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RESEARCH ARTICLE

Iraqi Genotyping of *Giardia lambila* (A,B,E,F) in Human Stool In AL-Muthanna Province –Iraq

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Abstract

This study was carried out in AL-Muthanna Province during the period from October 2014 to March 2015 for detect the prevalence of *G. lambila* and determining genotypes of this parasite(A,B,E,F,). A total of 200 stool samples (male and female), were taken from human aged (>1-40>) years who suffering from acute or persistent diarrhea and it examined by flotation methods and Conventional-PCR assay, that the results showed total infection percentage with *Giardia lamblia* was (25%).Also the present study showed the total number of *Giardia lamblia* genotypes (A,B,E,F) for positive samples from PCR was 73 samples that the highest infection of assemblage A was 30.14% more than assemblage B was 26.03% and assemblage E and assemblage F in percentage ratio 20.55% and 23.29% respectively, without significant differences at level (P>0.05). The results showed that the PCR product of *triosephosphate isomerase (TPI)* gene that using in detection *Giardia lamblia* genotype (Assemblage A) that the positive samples isolates at 576bp PCR product size and the results that the PCR product of *triosephosphate isomerase (TPI)* gene that using in detection *Giardia lamblia* genotype (Assemblage B) that the positive samples isolates at 208bp PCR product size. While the results the PCR product of *triosephosphate isomerase (TPI)* gene that using in detection *Giardia lamblia* genotype (Assemblage E) that the positive samples isolates at 416bp PCR product size and the results of the PCR product of *triosephosphate isomerase (TPI)* gene that using in detection *Giardia lamblia* genotype (Assemblage F) that the positive samples isolates at 323bp PCR product size. The results of current study showed non significant differences at level (P>0.05) between all age groups depended on molecular assay, also the results showed the highest percentage of infection in age group (>1-10) years in old was (60%) while the lower percentage of infection in both age groups (21-30) years and (31-40>) was (4%). The present study showed non significant differences at level (P>0.05) between male and female depending on molecular assay, also the results showed the highest percentage in female was (52%) while the lower percentage in male was(48%). The present study showed that percentage of *Giardia lamblia* infection in rural regions was (66%) which is higher than urban regions (34%) with significant differences at level (P<0.05).The results of the current study showed that the highest rate of infection with *Giardia Lamblia* depended on molecular assay was in November and January in percentage ratio (30%) while the lower rate was in March was (0.00%).

Introduction:

Giardia lamblia is a flagellated eukaryotic unicellular microorganism that is colonized and reproduced in the small intestine causing giardiasis (Samuel *et al.,*2001). Giardiasis is traditionally considered an epidemic and zoonosis disease between human and animals (farm animals, dogs, cats, birds and rodents) (Thompson *et al.*,2008).*Giardia* infection in humans is usually asymptomatic or mild enough to escape diagnosis , most cases are self – limited , yet significant acute and chronic infection can occur (Wicki *etal*, 2009). Acute infection can produce bloating , crampy abdominal pain and explosive diarrhea , with pale , frothy , steatorrheic stool (foul smelling, greasy stool often mixed with mucus but not blood) (Nyamngee, *et al*, 2009). Human giardiasis is considered a zoonotic infection because of these sub genotypes of *Giardia*, namely A1, A2, A3, A4 and B3, were found to be associated with infections of humans, dogs and calves, which supported the role of these animals as a source of infection for humans although the *Giardia* isolates are morphologically identical, they vary significantly in their biology, virulence and genetics **(**Lalle and Pozio, 2005).These genotypes can infect different hosts, the analysis of human isolates from different geographical locations, examined by PCR amplification of DNA extracted from stool samples, proved that in almost all cases only *Giardia lamblia* genotype A and B are associated with human infections (Caccio and Ryan, 2008). The assemblage A isolates have been more grouped into subgroups I and II, the assemblage B isolates have been divided into subgroups III and IV, these assemblages also infect a wide range of other hosts, including livestock, cats, dogs, and wild mammals (Babaei *et al*., 2008). The other assemblages appear to be host-adapted; assemblage C and D (dog), assemblage E (cattle and other ungulates), assemblage F (cats) and assemblage G (rats), and have not represented a potential public health concern (Monis, *et al.,* 1999).Genotypes A and E have been prevalent in beef cattle in Australia, United states, Denmark and Portugal (Xiao and Fayer 2008) .

Also there is no Molecular studies on the on this parasite prevalence in Al- Muthanna Province so that we needs for undertaking the study.

2-Material and Methods:

2-1.Patients:

This study was carried out in AL Muthanna province in the period from October 2014 to March 2015, to determine prevalence and genotypes of *Giardia lamblia,* a total of 200 stool samples (male and female), were taken from Human aged (>1-40>) years who suffering from with acute or chronic diarrhea in the Educational- AL-Hussein hospital in Samawa, General AL-Rumaiytha hospital, Feminine and Children's hospital in Samawah and Rumaiytha health centers, then it examined by Flotation and Conventional-PCR assay.

2-2.Fecal samples collection :

Fresh fecal samples were collected by using a sterile containers and transported in to a cooled box (temperature approximately 10°C) .Then , the samples were transported to the laboratory at College of Science - AL-Muthanna University , at the laboratory the fecal samples were divided into two portions , one portion was for the microscopic examination of parasites while the other portion was stored immediately at-20°C for molecular analysis(Conventional -PCR) .

2-2-1.Laboratory Tests :

2-2-1.1.Concentration Techniques :

Flotation Method By Sheather's Sugar Solution : (Schares *et al.,* 2005).

Five gram of feces were diluted in (10ml) of tap water , filtered using gauze and centrifuged at (2500 rpm) for (10 min) , then the supernatant was poured and the sediment was mixed in Sheather's solution in a (15ml) plastic tube , this suspension was centrifuged at (2500 rpm) for (10 min) , after that placed the cover slip was touched the surface of the solution for (10-15min) , then the cover slip was examined on a glass slide using microscope at 40x then 100x magnification .

2-3.Primers.

The PCR primers were using in this study for detection *Giardia lamblia*, genotype A, B, E, and F based on triosephosphate isomerase (TPI) gene and these primers was provided from Bioneer company, Korea as following Table (2-1).

Table (2-1): Primers of *G. lambila* genotypes (A,B,E,F).

Primer	Sequence		*PCR product*
Genotype A	**F**	**CGAGACAAGTGTTGAGATG**	*576 bp* (*Minvielle, et al., 2008).*
	R	**GGTCAAGAGCTTACAACACG**	
Genotype B	**F**	**GTTGCTCCCTCCTTTGTGC**	*208 bp(Minvielle, et al., 2008).*
	R	**CTCTGCTCATTGGTCTCGC**	
Genotype E	**F**	**GACGTTGTTGTTGCCCCTTC**	*416bp(Sulaiman, et al., 2003).*
	R	**CCAATGGACCATACGGGCTC**	
Genotype F	**F**	**AACGGCTCGCTCGACTTTAT**	*323bp* (*Suzuki, et al., 2011).*
	R	**ATCCCCTTTTCTAGAGCGCG**	

2-4. Multiplex Polymerase chain reaction (PCR):

The multiplex PCR technique was performed for detection *Giardia lamblia*, genotypes based triosephosphate isomerase (TPI) gene from human stool samples. This method was carried out according to method described by Amar *et al*. (2003) as following steps:

2-4-1. **Genomic DNA Extraction** :

Genomic DNA from feces samples were extracted by using AccuPrep® stool DNA Extraction Kit , Bioneer. Korea, and done according to company instructions as following steps:

A 200mg of the stool sample was transferred to sterile 1.5ml micro centrifuge tube, and then 20µl of proteinase K and 400 µl Stool lysis buffer (SL) were added mixed by vortex. And incubated at 60℃ for 10 minutes.

1. After 10 mins, the tube placed in centrifuge at 12,000rpm for 5 mins.
2. The supernatant was transferred in to a new tube and 200µl Binding buffer was added to each tubes.
3. The tubes were Incubated again for 10 min at 60℃.
4. 100 µl isopropanol was added and the samples mixed by lightly vortex for about 5 seconds, then spin down for 10 seconds to down the liquid clinging to the walls and lid of the tube.
5. DNA filter column was placed in a 2 ml collection tube and transferred all of the mixture (including any precipitate) to column. Then centrifuged at 8000rpm for 5 minutes. And the 2 ml collection tube containing the flow-through were discarded and placed the column in a new 2 ml collection tube.
6. 500µl W1 buffer were added to the DNA filter column, then placed in centrifuge 10000rpm for 30 seconds. The flow-through was discarded and placed the column back in the 2 ml collection tube.
7. 500µl W2 Buffer (ethanol) was added to each column. Then placed in centrifuge at 8000rpm for 30 seconds. The flow-through was discarded and placed the column back in the 2 ml collection tube.
8. All the tubes were centrifuged again for 1 minutes at 12000 rpm to dry the column matrix.
9. The dried DNA filter column was transferred to a clean 1.5 ml micro centrifuge tube and 50 µl of pre-heated elution buffer were added to the center of the column matrix.

10. The tubes were let stand for at least 5 minutes to ensure the elution buffer was absorbed by the matrix. Then centrifuged at 10000 rpm for 30 seconds to elute the purified DNA.

2-4-2. Genomic DNA Profile:

The extracted genomic DNA from feces samples was checked by using Nano drop spectrophotometer (THERMO. USA), which examines and measurement the purity of DNA by reading the absorbance in (260 /280 nm) as following steps:

1. After opening up the Nano drop software, chosen the appropriate application (Nucleic acid, DNA).
2. A dry wipe was taken and cleaned the measurement pedestals several times. Then carefully pipet 1µl of ddH2O onto the surface of the lower measurement pedestal.
3. The sampling arm was lowered and clicking OK to initialized the Nano drop, then cleaning off the pedestals and 1µl of the appropriate.
4. Blanking solution was added as black solution which is same elution buffer of DNA samples.
5. After that, the pedestals are cleaned and pipet 1µl of DNA sample for measurement.

2-4-3. Multiplex PCR master mix preparation:

Multiplex PCR master mix was prepared by using (**AccuPower® Gold Multiplex PCR PreMix Kit**) and this master mix done according to company instructions as following table(2-2):

Table (2-2): **.** Multiplex PCR master mix preparation

Multiplex PCR Master mix		Volume
DNA template		5µL
Forward primer (10pmol)	G.A	1µL
	G.B	1µL
	G.E	1µL
	G.F	1µL
Reverse primer (10pmol)	G.A	1µL
	G.B	1µL
	G.E	1µL
	G.F	1µL
PCR water		7 µL
Total volume		20µL

After that, these PCR master mix component that mentioned above placed in standard **AccuPower® Gold Multiplex PCR PreMix Kit** that containing all other components which needed to PCR reaction such as (Taq DNA polymerase, dNTPs, Tris-HCl pH: 9.0, KCl, $MgCl_2$,stabilizer, and tracking dye). Then, all the PCR tubes transferred into Exispin vortex centrifuge at 3000rpm for 3 minutes. Then placed in PCR Thermocycler (MyGene. Bioneer. Korea).

2-4-4. PCR Thermo cycler Conditions:

PCR thermo cycler conditions by using Convential PCR thermo cycler system as following Table(2-3):

Table(2-3):PCR Thermocycler Conditions.

PCR step	Temp.	Time	repeat
Initial Denaturation	95C	5min	1
Denaturation	95C	30sec.	30 cycle
Annealing	55C	30sec	
Extension	72C	1min	
Final extension	72C	5min	1
Hold	4C	Forever	-

2-4-5. PCR Product Analysis:

The PCR products of was analyzed by agarose gel electrophoresis following steps:

1-1.5% Agarose gel was prepared in using 1X TBE and dissolving in water bath at 100 °C for 15 minutes, after that, left to cool 50°C.

2- Then 3µ of ethidium bromide stain were added into agarose gel solution.

3- Agarose gel solution was poured in tray after fixed the comb in proper position after that, left to solidified for 15 minutes at room temperature, then the comb was removed gently from the tray and 10µl of PCR product were added in to each comb well and 5ul of (100bp Ladder) in one well.

4- The gel tray was fixed in electrophoresis chamber and fill by 1X TBE buffer. Then electric current was performed at 100 volt and 80 AM for 1hour.

5- PCR products were visualized by using UV Transilluminator.

2-5.Statistical Analysis: Chi-Square (X2)was used for detect statistical difference of data prevalence of disease and effect of other factors (Al-Rawi,2000).

3-Results :

3-1:Molecular Assay (Convential-PCR):

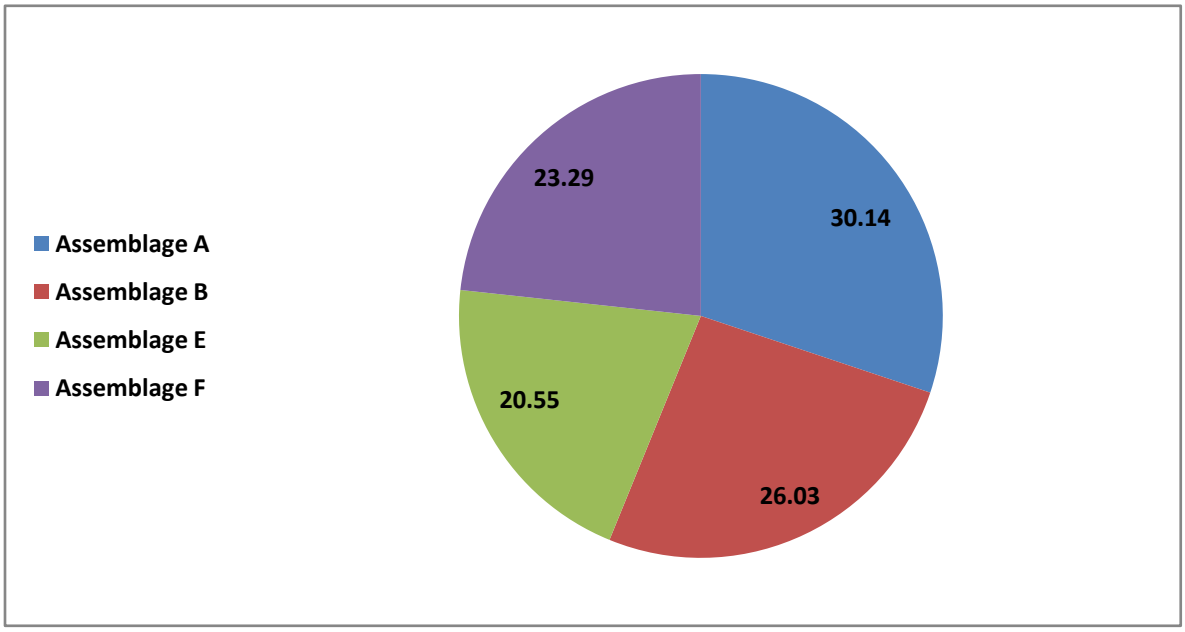
3-1-1. Infection rate for *Giardia lamblia* Genotypes by using Examination of PCR.

In table (3-1)and Figure (3-1),the present study showed the total number of *Giardia lamblia* genotypes (A,B,E,F) for Positive samples from PCR was 73 samples that the highest infection of assemblage A was 30.14% more than assemblage B was 26.03% and assemblage E and assemblage F in percentage ratio 20.55% and 23.29% respectively ,without significant differences at level (P>0.05).

Table (3-1): Infection rate of *Giardia lamblia* Genotypes

Giardia lamblia Genotypes	Positive samples from PCR tested samples	Percentage (%)	Chi-Value
Assemblage A	22	30.14	X^2.Calculated=1.954
Assemblage B	19	26.03	X^2.Table=15.507
Assemblage E	15	20.55	P-Value 0.05=0.582
Assemblage F	17	23.29	df=3
Total Number	73	100.00	

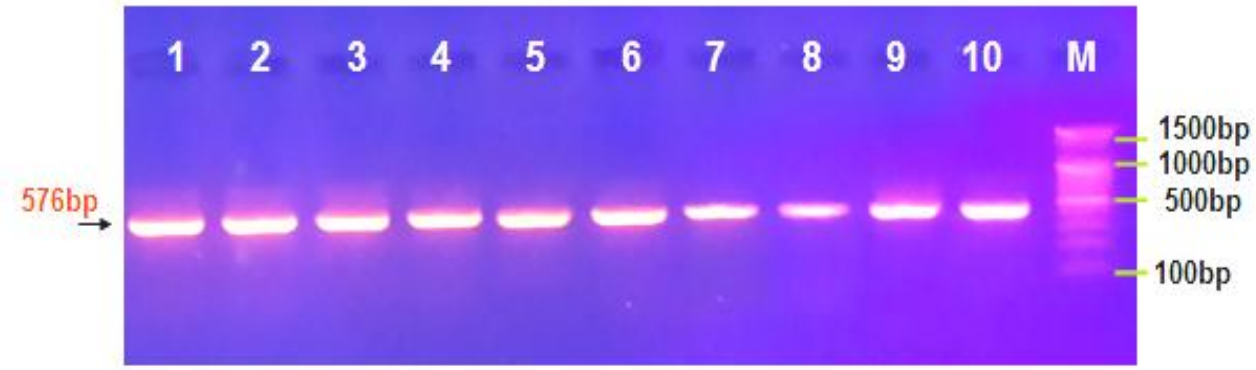
-Non significant differences at (P>0.05) .



Figure(3-1): Percentage of Genotyping of *Giardia lambila* Infection.

3-1-1-1.*Giardia lambila* Assemblage A:

In figure (3-2), Showed the results the PCR product of *triosephosphate isomerase (TPI)* gene that using in detection *Giardia lamblia* genotype (Assemblage A) that the positive samples isolates at 576bp PCR product size.



Figure(3-2): Agarose gel electrophoresis image that shown the PCR product of *triosephosphate isomerase (TPI)* gene that using in detection *Giardia lamblia* genotype (Assemblage A) . Where M: Marker (1500-100bp), lane (1- 10) some positive samples isolates at 576bp PCR product size.

3-1-1-2.*Giardia lambila* Assemblage B:

In figure (3-3), Showed the results that the PCR product of *triosephosphate isomerase (TPI)* gene that using in detection *Giardia lamblia* genotype (Assemblage B) that the positive samples isolates at 208bp PCR product size.

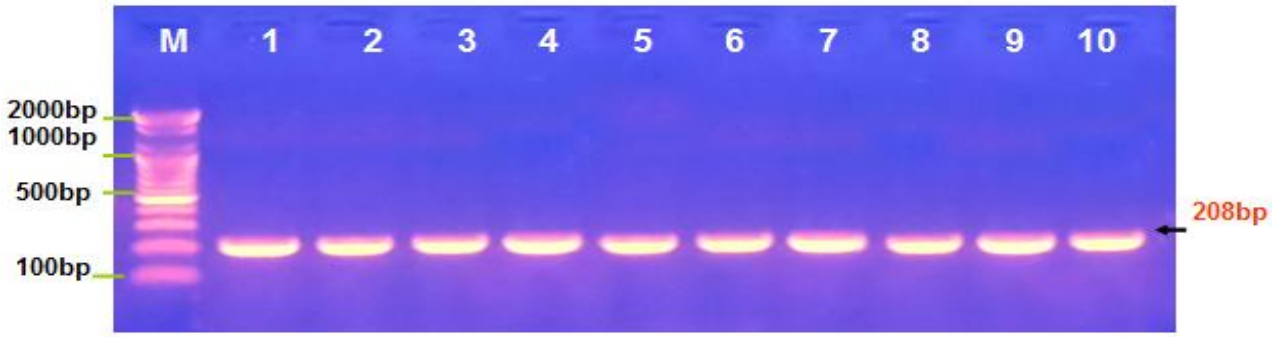


Figure (3-3): Agarose gel electrophoresis image that shown the PCR product of *triosephosphate isomerase (TPI)* gene that using in detection *Giardia lamblia* genotype (Assemblage B) . Where M: Marker (2000-100bp), lane (1-10) some positive samples isolates at 208bp PCR product size.

3-1-1-3.*Giardia lambila* Assemblage E:

In figure (3-4), Showed the results the PCR product of *triosephosphate isomerase (TPI)* gene that using in detection *Giardia lamblia* genotype (Assemblage E) that the positive samples isolates at 416bp PCR product size.

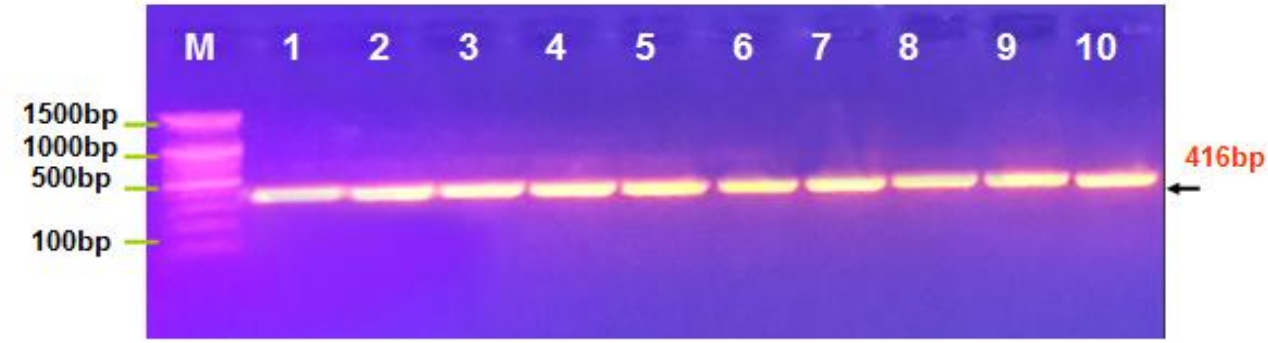


Figure (3-4): Agarose gel electrophoresis image that shown the PCR product of *triosephosphate isomerase (TPI)* gene that using in detection *Giardia lamblia* genotype (Assemblage E) . Where M: Marker (2000- 100bp), lane (1-10) some positive samples isolates at 416bp PCR product size.

3-1-1-4.*Giardia lambila* Assemblage F:

In figure (3-5), Showed the results the PCR product of *triosephosphate isomerase (TPI)* gene that using in detection *Giardia lamblia* genotype (Assemblage F) that the positive samples isolates at 323bp PCR product size.

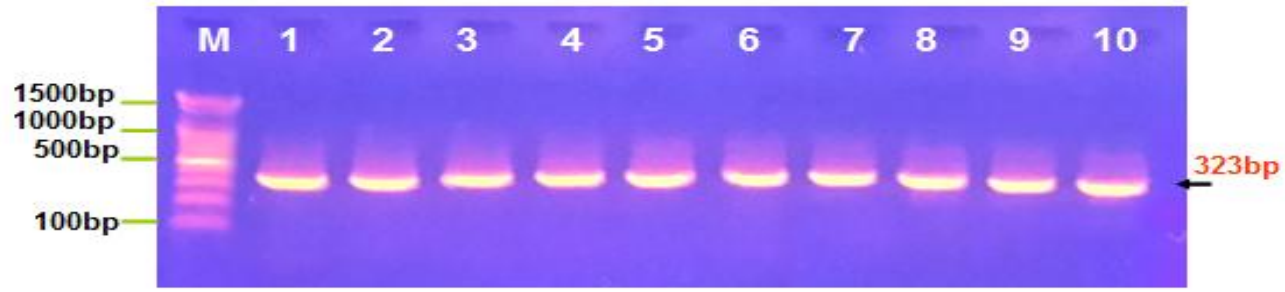


Figure (3-5): Agarose gel electrophoresis image that shown the PCR product of *triosephosphate isomerase (TPI)* gene that using in detection *Giardia lamblia* genotype (Assemblage F) . Where M: Marker (2000-100bp), lane (1-10) some positive samples isolates at 323bp PCR product size.

3-1-2.Relation of *Giardia lamblia* infection with Age depending on Molecular Assay (PCR):

The results of current study, showed non significant differences at level (P>0.05) between all age groups depended on molecular assay, that the total infection percentage with *Giardia lamblia* was (25%), also the results showed the highest percentage of infection in age group (>1-10) years in old was (60%) while the lower percentage of infection in both age groups (21-30) years and (31-40>) was (4%) Table (3-2) .

Table(3-2): Percentage of *Giardia lamblia* Infection according to Age depending on Molecular Assay .

Age	Number of sample	Number of patient diagnosed by PCR	Percentage (%) from total patients/age	Percentage (%) from infected patients	Chi-Value
>1-10	117	30	25.64	60	X^2.Calculated=2.919
11-20	51	16	31.37	32	X^2.Table=7.815
21-30	9	2	22.22	4	P-Value 0.05=0.404
31-40>	23	2	8.69	4	df=3
Total	200	50	25	100	

-Non significant differences at(P>0.05).

3-1-3: Relation of *Giardia lamblia* infection with Sex depending on Molecular Assay (PCR):

The present study showed non significant differences at level (P>0.05) between male and female depending on molecular assay , also the results showed the highest percentage in female was (52%) while the lower percentage in male was(48%) as in Table (3-3).

Table (3-3) :Percentage of *Giardia lamblia* infection according to Sex depending on Molecular Assay.

Sex	Number of samples	Number of patients diagnosed by PCR	Percentage (%) from total patients/sex	Percentage (%) from infected patients	Chi-Value
Male	106	24	22.64	48	X^2.Calculated=0.401
Female	94	26	27.65	52	P-Value 0.05=0.527
Total	200	50	25	100	df=1

-Non Significant differences at(P>0.05).

3-1-4:Relation of *Giardia lamblia* infection with location depending on Molecular Assay.

The present study showed that percentage of *Giardia lamblia* infection in rural regions was (66%) which is higher than urban regions (34%) with significant differences at level(P<0.05)as in table (3-4).

Table(3-4):Percentage of *Giardia lamblia* infection according to Location depending on Molecular Assay.

Location	Number of samples	Number of patients diagnosed by PCR	Percentage (%) from total patients/region	Percentage (%) from infected patients	Chi-value
Rural	108	33	30.55	66	X^2.Calculated=2.342
Urban	92	17	18.47	34	P-Value 0.05=0.126
Total	200	50	25	100	df=1

Significant differences at(P<0.05).

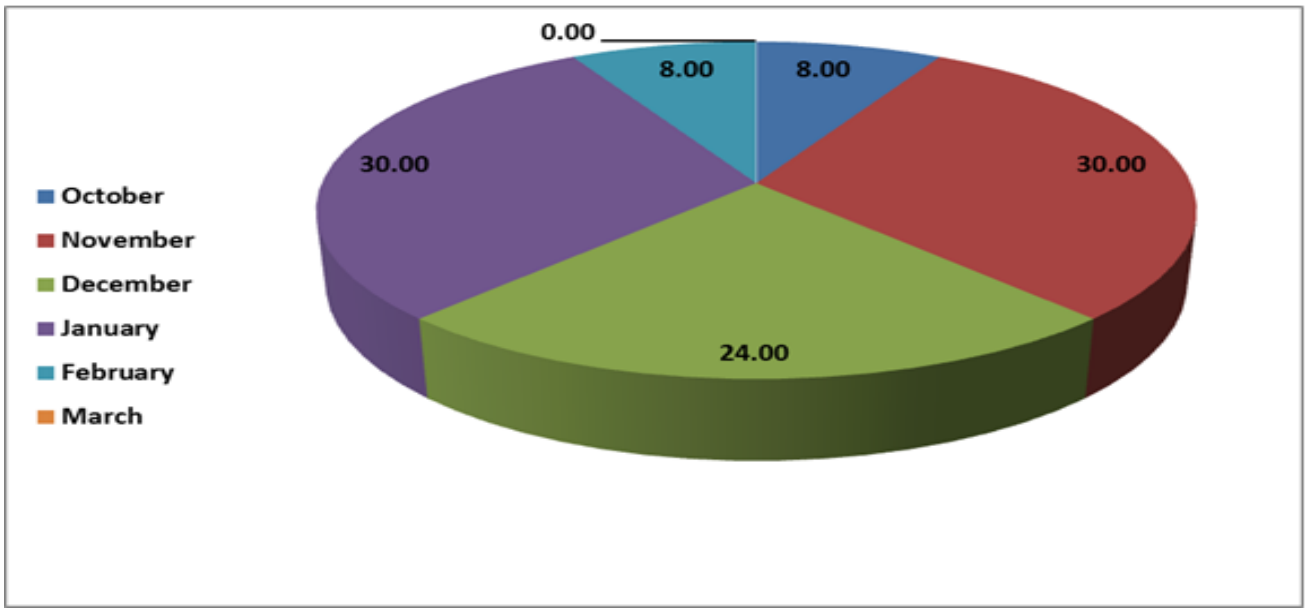
3-1-5: Relation of *Giardia lamblia* Infection with Year months Depending on Molecular Assay(PCR).

The results of the current study showed that the highest rate of infection with *Giardia Lamblia* depended on molecular assay was in November and January in percentage ratio (30%) while the lower rate was in March was (0.00%) as in table (3-5), figure (3-5).

Table (3-5) Percentage of *Giardia Lamblia* infection according to months of year Depending on Molecular Assay(PCR).

Month	Number of samples	Number of patients diagnosed by PCR	Percentage (%) from total patients/month	Percentage (%) from infected patients	Chi-Value
October	15	4	26.67	8.00	X^2.Calculated=11.53
November	55	15	27.27	30.00	X^2.Table=11.07
December	45	12	26.67	24.00	P-Value 0.05=0.042
January	32	15	46.88	30.00	df=5
February	30	4	13.33	8.00	
March	23	0	0.00	0.00	
Total	200	50	25.00	100.00	

-Significant differences at(P<0.05).



Figure(3-6): Relation of *Giardia lamblia* Infection with Year months depending on Molecular Assay(PCR).

4-Discussion :

Giardia lamblia occurs worldwide in human and animal, with greater human prevalence in regions with poor sanitation practices, crowding, harsh living conditions (Peter and Lisa,2010). *Giardia lamblia* is a universal and well-Known enteric flagellated protozoa that is found and reproduced in the small intestine, of mammalian hosts, including domestic, wild animals and humans causing giardiasis (Thompson and Monis ,2004; Plutzer *et al*., 2010). Giardiasis is traditionally considered an epidemic and zoonosis disease between human and animals(farm animals, dogs, cats, birds and rodents) (Thompson *et al.*,2008).

4-1. Molecular Assay (Conventional-PCR):

4-1-1. Infection rate for *Giardia lamblia* Genotypes by using Examination of PCR.

Used in the current study, polymer chain PCR reaction for the purpose of determining genotypes that are special for *Giardia lamblia* in human stool samples by using special primers for gene triose phosphate isomerase (Tpi) for the four genotypes A, B, E and F and to determine the prevalence of these types in the province of AL- Muthanna, the results of this study, showed presence of gene (Tpi) of the parasite in 50 sampleS from 200 stool specimens containing parasite *G. lamblia* through the examined PCR with percent 25%, while 150 sample found free of gene Tpi of the parasite. The failures in the amplification of some fecal samples may be due to the low quantity of DNA, either due to their degrading in time or may be presence of some of PCR inhibitors such as (lipids, hemoglobin, bile salts, polysaccharides from mucus, bacteria, and food degradation product) which can affect the results of amplification (Abbaszadegan, 2007; Cedilla-Rivera, 2003).

The purpose of used (Tpi) gene in the diagnosis genetic patterns of *Giardia lamblia* because of the higher genetic heterogeneity for this gene, as well as being a polymorphic so gene (Tpi) is one of milestones evolutionary history that was used in the analysis of molecular evolution and relationships taxonomic for types of *Giardia lambila* and through molecular analyzes for this gene was found that *Giardia lamblia* parasite is a complex type that is found in animals caribou and musk rats, mice and rabbits, which are one of the hosts that have a significant role in the transfer of infection to humans (Sulaiman *et al*., 2003).

Positive examination using a technique PCR depends on the quality and quantity of DNA and on the degree of purity by removing a large variety of the inhibitors in a stool sample, which has to be removed during the extraction such as bile salts, Bilirubin salt and Complex polysaccharides and Phenolic compounds, as well as the low amount of the parasite cysts can also affect on the concentration of the DNA sample, which will be subject to extraction or smashing of DNA during the process of keeping samples (David *et al*., 2011).

Identify the genetic patterns of *Giardia lamblia* pathogen by molecular methods that are highly sensitive has great importance to prevention the unnecessary medicines and treatment as well as for avoid patient the toxic effects of drugs and improve the effectiveness and give information on the biological processes of the parasite infection (Balcioglu *et al* 2012).

The results of the current study showed, that genotype (A) is the most common than genotype (B) where the number of infected samples with genetic pattern Tpi A was 22 (30.14%) while the number of infected samples with genetic pattern Tpi B was 19 (26.03%), while E was15 (20.55%) and F was 17 (23.29%).

This results is agreement with the following studies: a study conducted in Mancih province western Turkey on patients with parasite *Giardia lamblia* found that %70.4 % of patients were infected with the genetic pattern A, while there is 29 .6 % of the patients infected with the genetic pattern B (Balcioglu *et al*., 2012).

Also agreement with a recent study in Egypt by Helmy *et al*. (2009) found that infection with assemblage A was more prevalent (75.5%) than infection with assemblage B (19.5%).

Helmy, *et al.*(2009) revealed that the majority of giardiasis isolates were assemblage A genotype 31/41(76%).As in Italian study reported that 80% of samples were the assemblage A (Caccio` *et al*., 2002). In Egypt, Foronda *et al*. (2008) found that 15% of the positive samples belonging to assemblage E, this result is agreement with our results.

It can be the cause of human infection with the genetic patterns E, F due to lower the health and cultural awareness and consumption of non-sterile drinking water from contaminated rivers with human excrement or animals and animal husbandry reservoir of the disease within the home, such as cats, goats, sheep and direct contact with the reservoir animals for the disease (O' Handley *et al*. 2000).

Nader *et al.,* (2014) was reported that genotype A (81.8%) and genotype B (13.6%), and found that prevalence of genotype A is six times higher than genotype B in patients with diarrhea.

There is a strong correlation between severe diarrhea and permanent with genetic pattern (A) compared with the genetic pattern (B) who does not cause a symptoms of the giardia infection (Homan and Mank, 2001; Breathnach *et al.,* 2010). As In recent years many studies have been done in the different parts of the World including Bangladesh, Australia, Turkey, Spain, India and Iran and showed the correlation between genotype A and

diarrhea, this refers to assemblage A has virulence factors more than assemblage B and thus pathogenesis be severe, all these reasons confirm why assemblage A more Prevalent from assemblage B (Alam, *et al*., 2011).

Babaei, *et al*.,(2008) was noted 33 sample by rate (87%) contains on *G. lamblia* (genotype A), and 3 (7.8%) belonged to genotype B from total 38 sample.

While the present study, disagreement with the following studies: AL-Muhana, (2013) which noted genotype that the (B) is the most common than genotype (A), where the number of infected samples with the genetic pattern Tpi B (25/41) with percent 61.0% while the number of infected samples with genetic pattern Tpi A (16/41) with percent 39.1%.

A study carried out in India found that the genotype (B) of the parasite *Giardia lamblia* more prevalent than the genotype (A) in patients that suffer from giardiasis and by rate 61% and 39%, respectively (Traub *et al*., 2004).

In the Netherlands examined (100) stool specimens of patients with parasite *Giardia lamblia* where found that 65% of patients are infected with the genetic pattern(B) while there are 35% of patients infected by the genetic pattern (A), (Vander Giessen *et al*., 2006). A study has proved in Egypt that the infection rate with *Giardia lamblia* amounted 34.6% and the prevalence of genetic pattern (B) was 64% while the percentage of infection with genetic pattern (A) amounted 27% ,and the study proved that the cattle that harboring the genetic patterns A, B, may be a source of infection human (Foronda *et al* ., 2008).

Also found Minivielle *et al*.,(2008) the infection rate of genetic pattern (B) is more than type A and increased by 93.02% and 6.98%, respectively, where he was genotype (A) in patients with symptoms of a simple either type B was in patients with severe diarrhea in Argentina, South America. In Thailand, conducted a study on 61 samples of human feces that suffers from the giardiasis to know the genotypes of the parasite, where found 51% of infected samples type B while 8% were infected with the genetic pattern A (Tungtrongchitr *et. al*., 2010).

4-1-1-1.*Giardia lambila* Assemblage A:

The results of this study showed the that PCR product of *triosephosphate isomerase* (*TPI*) gene that using in detection *Giardia lamblia* genotype (Assemblage A) that the positive samples isolates at 576bp PCR product size, these findings are agreement with the results of AL -Muhana,(2013) that proved gene (*TPI*) for genetic pattern A is located at 576 bp, these findings are agreement with the results of (Molina *et al*., 2007 ; Minivielle *et al*, 2008 ; AL -Muhana, 2013;) that proved gene (*TPI*) for genetic pattern A is located at 576 bp.

4-1-1-2.*Giardia lambila* Assemblage B:

The results of this study showed that the PCR product of *triosephosphate isomerase (TPI)* gene that using in detection *Giardia lamblia* genotype (Assemblage B) that the positive samples isolates at 208bp PCR product size, these results are consistent with the results of AL -muhana, (2013) that proved gene (*TPI*) for genetic pattern B is located at 208 bp , these results are consistent with the results of (Molina *et al*., 2007 ; Minivielle *et al*, 2008; AL -Muhana, 2013).

4-1-1-3.*Giardia lambila* Assemblage E:

The results of this study showed that the PCR product of *triosephosphate isomerase (TPI)* gene that using in detection *Giardia lamblia* genotype (Assemblage E) that the positive samples isolates at 416bp PCR product size, where agreed with (Sulaiman, 2003).

4-1-1-4.*Giardia lambila* Assemblage F:

The results of this study showed that the PCR product of *triosephosphate isomerase (TPI)* gene that using in detection *Giardia lamblia* genotype (Assemblage F) that the positive samples isolates at 323bp PCR product size, where agreed with (Suzuki, 2011).

4-1-2: Relation of *Giardia lamblia* infection with Age depending on Molecular Assay (PCR).

The total infection percentage with *Giardia lamblia* in the present study, was(25%) also the highest percentage of infection in age groups (>1-10), where was 60%, while the lower percentage of infection in both age groups (21-30) years and (31-40>) was (4%). The present results are in agreement with similar findings from other studies, which reported by AL-Muhana, (2013) that explained the highest rate of infection was in the age groups less than one year, with ratio (85.7%), while the lowest rate was among the age group (36-40 year),where was (22.2%).

This result may be due to the bad habits such as putting fingers in the mouth and eat fruits and vegetables unwashed and not washing hands before eating and after defecation, with abstention a lot of children from taking treatment low education of children, low socio-economic status and climatic conditions (Ojonoma,2008)

Tungtrongchitr *et al* (2010) found that the age group (1-5 years) is the highest in the incidence with the genetic patterns of the parasite, with percent (92%) while the age group less than (50 years) was have the infection rate (8%).

Disclosed a Study in Ethiopia that younger age groups and elderly are the most exposed to infection *Giardia* parasite due to a weakened immune system in these age groups (Gorezynski and Terzioglu , 2008).

The high infection rate in the age group 46 years and present study can be attributed to exposure to chronic diseases more than others and that can weaken the immune system with age, Furthermore, the inability of some of them to take into account the rules of personal hygiene (Ouattara *et al*., 2010).

4-1-3. Relation of *Giardia lamblia* infection with Sex depending on Molecular Assay (PCR).

The current study, recorded high prevalence of infection (52%) among females depended on polymerase chain reaction(PCR) while in males the rate was lower 48%, but there is non-significant difference at level(P>0.05)

This study is agreement with Minivielle *et al*, (2008) that did not find significant differences for the rates of infection with genetic patterns of the parasite (*Giardia lamblia*) between male and female.

The present study disagree with AL-Muhana, (2013) where showed that the highest ratio of infection with genetic patterns A and B were in males, which was 43.7% compared to the females 37.8% and the statistical analysis showed that there were significant differences at the level (P< 0.05) between the sexes.

Also disagree with Bertrand *et al*, (2004) where proved in a study for the gene tpi of *Giardia lamblia* that the 64% of males were infected with the genetic pattern B for *Giardia lamblia* while 36% of them were infected with the genetic pattern A.

While Ahmed, (2014) confirmed that female are more exposure to giardiasis duo to the more dealing with children and spend most of their time in the cleaning and use of water liquefaction that may be contaminated of *Giardia* cysts, and most house wives do not have health awareness, therefore be more exposure to infection.

4-1-4. Relation of *Giardia lamblia* infection with location depending on Molecular Assay.

The results of the current study showed that the highest infection rate was in the rural region was 66% compared with urban region was 34% with significant differences at level(P<0.05).

This results is agreement with AL-Muhana, (2013) showed that the highest infection rate in the rural region was 45.5% compared to the urban region, which was 32.4%

Also is agreement with Gamboa, *et al* (2003) in his study in Argentina that the ratio of gene (Tpi) in the *Giardia lamblia* in the rural areas, higher than urban area where was amounted to 34% and 15% respectively and Thompson, (2000), has proven existence of the genetic patterns for *Giardia lamblia* in the rural areas more than the urban areas, and noted that the dogs which frequent their presence in rural areas contains genotypes of the parasite that common between human and animal (Zoonotic genotype).

While , Faronda, *et al*. (2008) that the cattle harbors the hereditary types A, B,E,F and thus be a source for human infection.

4-1-5. Relation of *Giardia lamblia* infection with Year months depending on Molecular Assay.

The results of the current study showed, that the highest rate of infection with *Giardia lamblia* depended on molecular assay was in November and January in percentage ratio (30%) while the lower rate was in March was **(**0.00%). The results agree with Wensaas, (2011) that showed high infection rate during November. Also the results were nearly agree with the results of Salman (2002), who proved the highest rate of infection was in Baghdad in month of October (65%), while the lowest infection was in the month of March (33.7%).While disagree with (AL-saeed *et al*., 2005) who showed that highest rate of infection during the month of July and stood at (2.7% ,5.4%) for both males and females respectively, while the lowest rate of infection was during the month of January (1.2% ,1.5%) and for both males and females respectively. Also disagree with results of (Salman,2012) who showed that cases of diarrhea was high during the summer months, where reaching in July to 47.70% while in August and September the infection rate was 41.50% and 19.10% respectively.

5-Refferences

Abbaszadegan, M.R.; Velayati, A.; Tavasoil, A. and Dadkhah, E. (2007). Rapid DNA extraction protocol from stool, suitable for molecular genetic diagnosis of colon cancer. I.B.J; 11(3): 203-208.

Abdul-Abbas, S. K. ; Abdul-Abbas, M. K. and Haider, AL-Rajab H. (2009). Protozoan Intestinal in patients with diarrhea in the area of the Abbasid /province of Najaf. Magazine University Kufa Department of Biology 0.1 (1): 89-91 Page.

Ahmed, H. S.(2014). Prevalence of Giardiasis in Human and Cattle in Al-Diwaniyah Province by Using Conventional and Immunological Methods Master. Thesis. college of veterinary medicine, University of Al-Qadissiya: 95 page.

Alam, M.M, Ilias, M. Siddique M.A, Kabir, M.M. Nazib, F and Khan, M.G.M. (2011). Genotype-specific detection of *Giardia lamblia* in stool samples of diarrhoeal and nondiarrhoeal patients in Dhaka, Bangladesh. Dhaka Univ *J Biol Sci*;20:183-89.

AL-Dulaimi, H. K. H. (2004). Study the effect of some chemical compounds on the parasite *Giardia lamblia*,. Master Thesis. Science College. University, Mustansiriya: 37.

AMAR, C. F., P. H. DEAR, and MCLAUCHLIN, **J**. (2003). Detection and genotyping by realtime PCR/RFLP analyses of *Giardia duodenalis* from human faeces. Journal of Medical Microbiology 52: 681–683.

Al-Muhana, W. H. Y. N. (2013). Epidemiological and Diagnostic Study of the *Giardia lamblia* Parasite Genotypes, which Causes Diarrhea Among the Patients in Najaf, province, by Using the PCR Technique Master Thesis College of Education For girls/ University of Kufa 85 page.

Al-Rawi, K.(2000). Introduction for Biostatic, Al-Mousul University.

Al-Saeed, A.T. and Issa, S.H. (2005). Frequency of *Giardia lamblia* among children in Dohuk, Northern Iraq. *Eastern Mediterranean Health J;* 12(5): 555–561.

Al-Shaheen, Z.; Al-Maki, A.K. and Kassim, H. K. (2005). A study on prevalence of *Entamoeba histolytica* and *Giardia lamblia* infection among patient attending Qurna hospital in Basrah. *Bas. J. Vet*. 30-36.

Al-Taie, K.L.L. and Ali, M.F.(2007). Epidimiology of giardiasis in sulaimaniya and chamchal with its effect on some biochemical parameters and pcv. QMJ. 5 (7): 45-53.

Al-Yassaree, H.F.A. (2004). Study of certain types of intestinal protozoa *E. hisolytica; G. lamblia*; and *Cryptosporidium spp*" in Hilla city/ Iraq M. Sc. Thesis, Coll. Sci., Kufa Univ., Iraq.

Babaei Z, Oormazdi H, Akhlaghi L, Rezaie S, Razmjou E, and Soltani A.S. SK. (2008). Molecular characterization of the Iranian isolates of *Giardia lamblia*: applicationofthe glutamate dehydrogenase gene. Iran *J. Pub Health*.;37(2).

Balcioglu, C.; Kurt, O.; Sevil, N.; Dagci, H.; Tetik, A.; Ergunay,A.,Yereli, K.; Ozilgn, A.; Turg, N. and Ozensoytoz , S. (2012). Genotping of *Giardia lamblia* in a Cohort of genotypes. Kafka between Symptoms and arelationsip Trkish Patients A Search for Univ Vet Fak Derg ., 18 .A125- A131.

Bertrand, I.; Gantzer C.; Chesnot, and J. Schwartzbrod.(2004). Improved specificity for *Giardia lamblia* cyst quantification in waste water by development of a real-time PCR method. J. Microbiol. Methods, **57:**41–53.

Breathnach AS. ; McHugh TD. and Butcher PD. (2010). Prevalence and clinical correlations of genetic subtypes of *Giardia lamblia* in an urban setting. Epidemiol. Infect. 138(10):1459-1467.

Caccio, S.M and Ryan, U.(2008). Molecular epidemiology of giardiasis. Mol Biochem. Parasitol. 2008; 160(2),75-80.

Caccio, S. M.; Giacomo, D.E and E. Pozio.(2002). Sequence analysis of the β giardin gene and development of a polymerase chain reaction restriction fragment length polymorphism assay to genotype *Giardia .duodenalis* cysts from human faecal samples. International Journal for Parasitology 32: 1023–1030.

Cedilla-Rivera, R.; Darby, J.M.; Enciso-Moreno, J.A.; Ortega-Pierres, G. and Ey, P.L. (2003). Genetic homogeneity of axenic isolates of *Giardia intestinalis* derived from acute and chronically infected individuals in Mexico. Parasitol. Res., 90(2): 119-123.

Cltunge R.N and Karumba P.N . (1991) Intestinal parasites in a rural community in Kenya . *East Afr. Med. J*. , ; 68(2) : 112-22 .

David, E.B; Coradi, S.T; Oliveira–Sequeira. T. C.G; Ribolla P.E.M.; Katagiri S. and Guimaraes, S. (2011). Diagnosis of Giardia infections by PCR-based methods in children of an endemic area . *J. Venom Anim Toxins incl Trop Dis*. ,17(2): 209- 215.

Faronda, P.; Bargues, M.D.; Abreu-Acosta, A.; Periago M.V.; Valero, M.A.; V alladares, B. and Mas-Coma, S. (2008). Identification of genotypes of *Giardia intestinalis* of human isolates in Egypt. Parasitol Res., 103: 1177-1181.

Gamboa, M.; Basualdo, J. ; Córdoba, M.; Pezzani, B.; Minvielle, M. and Lahitte, H.; (2003). Distribution of intestinal parasitoses in relation to environmental and sociocultural parameters in La Plata, Argentina. J Helminthol., 77: 15-20.

Gorczynski, R. M. and Terzioglu, E. (2008). Aging and the immune system. *Int. Urol. Nephrol*., 40:1117–1125.

Gorczynski, R. M. and Terzioglu, E. (2008). Aging and the immune system.Int. Urol. Nephrol., **40:**1117–1125.

Helmy, M. M. F., Abdel-Fattah, H.S. and Rashed, L. (2009). Real-time PCR/RFLP assay to detect *Giardia intestinalis* genotypes in human isolates with diarrhea in egypt. Department of Parasitology, College of Medicine, Misr University for Science and Technology, Al-Motamayez District 6th of October Governorate, *Egypt. J. Parasitol*., 95(4), pp.1–5

Homan, W. and Mank, T.(2001). Human giardiasis: genotype linked differences in clinical symptomatology. *Int J Parasitol*., 31: 822-826**.**

Lalle, M., Pozio, E., Capelli, G., Bruschi, F., Crotti, D.and Caccio, S. M. (2005). Genetic heterogeneity at the beta-giardin locus among human and animal isolates of *Giardia duodenalis* and identification of potentially zoonotic subgenotypes. *Int. J. for Parasitol.* 35, 207–213.

Markell, E.K.; John,D.T. and Krotoski, W.A.(1999). Medical parasitology.8thed.,W.B.SaundersCo.,Philadelphia. Pp:55-26;431-445.

Minvielle, M.C.; Molina, N.B.; Polverino, D. and Basualdo, J.A. (2008). First genotyping of *Giardia lamblia* from human and feces in Argentina, South America. Mem. Inst. Oswaldo. Cruz. Rio de Janeiro, 103(1): 98–103.

Molina, N. , Polverino, D., Minvielle, M., Basualdo, J. (2007). PCR amplification of triosephosphate isomerase gene of *Giardia lamblia* in formalin-fixed feces. Rev Latinoam Microbiol; 49 (1-2): 6-11.

Monis, P.T.; Andrews, R.H.; Mayrhofer, G. and Ey, P.L. (1999). Molecular systematics of the parasitic protozoan *Giardia intestinalis.* Mol. Biol. Evol; 16(9): 1135–1144.

Nader. P, Hamidullah. R,; Mohammad. R.N,; Hossein A. Y, and Ahmad H.S. (2014). Molecular identification of *Giardia lamblia*; is there any correlation between diarrhea and genotyping in Iranian population, Gastroenterol Hepatol Bed Bench. Summer; 7(3): 168–172.

Nayef ,H. Ch.; Macheed, Ch. L. and Abd-AL-Wahab. I.(2008). Epidemiological study in the prevalence of intestinal References protozoa in humans in the city of Baghdad. Nahrain University of Science magazine 14(3): 57-62.

Nyamngee, IA. ;Om Kolawole,G.; Durowade, K.A. and Kolawole, C.F.(2009). Prevalence of giardiasis among children in Guma refugee camp in GumaLga, Benue state, *Nigeria. SM.J.* 6(1): 32-39.

O' Handley, R.M.; Olson,; M.E.; Fraser, D.; Adams, P. and Thompson RC .(2000). Prevalence and genotypic characterisation of *Giardia* in dairy calves from Western Australia and Western Canada. *Vet Parasitol 90*: 193-200.

Ojonoma, O. L.(2008). A review of sexually transmitted diseases (STDs) ofparasitic origin: The case of giardiasis. *African J.Biotechnol. ;*7 :4979-4981.

Ouattara, M.; `Guessan, N.A.; Yapi, A. and Goran, E.K. (2010). Prevalence and spatial distribution of *Entamoeba histolytica*/*dispar* and *Giardia lamblia* among school children in Agboville area. Plos. Negl. Trop. Dis., 4 (1): 574-580.

Ouattara, M.; `Guessan, N.A.; Yapi, A. and Goran, E.K. (2010). Prevalence and spatial distribution of *Entamoeba histolytica*/*dispar* and *Giardia lamblia* among school children in Agboville area. Plos. Negl. Trop. Dis., 4 (1): 574-580.

Peter, M.R. and Lisa, A.C.(2010). Human-Animal Medicine.Zoonoses.9: 167-171.

Plutzer, J.;Ongerth, J. and Karanis, P. (2010). *Giardia* Taxanomy , Phylogeny and Epidemiology: Facts and Open Questions. *Int. J. Hyg. Environ. Health*. 213:321-333.

Samuel, W.M.; Pybus, M.J. and Kocan, A.A.(2001). Parasite diseases of Wild Mammals.2nd ed. Enteric Protozoans. *Giardia* and Giardiasis. (16) 399-404.

Schares,G.;Pantchev,N.;Barutzki,D.;Heydorn,A.O.;Bauer,C.andConraths, F.J. (2005) . Oocysts of *Neospora caninum*, *Hammondia heydorni*, *Toxoplasma gondii* and *Hammondia hammondi* in faeces collected from dogs in Germany. *Int. J. Parasitol*. 35**:** 1525–1537.

Sulaiman, I.M., Fayer, R., Bern, C., Gilman, R.H, Trout, J.M, Schantz, P.M., Das, P., Lal, A.A. and Xiao, L. (2003). Triosephosphate isomerase gene characterization and potential zoonotic transmission of *Giardia intestinalis*. Emerging infect. Dis.9 (11):1444-1452.

Suzuki, J., Murata, R., Kobayashi, S., Sadamasu, K., Kai, A and Takeuchi, T.(2011). Risk of human infection with *Giardia duodenalis* from cats in Japan and genotyping of the isolates to assess the route of infection in cats. Parasitol. 138(4).493-500.

Thompson, R.C. and Monis, P.T. (2004). Variation in *Giardia* implication for Taxonomy and Epidemiology. AdvParasitol. 58 :69- 137.

Thompson, R.C.; Hopk, R.M. and Homan, W.L. (2000). Nomenclature and genetic grouping of *Giardia* infecting mammals. Parasitology Today 16: 210-213

Thompson, R.C.; Palmer, C.S. and O'Handlely, R. (2008). Public health and clinical significance of *Giardia* and *Cryptosporidium* in domestic animals. *Vet. J.*, 177 (1): 18-25.

Traub, R.J.; Monis, P.T.; Robertson, I.; Irwin, P.; Mencke, N. and Thompson, R.C.A. (2004). Epidemiological and molecular evidence supports the zoonotic transmission of *Giardia* among humans and dogs living in the same community. Parasitol., 128: 253–262.

Tungtrongchitr.A.; Sookrury, N.; Indrawattan,N.; Kwangsi, S.; Ongrotchanakun, J. and Chaicumpa, W.; (2010) : *Giardia intestinalis* in Thailand: Identifiction of Genotypes. J Health Popul Nutr.28(1): 42-52.

Vander- Giessen, J.W.B.; De- Vries, A.; Roos, M.; Wielinga, P.; Kortbeek, L.M. and Mank, T.G. (2006). Genotyping of *Giardia* in Dutch patients and animals: A phylogenetic analysis of human and animal isolates. Int. J. Parasitol., 36(7): 849-858.

Wensaas, K.A.(2011). Giardiasis in Bergen. Outbreak and clinical consequences, dissertation for the degree philosophiae doctor (PhD) at the University of Bergen pp:82.

Wicki, M. ;Svoboda, P. and Tanner, M. (2009). Occurrence of *Giardia lamblia* in recreational streams in Basel Landschaft , Switzerland. Environ. Res.; Article in press.

Xiao L. and Fayer R. (2008). Molecular characterization of species and genotypes of Cryptosporidium and Giardia ana assessment of zoonotic transmission. Int. j for parasitol., 38, 1239-1255.
Zeid, I.K.A. and Abdullah, Y.AR. (2011). Relationship between the eating out home and infection with giardiasis. Primary health care center in Al –Najaf Al- Ashraf-Iraq.