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 enteroprotozoan 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 (≥5 parasites/field) [6], liquid stools, and brown color [7].
Blastocystis sp. and gastrointestinal symptoms

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. (≥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 ≥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 χ² 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%) cases.

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

| Characteristics | Total (%) | Blastocystis hominis | 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 (45) | 1.11 | 0.54 - 2.28 |
| Age (years)     | 0.263 | 0.98 | 0.96 - 1.01 | | |
| (mean ± SD)     | 50.9 ± 15.0 | 48.6 ± 16.3 | 51.7 ± 14.5 | | |
| Handwashing     | 0.066<sup>b</sup> | Ref. | | | |
| Before eating   | 147 (91.9) | 34 (85) | 113 (94.2) | 2.85 | 0.9 - 9.05 |
| No              | 13 (8.1) | 6 (15) | 7 (5.8) | Ref. | |
| After defecating| 0.258 | Ref. | | | |
| Yes             | 150 (93.7) | 36 (90) | 114 (95) | 2.11 | 0.56 - 7.9 |
| No              | 10 (6.3) | 4 (10) | 6 (5) | | |
| Access to sewage<sup>c</sup> | 0.414 | Ref. | | | |
| Yes             | 152 (95) | 37 (92.5) | 115 (95.8) | 1.86 | 0.43 - 8.18 |
| No              | 8 (5) | 3 (7.5) | 5 (4.2) | | |
| Drink boiled water | 0.113 | Ref. | | | |
| Yes             | 133 (83.1) | 30 (75) | 103 (85.8) | 2.02 | 0.84 - 4.87 |
| No              | 27 (16.9) | 10 (25) | 17 (42) | | |
| Regularly eats outside the household<sup>d</sup> | 0.188 | Ref. | | | |
| Yes             | 13 (8.1) | 1 (2.5) | 12 (10) | 4.33 | 0.55 - 34.4 |
| No              | 147 (91.9) | 39 (97.5) | 108 (90) | | |
| Dog owners      | 0.054 | Ref. | | | |
| Yes             | 122 (76.3) | 26 (65) | 96 (80) | 2.15 | 0.98 - 4.74 |
| No              | 38 (23.7) | 14 (35) | 24 (20) | | |
| Gastrointestinal symptoms | | | | | |
| Dyspepsia       | -0.001 | Ref. | | | |
| No              | 63 (39.4) | 6 (15) | 57 (47.5) | | |
| Yes             | 97 (60.6) | 34 (85) | 63 (52.5) | 5.12 | 2 - 13.11 |
| Abdominal distension | -0.001 | Ref. | | | |
| No              | 68 (42.5) | 6 (15) | 62 (51.7) | | |
| Yes             | 92 (57.5) | 34 (85) | 58 (48.3) | 6.05 | 2.37 - 15.49 |
| Abdominal pain  | 0.03 | Ref. | | | |
| No              | 50 (31.3) | 7 (17.5) | 43 (35.8) | | |
| Yes             | 110 (68.7) | 33 (82.5) | 77 (64.2) | 2.63 | 1.07 - 6.46 |
| Diarrhea        | 0.271 | Ref. | | | |
| No              | 88 (55) | 19 (47.5) | 69 (57.5) | | |
| Yes             | 72 (45) | 21 (52.5) | 51 (42.5) | 1.5 | 0.73 - 3.07 |
| Constipation    | 0.714 | Ref. | | | |
| No              | 72 (45) | 19 (47.5) | 53 (44.2) | | |
| 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.
<sup>c</sup>SD, standard deviation.
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

| 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* | Odd Ratio | 95% Confidence Intervals |
|-----------------|-----------|---------------------------|----------|-----------|-------------------------|
| Morphological stage |           | Gastrointestinal symptoms | P-value* | Odd Ratio | 95% Confidence Intervals |
| Vacular form    | 24 (60.0) | 22 (59.5) | 2 (66.7) | 0.806 | Ref. |
| Granular form   | 8 (20.0)  | 8 (66.7)  | 0 (0)    | ND      | ND |
| Ameboid shape   | 1 (2.5)   | 1 (2.7)   | 0 (0)    | ND      | 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 |

*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 determined.
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.

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