

## Brief Communication



# Blastocystis species and Gastrointestinal Symptoms in Peruvian Adults Attended in a Public Hospital

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## ABSTRACT

The objective of this study was to evaluate the role of *Blastocystis* sp. in gastrointestinal symptoms reported by adult patients in a Peruvian hospital. A case-control 3:1 study was performed at the outpatient clinic. Direct stool examinations were done. One hundred sixty patients were included, 40 cases and 120 controls. Positivity to *Blastocystis* sp. was associated with dyspepsia ( $P < 0.001$ ), bloating ( $P < 0.001$ ) and abdominal pain ( $P = 0.03$ ) in patients attending our hospital outpatient clinic.

**Keywords:** Blastocystis; Parasite Load; Intestinal Parasitosis; Stool Microscopy; Abdominal Pain

*Blastocystis hominis* is an enteroprotezoan distributed worldwide. In developed countries the prevalence is estimated at between 10 - 15% while in developing countries it is around 30 - 50% [1]. A small subset of cases progress into a self-limited "pathogenic" course known as "Blastocystosis or Zierdt-Garavelli Disease". Among its risk factors are travel to endemic areas, work contact with other people, bathroom hygiene and drinking water [2].

The genetic diversity among human and animal *Blastocystis* isolates is important and the symptoms vary among different strains [3]. However, the pathogenicity of *Blastocystis* sp. remains controversial.

Patients with *Blastocystis* present gastrointestinal discomfort including bloating, abdominal pain, constipation, and diarrhea [4]. *Blastocystis* is non-invasive and lacks cytophagocytotic capacity, with gastrointestinal symptoms developing during the amoeboid morphologic stage [5]. Factors associated with the symptoms include a high parasitic load ( $\geq 5$  parasites/field) [6], liquid stools, and brown color [7].

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**Ethical Approval**

Institutional Research Ethics Committee of the Hospital II EsSalud Huanuco.

**Conflict of Interest**

No conflicts of interest.

**Author Contributions**

Conceptualization: MXRC, JLM, LGH, VPC, KAL. Data curation: MXRC, VPC, KAL. Formal analysis: JLM, SPS, ACNS, AJRM, KAL. Writing - original draft: BDM, SPS, ACNS, KAL, JLM, ARR, AJRM. Writing - review & editing: MXRC, JLM, LGH, VPC, BDM, SPS, ACNS, AAR, AJRM, KAL.

In Latin-American countries, few studies have assessed the association between *Blastocystis* and gastrointestinal symptoms [8]. Indeed, even in mild cases, *Blastocystis* can be a cause of persistent discomfort and consultation. The present study assessed the role of *Blastocystis* in gastrointestinal symptoms among adult patients attending the EsSalud II Huánuco Hospital in Perú.

We conducted a case-control study, including patients from the Public Hospital II EsSalud Huánuco which attends patients of all economic levels. From June to September 2019, we recruited healthy individuals attending an annual follow-up, including medical consultation and laboratory analyses with coproparasitological screening. The sample size was calculated considering a 63 exposure and an estimated odds ratio (OR) of 3 [9], with three controls per case, alpha of 0.05 and beta of 0.20.

Patients meeting the inclusion criteria and agreeing to participate in the study were included. The presence of *B. hominis* was determined by direct stool examination of 3 samples collected on 3 different days. Samples were analyzed following the standard procedures recommended by the Peruvian National Institute of Health [10]. Stool examination was performed through direct observation with saline solution followed by lugol staining. The exclusion criteria were: the presence of other enteric pathogens; gastrointestinal symptomatology >1 month due to high probability of the presence of other digestive diseases; pregnancy; immunosuppression-associated diseases (human immunodeficiency virus [HIV], human T-cell lymphotropic virus, hepatitis B and C, rheumatic and chronic kidney diseases), and use of chronic corticosteroids and antibiotic/antiparasitic treatment during last month. Patients were defined as cases if they tested positive to *Blastocystis* sp. ( $\geq 1$  stool samples, in the absence of other parasites). Patients with negative results were defined as controls. The cases and controls were consecutively recruited when meeting the established criteria.

The demographic and clinical data were obtained by an interview conducted by two authors. Gastrointestinal symptoms, morphological stage, parasite load and stool consistency were considered as variables of interest. Parasite load was established according to international criteria: 1+ when 2 - 5 parasites were observed, 2+ when 6 - 10 parasites were observed and 3+ when >10 parasites were observed at 40 × magnification/microscopic field. Gastrointestinal symptoms included dyspepsia (abdominal discomfort), abdominal distension, abdominal pain, diarrhea (passing loose stools  $\geq 3$ /day), and constipation. Other variables included were age, sex, hand washing before meals and after defecation, the presence of dogs in the household, access to plumbing, eating habits, and consumption of boiled water.

Bivariate analysis with Mann-Whitney *U* and  $\chi^2$  tests was performed. ORs were calculated between the demographic characteristics and symptomatology in the presence of *Blastocystis*, parasite characteristics and the reporting of gastrointestinal symptoms. A *P*-value <0.05 was considered significant. Stata 16.0 software (Statacorp LLC, Lakeway Drive, College Station, TX, USA) was used for the analyses.

This study was approved by the Institutional Research Ethics Committee of the Hospital II EsSalud Huanuco (N°1333-D-RAHU-ESSALUD-2019). All patients provided signed informed consent.

From June 1 to September 30, 2019, 71 (44.4%) males and 89 (55.6%) females were recruited. The mean age was 50.9 years (standard deviation [SD]: 14.99). In relation to hygiene factors, 13 (8.1%) reported not washing hands before meals, and 10 (6.3%) reported not washing hands

after defecating. Boiling water for consumption was not performed by 27 participants (16.9%). Finally, only 8 (5.0%) people reported not having plumbing in their household.

We found associations among the presence of *Blastocystis* and dyspepsia ( $P < 0.001$ ), abdominal distension ( $P < 0.001$ ) and abdominal pain ( $P = 0.03$ ). However, no statistical association was found for sex or reported hygiene habits in the bivariate analysis. A greater number of dog owners tested negative for *Blastocystis* ( $n = 96$ ) compared to the subjects without dogs ( $n = 26$ ) (Table 1).

Forty patients (25.0%) tested positive for *Blastocystis* sp. The most prevalent morphological form was vacuolar (60.0%), followed by granular (20.0%). The parasitic load was 1+ in 29 (72.5%) cases and 2+ in 11 (27.5%). No case was 3+. Stool consistency was liquid in 19 (47.5%)

**Table 1.** Bivariate analysis of the presence of *Blastocystis* sp. in adult patients from the Hospital II Essalud Huánuco, Perú ( $n = 160$ )

Characteristics	Total (%)	<i>Blastocystis hominis</i>		P-value <sup>a</sup>	Odd Ratio	95% Confidence Intervals
		Positive (%)	Negative (%)			
Sex				0.783		
Male	71 (44.4)	17 (42.5)	54 (45)		Ref.	
Female	89 (55.6)	23 (57.5)	66 (55)		1.11	0.54 - 2.28
Age (years) (mean ± SD)	50.9 ± 15.0	48.6 ± 16.3	51.7 ± 14.5	0.263	0.98	0.96 - 1.01
Handwashing						
Before eating				0.066 <sup>b</sup>		
Yes	147 (91.9)	34 (85)	113 (94.2)		Ref.	
No	13 (8.1)	6 (15)	7 (5.8)		2.85	0.9 - 9.05
After defecating				0.258		
Yes	150 (93.7)	36 (90)	114 (95)		Ref.	
No	10 (6.3)	4 (10)	6 (5)		2.11	0.56 - 7.9
Access to sewage <sup>b</sup>				0.414		
Yes	152 (95)	37 (92.5)	115 (95.8)		Ref.	
No	8 (5)	3 (7.5)	5 (4.2)		1.86	0.43 - 8.18
Drink boiled water				0.113		
Yes	133 (83.1)	30 (75)	103 (85.8)		Ref.	
No	27 (16.9)	10 (25)	17 (14.2)		2.02	0.84 - 4.87
Regularly eats outside the household <sup>b</sup>				0.188		
Yes	13 (8.1)	1 (2.5)	12 (10)		Ref.	
No	147 (91.9)	39 (97.5)	108 (90)		4.33	0.55 - 34.4
Dog owners				0.054		
Yes	122 (76.3)	26 (65)	96 (80)		Ref.	
No	38 (23.7)	14 (35)	24 (20)		2.15	0.98 - 4.74
Gastrointestinal symptoms						
Dyspepsia				<0.001		
No	63 (39.4)	6 (15)	57 (47.5)		Ref.	
Yes	97 (60.6)	34 (85)	63 (52.5)		5.12	2 - 13.11
Abdominal distension				<0.001		
No	68 (42.5)	6 (15)	62 (51.7)		Ref.	
Yes	92 (57.5)	34 (85)	58 (48.3)		6.05	2.37 - 15.49
Abdominal pain				0.03		
No	50 (31.3)	7 (17.5)	43 (35.8)		Ref.	
Yes	110 (68.7)	33 (82.5)	77 (64.2)		2.63	1.07 - 6.46
Diarrhea				0.271		
No	88 (55)	19 (47.5)	69 (57.5)		Ref.	
Yes	72 (45)	21 (52.5)	51 (42.5)		1.5	0.73 - 3.07
Constipation				0.714		
No	72 (45)	19 (47.5)	53 (44.2)		Ref.	
Yes	88 (55)	21 (52.5)	67 (55.8)		0.87	0.43 - 1.79

<sup>a</sup>Fisher exact test.

<sup>b</sup>Mann Whitney U test.

SD, standard deviation.

**Table 2.** Bivariate analysis of the characteristics of the parasite and stool consistency and the presence of gastrointestinal symptoms in adults at the Hospital II EsSalud Huanuco, Perú (n = 40)

Characteristics	Total (%)	Gastrointestinal symptoms		P-value <sup>a</sup>	Odd Ratio	95% Confidence Intervals
		Yes (%)	No (%)			
Morphological stage						
Vacuolar form	24 (60.0)	22 (59.5)	2 (66.7)	0.806	Ref.	
Granular form	8 (20.0)	8 (66.7)	0 (0)		ND	
Ameboid shape	1 (2.5)	1 (2.7)	0 (0)		ND	
Cystic form	7 (17.5)	6 (16.2)	1 (33.3)		1.833	0.14 - 23.82
Parasitic load						
1 +	29 (72.5)	27 (73.0)	2 (66.7)		Ref.	
2 +	11 (27.5)	10 (27.0)	1 (33.3)	0.814	1.35	0.11 - 16.57
Stool consistency						
Solid	21 (52.5)	19 (51.4)	2 (66.7)		Ref.	
Liquid	19 (47.5)	18 (48.6)	1 (33.3)	0.609	0.53	0.04 - 6.34

<sup>a</sup>Fisher exact test.

1 +: 2 to 5 parasites per microscopic field observed.

2 +: 6 to 10 parasites per microscopic field observed.

3 +: >10 parasites per microscopic field observed.

ND, not determinated.

participants. Finally, there was no association between the morphological stage, parasite load, or stool consistency and *Blastocystis* sp. status (Table 2).

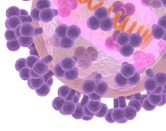
The Huanuco region can be divided in two areas, a mountainous region (Andes) and a tropical region (Amazonian jungle). The region is populated by inhabitants with diverse socio-economic and demographic characteristics and the prevalence of enteroparasitoses is higher than 20.0% [11]. According to the 2017 national census, this region is one with the lowest access to public water (59.0%) and sewage.

The vacuolar morphological stage of *Blastocystis* sp. was prevalent in both symptomatic and asymptomatic patients, as reported in other studies [12]. No association was found between its morphological stage and gastrointestinal symptom, in contrast to other studies that reported associations with the symptoms. This can be explained by the explosive growth of the amebic form after treatment with metronidazole in this population.

A case-control study in Iran, reported that parasite load in symptomatic patients can be as low as 1+ [13]. Another study in Mexico on the potential association with inflammatory bowel disease, showed no relationship between the parasitic load and symptoms [14]. A large study analyzing 37,000 samples, found positive correlations between parasite load and gastrointestinal symptoms [15]. Nevertheless, the authors failed to provide further details on the characteristics of immune response and nutrition, which are potentially confounding variables. Contrarily, parasite load could be affected by the techniques used, thereby making the studies non comparable.

The capacity of direct microscopy to adequately evaluate the presence of *Blastocystis* is of note. Compared with molecular biology techniques, the specificity and sensitivity of stains for *Blastocystis* detection are ~100% and 82%, respectively [5]. Another consideration is the possibility that other parasites cause the symptoms. Although microscopy can establish the presence of other parasites, the sensitivity and specificity of this test is similar to polymerase chain reaction in countries with a high prevalence of enteroparasites [16].

As in previous reports, we found no association between changes in stool consistency and the presence of gastrointestinal symptoms. Nonetheless, a study in Baghdad described a



relationship with diarrhea [17]. This difference is likely explained by the inclusion of only one symptom in the inclusion criteria of the Baghdad population and the greater number of symptoms observed in the present study.

No association was found between having dogs in the household and gastrointestinal symptoms, as reported previously. In contrast, a study in the Philippines found significant associations between these variables [18], in a urban population with adequate sanitation and care of their domestic animals. However, only 12.27 (12.3) % of the participants owned dogs, and this should be considered when interpreting the results. Additionally, this may be a confounding variable associated with socioeconomic level, exposure to children or occupation.

Boiled drinking water is considered a healthy habit and encouraged by local authorities to reduce gastrointestinal infections. However, we find no association between boiling drinking water and *Blastocystis*. This result differs from previous studies, including a systematic review showing that water treatment (consumption of boiled, filtered or treated water) is associated with lower odds of protozoa infection [19]. The study population was of medium to high socioeconomic status, which may explain this dissimilarity with the other populations.

Our study found that the likelihood of presenting dyspepsia, bloating, and abdominal pain increases with the presence of *Blastocystis*. However, several descriptive studies found no relationship between abdominal pain and *Blastocystis*. Nonetheless, our results correlate with previous reports from France and Spain, describing a significant association between abdominal pain and *Blastocystis*, coinciding with a meta-analysis of 5,882 participants (2,527 cases and 3,310 controls) [20].

The present study has multiple limitations that could affect the results and include memory bias during the interviews, the lack of 3+ parasite load, and also the high proportion of patients in the control group who presented episodes of diarrhea, which could have influenced the group of asymptomatic patients. Moreover, the recruitment of patients was performed over 4 months, hence, the potential role of climate seasonality of parasitic load or symptomatology could not be assessed. Another limitation of this study is that other possible pathogens or non-infectious causes such as *Helicobacter pylori*, *Clostridioides difficile*, *Salmonella*, among others, were not investigated. Nonetheless, their presence may be debatable since these pathogens regularly cause symptoms.

In conclusion, the presence of *Blastocystis* in stools is associated with a higher likelihood of presenting dyspepsia, abdominal distension, and abdominal pain, suggesting its potential pathogenic capacity.

## REFERENCES

1. Tan KS. New insights on classification, identification, and clinical relevance of *Blastocystis* spp. Clin Microbiol Rev 2008;21:639-65.  
[PUBMED](#) | [CROSSREF](#)
2. Deng Y, Zhang S, Ning C, Zhou Y, Teng X, Wu X, Chu Y, Yu Y, Chen J, Tian L, Wang W. Molecular Epidemiology and Risk Factors of *Blastocystis* sp. Infections Among General Populations in Yunnan Province, Southwestern China. Risk Manag Healthc Policy 2020;13:1791-801.  
[PUBMED](#) | [CROSSREF](#)

3. El Safadi D, Cian A, Nourrisson C, Pereira B, Morelle C, Bastien P, Bellanger AP, Botterel F, Candolfi E, Desoubieux G, Lachaud L, Morio F, Pomares C, Rabodonirina M, Wawrzyniak I, Delbac F, Gantois N, Certad G, Delhaes L, Poirier P, Viscogliosi E. Prevalence, risk factors for infection and subtype distribution of the intestinal parasite *Blastocystis* sp. from a large-scale multi-center study in France. *BMC Infect Dis* 2016;16:451.  
[PUBMED](#) | [CROSSREF](#)
4. Somocurcio J, Alvarez G, Lara Campos G, Cahuana Aparco J, Calongos Porras E, Bermejo Cataño P, Tejada Llaca P. Oclusión arterial aguda asociada a hidatidosis diseminada [Acute arterial occlusion associated disseminated hydatidosis]. *Rev Peru Med Exp Salud Publica* 2014;31:375-9.  
[PUBMED](#) | [CROSSREF](#)
5. Stensvold CR, Clark CG. Current status of *Blastocystis*: A personal view. *Parasitol Int* 2016;65:763-71.  
[PUBMED](#) | [CROSSREF](#)
6. Kumarasamy V, Anbazhagan D, Subramaniyan V, Vellasamy S. *Blastocystis* sp., parasite associated with gastrointestinal disorders: an overview of its pathogenesis, immune modulation and therapeutic strategies. *Curr Pharm Des* 2018;24:3172-5.  
[PUBMED](#) | [CROSSREF](#)
7. Vielma JR. Blastocystosis: Epidemiological, clinical, pathogenic, diagnostic, and therapeutic aspects. *Invest Clin* 2020;60:53-78.  
[CROSSREF](#)
8. Khorshidvand Z, Khazaei S, Amiri M, Taherkhani H, Mirzaei A. Worldwide prevalence of emerging parasite *Blastocystis* in immunocompromised patients: A systematic review and meta-analysis. *Microb Pathog* 2021;152:104615.  
[PUBMED](#) | [CROSSREF](#)
9. Shlim DR, Hoge CW, Rajah R, Rabold JG, Echeverria P. Is *Blastocystis hominis* a cause of diarrhea in travelers? A prospective controlled study in Nepal. *Clin Infect Dis* 1995;21:97-101.  
[PUBMED](#) | [CROSSREF](#)
10. Barahona Rondón L, Maguiña Vargas C, Náquira Velarde C, Terashima I A, Tello R. Blastocystosis humana: estudio prospectivo, sintomatología y factores epidemiológicos asociados [Human blastocystosis: prospective study symptomatology and associated epidemiological factors]. *Rev Gastroenterol Peru* 2003;23:29-35.  
[PUBMED](#)
11. Vidal-Anzardo M, Yagui M, Beltrán M. Parasitosis intestinal: Helmintos. Prevalencia y análisis de la tendencia de los años 2010 a 2017 en el Perú. *An Fac Med* 2020;81:26-32.
12. Mokhtar AB, Ahmed SA, Eltamany EE, Karanis P. Anti-*Blastocystis* Activity *In Vitro* of Egyptian Herbal Extracts (Family: Asteraceae) with Emphasis on *Artemisia judaica*. *Int J Environ Res Public Health* 2019;16:1555.  
[PUBMED](#) | [CROSSREF](#)
13. Moheemmi N, Moradi M, Khalilian A, Maghsood AH, Fallah M. The relationship between blastocystis hominis infection and Irritable Bowel Syndrome (IBS) and comparing direct wet mount, stool culture, Formalin- Ether and trichrome staining procedures for identifying organisms. *Hormozgan Med J* 2015;19:85-92.
14. Vargas-Sanchez GB, Romero-Valdovinos M, Ramirez-Guerrero C, Vargas-Hernandez I, Ramirez-Miranda ME, Martinez-Ocaña J, Valadez A, Ximenez C, Lopez-Escamilla E, Hernandez-Campos ME, Villalobos G, Martinez-Hernandez F, Maravilla P. *Blastocystis* isolates from patients with irritable bowel syndrome and from asymptomatic carriers exhibit similar parasitological loads, but significantly different generation times and genetic variability across multiple subtypes. *PLoS One* 2015;10:e0124006.  
[PUBMED](#) | [CROSSREF](#)
15. Tunalı V, Öztürk EA, Ünver A, Turgay N. The prevalence of Blastocystosis among patients with gastrointestinal and dermatologic complaints and effects of *Blastocystis* spp. density on symptomatology. *Türkiye Parazitol Derg* 2018;42:254-7.  
[PUBMED](#) | [CROSSREF](#)
16. Nazeer JT, El Sayed Khalifa K, von Thien H, El-Sibaei MM, Abdel-Hamid MY, Tawfik RA, Tannich E. Use of multiplex real-time PCR for detection of common diarrhea causing protozoan parasites in Egypt. *Parasitol Res* 2013;112:595-601.  
[PUBMED](#) | [CROSSREF](#)
17. Al-Kaissi E, Al-Magdi KJ. Pathogenicity of blastocystis hominis in relation to entropathogens in gastroenteritis cases in Baghdad. *Eur. J. Sci. Res.* 2009;25:606-13.
18. Belleza ML, Cadacio JL, Borja MP, Solon JA, Padilla MA, Tongol-Rivera PN, Rivera WL. Epidemiologic study of *Blastocystis* infection in an urban community in the Philippines. *J Environ Public Health* 2015;2015:894297.  
[PUBMED](#) | [CROSSREF](#)

19. Speich B, Croll D, Fürst T, Utzinger J, Keiser J. Effect of sanitation and water treatment on intestinal protozoa infection: a systematic review and meta-analysis. *Lancet Infect Dis* 2016;16:87-99.  
[PUBMED](#) | [CROSSREF](#)
20. Rostami A, Riahi SM, Haghighi A, Saber V, Armon B, Seyyedtabaei SJ. The role of *Blastocystis* sp. and *Dientamoeba fragilis* in irritable bowel syndrome: a systematic review and meta-analysis. *Parasitol Res* 2017;116:2361-71.  
[PUBMED](#) | [CROSSREF](#)