Prevalence of Gastrointestinal Parasites *Entamoeba* Species in Diarrheic Patients in Baqubah, Iraq

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Abstract: <u>Background</u>: Infection with intestinal parasites regard as the most important causative agent for diarrhea. <u>Object</u>: Study the infection rate of E. histolytica/dispar/moscovskii in fecal samples of patients with diarrhea, investigate the relationship between Entamoeba species infection and some socio-environmental factors and determine the possible co-infections between Entamoeba spp. and other parasites. Methods: The populations of this study included 326 patients with different age groups who came to the parasitology laboratory in BaqubahTeaching Hospital and AL-Batool Maternity Teaching Hospital/Diyala province, suffering from gastrointestinal complaints with acute diarrhea. All stool samples were laboratory diagnosed by microscopy. <u>Results</u>: The rates of the enteric protozoan detected by microscopy from patients with diarrhea wereE. histolytica /dispar/miscoviskii 150(46.1%), Giardia lamblia 27(8.28%), Entamoeba coli 10 (3.06%) Balantidium coli 4 (1.22%). The total Entamoeba positive patients, approximately half of the patients were under 6 years old, with male percentage was slightly greater than female (58% versus 42%). The high frequency of Entamoeba was demonstrated in patients from rural areas 81(54%). According to questioner 61 (40.66%) children's mother who are illiterate or in complete primary school. The data analysis showed that 104 patients were drinking tap water, the infection rate increased (62%) when the hand washing with water only, and most the children who fed on artificial milk were found infected with Entamoeba (12%), and in present study microscopic reveled that there were 10/150 (6.66%) cases of co-infection between Entamoeba spp. and other parasites. <u>Conclusion</u>: Higher infection rates of E. histolytica/ moshkovskii/dispar occurs in age group 2-5 years. Besides, the most potential risk factors are type of gender, residence in rural area, incomplete mother education for children, tap water sources, type of washing hands, and artificial milk.

1. Introduction

Diarrhea is the reversal of the normal net absorptive status of water and electrolyte absorption to secretion. Acute diarrhea is defined as the abrupt onset of 3 or more loose stools per day and lasts no longer than 14 days; chronic or persistent diarrhea is defined as an episode that lasts longer than 14 days (1).Diarrhea is the main cause of morbidity and mortality among infants and young children, particularly in low-resource settings(2). Diarrhea is a symptom of infections caused by several bacterial, viral and parasitic organisms. However, with regard to developed countries, the prevalence of intestinal protozoan parasites is higher than that of intestinal helminthes (3), every year over 350 million patients were infected with intestinal protozoan parasitic infection (4)Amoebiasis is a common intestinal protozoan infection of the human gastrointestinal tract produced by Entamoeba histolytica which causes widespread mortality and morbidity world wide through diarrheal disease and abscess establishment in parenchymal tissues suchas liver, lung, and brain. E. histolytica is a unicellular, protozoon parasite of humans. It moves by a jelly-like tongue-like protrusion of the cytoplasm (5).Symptoms, when present, range from mild abdominal discomfort with diarrhea containing blood or mucous to acute or fulminating dysentery with fever, chills and bloody or mucoid diarrhea. Complications of prolonged infection include extraintestinal disease such as ameboma or abscesses in the liver, lungs, heart, brain, skin or other organ (6,7). Humans and other primates are the only known reservoirs (8). Transmission is through the ingestion of fecally contaminated food or drinks, sexual exposure (usually anal sex) or through the unwashed hands of an infected food handler. The prevalence of E. histolytica/dispar in Iraq is 48% and 3.7% respectively. Najaf, Wasit, Basra, Diwaniya and Miasan provinces showed the highestprevalence rates, whilethe lowest prevalence was reported in Anbar, Diyala, Thiqar, and Erbil (9).

Microscopic examination of stool for cysts and trophozoites remains the most common test available for amoebiasis diagnosis however, symptomatic amoebiasis should be treated with antibiotics. A follow-up stool should be collected by the physician to ensure elimination of the organism (10). Contacts with positive stool specimens should be managed and treated as cases (11). Recent findings prevalence of amoebiasis differs greatly in different areas of the world reaching up to 50% (5).

For thus the current study to detect the infection rate of *E. histolytica/dispar/moscovskii*in fecal samples of patients with diarrhea, investigate the relationship between *Entamoeba* species infection and some socio-environmental factors and determine the possible co-infections between *Entamoeba spp.* and other parasites

2. Materials and Methods

Patients and samples

he study included 326 patients of different age groups who came to the Parasitology Laboratory in BaqubahTeaching Hospital and AL-Batool Maternity Teaching Hospital/Diyala province, suffering from gastrointestinal complaints with acute diarrhea. Stool samples were collected during the period from 1st August 2017 till 30thApril 2018.Ethical approval(MD8 December 2017 MAI).A questionnaire on personal information was prepared.

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Stool Samples Examinations

A-Macroscopic examination Stool samples were observed in terms of consistency, color, odor and presence of blood and mucus.

B- Microscopic examination

From each fecal samples, smears with normal saline and lugols iodine were examined by preparing two clean dry microscope slides, one with normal saline and the other with lugols iodine solutions. By using clean wood stick, the fecal specimens was touched in different regions, especially where streaks of blood or pus were noticed, then mixed completely with each drop of normal saline and lugols iodine solutions on the prepared slides, then each slide was covered with a cover slip. The smear was examined fully under the low (x10) and high (x40) powers of the microscope.

Statistical analysis

The Statistical Analysis System- (SAS) was used to find out of different factors in study parameters. The Chi-square- x^2 test was used to significant compare between the data (12).

3. Results

The present study enrolled 326 patients suffering from diarrhoea. Two hundred thirteen (65.33%) samples found to be positive for different intestinal parasites in patients with diarrhea. The rate of intestinal protozoan infection 191(58.58%) was higher than the rate of helminthes infection 22 (6.74%). *Entamoeba Spp*.and *G. lamblia* were the highest intestinal protozoan that were associated with diarrhea 150(46.01%) and 27 (8.28%) respectively. However, the highest rates of helminthes were: *Enterobius vermicularis* (3.06%) and *Hymenolepis nana* (2.45%), while the lowest was found to be with*Ascaris lumbricoides* (1.22%) as shown in Table (1).

Table 1: The intestinal parasites identified in studied groups by direct microscopic examination

<u> </u>		
Protozoan	No. of	Infection rate%
Tiotozoan	cases	of 326
E. histolytica/dispar/moscovskii	150	46.01%
Giardia lamblia	27	8.28%
Entamoeba coli	10	3.06%
Balantidium coli	4	1.22%
Sub-total	191	58.58%
Helminths		
Enterobius vermicularis	10	3.06%
Hymenolepis nana	8	2.45%
Ascaris lumbricoides	4	1.22%
Sub-total	22	6.74%
Total	213	65.33%

The study wasconducted on 150 patients which were positive for *Entamoeba* on microscopic examination, approximately half of the patients were under 6 years old, with male percentage was slightly greater than female (58% versus 42%). The high frequency of *Entamoeba* was demonstrated in patients from rural areas 81(54%).

According to questioner 61 (40.66%) children's mother who are illiterate or incomplete primary school. The data analysis showed that 104 patients were drinking tap water while 8 were drinking boiled water and 38 were drinking from different sources of water.

The study revealed that the infection rate increased (62%) when the hand washing with water only while decreased (38%) when the hand washing with water and soap. In this study most the children who fed on artificial milk were found infected with *Entamoeba* (12%) (Table 2).

Variable factors		Entamoeba	
		150	
		Positive	%
Age groups	<2 years	23	15.33
	2-5 years	51	34.00
	6-11 years	32	21.33
	12-18 years	16	10.66
	Above 19 years	28	18.66
Gender type	Male	87	58.00
	Female	63	42.00
Residence	Rural	81	54.00
	Urban	69	46.00
Mother education Illiterate or incomplete primary		61	40.66
for children	Primary or secondary	25	16.66
	High school or University	36	24.00
Water supply	Tap water	104	69.33
sources	Boiled	8	5.33
	Other sources*	38	25.33
Washing of	Water only	93	62.00
hands	Water and soap	57	38.00
Type of feeding	Breast	9	6.00
(1&2 years)	Artificial	18	12.00

Table 2: Variable factors of *Entamoeba* infection according to questionnaires obtained from patients with diarrhea..

Other source* river, well and tanker trucks

The present study showed 150 cases of *E.histolytica/* dispar/moshkovskii infections in stool samples diagnosed by microscopic reveled that there were 10/150 (6.66%) cases of co-infection. The diagnosis most common co-infection was between entamoebasis cases and *G. lamblia*, and entamoebiasis cases with*E. coli* with 5 cases (3.33%), and 4cases (2.66%) respectively, 'z while only 1 (0.66%) case of co-infection were among entamoebiasis with*H. nana* as is illustrated in Table (3).

Table 3:	Parasitic	co-infection	cases	among	amoebiasi	s
		nationt	c			

patients						
Double infection	No. of positive cases	%				
E.histolytica/dispar/moshkovskii and G. lamblia	5	3.33				
E. histolytica/dispar/moshkovskii andE. coli	4	2.66				
E. histolytica/dispar/moshkovskii andH.nana	1	0.66				
Total	10	6.66				

4. Discussion

Intestinal parasitic infections have been considered as the most common infections of humankind and more than any other causes, parasitic diseases are contributing significantly to the burden of illnesses, leading sometimes to death, and affecting people in developing and in developed world, even

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in regions that include high income countries (13). The intestinal parasites regarded as the most serious causative agent for diarrhea (14).

In the present study, the rate of protozoan infections (58.58%) was higher than rate of helminthic infections (6.74%) and most of these infections are related to protozoans like *E. histolytica/ dispar/ moskoveski* and *G. lamblia*. These two protozoans remain the most common enteric parasitic pathogens in the patients group. The high rate may be due to the existence of resistant cysts of the parasite in the study region (15).

However, the widespread of protozoal infections may be due to the easy transmission routes of these intestinal parasites happens via fecal- oral route, either directly from person-toperson or indirectly by eating or drinking fecally contaminated food and water, and the simple life cycle of protozoan that does not require intermediate host. Beside, many of these protozoans usually inhabit the human intestine and became pathogenic with the impairment of immune system (16).

In contrast to protozoan infections, the rate of helminthic infections in this study was low. The reason for this may be due to unsuitable ecological environment and other prevailing socio- environmental factors that influence helminthes survival and transmission. In addition, the high seasonal extremes in humidity and temperature from $(50^{\circ}C \text{ to } -1^{\circ}C)$ have a strong influence on the development and survival of helminthes in the environment (17).

The results of the study are compatible or close to the study which was conducted in Baghdad Province that found protozoans were more than helminthes infections (18). In India, a study found protozoans more than helminthes (77.4% and 23% respectively) (19). Another study in Senegal, found that protozoans (29.6%) were more than helminthes (0.8%) (20). Another study found higher prevalence rate of helminthes than the present study. It showed that the rate of helminthes infections was 17.47% among children reviser to some health centers and children's hospital in Zakho (21). In addition, in China it was found that the total prevalence of infection rate with helminthes was (17.33%) (22).

Amebiasis is a major cause of morbidity and mortality worldwide, mostly in tropical and sub-tropical countries characterized by inadequate health services and sanitation infrastructure (23).

There is no standardized method for the detection of *Entamoeba*. Traditionally and before the discernment between the pathogenic and the other species, microscopy was the only reliable diagnosis technique for fresh or fixed stool samples. However, the diagnosis of amebiasis by microscopic identification in stool was described as insensitive and unable to distinguish the invasive parasite *E*. *histolytica* from the other even if their trophozoites are more likely to contain ingested erythrocytes than *E. dispar*. This phenomenon is unfortunately rare in clinical samples (24). According to the WHO, screening by light microscopy

alone, should be reported as *E. histolytica/ dispar/ moshkowiskii.*

In present study, *Entamoeba*was detected from the 150 out of 326 patients with diarrhea (46.01%). This clearly indicates that *E. histolytica/ dispar/ moshkowiskii* is still very high present in the Diyala communities in Iraq. The high prevalence of *Entamoeba* parasites is a clear indicator of environmental contamination with infective stages, which are usually the result of poor sanitation.

The result of the present study agrees with the results of local study, conducted in Baghdad which showed that the most prevalent intestinal parasitic infection found among hospitalized children was *Entamoeba* with incidences 41.25% (25). In Babylon province, found that *Entamoeba* was the most prevalent parasite collected from patients in a primary health center with prevalence 36.7% (26).

Iraq is considered an endemic country for amebic infections which still a serious public health problem in most regions of Iraq. The overall infectivity rate of *Entamoeba* in Iraq was 3.78% at 2013, Diyala is the 2nd lowest prevalence was reported in Iraq with infectivity rate 1.9% (27).

However, other studies were reported higher or lower prevalence rates of *Entamoeba* than present study, in Diyala was 34% (28), in Erbil was (20.33%) (29), in Babylon which was (17.4%)(30), and in Diwaniyah, indicated very high incidence of amebic infection with 61.26% prevalence (31). While in Samarra city indicated that the incidence of *E. histolytica/ dispar/ moshkowiskii* infections with 12.8% prevalence (32). While in Nigeria and Ethiopia *E. histolytica/ dispar/ moshkowiskii* accounted 7.1% and12.9% respectively (33,34).

The variation in the prevalence rates of this protozoan from one study to another may due to different factors such as: environmental, nutritional, socio-economic, geographical conditions, demographic and health-related behavior, number of patients samples in screening study and diagnostic method used (35,36). In general, the prevalence of parasites was strongly associated with a variety of risky factors including host, sociodemograph environmental and zoonotic transmission (37).

Age and gender are important and significant factors affecting the prevalence of *Entamoeba* infections (27). The majority of positive cases were observed in children aged 2-5 years. This result is close to the results of the study conducted in Thi-Qar Province and in India (38,39), that found higher infection rates in age group < 6 years. The causes could be due to consuming unhygienic and contaminated food, especially that age of more contact with the ground as most parasites were belonging and they not realize the good sanitation in comparison with older ages. The lower immunity in young population could be another reason for higher parasitic infections (39).

Both gender, males and females of various ages, are exposed to the chance of amoebiasis because all of them were living under the same climates and conditions, but the result in the present study showed that males more were prone to

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infection than females. This may be due to the fact that males are more exposed to unhygienic conditions (contaminated soil or playing with animals and swimming in contaminated water) in fields during outdoor activity while females may remain indoors (40). This agrees with the results of a local study, conducted inBaghdad which showed that the rate of infection in males (58.50%) was higher than in females' (41.50%) (41), in Kirkuk, found higher infection in male (43.8%) more than infection in females (37.2%) (42). In India,infection in males (57.60%) was more than females (42.40%) (38).

Geographic location, place of residence in particular has been found to be another form of disparity. However, findings in this study are in agreement with other studies (43) that parasitic infections are more common in rural region. People in rural living under unsanitary socio environmental conditions were exposure to parasitic infections, due to poor sanitation, poor public health practices, live in large size family, relative crowded conditions, increasing of malnutrition cases and vectors in addition to using of river water directly for drinking and washing (44).

Clarifying the distribution of intestinal parasitic infection according to the maternal educational situation of the children, the higher infection rates of the *E. histolytica*, / *moshkovskii/ dispar* were among illiterate mothers or in those who did not attend schools. This may be due to the lack of health awareness, lack of practice good hygiene, as education may increase awareness or knowledge about the transmission and prevention methods of diarrhea, enhancing household health and sanitation practices, and encourages changes in behavior at the household level (45). Based on source of drinking water, it has been found the infection rate of parasites was higher in children who drink tap water. This high prevalence may be related to the poor quality of water, faulty of sewage line and deficient level of chlorine.

The present study found most *E. histolytica/ E.dispar/ E. moshkovskii* infections were related to non-washing of hands with soap after eating, using toilet and a player with animals or soil, due to cysts on the contaminated hands of infected individuals who do not practice hand washing after defecation may infect other individuals (person-to-person via the fecal–oral route) or spread over surroundings such as door bells and handles which then become sources of infection (46).

Based on the type of feeding, most of the prevalence of *E. histolytica/ moshkovskii,/ dispar* infection was detected among infants on artificial feeding. This finding agrees with the findings of other studies published worldwide (47,48). This can be explained by the fact that artificial milk may increase the exposure of children to different types of diseases caused by pathogenic microorganisms (49). Bottles feeding are highly susceptible and exposed to contamination by flies and focally contaminated hands (50), while low rate of amoebiasis in breast milk in this study is due to the fact that breast milk provides all the essential nutrients, vitamins and minerals an infant needs for growth and breast milk carries antibodies that help in combating diseases (51,52).

The present study found co-infection with *Entamoeba* in 10/150 cases. This may be due to the same transmission method (fecal-oral route) and unhygienic habits of patients. This is in agreement with various studies in tropical and sub-tropical areas which reported co-infections among amoebiasis patients which ranged from low to high prevalence rate among these patients (53,54).

Co-infection seems widespread in many developing regions (55). This trend in diarrhea is a clear indication that the origin of infection may be due to grossly contaminated food and water, or it could be related to the fact that patients were from low-income group and living in unhygienic conditions. However, the characterization of the polymicrobial interactions associated with carriage and invasions of pathogens is related to the host immune response patterns of single or multiple infections, wanted to elicit synergy or inhibition (55).

Indeed, the detection of parasitic co-infection can be considered the true first step in patient's recovery, as the treatment of only one parasite and neglect the other may lead to the failure of treatment (56). On the other hand, even if co-infection parasites can be killed by the same drug, the physician should know all species that infect the patient in order to describe the accurate dose and duration of treatment.

In conclusion: Higher infection rates of *E. histolytica/ moshkovskii /dispar* occurs in age group 2-5 years. Besides, the most potential risk factors are type of gender, residence in rural area, incomplete mother education for children, tap water sources, type of washing of hands, and artificial milk.

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