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RESEARCH ARTICLE

Molecular Detection and Prevalence of, *Cryptosporidium Parvum, Entamoeba histolytica* and *Giardia lamblia* among Patients with Diarrhea at Al- Rifea city/Thi-Qar Province

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Abstract

Diarrhea is defined as having extra stools than are usual for that individuals or as passage of watery stool for more than three times in 24 hours, caused by various pathogens such as bacteria, viruses and parasites and protozoa consider as important causes of diarrhea and other gastrointestinal infections in humans they include mostly *Cryptosporidium parvum, Entamoeba histolytica* and *Giardia lambella*, this study aimed to determined the prevalence of parasitic agents in stool samples of diarrheic patients, this study is carried out in Thi-Qar Province/Al-Refaie district in Al-Refaie general Hospital which included collection of stool samples from diarrheic patients at a period extended from October / 2017-January / 2018, (96) stool samples taken from patients with different ages to both sexes examined by PCR technique. The results showed The percentage of positive samples were 63.5% while 36.5% was negative *C. parvum* (11.9%), *E. histolytica* (22.6%) and *G. lamblia* (65.5%), no significant statistical differences between males, females and between Urban and Rural areas while there was significant statistical differences between age groups (the highest rate in less than 1-10 years and lowest age group was (11-20 years).

Keywords: *Cryptosporidium parvumm, Entamoeba histolytica and Gardia lamblia, Polymerase chain reaction (PCR), Protozoa.*

Introduction

Diarrhea is defined as passage of watery stool for more than three times in 24 hours [1]. Globally, it is estimated that there are 1.7- 4.6 billion case of diarrhea every year with 2.2 million associated deaths [2]. Diarrhea can be caused by various pathogens such as bacteria, viruses and parasites [3].Protozoan parasites are important causes of diarrhea and other gastrointestinal infection in humans they include mostly *Cryptosporidium parvum*, *Entamoeba histolytica* and *Giardia lambelia* [4].

C. parvum is a gastrointestinal tract of humans this parasite is spread by fecal-oral route either direct contact with infected human, animals or taken the Oocysts with contaminated food and drink [5].Cryptosporidiosis having Clinical signs includes watery diarrhea, vomiting, nausea, abdominal pain and fever [6].*E. histolytica* is enteric parasite that infects about 50 million human worldwide, causing widespread morbidity and mortality [7].

It transmitted by contaminated food or water, as well as potentially spread from person to person through fecal-oral contact [8].*E. histolytica* causing disease Amoebiasis the disease may remain restricted to intestinal lumen or invade intestinal lining causing Amoebic dysentery. It not only causes diarrhea but can also infect extra intestinal including rectal bleeding, peritonitis and abscesses in the intestine, liver, lung and other organs [9].*G. lamblia* is a gastrointestinal parasite that causes giardiasis, one of the causes of diarrhea in humans [10].

The parasite is distributed globally, and children are more at risk of infection than adults [11]. Transmission of *G. lamblia* generally occurs by fecal-oral through food, drinking water it can be infection many from animals such as cats or dogs [10].It is evaluation that approximately 200 million human infection per year by *G. lamblia* [12].

Materials and Methods

A total of 96 patients stool sample were collected from diarrhea who have referred to general Al- Rifae Hospital / Thi-Qar province from October / 2017-January / 2018. The ages were ranged from 1 months-70 years, and the sex of them was males 51 and females 45, fecal samples were collected by using a sterile containers and then transported in to the laboratory Hospital at the laboratory the fecal samples were divided into two portions one portion was for the microscopic examination of parasites while the other portion take it 200 mg and stored directly at -20 °C for molecular analysis conventional PCR [13].

Primer of *C. parvum, E.histolytica and.G.lamblia*

Primer		Sequence (5'-3')	Product Size
18SrRNAgene *G. intestinalis*	F	GGGCTAGAAGGCGATCAGAC	542 bp
	R	GGCGCCTACAAGACATTCCT	
18SrRNAgene *E.histolytica*	F	GGGGAGTATGGTCACAAGGC	667bp
	R	TGTGTACAAAGGGCAGGGAC	
18SrRNA gene *C. parvum*	F	TGGATGTCTTGGTTCTCATAACGA	504bp
	R	ACCCACTGATAGACGGATTTCC	

Gen bank: *Giardia intestinalis* (DQ157272.1), *Entamoeba histolytica* (GQ423750.1), *Cryptosporidium parvum* (AF015774.1)

Statistical Analysis

The statistical analysis proceeded in all groups of study, descriptive statistics analyzed by using one-way analysis of variance (ANOVA) were performed using means and standard deviations (SDs) with LSD test for continuous variables ($p \geq 0.05$) was considered to be significant, and X^2, (P-value 0.01) was considered to be significant. All analyses were performed with the Statistical Package for the Social Sciences SPSS for Windows (version 17.0, SPSS Inc, Chicago, III) [14].

Results

Percentage of Infected and Non-infected Patients with *C.parvum, E.hisolytca* and *G. lamblia* by using PCR

The current study included examination of 96 samples by conventional PCR the results showed percentage of infected patients which were 61 positive samples with percentage was (63.5%) and 35 negative samples, the percentage was (36.5%) Figure (1).

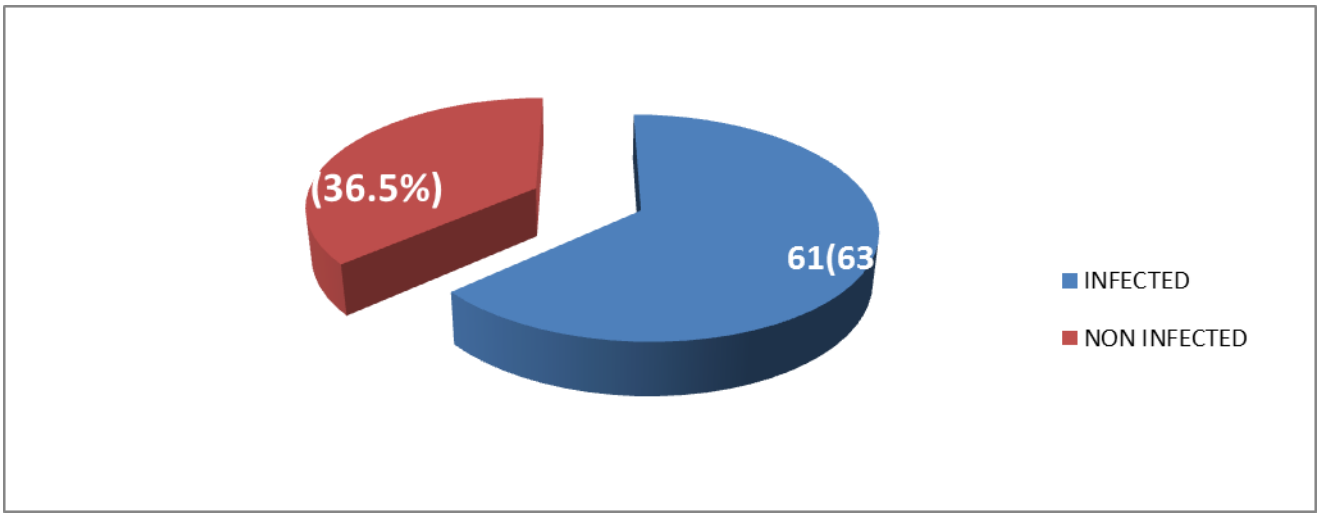

Figure 1: Percentage of infected and non- infected patients *with C. parvum , E.hisolytca* and *G. lamblia* by using PCR

Distribution of Infection with *C.parvum, E.histolytica and G.lamblia* according to the Type of Parasite by using PCR

Figure (2) showed distribution of infection according to the type of parasite by using PCR, the results were lowest infection with *C.parvum* (10) with (11.9%) percent, highest infection with *G.lamblia* (55) with (65.5%) percent and *E.histolytca* (19) with (22.6%) percent.

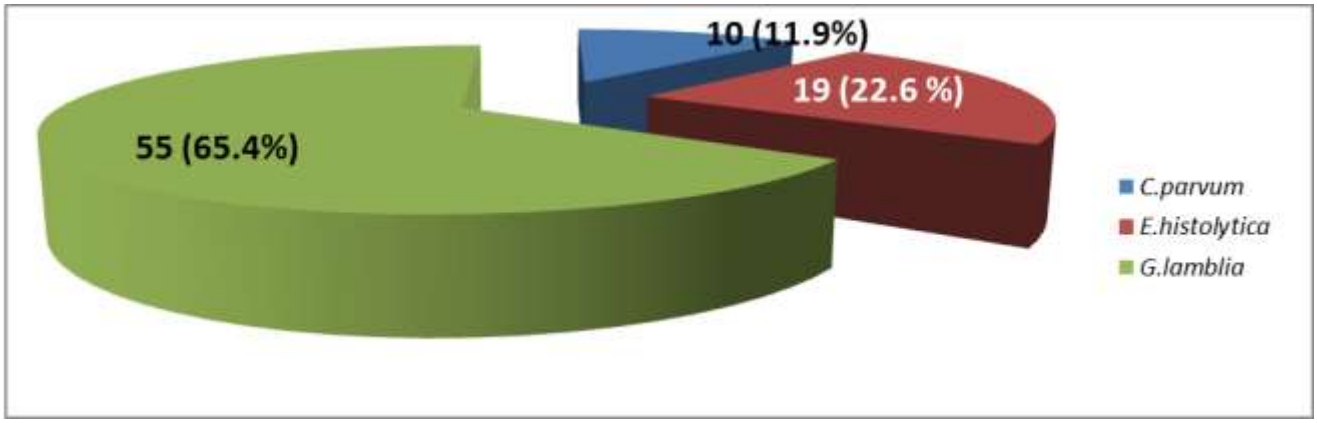

Figure 2: Distribution of infection with *C.parvum, E.histolytica and G.lamblia* according to the type of parasite by using PCR

Distribution of the Infected with *C.parvum, E. histolytica* and *G.lamblia* according to the type of Parasite by using PCR

Figure (3). Showed distribution of the infection according to the type of parasite by using PCR, the results were lowest infection *C.parvum* (2) with (3.3%) percent, highest infection with *G. lamblia* (37) with (60.6%) percent, *E.histolytca* (3) with (5.0%) percent, double infection was (15) with (24.5%) percent, and triple infection was (4) and its percent was (6.6%).

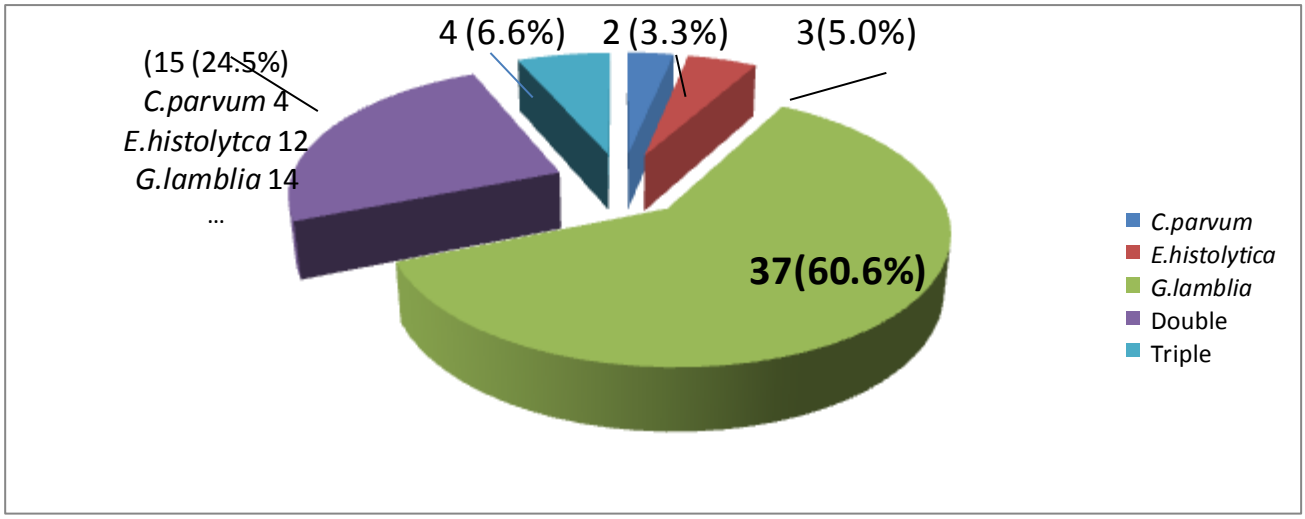

Figure3: Distribution of the infected with *C .parvum, E. histolytica* and *G.lamblia* According to the type of parasite by using PCR

Distribution of the Infected Patients with *C.parvum, E.histolytica* and *G.lamblia* according to Gender by PCR

Table (1) explained distribution of *C.parvum, E.histolytica* and *G.lamblia* according to gender of the infected patients found (59.0%) males and (41.0 %) females, where the infection rate in males (5.6%), *C.parvum* (5.6%) *E.histolytica*, (61.0%) *G.lamblia*, (22.2%) double infection, (5.6%) triple infection, while the infection rate in females was (0%) *C.parvum*, (4.0%) *E.histolytica*, (60.0%) *G.lamblia*, (28.0%) double infection, (8.0%) triple infection. This result showed no statistical significant differences between male and female.

Table 1: Distribution of the infected patients with *C.parvum* , *E.histolytica* and *G.lamblia* according to gender by PCR

Gender	No. of inf.with %	*C.parvum*		*E.histolytica*		*G.lamblia*		Double		Triple	
		Inf. No.	%	Inf. No.	%	Inf. No.	%	Inf. No.	%	Inf. No.	%
Male	36 (59.0%)	2	5.6	2	5.6	22	61.0	8	22.2	2	5.6
Female	25 (41.0%)	0	0.0	1	4.0	15	60.0	7	28.0	2	8.0
Total	61	2	3.3	3	4.9	37	60.6	15	24.6	4	6.6
Cal.X^2	1.799										

Tab.x2: 9.488 df: 4 P.value:0.05

Distribution of the Infected Patient with *C.parvum E.histolytica* and *G.lamblia* according to Habitation by PCR.

Table (2).Showed non-significant differences at level (P>0.05) between urban and rural, the highest rate of infection in rural area (60.7%) compared with urban (39.3%) where the infection rate in urban was (4.2%) *C.parvum*, (0%) *E.histolytica*, (62.5%) *G.lamblia*, (29.1%) double infection and (4.2%) triple infection While the infection rate in rural is (2.7%) *C.parvum*, (8.1%) *E.histolytica*, (59.5%) *G.lamblia*, (21.6%) for double infection and (8.1%) to the triple infection.

Table 2: Distribution of the infected patients with *C. parvum , E.histolytica and G.lamblia* according to Habitation by PCR

Habit-ation	No. of inf with%	*C.parvum*		*E.histolytica*		*G.lamblia*		Double		Triple	
		Inf. No.	%	Inf. No.	%	Inf. No.	%	Inf. No.	%	Inf. No.	%
Urban	24 (39.3%)	1	4.2	0	0	15	62.5	7	29.1	1	4.2
Rural	37 (60.7%)	1	2.7	3	8.1	22	59.5	8	21.6	3	8.1
Total	61	2	3.3	3	4.9	37	60.6	15	24.6	4	6.6
Cal.X^2		2.745									

Tab.X^2: 9.488 df: 4 P.value:0. 05

Distribution of the Infected Patient with *C.parvum E.histolytica* and *G.lamblia* according to Age Groups by PCR

Table (3) showed statistical significance in the distribution of *C.parvum, E.histolytica* and *G.lamblia* according to the age. The higher infection was, 29 (47.5%) in age groups (less than1-10) years while the lower infection was 6 (9.8%) in age groups (11-20) year.

Table 3: Distribution of the infected patient with *C.parvum, E.histolytica* and *G.lamblia* according to age groups by PCR

Age groups (Year)	No. of inf with %	*C.parvum*		*E.histolytica*		*G.lamblia*		Double		Triple	
		Inf. No.	%	Inf. No.	%	Inf. No.	%	Inf. No.	%	Inf. No.	%
Less than 1-10	29 (47.5%)	0	0	0	0	19	65.5	8	27.6	2	6.9
11-20	6 (9.8%)	0	0	0	0	2	33.3	2	33.3	2	33.3
21-30	9 (14.8%)	2	22.2	2	22.2	4	44.5	1	11.1	0	0
31-40	7 (11.5%)	0	0	1	14.3	5	71.4	1	14.3	0	0
More than 40	10 (16.4%)	0	0	0	0	7	70.0	3	30.0	0	0
Total	61	2	3.3	3	4.9	37	60.6	15	24.6	4	6.6
Cal.X2		31.665									

Tab.X2: 26.296 df: 16 P.value:0.05

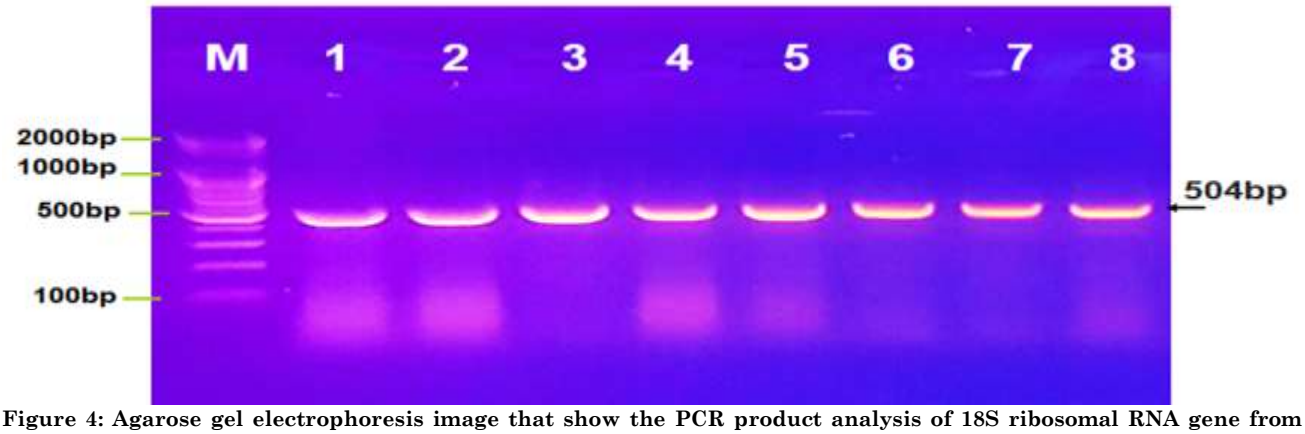

Figure 4: Agarose gel electrophoresis image that show the PCR product analysis of 18S ribosomal RNA gene from genomic DNA of human stool samples. Where M: Marker (2000-100bp), lane (1-8) positive samples C. *parvum* at 504bp

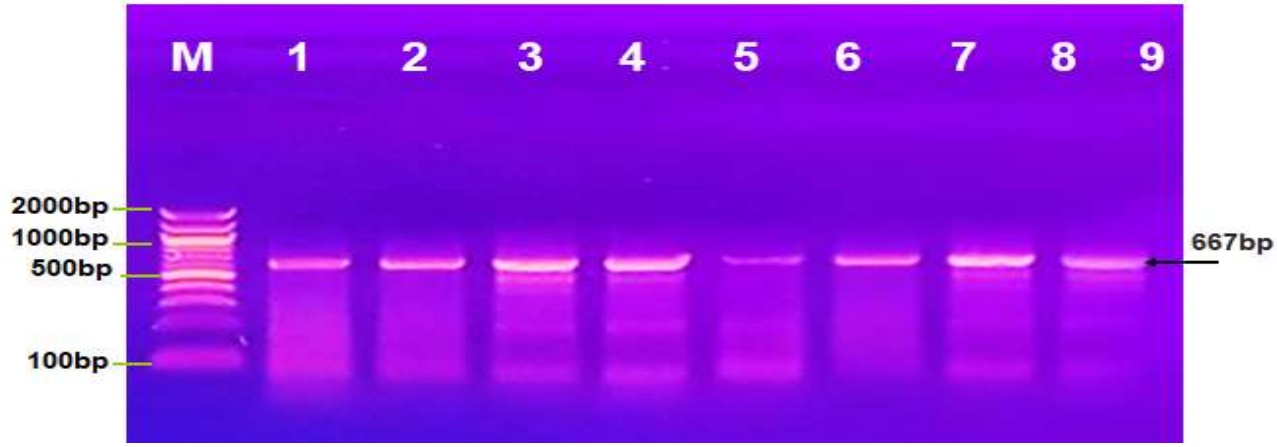

Figure 5: Agarose gel electrophoresis image that show the PCR product analysis of 18S ribosomal RNA gene from genomic DNA of human stool samples. Where M: Marker (2000-100bp), lane (1-8) positive samples E. *histolytica* at 667bp

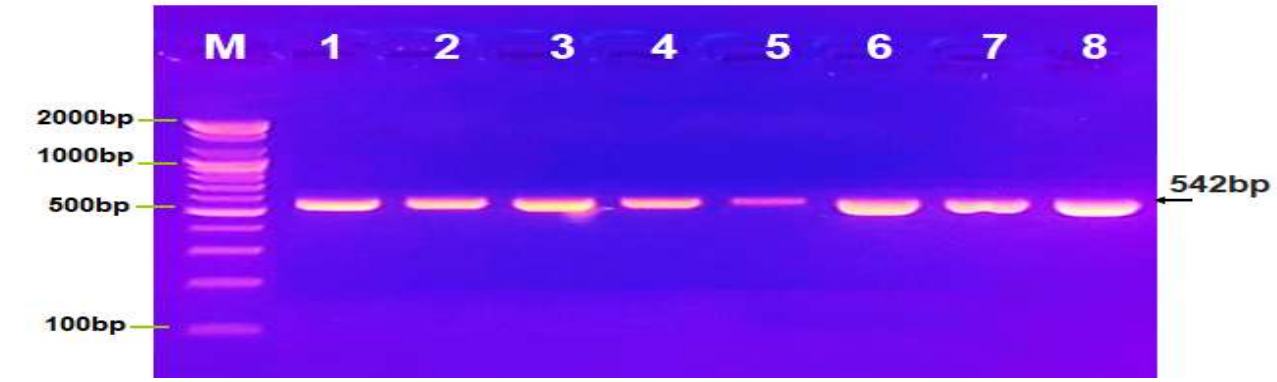

Figure 6: Agarose gel electrophoresis image that show the PCR product analysis of 18S ribosomal RNA gene from genomic DNA of human stool samples. Where M: Marker (2000-100bp), lane (1-8) positive samples *G.lamblia* at 542bp

Discussion

In the current study showed the percent of infected patients by PCR which were (61) positive samples with (63.5%) and negative samples were (36.5%) and this disagreed with [15], who found the positive PCR results (47.4%) and the negative PCR results (52.6%) from diarrheic stool samples and agreed with [16], who found positive PCR results (62.75%) and negative PCR results (37.25%). Also agreed with [17].The high infection with parasite may be related with worldwide distribution of this parasite comparing with other and the transmission of these parasites occurs via fecal-oral route, either directly from person to person or indirectly by eating or drinking fecal contaminated food and water.

Also this may be related to the poor living conditions and like of sanitation in studied area [18].In the current study showed distribution of infection according to the type of parasite by using PCR, the results were lowest infection with *C.parvum* (10) with (11.9%) percent, *E.histolytca* (19) with (22.6%) percent and highest infection with *G.lamblia* (55) with (65.4%) percent so that disagreed with[16].who found *C. parvum* was greater than other parasites (36.93%), *E. histolytica* (22.55%) and *G. lamblia* (20.92%), and disagreed with[15].who found *E. histolytica* (31.3%), *G. lamblia* (28.1%) and *C. parvum* (2.2%). Also agreed with [19]. Who found by using RT-PCR G. *lamblia* the highest infection (69%) and *C. parvum* was (17.5%) and *E. histolytica* 13.5% by examined of 200 diarrheic stool samples, and agreed with [20]. who found G. *lamblia* (%13.3), *E. histolytica* (%7.8). *G. lamblia* was major problem in the the world, especially in developing countries such as Iraq · *G. lamblia* infection in children more than in adults and many prospective studies have reported that the prevalence of parasitic infection in children under ten years of age was higher than for all other age group [21].

Person-to-person, zoonotic, water-borne, and food-borne transmissions can occur through the fecal-oral route after direct or indirect contact with the infective-stage cysts of *G. lamblia* [22]. In this study showed result were *C.parvum* (3.3%), *Glamblia* (60.6%),

E.histolytca (5.0%),double infection (24.5%) and triple infection (6.6%),this result disagree with [15].

Found poly parasitism with double and tribe infection was founded (26) and (13) out of 192 patients respectively, *C. parvum* was the most prevalent protozoan species (36.93%) and *G. lamblia* was less prevalent 22.52% so that no significant differences among protozoan species ,and disagreed with[15]. who found *E. histolytica* (31.3%) , *G. lamblia* (28.1%) and *C. parvum* 2.2%, also disagreed with[23].found in wide survey extended from 2000-2015 in Libya that the *E. histolytica* is major causative agents of diarrhea was 19.9% *G. lamblia* 4.6% and *C. parvum* (3.4%) among individual in Libya with gastroenteritis also In the current study found no statistical differences between males and females in case of infection with the three parasites that is agree with[24].in Baghdad as it did not find any significant differences between the gender, and agree with[25].

As it did not find any significant differences between the gender. Also agreed with [16].And agreed with [26].In his study that there was no statistical significant differences between gender in case of infection with the studied three parasites. and disagreed with [15], who found in their study that was significant difference between male and female, non-significant differences observed in total infection rate with intestinal parasites between males and females whose visitors of general hospital of Al-Rifeai because of presence of same opportunity perhaps to infected of both sex with intestinal parasites, or me be because these groups equally involved in out and indoor activities which might lead to the parasite transmission in both groups,and non-significant distribution in case of using of PCR technique as but remain the rural more than urban areas This study agree with what recorded by [27] in Al-Qadisyai as the rate of infection in the rural area (62.1%) higher than in the cities area (37.9%). And agree with what recorded by [28], in Al-Najaf as the rate of infection in the rural area higher than in the cities area.

This study agrees with [29] where he scored the highest rate of infection in the rural area, reaching 50.9% in Baghdad. Also agree with [30]. In Babylon province, who record the highest rate of infection in rural areas increased by 64.7% and less rate of infection in urban areas were 35.3% , Also agree with [20] whom found infection in rural areas was (34.1%) while infection in Urban areas was (31.1%), The reason for the high incidence of infection in rural areas due to several factors, including the lack of clean drinking water availability, and rely on river water directly as a source of water, and the lack of guidance and counseling by the authorities concerned as well as lower health and cultural level of the rural population as well as the lack of hospitals and health centers in those areas, as well as use of animal waste and human feces and sometimes as an organic fertilizer for the growth and plants and vegetables, socio- invar mental factor such as dejection level sanitation infrastructure used and water sources so that the difference in the protozoan infection in patients was insignificant with regards to the education level so, infection was less common in family with private sanitation as compared community sanitation[31].

In this study found statistical significance in compared with age groups, the age group (less than 1-10 years) was the highest (47.5%) and lowest infection in the age group (11-20) years was (9.8%) so that this study agree with [32] in his study on E. *histolytica* and *G. lamblia* that found the rate of *E. histolytica* was significantly ($p<0.05$) highest in 1-10 years (60%), and agree with [33] whom reported rate (38 %) in 1-12 years old, which may be attributed to defecation practices because these groups of children are fully independent in toilet use and are more involved in both outdoor activities and feeding [33]. So this agree with [34] who was found the same results while disagree with [32] in case of giardiasis who found no significant differences in the parasite distribution in relation to age groups, also disagree with [35] how found high infection in age groups(30-44 years),and disagree with[36]how found high infection in age groups(41-50 years), while [37] regard high infection in age groups more than (31-60),Giardiasis in this study in first age group (less than 1-10) was 65.5%,.

Giardiasis occurs in all ages but is most common in early childhood, since they eat indiscriminately and have less immunity to the parasite than adults who have been exposed during their childhood [38]. The

results related to unsanitary practice associated with child development (e.g playing in contaminated dirt and water, sucking on dirty Finger and other objects, etc). Their less mature immune system, especially in those < 6 years, can reduce their ability to mount strong immune defense to infectious agents [39].

Conclusions

The prevalence of the *G.lamblia* the main causative agents of diarrhea in the Al-Refai city.

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