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Molecular Detection and Prevalence of *Giardia lamblia* Among Patients with Diarrheia in Al-Rifai City/ Thi-Qar Province

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Abstract: Diarrhea the defined as the condition of having extra stools as normal for that than individual or as passage of watery stool for more than three times in 24 hours. *Giardia lambllia* is gastrointestinal parasite that causes giardiasis, one of the causes of diarrhea in humans .The parasite is distributed globally, and children are more at risk of infection than adults. This study aimed to determine the prevalence of *G. lamblia* in stool samples of diarrheic patients. This study is carried out in Al-Rifea district in Al-Rifai general Hospital / Thi –Qar Province which included collection of stool samples from diarrheic patients at a period extended from October / 2017 – January / 2018. 603 stool samples were taken from patients with different ages to for examined by microscopic examination and PCR technique**.** The results showed the percentage of positive samples *G. lamblia* by microscopic examination was (8.1%) and negative samples was(91.9%). The highest infected patients found (8.2%) in males and lowest infected patients found (7.0%) in females. The highest infected patients found (11.8%) in Rural area and lowest infected patients found (7.2%) in Urban area. According to age group the highest infected patients was(10.0%) in age group less than (1-10 years) and lowest infected patients found (4.5%) in age group (21-30) years**.** while results of PCR from (96) sample were positive in 55 samples with percentage (57.3)% and 41 negative samples with percentage of (42.7%). The highest infected patients found (55.5%) in males and lowest infected patients found (59.5%) in females. The highest infected patients found (64.4%) in rural area and lowest infected patients found (50.9%) in Urban area. Age group (1-10years) was the highest infected patients which was (81.2%) and lowest infected patients which was (31-40) years.

Keywords: *Giardia lambllia* ,polymerase chain reaction (PCR) ,protozoa, giardiasis

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Introduction

Historically, *Giardia lambllia* was a very common causative agent of diarrhea (1). More than 330 years ago, Antonio van Leeuwenhoek examined his own unformed stool under a single-lens microscope and was the first to detect a human infection with an entric protozoan, describing the organisms he observed as oval Larger than blood cells moving around (2). *G. lamblia* is flagellated, unicellular, an anaerobic organism ,found in avariety of mammalian hosts, including human (3). *G. lamblia* complex is found in the intestine and can infect a wide variety of vertebrate hosts. this complex is morphologically like, but with a genetically diverse assemblage(4).

G.lamblia favorably colonizes the duodenum and jejunum a hostile

environment in which few microorganisms survive because of the high concentrations of digestive enzymes and bile present during digestion, *G.lamblia* always move and re-attach to avoid being eliminated by peristalsis,the mucus layer protects epithelial cells from host digestive enzymes and prevents *G lamblia* from obtaining immediate access to the epithelium, it has been shown that intestinal mucins may protect the intestinal epithelium by binding pathogens such *G. lamblia* as and impeding microbial-epithelial interactions (5). *G. lamblia* transmission usually take place by fecal-oral transmission through food, drinking water, or recreational water contaminated with feces(1).

Materials and Methods

Samples

A total of 603 patients stool samples were collected from (603)patients with diarrhea who have referred to general Al- Rifae Hospital /Thi-Qar province from October / 2017 to January / 2018 . The ages were ranged from 1 months – 70 years, 347 were males and 256were females. Fecal samples were collected by using a sterile containers and then transported in to the laboratories hospital at the laboratory the fecal samples were divided into two the first portion was for the microscopic examination of parasites while the other portion of 200 mg and stored directly at -20 °C for molecular analysis by conventional PCR (6).

Direct wet

The stool is emulsified in normal saline to allow study the parasite shape and motility. Stool samples observed by the preparation of direct smear methods using clean glass slides , a small drop of normal saline (0.9%) or Iodine stain put was on slide glass and mixed well with a small portion of feces using wooden stick, then was put cover slides, and examined the sample under power amplify 40X (7).

Primer of *G. lamblia*

PCR primers were designed in this study for detection *G.lamblia* based subunit ribosomal rRNA gene by using NCBI-Genbank and primer plus design online. Then these primer was provided from Bioneer company, Korea as following table:

Primer	Sequence (5'-3')		Product Size
18SrRNA gene *C. parvum*	F	GGGCTAGAAGGCGATCAGAC	542 bp
	R	GGCGCCTACAAGACATTCCT	

Genbank: *G. lamblia* (DQ157272.1).

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)(8).

Results

Percentage of infected and non-infected patients with *G. lamblia* by direct smear

The result of examination 603 patient stool samples with diarrhea examined by a direct wet mount method a using light microscope. The percentage of infected patients which were (49) positive samples with percentage (8.1%) and (554) negative samples, with the percentage was (91.9%). As showed in figure (1). Stool my be watery semisolid, greasy, bulky and foul smelling (Figure-1).

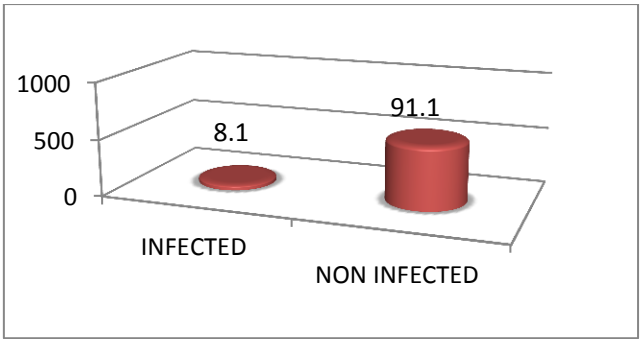

Figure (1): Showing the percentage of infected and non-infected patients with *G.lamblia* by direct smear.

Distribution of the infected patients with *G.lamblia* ,according to gender by Direct smear

Table (1) explained distribution of *G. lamblia* according to gender. The highest infected patients found (8.2%) in males and lowest infected patients found (7.0%) in females.

Table (1): Distribution of the infected patients with *G.lamblia* according to gender by Direct smear.

Gender	No. of sample examanation	No. of infected with *G. lamblia*	%
Male	347	31	8.9
Female	256	18	7.0
Total	603	49	8.1
Cal.X2	3.115		

Tab.X2: 9.488 , **df:** 2 , **P. value:** 0.05

Distribution of the infected patients with *G.lamblia* according to Habitation by Direct smear

Table (2) explained distribution of *G. lamblia* according to Habitation of the highest infected patients found (9.1%) in Rural area and lowest infected patients (6.7%) found in Urban area.

Table (2): Percentage of distribution of the infected patients with *G.lamblia* according to Habitation by Direct smear.

Habitation	No. of sample examination	No. of infected with *G.lamblia*	%
Rural	350	32	9.1
Urban	253	17	6.7
Total	603	49	8.1
Cal.x2	10.712		

Tab.X2: 9.488 , **df:** 2 , **P. value:** 0.05

Percentage of distribution of the infected patient with *G.lamblia* according to Age group by direct smear

Table (3) explained distribution of *G. lamblia* according to age groups of the highest percentage found age group (1-10years) which was(10.0%) and lowest percentage found in age group (21-30) years which was(4.5%).

Table (3): Distribution of *G. lamblia* according to age group by direct smear.

Age group / year	No. of sample examination	No. of infected with *G.lamblia*	%
1-10	140	14	10.0
11-20	127	9	7.0
21-30	109	5	4.5
31-40	102	10	9.8
More than 40	125	11	8.8
Total	603	49	8.1
Cal.X2		9.314	

Tab.X^2: 26.296 , **df:** 6 , **P. value:** 0.05

Percentage of infected and non-infected patients with *G. lamblia* by PCR

The current study included examination of 96 sample by conventional PCR, the results showed percentage of infected patients which were (55) positive samples with percentage (57.3%) and (41) negative samples, the percentage (42.7%) as shown in figure (2) The percentage of infected and non- infected patients with *G. lamblia* by using PCR.

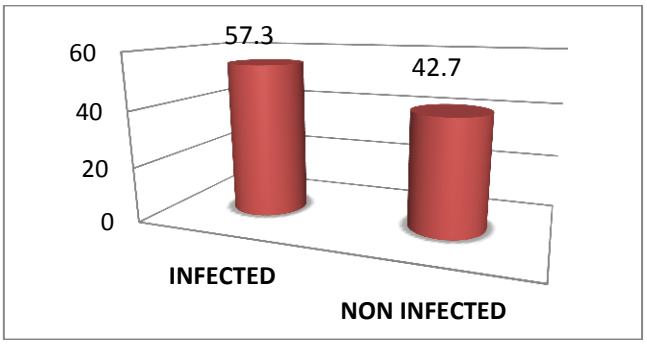

Figure (2): The percentage of infected and non- infected patients with *G.lamblia* by using PCR.

Distribution of the infected patients with *G. lamblia* according to gender by PCR

Table (4) explained the distribution of *G. lamblia* according to gender of the highest percentage was found in males (55.5%), and lowest percentage found in females (59.5%).

Table (4): Distribution of the infected patients with *G. lamblia* according to gender by using PCR .

Gender	No. of sample examination	No. of infected with *G. lamblia*	%
Male	54	30	55.5
Female	42	25	59.5
Total	96	55	57.2
Cal.X2		1.799	

Tab.X2: 9.488 , **df:** 2 , **P. value:** 0.05

Distribution of the infected patients with *G. lamblia* according to habitation using PCR

The highest infected patients found in Rural area (64.4%) and lowest infected patients found in Urban area (50.9%)(Table-5).

Table (5): The distribution of *G. lamblia* according to habitation.

Habitation	No. of sample examination	No. of infected with *G .lamblia*	%
Rural	45	29	64.4
Urban	51	26	50.9
Total	96	55	57.2
Cal.X2	2.745		

Tab.X2: 9.488 , **df:** 2 , **P. value:** 0.05

Distribution of the infected patients with *G.lamblia* according to age groupe by using PCR

The distribution of result of *G.lamblia* according to age groupe the highest infected patients found in age group (1-10years)which was (81.2%) and lowest infected patients found in age group (31-40) years which was (62.6%) as shown in table-6 and figure-4.

Table (6): Distribution of the infected patient with *G. lamblia* according to age group by PCR.

Age group/ year	No. of sample examination	No. of infected with *G. lambllia*	%
1-10	32	26	81.2
11-20	16	8	50.0
21-30	13	6	46.1
31-40	15	4	26.6
More than 40	20	11	55.0
Total	96	55	57.2
Cal.	31.665		

Tab.X2: 26.296 , **df:** 6 , **P. value:** 0.05

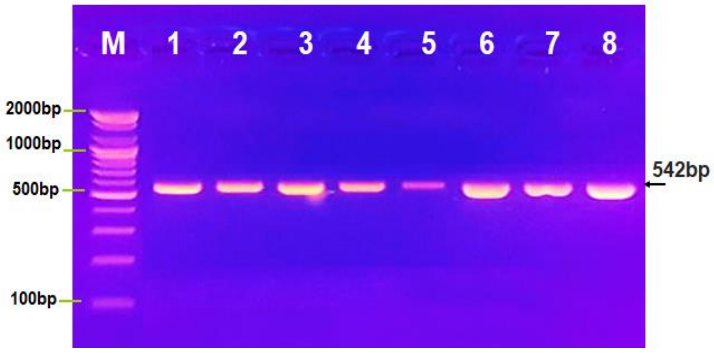

Figure (4): Agarose gel electrophoresis image that showed the PCR product analysis of 18S ribosomal RNA gene from genomic DNA of human stool samples. Where M: Marker (2000-100bp), lane(1-8) positive samples *G. lamblia* at 542bp.

Discussion

The microscopic examination showed that 49(8.1%) of total samples were positive samples and 55.4(91.9%) were negative samples. These results are very low than other study (9) who examined (987) diarrheic stool samples

by using of the direct smear to be an examination of enteric parasite and found (43.4%) which represent positive results and (56.6%) which represent negative results.

The current results are agreed with (10) who examined (92) diarrheic stool samples and found (15.3%) positive results and the remained (84.7%) was negative results.

Our results also found with less agreement with (11) who examined (240) diarrheic stool samples found (84.2%) positive results and the remained (15.8%) was negative results and with (12) who examined (160) diarrheic stool samples found (57.5%) positive result and (42.5%) was negative. The differences in the prevalence of parasite infection are supposed due to differences in methodology, geographical location, and type of study population, sensitivity and specificity of laboratory methods or stage of the disease (13).

While the current study shows the percent of infected patients by PCR which were (55) (57.3%) positive samples and (41) (42.7%) negative samples. This study very close to other studies (9)who found that positive PCR results (47.4%) and the negative PCR results (52.6%) from diarrheic stool samples and

agreed with (14) who found positive results (89.0%) and negative results (11%) and with (15) who found (86.6%) positive while negative results showed (13.4%). Also the current results agreed with (16, 17) who found positive PCR results in (62.75%) of their samples and (37.25%) negative PCR. But our results were disagreed with (18,19) who found that (34.5% and (35.9%) of samples are positive respectively.

The high infection with parasite may be related to 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 (20).

In the current study no statistical significant differences between males and females microscopic examination was seen. The present study agreed with study of (6,17,21) who found non-statistical significant differences between males and females and disagreed with the study of (22,23) in Baghdad, who scored the highest rate of infection in females and lowest rate of infection in males and disagreed with (24,25) study in the province of Babylon and Al- Diwaniya, who found that the highest rate of infection for males 80.6% , 81% respectively while the lowest infection for females.

Added to that by using of PCR technique, there were found no statistical differences between males and females in case of infection with the three parasites that is agreed with (17,26,27,28) who did not find any significant differences between the gender. These results are disagreed with (9) 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 Rifai 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.

In this study found there was statistically significant differences between urban and rural habitation areas in Al-Rifai city in case of infection by the *G.lambellia* microscopically, the rural area more than urban area this study agree with (6) where he scored the highest rate of infection in the rural area, reaching 50.9% in Baghdad. Also agree with agree with (29) whom found infection in rural areas was (34.1%) while infection in Urban areas was (31.1%), Also agree with (30) as the rate of infection in the rural area amounted to higher than in the cities area and disagree with (23), how found infection in rural areas was(25.2%) while infection in Urban areas was (28.3%). The reason for the high incidence of infection in rural areas due to several factors, including the absence of clean drinking water availability, and rely on river water directly as a source of water, and the absence 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-invarmental 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).

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 (32) 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 (33)in Al-Najaf as the rate of infection in the rural area higher than in the cities area.

This study show non- statistical significance in the distribution of the *G.lambellia* according to the ages by microscopically examination but the age group (less than1-10years) was the highest percent (48.8%) and the lowest percent (9.7) in age group (31-40 years) , this study agreed with (17) also, agreed with (20).

While in case of PCR technique 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 (34) and agree with (35)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 (35). So this agree with (36) who was found the same results while disagree with (19) how found high infection in age groups(30-44 years),and disagree with (37) how found high infection in age groups(41-50 years), while (38) 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 (39).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(37).

Infection with *G. lamblia*, on the other hand, was found to increase with age, reaching its highest in early age (39).Probably, indicating reduced parental personal, eating habit and activities linked with soil contaminated with infected fecal matters .the higher prevalence among children appears to be associated with their behavior. Children usually practice less strict hygiene and engage in more play activities with soil. They are also prone to contaminated food and drink. Again, they also buy a lot of food from streets vendors some of whom do not practice proper personal hygiene and may also be carriers of some of these infective parasites. This high rate of infection among children could be related to a number of factors such as poor health hygiene and toilet training, overcrowding, low education of children, low socio-economic status and climatic conditions(39).

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