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Investigation of intestinal protozoa parasites in patients attending basrah hospitals

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Abstract
Background: *Entamoeba histolytica, Balantidium coli, Giardia lamblia and Blastocystis hominis* are most popular invalid parasites in lower gastrointestinal tract of humans in this study in Basra which causing acute enteritis and diarrheal disease in patients.
Objective: Investigate the most important pathogenic parasites in the digestive system of patients coming to the hospitals of Basra province.
Methods: The research under study has been implemented in Dar Alshifaa investment hospitable in Basra Province southern of Iraq, A total of 1050 stool samples collected from patients from less than one month years old to over than 70 years. A direct saline smear preparation was made and examined by light microscopy.
Results: The highest rate of infection was represented by *E. histolytica* in October 20%, The ratios of *G. lamblia*, *B. colli* and *B. hominis* was 0.57%, 0.38, 0.19 respectively. The infection rate of *E. histolytica* between 2.7%- 38.46% was registered among children from one day even to ages above of 75 years, the highest infection in age 6 years old (38.46%) and less in children from one day to 3 years (2.7-%- 2.93%). This work was scored highest infection in the males 11.38% while the females was 10.34%. A Pus cells with blood were a common symptom in infection patients.
Conclusion: There are four species ofparasites in Basrah-Sothern of Iraq are: *Entamoeba histolytica, Balantidium coli Giardia lamblia* and *Blastocystis hominis***,** that have the ability to infect all age groups in humans. They can be infect males and females in close ratios.

Keywords: Intestinal Protozoa, *Entamoeba histolytica, Balantidium coli Giardia lamblia, Blastocystis hominis*

Introduction

The humans intestinal infection by a vast number protozoa (Kho & Lal, 2018) [1], most of them cause several gut systemic diseases for example: inflammatory digestive system, disorder of metabolic, and cancer (Gopalakrishnan *et al*., 2018) [2]. Protozoa are a group of unicellular eukaryotic, that can be found in a many of environments: symbiotic, free-living or most likely are parasitic (Issa, 2014) [3]. *Entamoeba histolytica*, *Balantidium coli, and Giardia lamblia* are common parasites in gut of mammals which cases to acute bowels and diarrheal disease in all over the world, the life cycles and the pathogenic of *E. histolytica* (amoebiasis), *G. lamblia* (giardiasis), *B. coli* (balantidiasis), and *B. hominis* parasites are similar, as humans are infected by entering the cystic phases and then the vegetative phase (trophozoite) in the intestine alternates, cysts found in contaminated vegetative or water, sometimes through person to other especially in endemic areas, and even when swimming in water contaminated with parasitic cysts (Adam, 2001) [4]. (Duc *et al*., 2011) [5] observed lower risk in farmers handling animal feces, but washing their hands with detergents compared with who didn't washing their hands.

A treatment of infections by protozoa parasites, the most important of which is caused by *E. histolytica* (even in asymptomatic patients) to prevent developing of invasive disease, also to reduce the spread of its, noninvasive infection can be treated by paromycin, to get rid of intestinal parasite cysts (Haque *et al*., 2003) [6]. For invasive case and extra intestinal amoebiosis may be therapy by nitroimidazoles (metronidazole) are active against amoeba trophozoite form only (Haque *et al*., 2003; Gardiner *et al*., 2015) [6,7]. For a definitive treatment and to reduce the possibility of the disease returning, the second line of treatment is used with or after nitroimidazoles include diloxanide furoate or diiodohydroxyquin that good effective on the cysts and trophozoites stages (Haque *et al*., 2003; Gardiner *et al*., 2015) [6,7]. The existing work aimed to investigate the most important pathogenic parasites in the digestive system of patients coming to the hospitals of Basrah province, to complement other similar studies and find appropriate solutions, as well as prevent infection or to reduce infection in future and treat it before the development of the pathological condition that may expose the individual to serious secondary infection that may lead to the life of the infected individual.

Methods

Study design

The current study was carried out in Dar Alshifaa investment hospitable in Basrah Province southern of Iraq in the period between August 2024 until July 2025, the fieldwork involved the collection of stool samples from all patients attending this hospital complaining of abdominal pain, diarrhea, and stomach complaints. The age groups ranged from less than one month to over than 70 years, from various socioeconomic status. A questionnaire was design with all necessary general information's for each patient included name, age, gender, economic situation, living area, type of pain, and any medical history.

Stool samples collection, preservation, and transportation

A total of 1050 stool samples were collected in clean plastic containers. Firstly, the samples were macroscopically examined for blood, mucus, segments of worms, color and consistency of stools were recorded. Then all samples were examined microscopically with saline solution to observe the motile stage as much as possible. Then kept in cold box and transferred to the Parasitology Laboratory at Southern Technique University in Basrah, where the laboratory diagnosis work was done by using direct smear. Stool specimens were examined for intestinal parasites using the standard routine methods used by hospitals and microbiological laboratories for diagnosing parasite, as described by (Cheesbrough, 2009) [8].

Direct wet mount technique

A direct saline smear preparation was made and examined by light microscopy for detection the motile form of parasite, another preparation was made using Lugol's solution. About 2 gram of the faecal sample was kept to being ready for examination by direct smear method, approximately 0.2 gm of the feces (depending on consistency of stool) mixed carefully with drop of normal slain or Lugol's iodine solution on glass slide by stick until reach.

Statistical analysis

The SPSS program was used in this study for the purpose of statistical analysis of data which represented by using the Chi square, each analysis was made under probability level $p \leq 0.05$.

Results

(Table 1) and (Fig 1) were showed four different protozoan species were identified, with varying rates of infection, *Entamoeba histolytica* was the most prevalent parasite, detected in 115 patients, with rate of infection 10.95%. followed by *Giardia lamblia* which found in 6 patients, with low infection rate of 0.57%. Although less common than *E. histolytica*, giardiasis remains an important cause of intestinal disturbance. *Balantidium coli*, a less frequently, was observed in 4 patients, with infection rate of 0.38%, this parasite is often associated with exposure to pigs or contaminated water sources, *Blastocystis hominis* was the least common, detected in only 2 patients, with infection rate of 0.19%.

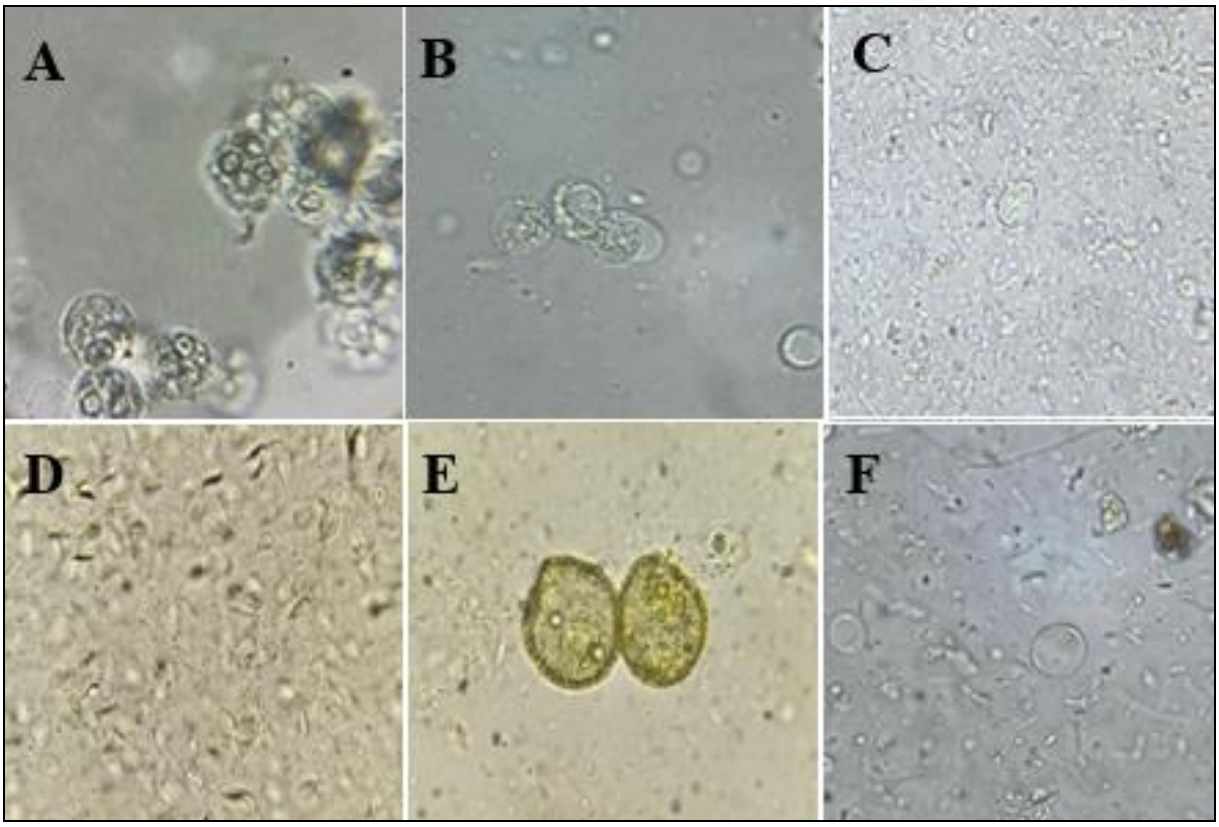

Fig 1: Intestine parasite under the microscope (40x), A: *E. histolytica* cyst B: *E. histolytica* trophozoite pseudopodia C: *G. lamblia* (cyst). D: *G. lamblia* heavy infection (trophozoite) E: *B. coli.* (cyst) F*: B hominis* (Vacuolar form)

Table 1. Prevalence of intestinal protozoan parasites among patients: infection rates in a sample of 1050 patients

Parasites	Total patients = 1050		
	Total no. Infection	Infection rate %	p-value
E. histolytica	115	10.95	< 0.0001
G. lamblia	6	0.57	
B. coli	4	0.38	
B. hominis	2	0.19	

According to the age (Table 2) and (Fig 2) were showed a variation in infection rates in different age groups. Patients aged 1 to 3 years showed low infection rates, ranging from 2.7% to 2.93%. The highest infection rates were observed in children aged 4 to 6 years, with the highest rate recorded at 38.46% in 6-year-old patients. The 7–15 years group showed a moderate infection rate (14.1%), Similarly, patients aged ≥16 years with infection rate of 14.99%.

Table 2. Distribution of infection rates according to age group and sex in the patients

Demographic characteristics		Total patients	Total no. Infection	Infection rate %	p-value
Age	1 year	239	7	2.93	< 0.000
	2 years	103	3	2.91	
	3 years	37	1	2.7	
	4 years	23	4	17.39	
	5 years	30	5	16.67	
	6 years	13	5	38.46	
	7- 15	78	11	14.1	
	≥16 years	527	79	14.99	
Sex	Male	615	70	11.38	*0.66*
	Female	435	45	10.34	

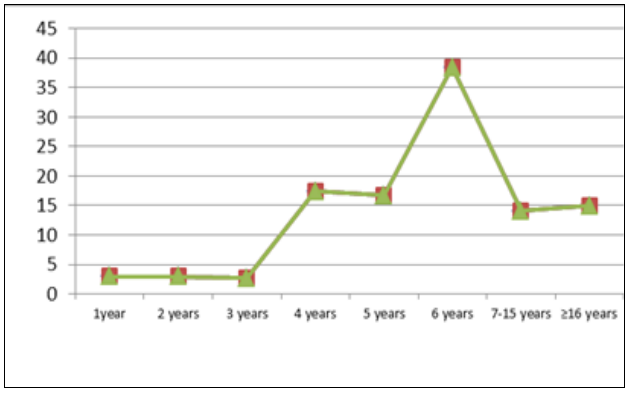

Fig 2: Distribution of infection rates according to age group

According to the sex the results showed no difference in infection rates between male and female patients. Males (n=615) had an infection rate of 11.36%, whereas females (n=435) had a slightly lower rate of 10.34%, despite the higher infected rate recorded in males, but with no significant p>0.05 (Fig 3).

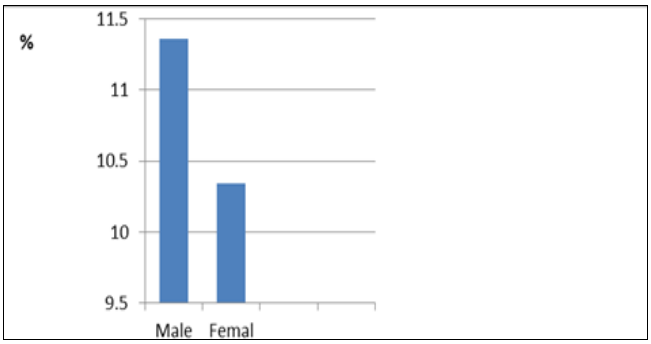

Fig 3: Distribution of infection rates according to sex group

(Table 3) and (Fig 4) were showed the distribution of infection rates across the months for a total of 1,050 patients, with 115 infections, (10.95%). A statistically significant difference was observed among the months (p<0.05), indicating that infection rates vary depending on the time of year. The highest infection rate was observed in October (20%), followed by November (14.7%) and September (13.41%). The lowest infection rate was observed in July (3.03%), followed by January (6%) and February (6.15%). Infection rates were moderate in August (11.11%), December (10.64%), April (10.40%), and May (10.98%).

Table 3: Monthly distribution of infection rates of intestinal parasite among patients

Months	Total patients	Total no. Infection	Infection rate %	p-value
August	45	5	11.11	0.12
September	82	11	13.41	
October	75	15	20.00	
November	102	15	14.70	
December	94	10	10.64	
January	50	3	6.00	
February	65	4	6.15	
March	79	6	7.59	
April	128	14	10.40	
May	173	18	10.98	
June	92	12	13.04	
July	65	2	3.08	
Total	1050	115	10.95	

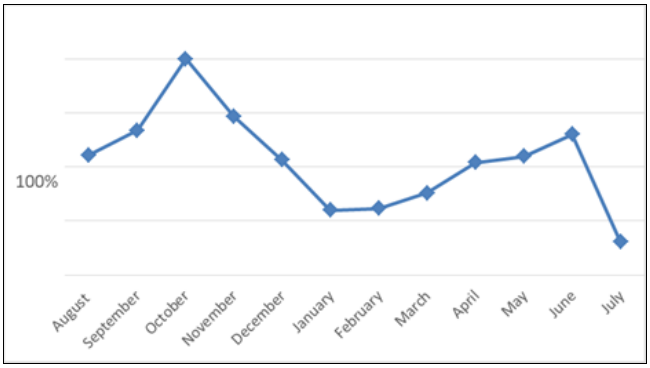

Fig 4: Monthly distribution of infection rates of intestinal parasite among patients

Discussion

The idea of the current study came because the large number of patients who complain of intestinal pain and the presence of pathological symptoms such as diarrhea and bloody stools, the general parasites infection due to widespread pollution in drinking water and lack of personal hygiene for some restaurants, especially for patients who travel frequently outside the country and eat some meat of unknown origin and may be mixed with pork meat, which is a reservoir for most parasites that infect humans (Slack, 2012) [9]. Four different species were isolated in this study among a sample of 1050 patients, *E. histolytica* was the most detected parasite, they were recorded among children from less than one year even to above of 75 years, with an infection rate of 10.95% (115 cases), indicating that it is the most prevalent protozoan in this study. This suggests that *E. histolytica* still poses a threat to public health due to its high transmission potential and common presence in areas with poor sanitation (Hasan *et al*., 2023) [10].

In contrast, the prevalence of *Giardia lamblia* was notably lower (0.57%) (6 cases). Despite its cause of intestinal infections worldwide, its low occurrence in this study may reflect variances in water sources, hygiene practices, or diagnostic sensitivity (Hajare *et al*., 2022) [11]. On the other hand, the ongoing work recorded low rates of *Balantidium coli* and *Blastocystis hominis* which identified in only 4 and 2 patients, respectively, with infection rates of 0.38% and 0.19%. These results indicate that these protozoa are rare among the examined patients, possibly due to their limited transmission routes or less common exposure in the studied environment. (Al-Tai, 2009) [12], pointed out the *E.*

histolytica was highest percentage 12.14% followed by *G. lambilia* 4.9% and *B. hominis* 0.61%. The infection rates of *E. histolytica* during the months (3.03-20%) less than (11) were (31%-69.8%). The statistical analysis shows clear differences in infection rates between age groups, while no statistical differences between sexes, According to the ages, in this study the patients were distributed in the early years in isolation for each year of life to find out the impact of the way of eating and the intellectual level of each age stage and the impact of personal hygiene of parents on the rates of infection, The highest infection rate was observed in children aged 6 years, with 38.46% (5 out of 13 patients), followed by children aged 4 and 5 years, who also showed relatively high infection rates (17.39% and 16.67%, respectively). These findings suggest that younger children, around the age of 4 to 6 years although they are more aware, this does not prevent them from being exposed to parasites, may be to intestinal protozoan infections. This could be due to weaker immune systems, close contact with contaminated environments (like in nurseries or schools), or poor hygiene practices at this age, in this way transmitting parasitic cysts between them became easily (Gebru *et al*., 2023; Taheri *et al*., 2011) [13,14].

The infection rate among infants (1 to 3 years old) was low, ranging from 2.7% to 2.93%, which might reflect closer parental supervision and more controlled food and water sources, especially in the first year the child depends on breastfeeding to product him or her from several diseases (Lopez *et al*., 2006) [15]. In older children (7–15 years), the infection rate was 14.1%, while in individuals aged 16 years and above, it was also 14.96%. Although not the highest, these rates indicate that infections are still present in older age groups but less than in the younger children. During the age period of 7-15,children are more aware, but this period is critical due to the rapid hormonal changes of their maturity, so it is difficult to prevent them from eating or drinking some foods that may be contaminated with cysts of parasites, (Mroczek & Little, 2006) [16], was confirm that personality growth is an important for research, the behavior of individual varies depended on several factors including genetic and environmental foundations of personality traits. (Krueger & Johnson, 2008) [17], which plays an important role in the overall health outcomes of a person (Ozer & Benet-Martínez, 2006) [18], the personality changes and social developments persists well after adulthood period to reach its peak in middle age of life (Srivastava *et al*., 2003) [19].

According to sex, males had a slightly higher infection rate (11.38%) compared to females (10.34%) with no statistically significant (p=0.66), indicating that sex does not appear to play role in the risk of infection in this population, this results identical to study of (Acuna-Soto *et al*, 2000) [20], that recorded infections in male higher (14.18%) than female (11.42%) without significant (ages between 1-6 years) by used PCR to diagnoses the samples, (Al-Tai, 2009) [12], was recorded *E. histilytica* in male highest than female by using microscopic examination. According to the monthly distribution of intestinal parasite infections, although there is some variation in infection rates across the months, but there is not statistically significant (p=0.1205), the highest infection rate was recorded in October (20.0%), followed closely by November (14.7%) and September (13.41%). In contrast, the lowest rate was seen in July (3.03%), suggesting a possible decrease in transmission during mid-summer and the ratio may affected by seasonal changes, potentially influenced by environmental factors, or increased exposure due to the beginning of the school year and seasonal viral activity (Pawestri *et al*., 2021) [21].

E. histolytica is an important cause of traveler's diarrhea, the highly of infections happen at the South and Central America, the Indian subcontinent and Africa. The risk to travelers humans is paralleled to the local endemicity (Slack, 2012) [9]. *E. histolytica* is a major type of parasites cause mortality in world, especially in countries that characterized by less of health services (Ximénez *et al*., 2009) [22]. About 4 to 10% of the infection with Amoebiosis develop essential clinical symptoms through a year, *E. histolytica* dysentery is a description as the third most dangerous disease can be cause of humans death after Malaria and Schistosomiasis (Mortimer & Chadee, 2010; Ghasemi *et al*., 2015) [23,24]. Amoebiasis infection of highest encumbrance in Asia, It is mainly recorded in the poor hygienic conditions. The cysts and few of Amoeba trophozoits stage are release with human feces to environment and lack of good sewage system, this ensures the spread of amoebiasis (Forson *et al*., 2018; Shrestha *et al*., 2019) [25, 26]. The prevalence of intestinal *E. histolytica* In Indian subcontinent was found 11.7% (Samie *et al*., 2012) [27]. In Bangladesh, it was the most prevalent parasite species as well also (Haque *et al*., 2003) [6]. By PCR, in urban slums in Dhaka was reported 4.2% among children when examining 2000 samples of stool (Haque *et al*., 2006) [28]. 11% among Bangladeshi children in the first year of life, this same to current study that recorded amoebiosis less than one year (Mondal *et al*., 2012) [29].

On the other side, *E. histolytica* was prevalent in Persian Gulf areas, 30% for it was recorded in United Arab Emirates (El-Bakri *et al*., 2013) [30]. As well as reported in Jeddah and Saudi Arabia, among humans less than 16 years old (Hegazi *et al*., 2013) [31]. In Iran a prevalence was 3.45% by using PCR (Stauffer *et al*., 2006) [32]. In China, recorded prevalence 11% in a study conducted on this disease in 7 provinces (Yang *et al*., 2012) [33]. The rate of it in Gaza from 2008 to 2017 were 358.8/100,000 inhabitants (Hamarsheh & Amro, 2020) [34]. When compare this study with other studies in Iraq, we find incidence rate for *E. histolytica* (13.17%), (Al Saqur *et al*., 2017) [35], that have recorded total infection 3.78% in all governorates of Iraq (the lowest infection rate was recorded in Anbar 0.5% and the highest in Al-Najaf Al-Ashraf 18.6%).

Conclusion

There are four species of parasites in Basrah-Sothern of Iraq are: *Entamoeba histolytica, Balantidium coli Giardia lamblia* and *Blastocystis hominis***,** That have the ability to infect digestive system of all age groups in humans. They can be infect males and females in close ratios.

Ethical Approval

This study was conducted with the approval of the Research Ethics Committee of the Basra Health Directorate, in accordance with protocol EC/*82* dated July 28, 2024. Informed consent was also obtained from all participating patients.

Conflict of Interest

All authors declare that there are no conflicts of interest in this article.

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