ORIGINAL ARTICLE

Evaluation of risk factors associated to detection of *Blastocystis* sp. in fecal samples in population from Barcelona, Spain: a case-control study

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Abstract

Blastocystis sp. is the most common intestinal parasite isolated in humans. The aim of the study was to describe the risk factors associated to *Blastocystis* sp. detection. A case-control retrospective study was carried out at Vall d'Hebron University Hospital (Barcelona, Spain), which receives all fecal samples collected in Barcelona at primary care level. Eligible patients were patients older than 18 years in whom three consecutive stool samples were examined for parasitic diagnosis from January to December 2017. Positive patients for *Blastocystis* sp. were assigned as cases whereas negative patients were assigned as controls. Overall, 4174 patients were eligible for the study, from whom 724 (17.3%) had *Blastocystis* sp. detection. From these, 170 cases (*Blastocystis* sp. positive) and 170 controls (*Blastocystis* sp. negative) were randomly selected for inclusion. One hundred and twenty-six (37.1%) of them were immigrants, and 171 (50.3%) patients had traveled out of Spain the year before. The majority of individuals had jobs with no direct contact with other people (health personnel, teachers, and caregivers) (85.6%), and 29.4% were in usual contact with animals. Regarding clinical information, 68.2% of patients presented digestive symptoms, 3.5% presented an immunosuppressant condition, and 6.5% were infected by other intestinal parasites. Variables associated to *Blastocystis* sp. detection were being born in Africa, having traveled abroad, and working in direct contact with other people. Having other intestinal parasitic infections had a protective value. Our study provides new insights into the epidemiology of *Blastocystis* sp. in industrialized countries.

Keywords *Blastocystis* sp. · Intestinal protozoa · Risk factors · Spain

Introduction

Blastocystis sp. is the most common intestinal parasite isolated in humans, with up to one billion humans colonized [1–3].

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Transmission can occur from human to human, human to animal, or animal to human [4]. Recent studies based on the comparison of the nuclear small subunit rRNA gene show that *Blastocystis* has an extensive molecular diversity, with up to 17 genetic distinct ribosomal lineages described [5].

Blastocystis sp. distribution around the world is not homogeneous, as prevalence can vary from up to 10% in developed countries to 50–60% in developing countries [6]. The highest prevalence ever observed was seen in Senegal, in the year 2014 [7]. Moreover, differences in subtype distribution have been observed regarding geographic distribution [8]. This large variance may be due to misreporting and misdetection in some clinical laboratories, which suggests that prevalence may be higher than recorded in some studies [9]. The high prevalence in developing countries is usually related to poor hygiene practices and to consumption of contaminated food or water [10]. In addition, recent studies have also strongly related its distribution to the socioeconomic and geographic

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situation. However, developed countries also show high prevalence rates of detection. Animals have been suggested as the sources of infection since genetic similarity has been demonstrated [11]. Immigrants, travelers, and people who live in close contact with animals are also susceptible to be detected with *Blastocystis* sp.

Nowadays, there is a growing recognition about *Blastocystis* sp. pathogenic potential, although its virulence mechanisms are not fully understood [1]. However, no consensus has been reached yet, and many authors still put doubt on the *Blastocystis* sp. role in human disease, making its real human health impact uncertain [12, 13]. Even so, it is accepted to be a potential pathogen for immunocompromised people.

Transmission is mostly accepted to happen fecal-orally both by direct or indirect ways, like contact with infected people or animals or through the ingestion of contaminated water, respectively [14]. Foodborne transmission has also been reported as a source of infection. However and even if detected in asymptomatic patients, it is thought to cause gastrointestinal symptoms including diarrhea, abdominal pain, flatulence, dyspepsia, or vomiting [8]. Moreover, cutaneous manifestations such as chronic urticaria have been reported and have been associated to blastocystosis, especially in patients with irritable bowel syndrome (IBS) [15–17].

Despite its prevalence and all consequences associated to *Blastocystis* sp. infection, current knowledge about this organism in Spain is scarce. However, *Blastocystis* sp. zoonotic transmission risk has been recently studied for individuals in the Basque Country [18] and for HIV-positive children in Madrid [19].

Therefore, the main objective of the present study was to determine the risk factors associated to *Blastocystis* sp. detection in symptomatic and asymptomatic patients in Barcelona, Spain.

Methods

A retrospective case and control study was performed at the Vall d'Hebron University Hospital, a tertiary hospital included in the International Health Program of the Catalan Health Institute (PROSICS, Barcelona, Spain). The hospital receives all stool samples collected in Barcelona at primary care level. After consulting the Microbiology Department registry, 34,158 stool samples were registered at the hospital database from January to December 2017. Eligible patients were those older than 18 years old in whom three consecutive stool samples had been examined for parasitic diagnostic for any reason (gastrointestinal symptoms, eosinophilia, and screening of asymptomatic carriers) and those who had not received any previous treatment. Only patients with three stool samples were selected because of an agreement with earlier studies that state the need for multiple stool examinations with microscopy before reporting a negative result for *Blastocystis* sp. [20].
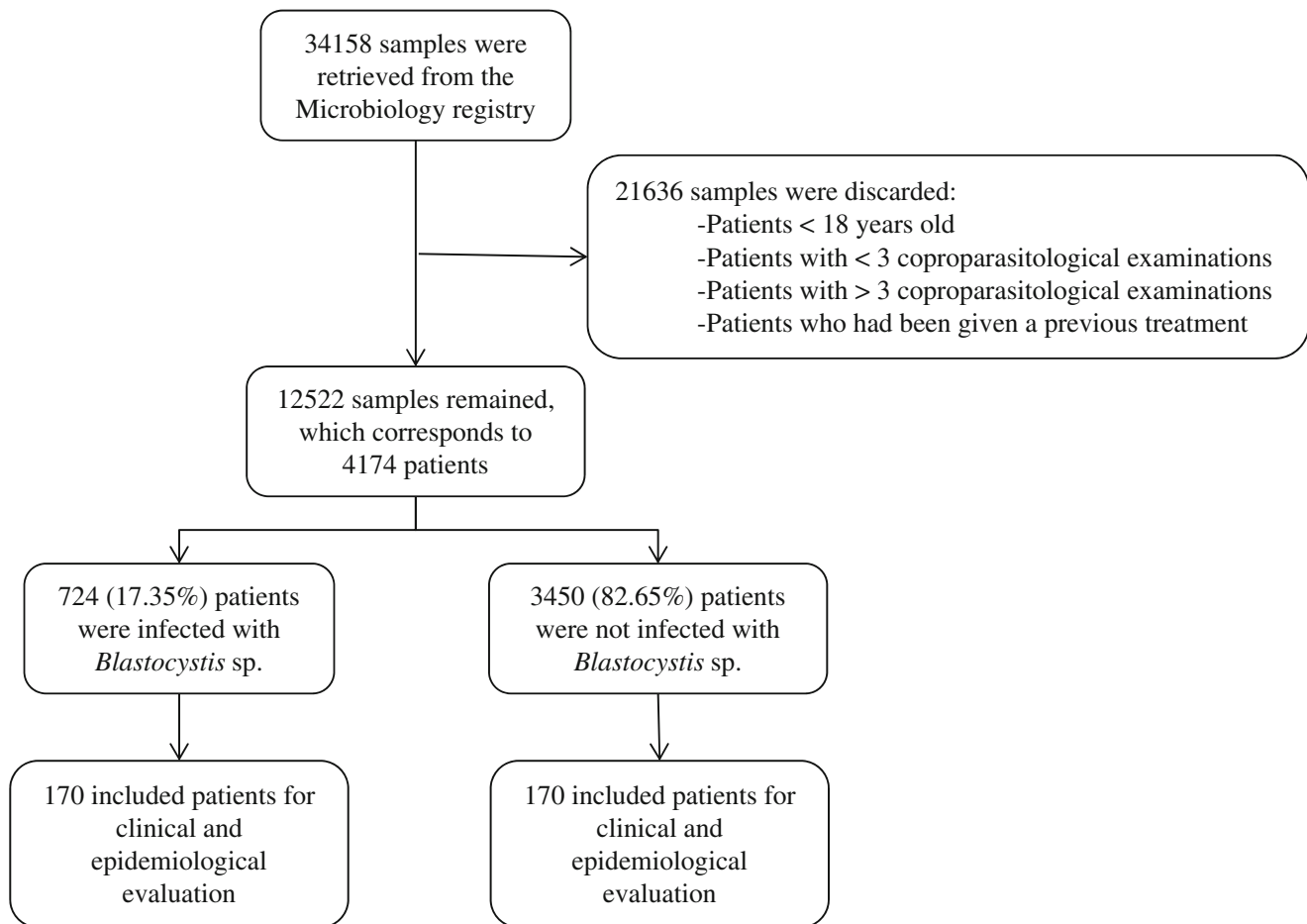
A total of 12,522 samples fulfilled the inclusion criteria, which corresponds to 4174 patients. From this, 724 (17.3%) were infected with *Blastocystis* sp. (see Fig. 1). During the study period, the diagnosis of intestinal parasites, including *Blastocystis* sp., was performed by microscopic examination of concentrated stool samples using a commercial dispositive (Midi Parasep SF, APACOR, England).

To calculate the sample size for a case-control study, a proportion of 50% of cases exposed to risk factors of *Blastocystis* sp. detection was selected, whereas a proportion of 35% was selected for controls. The ratio among cases (at least one positive stool examination for *Blastocystis* sp.) and controls (all three stool samples negative for *Blastocystis* sp.) was 1:1; the power of the study was established at 80% with a confidence level of 95%. With the previous data, a sample size of 170 cases and 170 controls was obtained, which were randomly selected from the 4174 included participants.

Based on reviews of the literature on intestinal parasitic infections, we developed a standardized questionnaire to determine the demographic and clinical characteristics of patients, in order to be able to later assess the correlation between the questionnaire's answers and potential detection risk factors (Supplementary material) [13, 15–17, 21]. It included questions regarding date of inclusion, age, sex, origin, type of work, type of environment, travels, regular contact with animals, and gastrointestinal symptoms. Jobs were subdivided into two categories, depending on the presence of direct contact with other people or not. Hospital personnel, teachers, restaurant workers, and caregivers were included in the first group. The questionnaire also included questions about clinical issues such as number of positive stools, diabetes mellitus, immunosuppression, and coinfection with other parasites, which were obtained by looking at the medical record of patients. All questions were asked related to the moment of the stool sampling. As it has been demonstrated that *Blastocystis* sp. can persist for months and colonize the gut [22], questions referring to previous travels took into account those done from 2016 until when stool sampling was done.

Eligible patients were contacted by telephone, and after a brief description of our research, they gave their oral consent to participate in the project. Contact telephones were obtained from the electronic medical record at our institution. The study protocol was reviewed and approved by the Ethics Committee of the Vall d'Hebron University Hospital (reference number PR (AG) 99/2018).

IBM SPSS software platform version 15 was used to perform the statistical analysis. Categorical data were presented as absolute numbers and proportions, and continuous variables were expressed as means and standard deviations (SD) or medians and interquartile ranges (IQR) depending on the distribution. The Kolmogorov-Smirnov test was used to evaluate the normal distribution of variables. Intergroup differences for continuous parameters were assessed by the Mann-Whitney *U* test

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│ 34158 samples were      │
│ retrieved from the      │
│ Microbiology registry   │
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│ 21636 samples were discarded:                      │
│   -Patients < 18 years old                         │
│   -Patients with < 3 coproparasitological examinations │
│   -Patients with > 3 coproparasitological examinations │
│   -Patients who had been given a previous treatment│
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│ 12522 samples remained, │
│ which corresponds to    │
│ 4174 patients           │
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│ 724 (17.35%) patients   │   │ 3450 (82.65%) patients  │
│ were infected with      │   │ were not infected with  │
│ Blastocystis sp.        │   │ Blastocystis sp.        │
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┌─────────────────────────┐   ┌─────────────────────────┐
│ 170 included patients for│  │ 170 included patients for│
│ clinical and            │   │ clinical and            │
│ epidemiological         │   │ epidemiological         │
│ evaluation              │   │ evaluation              │
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Fig. 1 Flow diagram depicting study cohort size and selection

if they did not present a normal distribution. The χ^2 test or the Fisher exact test, when appropriate, was used to compare the distribution of categorical variables. Multivariate logistic regression analysis was made to identify factors associated to *Blastocystis* sp. detection. Variables were entered in the model if $p < 0.20$ on univariate analysis. Results were considered statistically significant if the two-tailed p value was < 0.05.

Results

The epidemiological characteristics of patients are summarized in Table 1. A total of 340 individuals aged 18 to 88 were interviewed in this study with an overall mean age of 46.7 ± 18.24 years, and most of them were females (60.6%). The majority of the participants lived in Barcelona city (95.9%), whereas the rest lived mainly in the surrounding areas. One hundred and twenty-six (37.1%) of participants were immigrants and had been living in Spain for 11 years on average. Among them, the majority of immigrants came from America, encompassing 69 (54.8% among immigrants) patients. Specifically, 54 (15.9%) came from South America, 14 (4.1%) from Central America, and 1 (0.3%) from North America. Twenty-four (7.1%)

immigrants came from Asiatic countries and 16 (6.9%) from European countries. Finally, 17 (5%) patients came from Africa.

Most of the people did not have direct contact with people at work ($n = 291$, 85.6%). Only four (1.2%) people were in contact with the rural environment (villages with less than 10,000 inhabitants). Regarding daily life, 100 (29.4%) patients were in usual contact with animals, 70 (20.6%) of them with dogs and 28 (8.2%) with cats. Other pets included birds ($n = 6$, 1.8%), rabbits ($n = 7$, 2.1%), turtles ($n = 2$, 0.6%), fishes ($n = 1$, 0.3%), hamsters ($n = 3$, 0.3%), and chickens ($n = 3$, 0.3%). In addition, 171 (50.3%) patients traveled abroad during the year before the stool sampling. The most visited continent was Europe, with 66 (38.6% among all destinations) individuals, followed by 62 (18.9%) visitors to America and 34 (10%) to Asia.

Clinical characteristics of patients are summarized in Table 2. Most of the patients presented digestive symptoms ($n = 232$, 68.2%), and only 22 (6.5%) of participants were infected with other intestinal parasites, mainly *Endolimax nana* ($n = 9$, 2.6%) and *Giardia lamblia* ($n = 6$, 1.8%). Others included *Dientamoeba fragilis* ($n = 4$, 1.2%), *Entamoeba coli* ($n = 3$, 0.9%), *Entamoeba histolytica/ Entamoeba dispar* ($n = 3$, 0.9%), *Strongyloides stercoralis* ($n = 2$, 0.6%), and *Enterobius vermicularis* ($n = 1$, 0.3%).

Table 1 Univariate and multivariate analysis for epidemiological characteristics of patients with and without **Blastocystis** sp. detection attended in Barcelona (2017)

Characteristics	Overall (**n** = 340)	B+ (**n** = 170)	B− (**n** = 170)	Univariate analysis (**p** value)	Multivariate analysis (OR, 95% CI, and **p** value)
Age, years	46.69 (17–88)	43.95 (18–85)	49.44 (17–88)	0.005	OR 0.994 (0.98–1.008), **p** = 0.396
Gender, female	206 (60.6%)	98 (57.6%)	108 (63.5%)	0.267	–
Immigrant	126 (37.1%)	77 (45.3%)	49 (28.8%)	0.002	OR 1.301 (0.753–2.2251), **p** = 0.346
North America	1 (0.3%)	1 (0.58%)	0 (0%)	0.317	
Central America	14 (4.1%)	11 (6.5%)	3 (1.8%)	0.029	OR 3.418 (0.881–13.256), **p** = 0.075
South America	54 (15.9%)	30 (17.6%)	24 (14.1%)	0.373	–
Africa	17 (5%)	14 (8.2%)	3 (1.8%)	0.006	**OR 5.216 (1.441–18.879), p = 0.012**
Asia	24 (7.1%)	13 (7.6%)	11 (6.5%)	0.672	–
Other European countries	16 (6.96%)	8 (4.7%)	8 (4.7%)	1.000	–
Traveling	171 (50.3%)	99 (58.2%)	72 (42.4%)	0.003	**OR 1.932 (1.229–3.037), p = 0.004**
North America	10 (2.9%)	6 (3.5%)	4 (2.4%)	0.521	–
Central America	13 (3.8%)	6 (3.5%)	7 (4.1%)	0.777	–
South America	39 (11.5%)	24 (14.1%)	15 (8.8%)	0.126	OR 1.692 (0.790–3.624), **p** = 0.176
Africa	24 (7.1%)	17 (10%)	7 (4.1%)	0.034	OR 1.346 (0.438–4.131), **p** = 0.604
Asia	34 (10%)	20 (11.8%)	14 (8.2%)	0.278	–
Europe	66 (19.4%)	36 (21.2%)	30 (17.6%)	0.411	–
Working in contact with people	49 (14.4%)	33 (19.4%)	16 (9.4%)	0.009	**OR 2.654 (1.356–5.197), p = 0.004**
Contact with animals	100 (29.4%)	47 (27.6%)	53 (31.2%)	0.475	–
Dog	70 (20.6%)	31 (18.2%)	39 (22.9%)	0.283	–
Cat	28 (8.2%)	13 (7.6%)	15 (8.8%)	0.693	–
Bird	6 (1.8%)	3 (1.8%)	3 (1.8%)	–	–
Rabbit	7 (2.1%)	4 (2.4%)	3 (1.8%)	–	–
Chicken	1 (0.3%)	1 (0.6%)	0 (0%)	–	–
Fish	1 (0.3%)	1 (0.6%)	0 (0%)	–	–
Turtle	2 (0.6%)	1 (0.6%)	1 (0.6%)	–	–
Hamster	3 (0.9%)	1 (0.6%)	2 (1.2%)	–	–

Data are reported as number of patients (%) or median (range). Abbreviations: **B+**, **Blastocystis** species–positive patients; **B−**, **Blastocystis** species–negative patients

Data set in bold are those results in the multivariate analysis that are significant statistically.

Twenty-five (7.4%) patients were diabetic, and 12 (3.5%) presented an immunosuppressant condition; among these, most patients had HIV infection ($n = 6$, 1.8%). There were 14 (4.1%) patients with a diagnosis of irritable bowel syndrome.

After doing the multivariate analysis, the variables associated to *Blastocystis* sp. detection were coming from Africa (OR 5.216, $p = 0.012$), having traveled abroad (OR 1.932, $p = 0.004$), and working with the public (OR 2.654, $p = 0.004$). Moreover, being infected by other parasites resulted to be a protective factor (OR 0.370, $p = 0.046$).

Discussion

We retrospectively studied 340 patients, divided into cases and controls, whose stool samples were analyzed at the Vall d'Hebron University Hospital during year 2017. We used standardized questionnaires and medical records with the aim to gather information to detect risk factors related to *Blastocystis* sp. detection. Most of the samples belonged to Spanish women who lived in Barcelona and did not work directly with the public. Among all the variables studied, we found out being African, working with the public, and traveling to other countries significantly increase the risk of having a positive detection for *Blastocystis* sp. Moreover, being infected by other parasites was found out to be a protective factor. In addition, IBS patients had a lower *Blastocystis* carriage rather than non-IBS patients. However, no consensus has been reached for this fact, as there are studies which suggest correlation and studies which refuse it [23].

Throughout these lines, it has been stated that the public health burden of *Blastocystis* sp. is still underestimated, and so there is an interest in performing large-scale epidemiological surveys in industrialized countries. Parasitological studies in

Table 2 Univariate and multivariate analysis for clinical characteristics of patients with and without *Blastocystis* sp. detection attended in Barcelona (2017)

Characteristics	Overall ($n = 340$)	B+ ($n = 170$)	B− ($n = 170$)	Univariate analysis (p value)	Multivariate analysis (OR, 95% CI, and p value)
Presence of digestive symptoms	232 (68.2%)	116 (68.2%)	116 (68.2%)	1.000	–
Other intestinal parasites	22 (6.5%)	7 (4.1%)	15 (8.8%)	0.078	*OR 0.370 (0.14–0.98), p = 0.046*
E. nana	9 (2.6%)	5 (2.9%)	4 (2.4%)	–	–
G. lamblia	6 (1.8%)	1 (0.6%)	5 (2.9%)	–	–
D. fragilis	4 (1.2%)	2 (1.2%)	2 (1.2%)	–	–
E. coli	3 (0.9%)	2 (1.2%)	1 (0.6%)	–	–
E. histolytica/E. dispar	3 (0.9%)	1 (0.6%)	2 (1.2%)	–	–
S. stercoralis	2 (0.6%)	0 (0%)	2 (1.2%)	–	–
E. vermicularis	1 (0.3%)	0 (0%)	1 (0.6%)	–	–
Diabetes mellitus	25 (7.4%)	9 (5.3%)	16 (9.4%)	0.146	OR 0.586 (0.236–1.452), $p = 0.248$
Presence of immunosuppressant condition	12 (3.5%)	6 (3.5%)	6 (3.5%)	1.000	–
Irritable bowel syndrome	14 (4.1%)	4 (2.4%)	10 (5.9%)	0.101	OR 0.334 (0.98–1.137), $p = 0.079$

Data are reported as number of patients (%). Abbreviations: *B+*, *Blastocystis* species–positive patients; *B−*, *Blastocystis* species–negative patients

developed countries are not common. In Spain, studies regarding *Blastocystis* sp. detection are limited [15, 24, 25] and focused on specific population groups [26]. Up to date, only one molecular-based study has been conducted in asymptomatic population in Spain [18].

Regarding our study, only people from Africa were reported to have a stronger risk of detection. A higher incidence, however, not significant, was also seen among people coming from Central America. No differences were stablished regarding living in Spain.

A story of recent travel has been stated to be a risk factor to detect the parasite, with those in tropical and subtropical destinations being at increased risk [27]. Among all possible destinations, we found out Southern American and African countries had a higher risk of detection, even if it was not significant enough. It is interesting to point out that immigrants coming from Africa and people traveling to Africa are both at a higher risk to get infected. However, the latter is not statistically significant.

Age has sometimes been stated as a risk factor, usually describing children in developing countries as the most prevalent group [6]. Children were not included in our study, but, even if it was not statistically significant, younger adult age groups were at a higher risk, in accordance with a study conducted in France. However, the prevalence could be overestimated by fact that young people usually travel more [27].

Some studies have associated *Blastocystis* sp. detection to female gender [28], relating it to differences on the jobs but also to environmental, ecological, economical, and cultural factors [29]. In our case, women presented a higher prevalence of detection than men, but it was not statistically significant.

Contact with animals has been stated to be a risk factor for *Blastocystis* sp. detection [9, 30, 31] but not in our case. However, most of the studies which have proven this fact have been conducted in developing countries, where animal hygienic conditions and treatment may be different. In these studies, population is mostly rural and is in contact with livestock animals, whereas, in our case, dogs and cats were the main pets reported. A study performed in Spain suggested that dogs and cats do not play a relevant role as a natural source of human infections [17]. The fact that the present study was conducted by using samples which had been analyzed in Barcelona conditioned the origin of our samples, so most people came mostly from an urban environment. This finding makes it difficult to evaluate this variable and limits our study. Therefore, our results are based on an urban population, from an industrialized country.

We found out that people working with other people were at a higher risk than those who did not. The occupations that were taken into account (hospital personnel, caregivers, teachers, and restaurant workers) can be related to working with groups of people who are at a higher risk to be positive for *Blastocystis* sp., meaning that the risk of those surrounding them may also be increased. Therefore, not taking the adequate prevention may significantly increase the risk of having a positive isolate. Unfortunately, from all the participants, almost no one worked in contact with animals or in the rural (only two people did), so we cannot make any elucidation regarding this possible risk factor.

Blastocystis sp. pathogenicity has been one of the main controversial issues regarding this parasite. Even so, this was not a significant factor to consider in our study. Curiously, the same number of participants among cases and controls

reported presence of digestive symptoms. However, some studies suggest that pathogenicity may be related to different *Blastocystis* sp. subtypes, and it has also been demonstrated that these subtypes vary geographically [21, 32]. Interestingly, ST4 has been suggested to be more pathogenic than other *Blastocystis* subtypes [32].

Extensive research shows that immunosuppression and use of immunosuppressive drugs increase the risk of detecting *Blastocystis* sp. [6, 28, 33]. However, in this study, no correlation was found among these factors. Some studies have stated that [20] *Blastocystis* sp. may be linked to irritable bowel syndrome (IBS), but this association was not observed in our case. A significantly lower prevalence of *Blastocystis* sp. was reported among patients who were infected by other parasites than those who were not. Enteric pathogen infection by different parasites has commonly been detected in different studies [23, 34]. Although in our study the presence of other parasites acted as a protective factor, it could be the result of a bias due to the fact that all participants were patients asked for coproparasitological examination, with a higher chance to find a pathogen, rather than if they had been randomly selected from population. Furthermore, infection by other parasites could produce changes in the intestinal flora that could hinder *Blastocystis* sp. colonization.

Given its retrospective nature, our study presents some limitations. Firstly, conventional microscopic methods were used for diagnosis rather than molecular methods even though there has been stated a relatively low sensitivity of estimation of the presence or absence of *Blastocystis* [35]. Besides, the small sample size could also be a limiting factor, but the power of the study was high enough to show differences among cases and controls. In addition, it is not known if patients were already infected before traveling. Moreover, the immigrant population group had been living in Spain for a long time and therefore could have acquired the infection in Spain. However, this cannot be confirmed, as the incubation period is not clearly known. Finally, recall bias was identified during the telephonic interviews (mostly related to traveling or to the presence of digestive symptoms); however, it only affects a small percentage of participants.

The results of the present study suggest that African people, recent traveling, and working with the public are risk factors for acquiring *Blastocystis* sp. On the other side, being infected by other intestinal parasite was a protective factor. Our study provides new insights into the epidemiology of *Blastocystis* sp. in industrialized countries in an urban population. Further research including molecular and epidemiological studies should be done. This would help to map prevalence and subtype distribution and to clarify the importance of risk factors depending on the area and would improve intervention strategies that could help to mitigate *Blastocystis* sp. transmission.

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