which means that a significant percentage of the apparent mono-infections would actually have an undetected pathogenic parasite.

Researchers report that *Blastocystis sp.* is the most common parasite encountered in symptomatic patients based on two main reasons. Firstly, clinicians are reluctant to treat this parasite due to its low pathogenicity and self-restricting symptomatology; and secondly, *Blastocystis sp.* that are resistant to other antiparasitic medications have the ability to colonize easily into empty intestinal niches after the treatment of other pathogenic protozoa with conventional medications [2].

In our study, the most common symptoms in all symptomatic patients were abdominal pain, diarrhoea, nausea and flatulence. In Saudi Arabia, abdominal pain, constipation and diarrhoea were the most common symptoms reported among 12,136 Blastocystis-infected patients [32]. Moreover, abdominal pain, diarrhoea and abdominal distension were found to be associated with *Blastocystis* infection among hospitalized children in Turkey [33].

Although the number of patients infected only with *Blastocystis sp.* was not high in our investigation, the probability to develop gastrointestinal symptoms was almost similar with those patients infected with parasites of medical importance, showing the clinical significance of this controversial parasite.

The presence of *Blastocystis* representatives has also been reported in a variety of mammals, birds, reptiles, and even insects [7]. This protozoan exhibits extensive genetic diversity, and on the basis of molecular analysis of the small subunit RNA gene, up to 17 subtypes have been described with subtype (ST) 1–9 being found in humans, and ST3 is the predominant ST found in most human epidemiological studies [34].

As main limitations of the present study, data on other bacterial and viral agents were not studied. In addition the study may have incorporated a selection bias, as only one sample from patients submitted to "Pedro Kouri" Institute were examined, so the true odds ratio found for *Blastocystis* alone is likely to be significantly lower, although it is not possible to know by how much. However, the National Reference Laboratory of Intestinal Parasitic Infections from "Pedro Kouri" Institute receives samples from symptomatic and asymptomatic individuals including those who come for routine medical check-ups requested by public health institutions. It is necessary to do further studies to introduce molecular techniques for the genetic characterization of *Blastocystis* isolates and correlate those results with clinical data.

Conclusions

The present study is the first to provide information about the correlation of *Blastocystis* infection and the symptomatology in a group of Cuban individuals. This study reveals a low prevalence of *Blastocystis* infection among individuals seeking health care in "Pedro Kouri" Institute, although it was the fourth most common parasite in frequency diagnosed. Despite the fact that age was not a controlled variable, it was remarkable that the age group between 5 and 14 years old presented the highest risk for infection and a significant association with symptoms in individuals infected with Blastocystis. More research, especially with the use of advanced molecular techniques would be highly recommended in future attempts to reveal the possible clinical significance of the different *Blastocystis* spp. subtypes in Cuban infected individuals.

Authors' Contributions

All authors listed on the manuscript have contributed significantly to the experimental design, its implementation, or analysis and interpretation of the data. Lic. Luis Enrique Jerez (LEJ) and Dr. Fidel Núñez (FN) designed the study, and LEJ and Dr. Iraís Atencio (IA) carried out the parasitological work on stool samples. FA and LEJ did the analysis and interpretation of data results, and FA critically revised the manuscript for intellectual content. All authors read and approved the final manuscript.

Competing Interests

The authors declare that they have no competing interests.

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