Republic of Iraq Ministry of Higher Education and Scientific Research University of AL-Qadisiyah College of pharmacy



Detection of *Giardia lamblia* in human by Conventional methods in AL-Qadisiyah Province

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DEDICATIONS

To my mother

To my father

To my sisters

To my brothers

Mohammed and Baraa

ACKNOWLEDMENT

First of all, thank God for helping us in performing this work. We would like to introduce our deepest thanks to our supervisor Dr. Amal AL.shapainy ,for her guidance and kindness throughout the study.

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Mohammed and Baraa

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summary

Summary

The present study was conducted during the period from September 2017 until February 2018, 50 fecal samples was collected from human in different age and from both sexes, the sample collected from different region in AL-Diwianyah teaching hospital, Baghdad medical lab and AL-Belad medical lab,. The study designed to evaluate the microscopic features of the parasite by Lougal iodine (1%) and flotation.

The results of the current study recorded the percent of infection to the *Giardia* is(20%) 10 positive samples out of 50 samples also the results showed that the highest rate of infections (29.41%) was observed in the ages (1-5year) and highest rate of infection in male (22%) while the female show lowest infection (15.78%). Also the result depend of months of the year are ranged between (0%-27%)

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Chapter one introduction

Introduction

Giardiasis is the most common parasitic infection of the human intestine worldwide, caused by infection with flagellated, bi-nucleated protozoan parasite *Giardia lamblia*, disease ranges in seriousness from asymptomatic, to acute or chronic diarrheal disease associated with abdominal pain and nausea (Halliez and Buret, 2013). *Giardia lamblia* (also known as *G. duodenalis* and *G.intestinalis*) (Feng and Xiao, 2011). First discovered in 1681 by Antonie van Leeuwenhoek (Raza *et al.*, 2013). *Giardia lamblia* has a simple life cycle comprising rapidly multiplying, non-invasive trophozoite on the mucosal surface of the intestine, and production of environmentally resist at cysts that are shed with host feces (Adam, 2001).

Aim of the study

- 1- study the prevalence of *Giardia lamblia* in human by using conventional method in AlQadisiyah province.
- 2- Studied the effect of some factors like age, sex and months on infection rate of the parasite.

Chapter two review of literature

2.1 Morphology:

Giardia parasite appears two forms, a vegetative trophozoite form, that lives principally in the upper small intestine and transmissible cyst that excreted in feces (Peter and Lisa, 2010). *Giardia lamblia* trophozoite characteristic tear-drop shape and measures 10-15 micron in length (Al-Fetly, 2008). It has twin nuclei and an adhesive disk which is a sold structure supported by subpelicular microtubules, there are two median bodies of uncharted functions but their shape is paramount for recognitation between species, there are 4 pairs of flagella, one anterior pair, two posterior pairs and a caudal pair The dorsal surface is convex and the ventral side is concave (John and petri, 2006; Taylor *et al.*, 2007).

The cysts are oval, ellipsoidal or circle shape, measure 8-19 micron long and 7-10micron width, they have four or twin nuclei and four median bodies (Brook *et al.*, 2004). The cyst may able to stay for long time in the environment, especially in cold water (Khudier, 2011).

GIARDIA

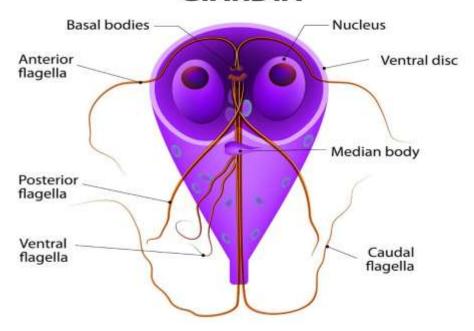


Figure (2.1): Trophozoites of Giardia (Roberts et al., 2013)

2.2 Classfication:

The genus of giardia has been classified as shown in ((Urquhart et al., 2003)

Kingdom: Protista

Subkingdom: protozoa

Phylum: Sarcomastigophora

Subphylum: Mastigophora

Class: Zommastigophora

Order: Diplomonadida

Family: Hexamitidae

Genus: Giardia

Species: lamblia

2.3 Life cycle:

Giardia lamblia lifecycle is extracellular at all phases ,the cysts have to be ingested, after which they travel to the upper small intestine where the thick walls are broken down presumably with the help of bile salts, proteases and lipases, this phase is called excystation , The released excyzoites differentiate into trophozoites which in turn start to replicate (Huang and White, 2006). Once a certain threshold is surpassed, the symptoms are induced, the trophozoites adhere to the epithelia of the small intestine via an adherent disk (Cotton et al., 2011) and they have to constantly detach and reattach to avoid elimination by peristalsis ,in the next step, the trophozoites either disintegrate or encyst to form new cysts, which are excreted with the faeces, some trophozoites might also be excreted, but they are not viable outside the body (Huang and White, 2006).

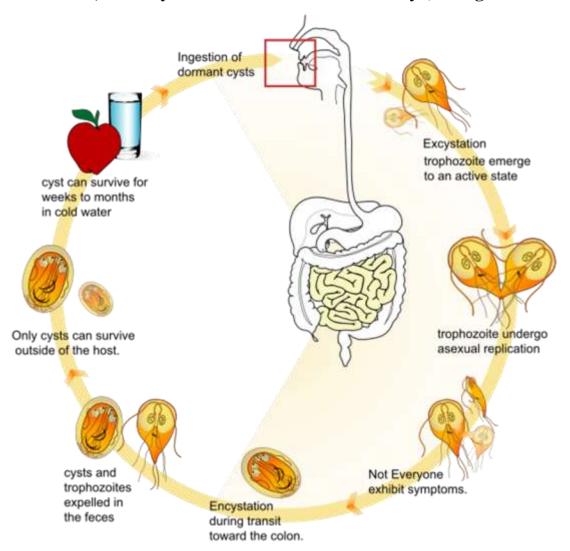


Figure (2-2): Life cycle of Giardia lamblia (Rochelle, 2002).

2.4 Clinical sign:

The clinical sign of Giardia infection varies from an asymptomatic carrier state to a severe disease which is associated with fat malabsorption and lactose intolerance due to disaccharidase deficiency (Sweeny et al., 2011). Furthermore, Giardia infection contributes substantially to the 2.5 million annual deaths from diarrhoeal disease (Adam, 2001). Several studies have revealed that a chronic infection of Giardia during childhood contributes to protein-energy malnutrition, vitamin A, B12 deficiency, iron deficiency anaemia, zinc deficiency and poor cognitive and educational performance (Robertson et al., 2007). Giardia induced diffuse shortening of epithelial brush border microvilli represents a key factor in the production of diarrheal disease via malabsorption and maldigestion (Scott et al., 2004).

2.5 Diagnosis:

In general, *Giardia* infections are diagnosed by microscopy and / or antigen detection in fecal samples, these methods have also been selected in previous mentioned epidemiological studies for the initial recognition of the study subjects, real-time PCR on DNA isolated from stool samples has the potential to detect a signification number of *Giardia lamblia* infections, that would have remained undetected by microscopy and / or antigen detection (Teodororic *et al.*, 2009).

2.5.1 Microscopic Diagnosis:

Conventional microscopy of three stool samples (with or without concentration techniques) is still being recommended as the reference standard (gold standard), disappearance of the parasite cysts from fecal samples will not always mean cure of infection (Younas *et al.*, 2008). The methods include:

A- Direct Smear Method:

The direct smear should be performed on fresh fecal sample as soon as possible after being passed, diagnosis involves testing for *Giardia* trophozoites and cysts in

direct unstained fecal smears to look for motile trophozoites or using Lugol's iodine to help distinguish the cysts and trophozoites (Barr and Bowman, 2006).

B- Sedimentation method:

This method used to detect of *Giardia* cysts in greasy or fatty stool collect from children, the sensitivity of this method reach to 97% of each single case has been examining at least three times (Stringer and Babyn, 2000).

C- Flotation method:

This method is used when *Giardia* cysts in stool exist in few numbers which is can't appear by direct smear method, it is concentrate the number of cysts in fecal or stool samples by use saturated zinc sulphate solution and sheather solution (Dryden *et al.*, 2006).

2-5-2- Immunological diagnosis:

Use to detect the parasite antigens in feces or stool samples, the most important are immunofluorescence assays (IFA), enzyme linked immunosorbent assays (ELISA) (Garceia, 2003). And rapid solid phase qualitative immune chromatography assays (Garceia *et al.*, 2000).

2.6 Treatment:

Several drugs can be used to treat *Giardia* infection, effective treatments include metronidazole(Flagyl), tinidazole(Tindamax), and nitazoxanide(Alinia), alternatives to these medications include paromomycin, quinacrine, and furazolidone (Escobedo, 2007).

Chapter three materials and methods

3.Materials and Methods

3.1 Materials

3.1.1- Chemical materials

Table (3-1) chemicals with their companies and origin:

No.	Chemical	Company and Origin
9	Normal Salin	Haidylena (Egypt)
10	Oil immersion	BDH (Englan
13	Hydrochloric Acid 100%	BDH (England)
15	Xylol	BDH (England)

3.1.2-Instuments and Equipment:-

Table (3-2) Instruments and equipment with their companies and countries of origin

No.	Equipment & instrument	Company
2	Compound light	Olympus (Japan)
	microscope	
3	Digital camera	Samsung/ china
4	Disposable syringe 10 ml,	Sterile EO. / China
	5ml and 3ml	
13	Plastic Containers	Kaeaho(Russian)
16	Slides and cover slipes	Superestar(India)
17	Sterile test tube	Superestar/ India
21	Wach timer	Japan

3.1.3- Solutions :

1-Formal saline (10 %)

Prepared by mixed 100 ml of formaldehyde (40 %) were added to 900 ml of normal saline (0.85 %) (Levine, 1961).

2-Lougal iodine (1 %)

One gram of crystal iodide was dissolved in 100 ml of distal water and mixed thoroughly, then 2 grams of potassium iodide were added to the solution and mixed until dissolved completely (Coles, 1986).

3.2- Method:

3.2. 1-Sample collection: The samples were collected randomly from 50 of human ,in different age ,from both sexes during the period from the beginning from September to the end of February, All sample were collected in a clean plastic containers (100 ml) and tightly closed and labeled it with writing the number ,age and sex with taking of protective measure such as wearing disposable gloves and change in each sample to avoid contamination, the samples were transported in refrigerated bag ,than kept cool until the sample exanimated.

3.2.2-Investigation of stool samples:

- 1- Macroscopic Examination: The inspection of stool samples involve examination of the amount of feces, form it, Consistency & color it. In liquid or soft samples often trophozoites appear, while cystic phases appear in semisoft samples (Turgeon, 2001).
- 2-Microscopically examination

A-The direct smear method by Lougal Iodine:

According to Coles (1986) the method was done was following:

- 1. A drop of lougal iodine solution was placed on a glass slide.
- 2. Small amount (about 1 gm) was put on lougal iodine drop and mixed thoroughly using wooden stick.
- 3. Cover slide was applied with forceps or fingers.
- 4. Examined of slide under (40 x) and (100 x) powers with oil emersion lens.

B-Floatation method:

Zinc sulfate solution consider the best solution usage for floatation method, according to mixed the feces with high qualitative weight as saturated zinc sulfate solution which prepared from dissolve 33 gm of saturated zinc sulfate solution in 100 millilitters of distaled water . the examination by floatation method as following steps: (Coles,1986)

1- Mixed 3 gm of feces with distaled water weller then filtered through two layers of lint in picker .

- 2- Put the solution in test tubes and entered in centrifuge at speed 3000 rpm for two minute, the sediment was taken and discard the floater.
- 3- Diluted the sediment with distaled water and repeated centrifuge for 3 times at speed 3000 rpm for two minute.
- 4- Added saturated zinc sulfate solution to sediment then centrifuged at speed 3000 rpm for two minute .
- 5- Taken the foam in upper by pipette then put on the slide for microscopic examination .
- 3.2.3 statistical analysis: The results of the present study were analyzed by SPSS program (version) software 2010, using Chi-square test(X^2) and values of $p \le 0.05$ were considered to record statistical significance (Leech *et al.*, 2011).

Chapter four result

4- Results

4-1 Results of Microscopically Examination

Diagnostic characterization of Giardia limbla.

Giardia parasite was seen in human and when they are examined under high oil emersion $(100\times)$ lens of light microscopic as in figure



Figure (4-1) Giardia lamblia cyst (magnification x100) using Floatation method with Zinc Sulfate solution.

4-2 Prevalence of Giardia in human according to the age:

The ages of human were divided into four groups which were, from (1month-1year), from (1-5) years, from (6-10) years and up (>10), years. The results showed that the highest rate of infections (29.41 %) was observed in the ages (1-5)year).

Ages	No.	Positive	Percentage
1month-1year	16	4	(25 %) b
1-5 year	17	5	(29.41 %) ^a
6-10 year	6	1	(16.67 %) ^c
>10 year	11	0	(0 %) ^d
Total	50	10	20%

Table (4-1) Prevalence of Giardia according to age

Differences in small letters, vertically, referred to significant differences at level of P≤ 0.05

4-3 Prevalence of Giardia in human according to the sex:

Our results showed that the highest rate of infection in male (22%) while the female show lowest infection (15.78%)

Table (4-2) Prevalence of Giardia according to sex

Sex	No.	Positive	Percentage
Male	31	7	(22.58 %) ^a
Female	19	3	(15.79 %) b
Total	50	10	20%

Differences in small letters, vertically, referred to significant differences at level of $P \le 0.05$

4-4 Prevalence of *Giardia* according to the month of study:

According to the results, it was observed that the rates of infection in a different study months were relatively different, and ranged between (0%-27%), and the months from September toward February showed different results

Table (4-5) Prevalence of Giardia according to the month of study

Month	No.	Positives	Percentage
September	2	-	(0 %) ^d
October	5	1	(20 %) b
November	10	1	(10 %) °
December	10	2	(20 %) b
January	11	3	(27 %) ^a
February	12	3	(25 %) ^a
Total	50	10	20%

Differences in small letters, vertically, referred to significant differences at level of P≤ 0.05

Chapter five discussion

Discussion:

The results of the current study recorded the percent of infection to the Giardia is 20% of 10 positive samples from total 50 samples examined microscopically using Lougal iodine that this percentage is a health risk in the province has been attributed to several reasons, including poor health awareness among children and their families as well as the lack of services municipal and health in the province, which which accordance with the results of Salman (2001) and congruence with the result of Al-Ammash (2015) who recorded the percent of 19.7% after examining 304 sample of feces .

The current study also recorded a higher percentage infection in male (22.58 %) and (15.79 %) in female also agree with the result of Al-Ammash (2015) who recorded the percentage of infection in males more than in females (63.64%) (46.94%) respectively. The cause of increase in male infected rate belong the males effective more & contact without environment than females Mahdi (1996). And disagreement with Yahya (2016) who recorded higher infection rate in female than male.

The high infection rate in the current study for several reasons, foremost among which poor social and economic conditions as well as malnutrition and lack of attention to personal hygiene and surrounding, the environment ignorance and poor health culture.

The results of the current study parasite Giardia For months the study, as the highest percentage found the parasite in the winter, specifically in the month (1) amounting to 27% while the lowest rate of infection to the parasite in the summer months, especially in the month of September, amounting to 0%. This study

agreement with Nabaa (2015) who recorded high percent of infection in January month reach to the (46.88%). The reason in high percentage of infection in January may be attributable to high humidity & low temperature which cysts help to life for long period & thus be biggest infection chance with it Mahdi (1996).

The results of the present study was that the parasite *Giardia* affects all age groups studied, as it recorded the highest rate of infected parasite within the age group (1-5year), amounting to 29.41%, while the lowest rate of infection recorded in the age group (>10) years and amounted to 0%. Attributed infection concentration in children have one year of age for non-completion of the immune system to have as the low-dose parasite cyst enough to cause infection as well as leaving breastfeeding to artificial feeding and the ignorance of many mothers things sterilization and cleanliness of the water and bottles of milk so suffer more than others (Areeshi *et al.*, 2007).

Conclusion and recommendations

Conclusion

- 1-The Giardia lamblia is a common parasite spread in Qadisiyah province
- 2. Factors studied, such as age, sex, and months, and clinical symptoms influential in the development of parasite *Giardia lamblia*.
- 3- Giardia lamblia parasite infection that may lead to the emergence of signs of diarrhea, but the absence of diarrhea not mean absence the presence of the parasite in children, especially at the beginning of the infection.

Recommendations:

- 1. New techniques as polymerase chain reaction(PCR) can be used to get accurate result about *Giardia lamblia* because its faster, sensitive and specific method.
- 2. Start implementing a health education program through health education to people in areas infected with the parasite programs about the symptoms of the disease and the mode of transmission
- 3. Conducting epidemiological surveys between the duration and the other to get to know the real reasons that contribute to the spread of the parasite for the purpose of identifying infected areas.

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