

PREVALENCE OF INTESTINAL PARASITES *BLASTOCYSTIS HOMINIS* AND *GIARDIA LAMBLIA* AMONG DIARRHEAL PATIENTS AT WASIT PROVINCE , IRAQ

Prof. Dr. Abdulsada A. Rahi ^[1]

Suadad B. Khairi ^[2]

^[1,2] Department of Biology, College of Science, Wasit University

Abstract:

Giardia lamblia and *Blastocystis hominis* are two common zoonotic intestinal parasites responsible for the majority of diarrheic cases, especially in children. The study was planned to determine the prevalence of both *Giardia* and *Blastocystis* co-infection in diarrheal patients and risk factors associated with these parasites at Wasit province, Iraq. Stool samples collected from 135 patients divided into four groups *G. lamblia* group, *Blastocystis hominis* group, mixed infection group, and healthy control group , within a period of October 2022 to October 2023, were examined using direct wet mount for detecting *Blastocystis hominis* and *Giardia lamblia* . By microscopic examination the results of the 135 ; 60 were positive of *Blastocystis hominis* ; 20 of *G. lamblia* and 30 of mixed infection. Two risk factors were significantly associated with higher risk of *Giardia lamblia* , *Blastocystis hominis* and co-infection including age category (6-17) years old and family size (≥ 5 members) by univariate analysis (P = 0.001, 0.001 respectively). Abdominal pain was the only gastrointestinal manifestation associated with the co-infection using the univariate analysis (P = 0.001).

Keywords: Intestinal parasites , stool , *Giardia* , *Blastocystis* , Wasit .

Introduction

Diarrhea in children is still one of the most common and serious health problems, particularly in low income developing countries. Not only bacteria and viruses are causative agents of diarrhea, but protozoan parasites are also accused. Protozoan diarrhea is found to be the second cause of gastroenteritis causing 100,000 deaths in infants and children (Lozano *et al.*, 2012). *Blastocystis hominis* and *Giardia lamblia* are protozoan parasites of many animals and humans. Both parasites are sharing the same pattern of transmission where they are typically transmitted indirectly through the fecal-oral route. On the other hand, both parasites are sharing the host response, age-prevalence curves, and environmental factors. Moreover, both zoonotic anaerobic protozoa have been reported in cases of traveler's diarrhea (Paschke *et al.*, 2011).

Blastocystis hominis is currently the most common intestinal protozoan identified in the human population worldwide (Nemati *et al.*, 2021) since its prevalence has often been reported to exceed 50% in developing countries from diverse geographical areas and, in particular, in the African continent (Guilavogui *et al.* , 2022) . This enteric microorganism has also been frequently detected in the gastrointestinal tract of a wide range of animals, including numerous groups of mammals, birds, fish, reptiles and insects (Hublin *et al.*, 2021) . Like other intestinal protozoa, the main mode

of transmission of *Blastocystis hominis* is the indirect fecal–oral route through the consumption of water and eventually food contaminated by resistant cystic forms of the parasite excreted by various hosts (Rauff-Adetotun *et al.*, 2020). Transmission can also occur through direct contact, either with infected humans or animals, due to the zoonotic potential of this microorganism. Based on its observed frequency, an estimated 1 billion people could be infected with *B. hominis* across the world, with a large majority of them asymptomatic carriers (Andersen and Stensvold, 2016).

Giardia lamblia is one of the most widespread intestinal protozoa that infects humans all over the world causing giardiasis (Escobedo *et al.*, 2018), it affects more than 280 million people annually worldwide transmitted by the fecal-oral route to maintain the life cycle (Thompson, 2016). Giardiasis can be asymptomatic or even symptomatic, clinical symptoms are very diverse which include weakness, weight loss, watery diarrhea, stinky stools, fatty diarrhea, abdominal cramps and bloating, nausea, vomiting, and mal-absorption syndrome (García *et al.* , 2017) . Among diarrheic subjects, both parasites were common findings. This is an interesting background to the debate concerning the role of those enteric parasites in causing gastrointestinal symptoms and the pathogenicity of *Blastocystis* (Forsell *et al.* 2016).

Materials and methods

Study subjects and collection of stool samples

A hospital-based cross sectional study was carried out on fecal specimens from 135 individuals attending AL-Karamah Teaching Hospital and AL-Zahraa Hospital from October 2022 to October 2023, for screening for parasite as part of routine check-up (asymptomatic group) or have GIT symptoms (symptomatic group). Demographic and clinical data of all participants were recorded. Stool specimens were collected in clean, dry, labeled plastic containers, sent immediately to the parasitology laboratory .

Direct Wet Mount Using Normal Saline (0.9%):

The stool was emulsified in normal saline to allow study the parasite shape and motility. Stool samples observed by the preparation of direct smear methods using clean glass slides , a small drop of normal saline (0.9%) or Iodine stain put on slide glass and mix well with a small portion of stool using wooden stick, then put a cover slide, and examine the sample under power amplify 40X (Tanyuksel and Petri,2003).

Statistical analysis

Data were collected, summarized, analyzed and presented using statistical package for social sciences (SPSS) version 2 and Microsoft Office Excel 2010. **One way anova test** was used to study difference in mean among more than two groups provided that the variable is normally distributed. **Chi-square test** was used to study association between any two categorical variables. The level of significance was considered at *P*-value of less 0.05 and highly significant level at 0.01 or less (Daniel, 2018) .

Results

Overall, the total prevalence rate of parasitic infections in the present study by light microscope examination was 60 (44.5%) of patients with diarrhea have *B. hominis* infection and 20 (14.8%) have *G. lamblia* infection, while 30 (22.2%) of patients with diarrhea have mixed infection with *B. hominis* and *G. lamblia* as shown in table and figure (1).

Table (1): Prevalence of intestinal parasitic infection by light microscope examination.

| Total number | <i>B. hominis</i> | | <i>G. lamblia</i> | | Mixed | | <i>P</i> |
|-------------------------|-------------------|------|-------------------|------|----------|------|----------|
| | <i>n</i> | % | <i>n</i> | % | <i>n</i> | % | |
| Microscopic examination | | | | | | | |
| 135 | 60 | 44.5 | 20 | 14.8 | 30 | 22.2 | 0.001 |
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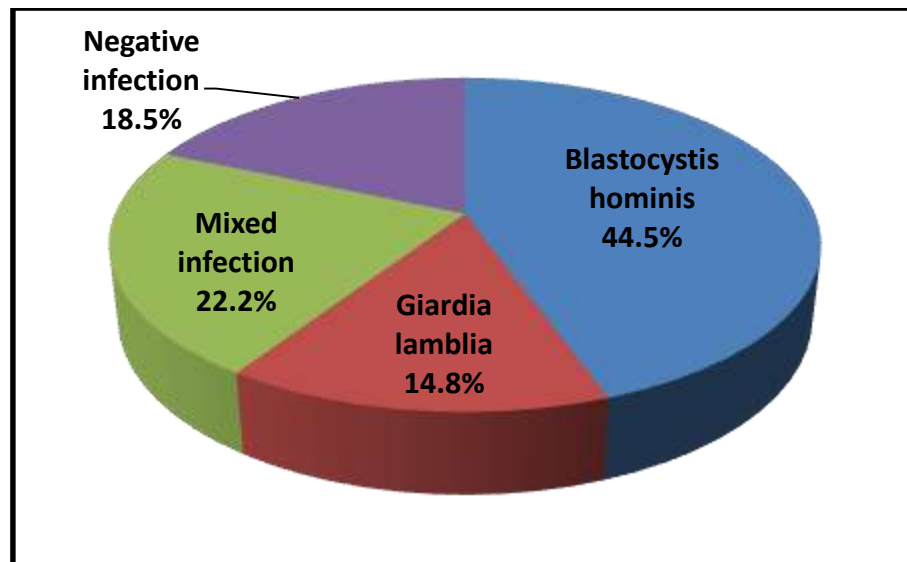


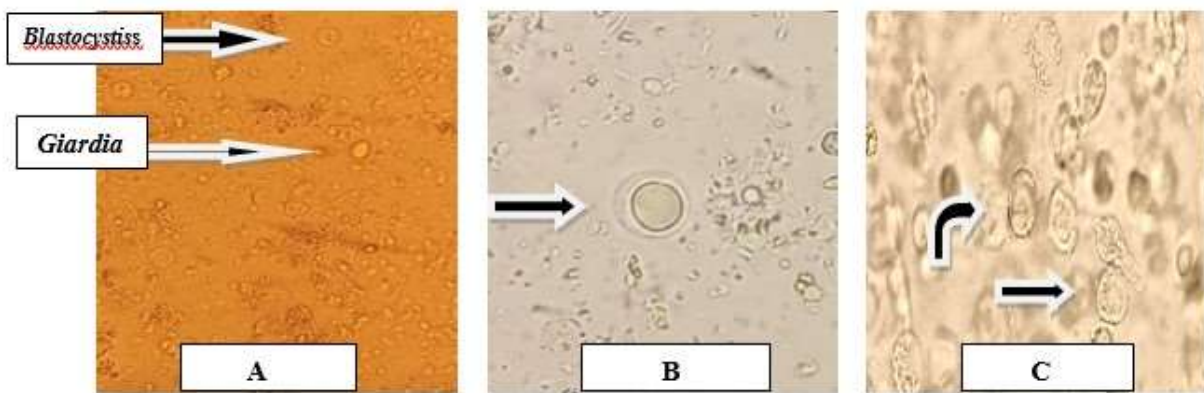
Figure (1) : Diagnosis of intestinal parasite in stool samples of diarrheal patients under light microscope.

The age of the studied sample ranged from <6 to ≥18 years. Out of 135 patients recruited in the study. In general, the highest incidence was recorded in the age group 6-17 with *Giardia lamblia* and co-infection (20.8% and 27.1%) respectively.

Regarding of gender male participants were slightly higher infection than females when infection of *G. lamblia* and Co-infection (18.1% and 23.6%) respectively. Depending of residence,

there is an increase of *B. hominis* and *G. lamblia* infections in patients living in rural areas, which amounted to (50% , 33%) respectively compared to (44.2 % , 14%) in urban areas respectively. The most common clinical manifestation associated with diarrhea was abdominal pain (46.7%), (19%) and (28.6%) in *Blastocystis* , *Giardia* and co-infection respectively. Interestingly, the occurrence of more than one clinical presentation was common.

Results of the association between light microscope finding and fathers education of the study showed that out of 135 patients (73) have father education while (37) have no father education . The infection rate is proportional to the size of the family, patients from tiny families (< 5) had the lowest infection rate of *Blastocystis*, *Giardia* and co-infection (29.2% , 4. 2% and 16.7%) respectively . The infection rate gradually increased as family size grew, reaching (47.7%, 17.1% and 26.1%) respectively in patients from big households (≥ 5). Associations of *G. lamblia* and *B. hominis* infections with different demographic, socioeconomic, and environmental factors were investigated and the results are presented in (Table 2) .



Figure(2) : A: Co-infection of *B. hominis* and *G. lamblia* in stool exam (40x) iodine mount , B: vascular shape of *B. hominis* in stool exam ,wet mount , C: granular shape of *B. hominis* in stool exam, wet mount.

Table 2 : Association of different variables with positive cases of *B. hominis*, *G. lamblia* and co-infection between both parasites by light microscope

| Variables | Examined number | <i>B. hominis</i> | <i>G. lamblia</i> | Co-infection | P |
|-----------|-----------------|-------------------|-------------------|--------------|---|
|-----------|-----------------|-------------------|-------------------|--------------|---|

Age groups in years

| | | | | | |
|------------------------------|-----|-----------|-----------|-----------|-------|
| < 6 | 16 | 10(62.5%) | 3(18.8%) | 2(12.5%) | 0.022 |
| 6-17 | 48 | 18(37.5%) | 10(20.8%) | 13(27.1%) | 0.303 |
| ≥18 | 71 | 32(45.1%) | 7(9.9%) | 15(21.1%) | 0.001 |
| Gender | | | | | |
| Male | 72 | 28(38.9%) | 13(18.1%) | 17(23.6%) | 0.044 |
| Female | 63 | 32(50.8%) | 7(11.1%) | 13(20.6%) | 0.001 |
| Residency | | | | | |
| Urban | 129 | 57(44.2%) | 18(14.0%) | 29(22.5%) | 0.001 |
| Rural | 6 | 3(50.0%) | 2(33.3%) | 1(16.7%) | 0.607 |
| Type of stool samples | | | | | |
| Watery | 28 | 15(53.6%) | 5(17.9%) | 6(21.4%) | 0.030 |
| Diarrhea | 54 | 29(53.7%) | 7(13.0%) | 13(24.1%) | 0.001 |
| Mucoid | 20 | 8(40.0%) | 3(15.0%) | 5(25.0%) | 0.305 |
| Normal | 33 | 8(26.7%) | 5(15.2%) | 6(18.2%) | 0.607 |
| Abdominal pain | | | | | |
| Yes | 105 | 49(46.7%) | 20(19.0%) | 30(28.6%) | 0.001 |
| No | 30 | 11(36.7%) | 0 | 0 | |
| Father education | | | | | |
| Yes | 93 | 39(41.9%) | 12(12.9%) | 22(23.7%) | 0.001 |
| No | 42 | 21(50.0%) | 8(19.0%) | 8(19.0%) | 0.010 |
| Family size | | | | | |
| <5 | 24 | 7(29.2%) | 1(4.2%) | 4(16.7%) | 0.105 |
| ≥5 | 111 | 53(47.7%) | 19(17.1%) | 29(26.1%) | 0.001 |

Discussion

This study recorded a high prevalence of intestinal parasitic infections in the stool samples with one or more detected parasites. Two protozoan parasites, *B. hominis* and *G. lamblia* were found to be responsible for human infection in the study of affected patients in Wasit province. Both *B.*

hominis and *G. lamblia* were common findings, among both diarrheic and non-diarrheic individuals. .

The results of the current study were similar to what (Doaa *et al.*, 2020) about the *B. hominis*, *G. lamblia* and mixed infection prevalence in Egypt which reported the prevalence of this parasite (44% , 10% and 20%) respectively were examined by direct wet mount. Our study disagreed with another study in Spain carried out by (Matovelle *et al.* , 2022) who recorded only (9.18% and 1.2 %) of the samples were identified positive for *B. hominis* and mixed infection respectively by microscope examination. This co-infection may be due to sharing the same environmental ,social condition , the pattern of transmission and the age prevalence curves between both parasites. Such differences in the prevalence rate may be due to variance in socioeconomic, cultural conditions, the pattern of transmission of the disease and techniques used in each study.

In the present study the prevalence of all parasite infection is higher in age between 6-17 years (20%, 27%) respectively compared with other age groups with statistical significance, which is consistent with the study of (Shurook and Mohammad , 2023) where they are found highest rate (58.62%) in age group (5-14) years old in Babylon province, Iraq. Another study agreed with our study, in Ghana (Nkrumah and Nguah , 2011) found the highest rate (18.6%) in age group (5-17) years old . Shatha and Nada , (2011) did not agreed with our study where found the highest rate (92%) in age group (5-10) years old in Baghdad . Parasite frequently observed included *G. lamblia* and co- infection, with observed overall prevalence rates in male (18% , 23%) than female (11% , 13%) respectively. These findings agreed with another study in Babylon province , were males had higher infection rate compared with females in *G. lamblia* (37.25%), (30.61%) (Shurook and Mohammad , 2023) . The finding was in accordance with (Doaa *et al.* , 2020) in Egypt that found high infection rate in *G. lamblia* and co-infection patients in male (64. 7% , 68%) than female (35.3% , 32%) respectively. Shatha and Nada , (2011) in Baghdad province found high prevalence of *G. lamblia* in female (26.7%) than male (0.9%) and this results did not agreed with our study.

The high prevalence of parasite found in rural than in urban, the studies conducted in Iraq reported the same results (94.83% and 72.5%) in Babylon and Wasit provinces (Mohamed, 2016) ; (Magda and Donia , 2022). The main symptoms of patients were diarrhea with abdominal pain , these results agree with (Magda and Donia , 2022) in Wasit province. Darwish *et al.* , (2021) in Syria that found the same results of infection. Patients with *B.hominis* infection may not show any symptoms (Kumarasamy *et al.*, 2018), while other patients may show the above symptoms, and this depends on the physiological and immune status of the patients. The highest rates of infection among parents whose had the education level (illiterate) and the lowest level of infection were among patients whose parents have education reached.

Results of this study consistent with other studies done by (Viesy *et al.*, 2022) in Ilam / Iran , but did not agreed with our results (Khalili *et al.* , 2012) in Iran . The infection rate is proportional to the size of the family. patients from small families (< 5) had the lowest infection rate of *B. hominis*

, *G. lamblia* and co-infection (29.2% , 4.2% and 16.7%) respectively . This finding was in accordance with (Mohy *et al .* , 2022) in Al- Najaf , who found the lowest rate in small families (18%) and highest rate in big families (83%). Also Doaa *et al .* , (2020) in Egypt found the same results.

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