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DIRECT SMEAR DETECTION OF PARASITIC INFECTION CAUSES DIARRHEA IN PATIENTS OF WASIT PROVINCE, IRAQ

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Abstract

Diarrhea is a common symptom of many diseases of the digestive system. Infectious pathogens are the most common cause of diarrhea in developing countries where sanitation and hygiene are inadequate. Due to the lack of preventive measures and limited medical resources in these countries, there has been a significant increase in the incidence, morbidity and mortality of infectious diseases such as diarrhea. Poverty, illiteracy, lack of sanitation, lack of clean water, and the hot and humid tropical climate are all factors that contribute to intestinal parasitic infections. Infectious diarrhea is a major cause of morbidity worldwide. The objective of this cross-sectional study is to detect diarrhoea-causing protozoan parasitic infection by direct smear test in Wasit district. Between January and March 2023, 206 diarrheal stool samples (97 men and 109 women) were randomly collected from patients in Wasit Province. The samples studied were used to detect the presence of intestinal parasite infection, and pre-tested formulations were used in the study. Prognosis was adjusted for demographic parameters such as age, sex and place of residence, and the history of diarrhea was recorded by direct smear microscopy to detect intestinal parasite infection. The results showed that protozoa included Cryptosporidium parvum (2.4%), Blastocystis hominis (4.8%), Giardia lamblia (6.7%) and Entamoeba histolytica (47.5%). Finally, the incidence of parasitic infections is high and is influenced by personal hygiene. Comprehensive health education is therefore essential with the aim of reducing parasitic infections.

Keywords: Protozoa, *Cryptosporidium parvum*, *Blastocystis hominis*, *Giardia lamblia*, *Entamoeba histolytica*

Introduction

Gastrointestinal parasite infection is a common problem in third world countries [1]. It is important to note that most cases of parasitic diarrhea are short-lived, mild, and self-limiting, whereas others may be associated with chronic diarrhea and severe morbidity and mortality [2]. Socioeconomic factors such as poor sanitation, lack of clean water and sanitation, and low socioeconomic status are known to play a major role in susceptibility to infection [3].

Despite significant improvements in access to basic services, in 2020 2 billion people worldwide will still lack access to safe drinking water, 40% will have access to basic handwashing facilities and 3.6 billion people will have access to safe drinking water. estimated to have no access to managed sanitation. It will never be 1.7 billion. No basic sanitation facilities. Of particular concern is the lack of progress in global hand-washing practices. The incidence and prevalence of intestinal parasites varies by country and several environmental, social and geographic factors are major risk factors for the prevalence of parasitic infections, especially protozoan infections. In this regard, age, gender, lack of sanitation, source of drinking water, personal hygiene, location, contact with animals, and seasonal changes are the most commonly reported risk factors for parasitic protozoan infections in humans [4]. Intestinal parasitic infections are common worldwide, with a higher incidence in poor and socioeconomically disadvantaged tropical and subtropical regions. These insect invasions are clearly associated with human malnutrition and pose a serious threat to public health worldwide [2]. Infections with this parasite are common worldwide, but are more common in tropical/subtropical climates, the Middle East, and areas with poor sanitation [5]. In developing regions, the lack of diagnostic tests can make it difficult to identify the etiology of diarrheal disease, and often requires empirical treatment [6]. Amebiasis is a parasitic disease caused by the extraintestinal protozoan Entamoeba histolytica. The infection mainly affects people in developing countries where sanitation is poor, where it is endemic. Infected cysts are transmitted by the fecal-oral route and the cysts are eventually excreted in the ileum, forming invasive trophozoites (amoeba). E. histolytica resides primarily in the large intestine and causes no symptoms, but amoebic invasion of mucous membranes and epithelial cells leads to intestinal embolism, possibly by unknown signs [7]. Parasites, especially those that live in the human digestive tract, cause an estimated 3.5 billion infections worldwide. Coccidia is one of the causes of protozoan intestinal infections in humans and animals. The main symptom is diarrhea, which can be bloody in severe cases. Most cases of coccidiosis are asymptomatic, but severe symptoms and death can occur in young and immunocompromised patients [8]. Blastocystus is a zoonotic parasite of the large intestine found in humans and animals. Parasites can cause a variety of digestive tract disorders, including indigestion, diarrhea, abdominal pain, bloating, nausea, and vomiting [3]. Common causes of diarrheal disease in low-income countries are rotavirus, *Shigella*, *Cryptosporidium*, *Campylobacter*, *Salmonella*, and enterotoxin-producing Escherichia coli (ET-STEC) and Ciga [6]. *Schistospora bailii* (formerly *Isospora bailii*) is a single-celled protozoan belonging to the phylum Apicomplexa that lives in the epithelium of the human small intestine and causes diarrhea [8]. Protozoa are responsible for many chronic and serious human diseases worldwide. They are spread through food, water, blood transfusion and organ transplant. Subgroups of tropical parasites include *Entamoeba histolytica*, *Cryptosporidium*, and *Giardia*, which cause debilitating and fatal human diseases [9]. This study was conducted to determine the prevalence of intestinal parasites in Wasit district and determining the factors that contributing in prevalence of intestinal parasites.

Materials and Methods

Study design and patients

A cross-sectional study was conducted in Wasit district. A total of 206 faecal samples (97 men and 109 women) randomly collected from patients in Wasit district between January and March 2023 were included in the study. The samples used in the study used a pre-test formulation to detect the presence of intestinal parasite infection. The cases were matched by demographic parameters such as age, sex and place of residence and history of diarrhea was recorded by direct smear microscopy. detection of intestinal parasite infection.

Stool Samples Examinations

1. *Direct wet mount by using normal saline (0.9%) method*

To study parasite size and movement, fecal samples were excised in saline [10, 11], saline was placed in the center of a slide, and a small portion of the fecal sample was sterilized with saline solution. The lower stem was then removed and examined under a microscope under X10 and X40 objective [10, 12]

2. *Direct Smear by using Lugol's Iodine*

This method was demonstrated as follows [10, 13]. Briefly, place a drop of Lugol's iodine solution on a glass slide, place a small amount of human feces (1 g) on the Lugol's iodine drop, mix thoroughly with a wooden stick and press the blade with tweezers or your fingers, cover it with cover slip and examined at X40.

3. *Formal-Ether concentration technique*

This technique has been recommended as the best comprehensive technique for culturing fecal parasites [10, 14]. Add 1 g of feces to about 10 ml of 10% ether formaldehyde, emulsify with glass beads and mix well by shaking for about 20 seconds.

4. *Modified Acid-Fast staining technique (Z-N Technique)*

The method is as follows [10]. Briefly, smears are prepared from fresh feces, air-dried and fixed in methanol. Fixed smears were stained with phenolphoxine for 10 min, acid alcohol for 10–15 sec and malachite green for 3 min. Examine the stained smear using a light microscope at X10 magnification to detect *C. parvum* oocysts that appeared as a red or pink in color.

Statistical analysis

Chi-square (x^2) test and One-Way ANOVA in the GraphPad prism Software were used to detect significant differences between the different groups of the study. Values were represented as percentage and variation was considered significant at $P<0.05$ (*), [15].

Results

Among totally 206 fecal samples, the prevalence rate of intestinal parasites was 61.6% (127/206) including 8.7% in bloody diarrheic samples and 52.9% in watery diarrheic samples (Table 1).

Table (1): Prevalence of intestinal parasites among totally 206 fecal samples

Test	Infected		Not infected		Total	
	No.	%	No.	%	No.	%
Bloody diarrhea	18	8.7	2	0.9	20	8.7
Watery diarrhea	109	52.9 *	77	37.3	186	52.9
Total sample	127	61.6	79	38.3	206	61.6

To detect the type of intestinal parasites, microscopic examination of fecal positive samples revealed that the prevalence rate of *E. histolytica*, *G. lamblia*, *Cryptosporidium* spp. and *Blastocystis* spp. were 77.1%, 11%, 3.9% and 7.8%, respectively (Table 2). In addition, the findings showed that *E. histolytica* was increased significantly (P<0.05) in both males and females of bloody (6.2% and 7.8%, respectively) and watery (27.5% and 35.4%, respectively) diarrhea. Significantly, *E. histolytica* was more prevalent (P<0.05) in female (35.4%) than males (27.5%) of watery diarrheic patients, but not in bloody diarrhea patients who revealed no significant differences (P>0.05) in prevalence of *E. histolytica* between females (7.8%) and males (6.2%).

Table (2): Type of intestinal parasites among totally 127 positive fecal samples

Type of parasite	Bloody diarrhea				Watery diarrhea				Total	
	Male		Female		Male		Female			
	No.	%	No.	%	No.	%	No.	%	No.	%
E. histolytica	8	6.2	10	7.8	35	27.5	45	35.4 *	98	77.1 *
G. lamblia	0	0	0	0	5	3.9	9	7	14	11
Cryptospordium	0	0	0	0	0	0	5	3.9	5	3.9
Blastocystis	0	0	0	0	6	4.7	4	3.1	10	7.8
Total	8	6.2	10	7.8	46	36.2	63	49.6	127	100

Statistical analysis to detect association between the type of intestinal parasite and age showed a significant difference (P<0.05) in prevalence rate of *E. histolytica* and *Blastocystis* spp. between age groups but not in other parasitic infection (Table 3). However, significant higher *E. histolytica* and *Blastocystis* spp. infections were seen in patients of <18 years (43.3% and 5.5%, respectively), (P<0.05).

Table (3): Distribution of intestinal parasites according to age and gender of study patients

Type of parasite	Age	Bloody diarrhea		Watery diarrhea		Total	
		Male	Female	Male	Female		
		No.	No.	No.	No.	No.	%
E. Histolytica	≥5	3	1	9	8	21	16.5
	<5 ≥ 18	1	3	7	11	22	17.3
	<18	4	6	19	26	55	43.3 *
G. lamblia	≥5	0	0	2	1	3	2.3

	<5 ≥ 18	0	0	3	5	8	6.2
	<18	0	0	0	2	2	1.5
Cryptospordium	≥5	0	0	0	1	1	0.7
	<5 ≥ 18	0	0	0	4	4	3.1
	<18	0	0	0	1	1	0.7
Blastocystis	≥5	0	0	0	0	0	0
	<5 ≥ 18	0	0	3	0	3	2.3
	<18	0	0	3	4	7	5.5 *
Total samples		8	10	46	63	127	100

Regarding the source of water, significant increases (P<0.05) in parasitic infections were recorded in patients who ingested purified water (37.8%) more than those of station water (23.7%), (Table 4).

Table (4): Association of water source to parasitic infections in study patients

Source of water	Infected		Not infected		Total	
	No.	%	No.	%	No.	%
Station water	49	23.7	33	16	82	39.8
Purified water	78	37.8 *	46	22.3	124	60.1
Total	127	61.6	79	38.3	206	100

Concerning the efficacy of nutrition, prevalence rate of intestinal parasites was increased significantly (P<0.05) in patients undergo malnutrition (44.6%) when compared to other group of patient who received sufficient amount of nutrition (16.9%), (Table 5).

Table (5): Association of malnutrition to parasitic infection in study patients

Malnutrition	Infected		Not infected		Total	
	No.	%	No.	%	No.	%
Yes	92	44.6 *	27	13.1	119	57.7
No	35	16.9	52	25.2	87	42.2
Total samples	127	61.6	79	38.3	206	100

In this study, intestinal parasites were showed a significant higher prevalence (P<0.05) in patients of rural areas (48.5%) when compared to those of urban areas (13.1%), (Table 6).

Table (6): Association of residence to parasitic infection in study patients

Residence	Infected		Not infected		Total	
	No.	%	No.	%	No.	%
Urban	27	13.1	27	13.1	54	26.2
Rural	100	48.5 *	52	25.2	152	73.7
Total samples	127	61.6	79	38.3	206	100

Discussion

Intestinal parasitic infections and related diseases are more prevalent in tropical and subtropical regions of the world and more common in developing countries. This high infection rate can be attributed to poor sanitation standards in developing countries. The prevalence of intestinal parasites is mainly due to poor sanitation, inadequate supply of clean water and ignorance of health promotion methods. Poor hygiene results in a high individual incidence of intestinal parasitosis. The high prevalence of intestinal protozoan infection in Wasit district can be linked to contamination of drinking water. Because the infective dose is relatively low, about 10 out of 1000 in healthy people, eggs and other infective stages can spread from person to person, especially within families, through small amounts of water or contaminated food. Consumption of freshly squeezed apple juice has been associated with transmission of food-borne infections and consumption of cooked canned foods and raw milk have been reported as risk factors for food-borne infections [17, 16].

This study assessed the prevalence of intestinal parasites in patients attending a waste and health hospital. Microscopic analysis of stool samples showed a prevalence of 61.6% in diarrhea cases and 38.3% in uninfected cases. The results of this study indicated parasite prevalence in the study close to 57.5% [17]. In contrast, this study showed that the prevalence of parasitic infection was low in southwestern Ethiopia (83%) and 62.2% in TEDA health center in northwestern Ethiopia [43]. This indicates that parasite infection varies by location and country. Differences may be due to differences in study population characteristics, geographic distribution, and diagnostic methods used in these and other studies.

Our results are in line with Shekar and Hussain (2015), who found an overall parasite infestation rate of 52.77% in Diyala district [18]. Furthermore, the present results are consistent with the findings of 54.9% of kidneys infected with intestinal parasites in Ethiopia [19] and 42.3% of kidneys infected with intestinal parasites in Iran [20]. , a study in Libya found 38% of patients in the city of Benghazi [21]. Our results show 6.2% [22] in Saudi Arabia, 73% [23] in Iraq, 15.5% [24] in Iran, 11.75% [25] and 28.9% in Saudi Arabia, which is consistent with the findings of other researchers in second Ethiopia. [26].

In this study, the prevalence of protozoa and intestinal parasites was assessed according to the gender of the study participants. The results showed that there was a significant difference in the prevalence of parasite infection between male and female study participants. Related to this, the prevalence in females and males was 57.4% and 42.5%, respectively. This may indicate that the risk of infection is not the same for men and women. The highest prevalence of intestinal worms found in females in this study was Muhammad [27] Male patients (65.7%) had a higher prevalence of intestinal worms than females (57.4%). [1] Male patients (42.5%) were more likely than female patients (20%). However, this finding is in line with Acharya et al. [28] Their study showed that the prevalence of parasitic infection was highest among females (32.11%) as compared to males (21.99%). Additionally, Patel et al. [29] and Zemene and Schifferau [30] reported similar results in their study that women have a predominance of parasitic infections. In Ethiopia, women are more involved than men in caring for children, cleaning the home, processing raw fruits and

vegetables, and cleaning and washing home gardens, therefore a major cause of exposure to contaminated soil diseases or water. Our study contradicts the results of Rahi and Majeed (2019) [17] who estimated that *E. histolyticus* infected (25%) males and 33 (16.5%) females.

In this study, the prevalence of intestinal protozoa and parasites was assessed based on the study participants' place of residence. The results showed that there was a significant difference in the prevalence of parasitic infections between rural and urban study participants. To put this in context, the prevalence in rural and urban areas was 48.5% and 13.1%, respectively. Permanent residence can also affect people infected with the parasites. People who live mainly in rural areas are engaged in agricultural activities (crops and animals) and are exposed to soil, animals, manure and other animal waste. This suggests that it is related to Cryptosporidium. Pets were a significant risk factor for infection among study participants. Cryptosporidiosis is a common parasitic disease of the gastrointestinal tract caused by protozoa of the genus Cryptosporidium that infects a wide variety of animals worldwide, including humans. Cryptosporidium parvum is also a common enteritis in young sheep and goats. Diarrhea can be caused by a single infection, but more often it is associated with mixed infections. The infection may be associated with episodes of severe diarrhea. Mortality is high in sheep aged 4 to 10 days and goats aged 5 to 21 days [31]. These results are in line with those of Alkhanak et al. (2022), [32], (84.4%) and El-Settawy and Fathi, (2012), [33] reported the highest infection rate of 80.9% in rural Egypt. Other similar results have been reported by Gawad et al. (2018), [34] reported a positivity rate of 79.8% in immunosuppressed patients with diarrhea using nested PCR in a rural area of Bani Sufa, Egypt. This result contradicts another study conducted by Muhammad (2018) [35] in Al-Diwaniya district. The study documented 63.3% of patients and children in a rural teaching and maternity hospital in Diwaniya district using conventional PCR techniques. Furthermore, Gabor et al. (2019), [36] documented a high infection rate (47.8%) in humans in rural areas by nested PCR [37] in Minya/Egypt. The results of this study showed an overall prevalence of E. coli. histolytica 77.1%. These results are in line with those of Rahi and Majeed (2019) [17] and Aza et al, who reported the prevalence of E. coli. histolytica in 41% patients of Vasit district. (2003), [38] reported an intestinal parasite prevalence of 21.0% in seven villages in Malaysia [39, 40]. Gawood et al. (2018) documented an 11% prevalence for E. historica in children in Delhi, India, and Pakistan, respectively [34, 38, 40]. 14 out of 127 (11%) in the present study showed proliferation of G. lamblia. Previous studies have associated high levels of intestinal parasites with poor environmental and personal hygiene, inadequate water supply, and poor service practices [42]. , In the present study, the prevalence of Cryptosporidium was 3.9%, a result consistent with that of Abdul-Sada (2015) [43] [43] The rate of Cryptosporidium infection among children hospitalized via the MZN in the Middle Euphrates region was 8.35% . In a previous study by Jawad (2015) [44] in the city of Al-Dewaniya, M.Z.N. showed a positivity rate of 0.625% in children with diarrhea using . (2018), [45] reported 3.5% of immunosuppressive and immunocompetent Turkish patients with diarrhea using M.Z.N. Rahi and Majeed (2019), [12] reported using M.Z.N staining in 12.5% of patients with diarrhea in Wasit district and al. -Abudi, (2019), [46] 5.1% of diarrheal patients reported in Al-Rifai city/Thi-Kar district M.Z.N. Saha et al. (2019), [47] documented 20% of symptomatic immunocompromised children in Delhi/India

and made a comparative clinical assessment by Ziehl-Nielsen stain. Prevalence of cryptosporidiosis in different patients with diarrhea was being vary widely from country to country and region to region. This may be due to differences in socio-demographic factors within the country.

Conclusions

The prevalence of intestinal protozoan infection varied according to the age, sex, and place of residence of study participants. Therefore, sensitization training should be conducted in the communities of Seth district to reduce the parasite infestation.

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