How to Cite:
Ali, M. A., & Abass, D. F. (2022). Molecular diagnosis and identification of Blastocystis hominis at Wasit Province. *International Journal of Health Sciences*, *6*(S2), 14137–14143. https://doi.org/10.53730/ijhs.v6nS2.8693

Molecular diagnosis and identification of Blastocystis hominis at Wasit Province

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Abstract*---Blastocystis hominis*** is an enteric protozoan. It is frequently found in children and may cause chronic diarrhea. The present cross-sectional study was carried out during the period from October to December, 2021 in Wasit Province of Iraq. A total 100 stool samples from both gender and different ages (two years to the 60 years) suffering from primary gastro-intestinal disorders. All stool samples were examined microscopically by the direct method for identification of *B. hominis* and for the presence of other intestinal parasites. The number and percent of infection rate with *B. hominis* was 34(34%) in both of adults and children groups, the age group (14 – 35) years showed the highest rate of infection 12 (35.29 %) and the lowest rate was in ages (51-60 years) 3 (15%). The number and percent of infection among children were 4(11.76 %) and among age group of (36 – 50 year) were 10(29.41%). The number and percent of the infection among males were 24 (70.58%) while among females were 10 (29.41%).. According to the residence, urban areas were reported 24(70.58%) and rural areas were 10 (29.41%). Out of 100 stool samples examined 34 (34%) were positive with other intestinal protozoa. The most prevalent pathogenic intestinal parasites were *Entameba histolytica* and *Giardia lamblia* . The abdominal pain and distention were the most frequent symptoms associated with *B. hominis* infection followed by diarrhea.

***Keywords*---**Blastocystis hominis, PCR, feces, Wasit.

Introduction

Blastocystis spp. are anaerobic parasites that inhabit human intestinal tract, besides a wide range of animals (1). *Blastocystis spp.* are reported as the most common eukaryotic organism reported in human fecal samples, as infection is common in tropical, subtropical, and developing countries, with a prevalence rate

ranging from 30 to 50% in some developing countries compared with 1.5-10% in some developed countries (2,3,4). Regarding the pathogenic potential of *Blastocystis* spp., it was widely debated in the literature during the last two decades because it may be found in both symptomatic and asymptomatic patients (5) . Throughout the literature, the organism has been reported as a commensal organism, but also as a pathogen. In recent years, numerous studies have been published reporting that the infection with *B. hominis* is common in immunocompromised individuals (6,7). *Blastocystis spp* is a eukaryotic protozoan. It is a single-celled intestinal parasite found in the intestinal tract of humans and various animals. It is clear that most carriers of *Blastocystis hominis* appear asymptomatic, its place in human health remains very controversial; commensal or opportunistic. At the morphological level, *Blastocystis spp*. is a polymorphic protozoon with four main forms described in stools and/or in-vitro cultures: vacuolar, granular, amoeboid, and cyst forms (8) . The water-resistant infective cyst represents its transmissible form (9) , whereas the irregular amoeboid form has been suggested as the pathogenic form (10).

Blastocystis hominis is the most common intestinal parasite isolated in humans. However, a lot of controversies still surround it. Even it has a worldwide distribution, with a higher prevalence in developing countries, its burden is still under-estimated. Nowadays, interest concerning it is increasing due to its potential role as a human pathogen**,** a number of clinical and epidemiological studies implicate the parasite as a potential pathogen, while others exonerate it as an etiology of intestinal disease (11). Significant progress has been achieved on descriptions of the morphology and genetic diversity of *Blastocystis* but most aspects of its life cycle, molecular biology, and pathogenicity remain unresolved. Hence, the incidence of enteric infection in patients there is few studies focused, thus current study was one of these studies. The present study aimed to investigate the prevalence of *B. hominis* in patients suffering of gastroenteritis symptoms and to determine the effect of age, gender, district , living conditions and water sources and molecular detection of specific gene for *B. hominis* at Wasit province, Iraq.

Materials and Methods

Ethical Consideration

The proposal of the study was approved by the Ethical Committee of Wasit University, the patients were interviewed and the ones who were willing to participate and sign an informed consent were recruited.

Samples collection

Fresh stool samples were obtained randomly in patients who suffering from diarrhea in Al Karama Teaching Hospital in Wassit Province from October to December, 2021. Before analyzing the fecal samples the special questionnaire form was prepared to denote full information from each patient which was relevant to various epidemiological factors that might be responsible for parasite infection which included patient name, age, gender, region, period of infection.

The stool samples were collected without preservatives in dry, clean, plastic containers with tight fitting lids. Each sample was divided into two parts: First part was submitted to direct saline and iodine wet mounts examined microscopically for *B. hominis* (12). Second part was stored at -20°C for DNA extraction to detect *B. hominis* using PCR technique (13).

Genomic DNA preparation

The genomic DNA extraction of *B.homonis* from stool samples was done by using I-genomic Stool DNA Extraction Mini Kit (Bioneer, Korea, and Cart Number17451) , according to the manufacturers protocol.

Polymerase chain reaction for detection of *B. hominis*

Frozen stool samples was carried out by PCR thermocycler and GoTaq®Green Master Mix kit (Promega, U.S.A) according to manufacturer's instructions in final volume 25 µl reactions. Two pairs of different primers were used for detection of 1770 bp and1100bp16S-like ribosomal RNA gene of *B.hominis* , the first pair included the forward BH1 (5 TATCTGGTTGATCCTGCCAGT) and the reverse BH2 (3 TGATCCTTCCGCAGGTTCACCTAC). This pair of primers have been established by Silberman *et al.*(12) and also used by Init *et al.*(13), the PCR conditions consisted of on cycle denaturing at 94°C for 5 min, 35 cycles including annealing at 54°C for 1s, extending at 72°C for 1s,denaturing at 94°C for 30s, and additional cycle with a 5-min chain elongation at 72°C. the second pair of primers included the forward BH3 (5GGAGGTAGTGACAATAAATC 3) and the reverse BH4 (3` CGTTCATGAACAATTAC 5) which was used for Nested PCR as Böhm-Gloning *et al.*(1997) done, the PCR conditions consisted of one cycle denaturing at 94°C for 4 min,35 cycles including annealing at 54°C for 30s,extending at 72°C for30s, denaturing at 94°C for30s, and additional cycle witha5-minchaine longation at 72°C.The PCR products were electrophoresed in1% agarose gel with Tris-boric-EDTA buffer (Bio Basic, Canada),the fragments were visualized by UV light and fragment sizes were confirmed with bands of a DNA length standard (Promega, U.S.A) .

Statistical analysis

Descriptive analysis was used with percentages to express the positive samples of *Blastocystis*.

Results and Discussion:

By the examination of (100) stool sample of outpatient in Al-Karama-Teaching Hospital in Wasit province , from those patients only 34 (34%) samples were positive for *B.hominis* according to the two groups was mentioned in (Table1),which is examined using PCR technique.

Table 1. Percentage of *B,hominis* infection

Groups	Samples	Percentage

Positive *B,hominis*	34	(34%)
Negative *B,hominis*	66	(66%)
Total	100	(100%)

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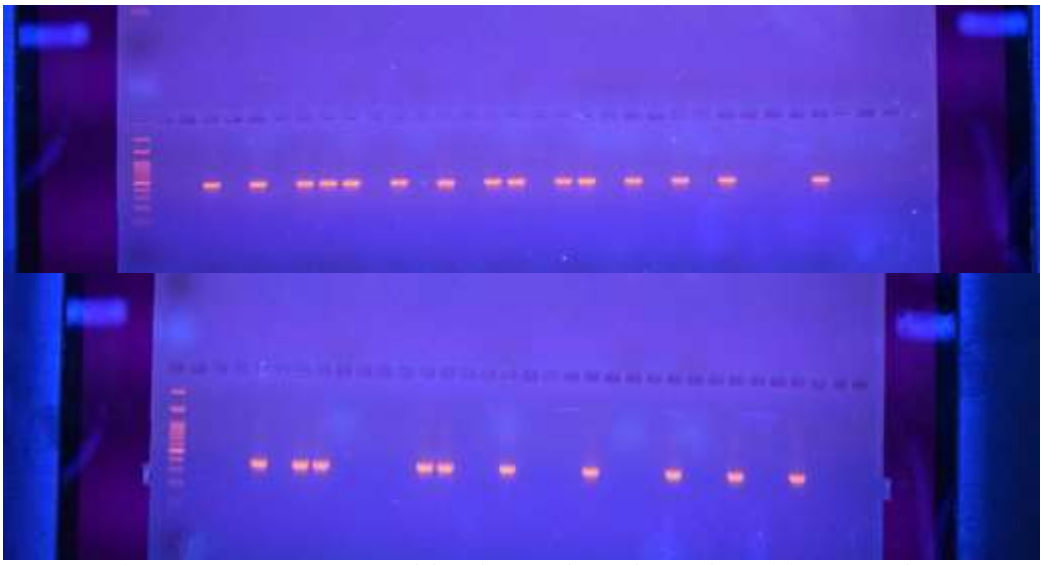

Figure (1): DNA assay of fecal samples, showed positive samples

The study showed that the rate of infection with intestinal parasites was 34% among positive sample for *B. hominis* . and 66% among negative subjects. The results of the statistical analysis showed that there is a significant difference at the level ($P \leq 0.05$) between the two groups as shown in(Table 1). By the examination of (100) stool sample the present study recorded (34) persons infected with gastrointestinal parasites (infection percentage (34 %) The results of this study are consistent with the result of previous studies in Samarra city, Iraq (13), Indonesia (14), and Egypt (15) . The reason for the difference may be attributed to many reasons, the most important of which are the service and environmental situation, as well as public hygiene, attention to personal culture, and the difference in the standard of living and social.

Categories representation in positive sample stool with *Blastocystis hominis*, recorded the highest infection 12 (35.29%). cases at age (14-35) years old, Percentage of infection. While the lowest infection were 4 (11.76%) cases at age (51 - 60) years old as in (Table 2).

Table 2. Distribution of *B. hominis* infection by age groups

Age groups Years	Positive(%)	Rural(%)	Urban(%)
2-13	8(23.52%)	2(25%)	6(75%)
14-35	12(35.29%)	4(33.33%)	8(66.66%)
36-50	10(29.41%)	3(30%)	7(70%)
51-60	4(11.76%)	1(25%)	3(75%)

Total (%)	34(100%)	10(29.41%)	24(70.58%)

The results of present study were discovered that the age group (14-35) years old had the highest prevalence of infection (35.29 %), followed by (36-50) years old (29.41 %), followed by (2-13) years (23.52 %), and finally by (51-60) years (11.76 %), with no statistically significant difference across age groups table (2). The finding results were similar to Termmathurapoj *et al* (16), but were different with Parker *et al* (17). The prevalence rate of infection with *B.hominis* in rural area was slower than in the urban area this may be due to the differences in sample sizes, patients in rural area may be treated in the inhabitants hospitals too far to reach the city hospitals, the result agrees with Wang *et al* (18) who found a relationship between urban and rural places in the infection rates with *B.hominis*, but it was different with Lee *et al* (19) who found that *Blastocystis* was prevalent among rural because of their close contact with animals.

Table 3 show the positive stool sample with gastrointestinal parasites according to the gender, the highest parasite infections were (24) cases in male (70.58 %) and 10 cases in female , (29.41%).

Table 3. Percentages of *B.hominis* infection by gender

Gender	Number of examined samples	*B.hominis* infection %
Male	70 (70 %)	24 (70.58%)
Female	30 (30%)	10 (29.41%)
Total	100 (100%)	34 (100%)

In the current study, the results showed that the prevalence of *B. hominis* in the males (70.58%) were more exposed to infection than females (29.41%) with no statistically significant difference between them (table3) . The results appeared that in the rural areas the prevalence rate was higher than in the urban areas. This may be return to practicing habits of both genders in both places, this finding was similar to the results found by Wang *et al.* (18); Ali and Mohammed (21) ,but disagreed with Parker *et al.*(17).On the other hand, our study disagreed with Al Saeed *et al.* (2013) in Duhok (22), who reported that the infection rate among children and adult males (4.63%) and (7.29%) respectively was higher than among children and adult females (3.19%) and (5.69%). In Kirkuk, Hammood *et al.* (2016) (23) reported that males [M:F ratio (1.58:1)] were more infected with *B. hominis* due to more outdoor activities (24, 25).

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