

Phylogenic study of Genotypeing *Giardia duodenalis* from Cattle in Wasit province

Zainab A. Makawi Athmar Khudair Abbas

Department of Parasitology/ College of Veterinary Medicine/ University of Baghdad/ Iraq.

E-mail: Zainabalwan540@yahoo.com

Received date:15Dec 2019 Accepted:(463) 24Dec 2019 page (65-73) Pubished:31Dec 2019

DOI: <https://doi.org/10.36326/kjvs/2019/v10i23315>

Abstract

The present study aimed to investigated *Giardia duodenalis* in cattle in some different areas of Wasit province by using molecular study and verification of the genotype of *Giardia duodenalis*. Collected one hundred fecal samples from cattle, the result showed that the rate of infection was 83% (100) . DNA was extracted from the 100 positive samples from the cattle then amplified using the special tris-phosphatesomerase gene for genotyping A and B. The result of type A infection was (69%) and (45%) of the genotype B. The purpose of this study was to investigate the genotypes of cattle in Wasit province and compare them with previous sources at the NCBI data bank.

Keywords: *Giardia duodenalis* ,genotypes ,cattle, Molecular diagnosis, phylogenic study.

دراسة وراثية لانماط الجيارديا في الابقار في محافظة واسط

زينب ن اوعل مكناوي
فرع الطفيليات / كلية الطب البيطري / جامعة بغداد

الخلاصة:

تهدف الدراسة الى التحقق من طفيلي الجيارديا في الابقار في بعض مناطق محافظة واسط باستخدام الدراسة الجينية في طفيلي الجيارديا، حيث جمعت 100 عينة براز الابقار، أظهرت النتيجة أن معدل الإصابة بالعدوى وبيئ التحقق من انماط الإصابة كان 83% (100). حيث تم استخراج الحمض النووي من العينات الموجبة وبالغلة 100 عينة من الابقار ثم بعد تضخيمها باستخدام الجين ثلاثي الفوسفاتيزوميراز الخاص للنمطين A and B . كانت نتيجة الإصابة بالنمط A هو (69%) و(45%) من النمط الوراثي B وكان الغرض من هذه الدراسة هو التحقيق في الأنماط الوراثية من الابقار في محافظة واسط ومقارنتها مع المصادر السابقة في بنك المعلومات NCBI .

Introduction

Giardia duodenalis (syn. *Giardia lamblia*, *Giardia intestinalis*) is a widespread and prevalent intestinal protozoa with a broad host range that includes humans, domestic animals, and wildlife. *G. duodenalis* is one of the most frequently identified parasites

causing diarrhea worldwide (1). Giardiasis clinical manifestations in cattle are relatively

variable, ranging from the absence of symptoms to persistent diarrhea, mucous and fatty stool, weight loss and growth rate reduction. (2).Cattle have been considered as potential sources of giardiasis in humans

through direct contact and/or surface water supplies contamination. (3 and 4). In Asia, Africa, and Latin America, about 200 million people have symptomatic giardiasis with some 500,000 new cases reported each year (5). The cyst is the infective stage and represents the resting stage of the organism. Its rigid outer wall protects the parasite against changes in environmental temperature, dehydration and chlorination, all of which would destroy the trophozoite (6 and 7). Transmission occurs by the faecal-oral route, either by direct contact with an infected host, or through contaminated food or water (8 and 9). Mechanical transmission of the parasite through insect vectors has also been reported (10). Factors that facilitate infection include overcrowding and high excretion of cysts by infected animals (11 and 12).

The molecular analysis of cattle isolates from different geographical locations has demonstrated that only *G. duodenalis* genotype E and the zoonotic genotypes (A and B) are associated with cattle infections. (13 and 14). The taxonomy of the genus is mainly based on morphology and genetic evidence. According to these criteria, six species have been recognised in the genus *Giardia* and these include *G. duodenalis* in humans and other mammals, *G. agilis* in amphibians, *G. muris* and *G. microti* in rodents, *G. psittaci* and *G. ardeae* in birds. In recent years, phylogenetic analysis and enzyme electrophoresis have revealed the existence of eight assemblages A–H within the species *G. duodenalis* (15 and 16).

Materials and Methods

1. Samples collections

One hundred fecal samples from cattle were collected from different area of wasit province include (Al-Hafrea, Al-swearea, Al-Azezea) , during the period from December 2018 to August 2019. The fecal sample are placed in sterile container then transported to laboratory of collage veterinary medicine in Baghdad university to examine then stored samples in refrigerator until genomic DNA extraction step.

2. Genomic DNA extraction

Genomic DNA was extracted from fecal samples by using (fecal DNA extraction Kit, Bioneer. Korea). and checked by Nanodrop spectrophotometer (Bioneer. Korea) ,to calculate the quantity and purity of the extracted DNA then stored at refrigerator(-20C) until used in PCR amplification.

3. Nested PCR amplification

Nested PCR assay was performed for detection and genotyping of *Giardia duodenalis* from cattle. The PCR assay was carried out according to Minvielle *etal.*,(2008) ,using primers for amplified triosephosphateisomerase (*tpi*) gene that specific for genotyping A and B were provided by (Bioneer company . Korea) (Table-1). The PCR products were examined by electrophoresis 1.5% agarose gel, stained with ethidium bromide, and visualized under UV transilluminator using .

Table (1) show gene, sequence and base pare of Nested PCR

Parasite	Gene		Sequence	Base pare
<i>Giardia duodenalis</i>	Genotype A TPI gene First step	F	CGAGACAAGTGTTGAGATG	576 bp
		R	GGTCAAGAGCTTACAACACG	
	Genotype A TPI gene Second step	F	CCAAGAAGGCTAAGCGTGC	476 bp
		R	GGTCAAGAGCTTACAACACG	
	Genotype B TPI gene First step	F	GTTGCTCCCTCCTTTGTGC	208 bp
		R	CTCTGCTCATTGGTCTCGC	
	Genotype B TPI gene Second step	F	GCACAGAACGTGTATCTGG	140bp

4-Sequence analysis of TPI gene of *G.duodinalis* and Phylogenetic analysis

Genotyping was performed using sequence analysis on the 10 PCR products of *G.duodinalis* nucleotide. Sequence information was obtained for a representative isolate of each of the Assemblages A and B from the NCBI database. The resulting sequences were analyzed and compared with similar *G.duodinalis* sequences deposited in Gen-Bank using the Basic Local Alignment Search Tool (BLAST) program. phylogenetic tree was constructed using

Unweighted Pair Group method with Arithmetic Mean (UPGMA tree) in (MEGA 6.0 version).

Results

1-Identification of *G. duodinalis* genotypes

Among 100 fecal samples from cattle diagnosed by Nested PCR, amplification of tpi gene of *G. duodinalis* was successful 83/100 (83%) samples. However, the amplification of these samples showed that 69/100 (69%) contained genotype A (fig.1and2) and 45/100 (45%) samples contained genotype B (fig.3and4) Table (2 and 3).

Table(2): Total infection rate with *Giardia duodenalis* in cattle by Nested PCR:

Host	No.of Samples examined	No.of positive	Percentage (%)
Cattle	100	83	83

Table(3).The results of nested PCR technique for detection of genotyping *Giardia duodnalis* of cattle

Genotype of <i>Giardia duodnalis</i>		No. of Samples examined	No. of positive	Percentage (%)
Assemblage A	100	69	69	
Assemblage B		45	45	

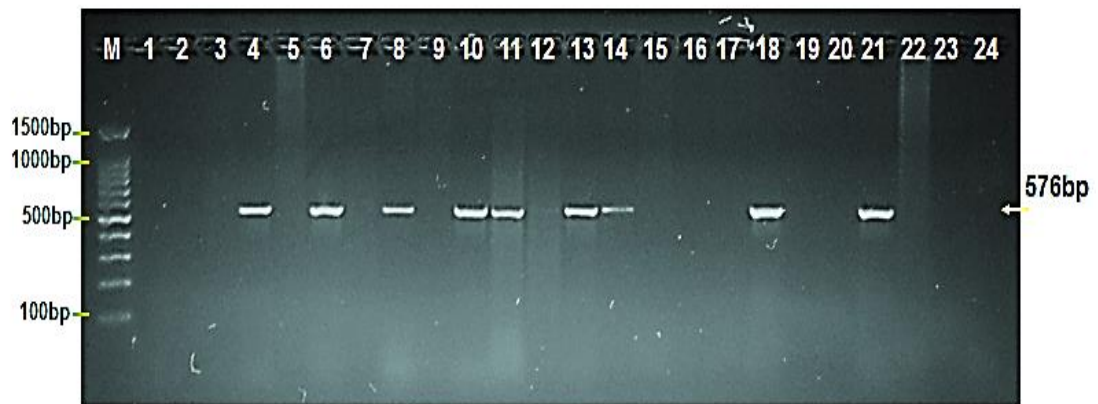


Figure (1): Agarose gel electrophoresis image that showed PCR product analysis for triosephosphate isomerase gene (TPIA) gene in *Giardia duodnalis* genotype (A) isolates. M (Marker ladder 1500-100bp). Lane (4,6,8,10,11,13,14,18,21) positive *Giardia duodnalis* genotype A at 576bp PCR product size.

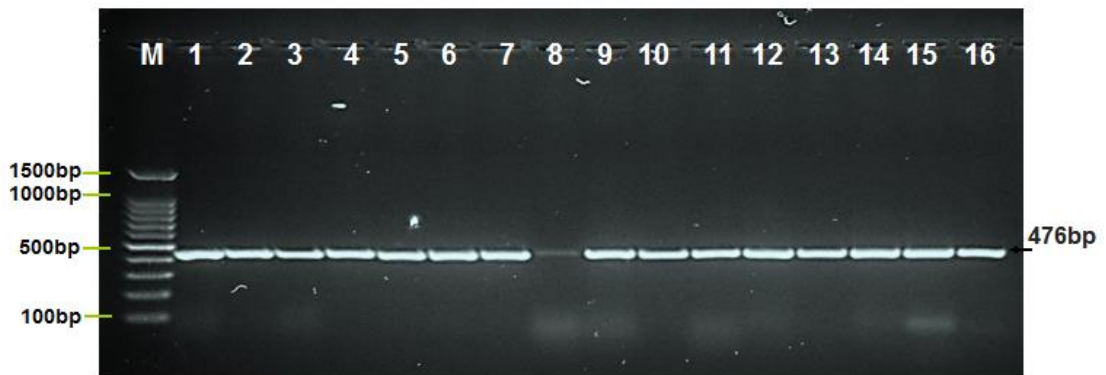
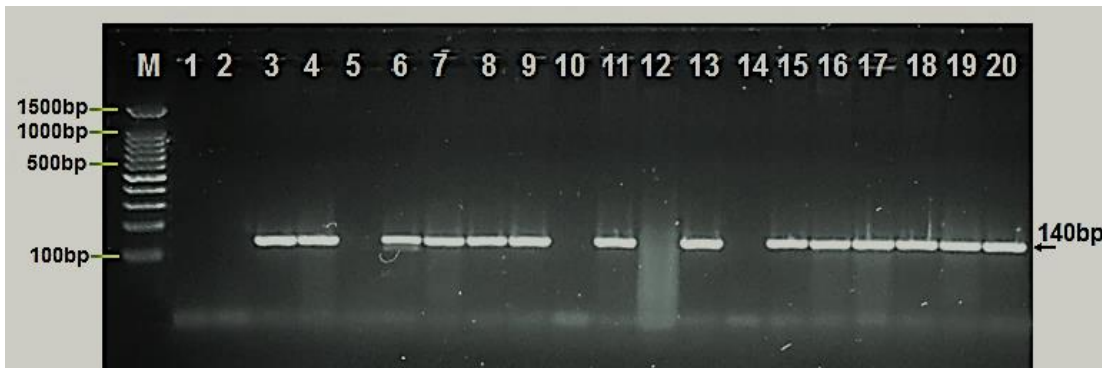


Figure (2): Agarose gel electrophoresis image showed Nested PCR product analysis for triosephosphate isomerase gene (TPIA) gene in *Giardia duodnalis* genotype (A) isolates. M (Marker ladder 1500-100bp). Lane (1-16) some positive for genotype A at 476bp .



Figure (3): Agarose gel electrophoresis image that showed PCR product analysis for triosephosphate isomerase gene (TPIA) gene in *Giardia duodenalis* genotype (B) isolates. M (Marker ladder 1500-100bp). Lane (4,5,7,8,11,14) positive *Giardia duodenalis* genotype B at 208bp PCR product size.



Figure(4): Agarose gel electrophoresis image showed Nested PCR product analysis for triosephosphate isomerase gene (TPIA) gene in *Giardia duodenalis* genotype (B) isolates. M (Marker ladder 1500-100bp). Lane (3,4,6,7,8,9,11,13,15,16,17,18,19,20) positive *Giardia duodenalis* genotype B at 140bp .

2-Result of sequence analysis of TPI gene of *G.duodenalis* and Phylogenetic tree

The result of Sequences of 10 PCR products (5 PCR products for assemblage A and 5 PCR products for assemblage B) of *G.duodenalis* alignment compared with references for *G duodenalis* which previous recorded in NCBI genbank. Five *G duodenalis* of genotype A isolate IQS (No.1, No.2, No.3, No.4, No.5) were showed

closed related to Ncbi blast *G duodenalis* of **Japan** with identity (99.55%, 99.66%, 99.64%, 99.61% and 99.62%) whereas isolate IQS (No1, No2, No3, No4 and No5), respectively. While Five sample of *G duodenalis* genotype B isolate (No.6, No.7, No.8, No.9, No.10) were showed closed related to Ncbi blast *G duodenalis* of **Iran** with identity (99.03%, 97.58%, 99.51%,

99.03% and 99.51%) respectively. (Table 4 and 5)

Phylogenetic tree of local *Giardia duodinalis* (No.1-No.5) were showed closed related to NCBI-BLAST *Giardia duodinalis* TPI gene for triosephosphate isomerase, genotype: assemblage A (LC437479.1) at

total genetic changes (0.0010%). Also The local *Giardia duodinalis* (No.1-No.5) were showed closed related to NCBI-BLAST *Giardia duodinalis* TPI gene for triose phosphate isomerase, genotype: assemblage B (LC505049.1) at total genetic changes (0.0020%) fig(5 and 6).

Table (4). The NCBI-BLAST Homology Sequence identity (%) between local *Giardia duodinalis* IQS-No.1 isolates and NCBI-BLAST submitted *Giardia duodinalis* isolates:

<i>G duodinalis</i> isolate No.	Genbank Accession number	NCBI-BLAST Homology Sequence identity (%)			
		Identical NCBI genotype	Genbank Accession number	County	Identity (%)
<i>G duodinalis</i> No.1	MN815121	Assemblage A	LC437479.1	Japan	99.55%
<i>G duodinalis</i> No.2	MN815122	Assemblage A	LC437479.1	Japan	99.66%
<i>G duodinalis</i> No.3	MN815123	Assemblage A	LC437479.1	Japan	99.64%
<i>G duodinalis</i> No.4	MN815124	Assemblage A	LC437479.1	Japan	99.61%
<i>G duodinalis</i> No.5	MN815125	Assemblage A	LC437479.1	Japan	99.62%

Table (5) the NCBI-BLAST Homology Sequence identity (%) between local *G duodinalis* IQS-No.1 isolates and NCBI-BLAST submitted *G duodinalis* isolates:

<i>G duodinalis</i> isolate No.	Genbank Accession number	NCBI-BLAST Homology Sequence identity (%)			
		Identical NCBI genotype	Genbank Accession number	County	Identity (%)
<i>G duodinalis</i> No.6	MN815126	Assemblage B	LC505049.1	Iran	99.03%
<i>G duodinalis</i> No.7	MN815127	Assemblage B	LC505049.1	Iran	97.58%
<i>G duodinalis</i> No.8	MN815128	Assemblage B	LC505049.1	Iran	99.51%
<i>G duodinalis</i> No.9	MN815129	Assemblage B	LC505049.1	Iran	99.03%
<i>G duodinalis</i> No.10	MN815130	Assemblage B	LC505049.1	Iran	99.51%

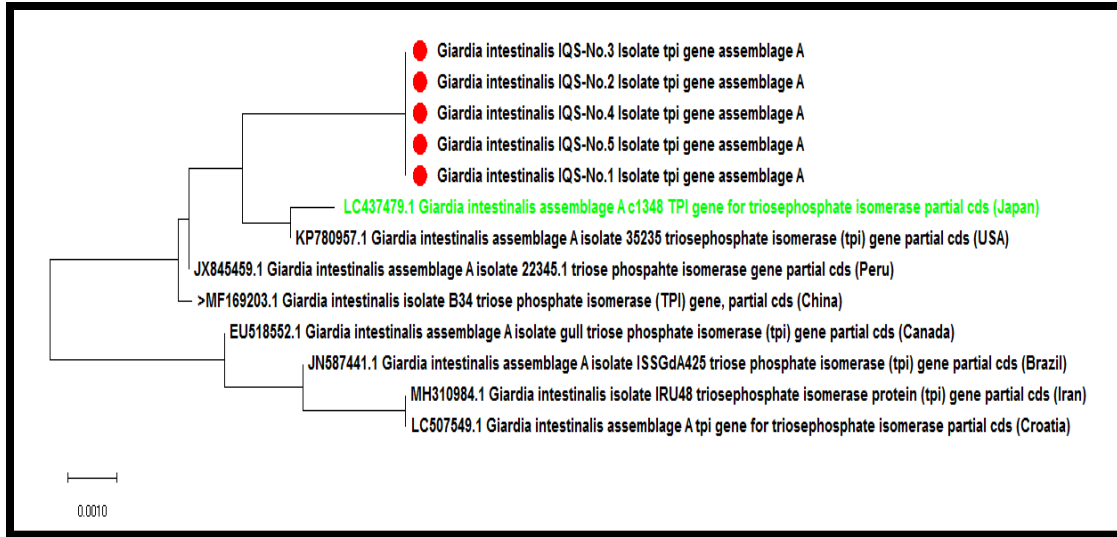


Figure (5): Phylogenetic tree analysis based on triose phosphate isomerase (tpi) gene partial sequence in local *Giardia duodenalis* IQS-No.1-No.5 that used for genetic relationship analysis . The phylogenetic tree was constructed using Unweighted Pair Group method with Arithmetic Mean (UPGMA tree) in (MEGA 6.0 version). The local *Giardia duodenalis* IQS-No.1-No.5 were showed closed related to NCBI-BLAST *Giardia duodenalis* assemblage A c1348 (LC437479.1) at total genetic changes (0.0010%).

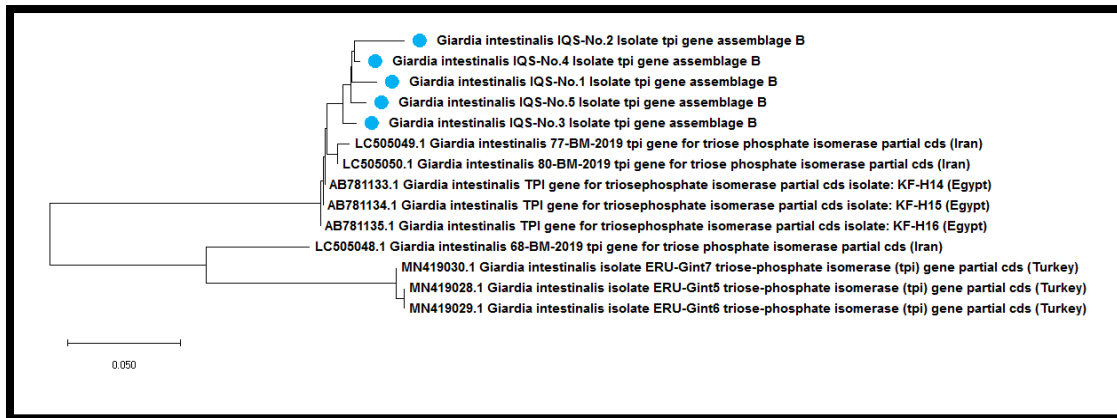


Figure (6): Phylogenetic tree analysis based on triose phosphate isomerase (tpi) gene partial sequence in local *G. duodenalis* IQS-No.1-No.5 that used for genetic relationship analysis . The phylogenetic tree was constructed using Unweighted Pair Group method with Arithmetic Mean (UPGMA tree) in (MEGA 6.0 version). The local *G. duodenalis* IQS-No.1-No.5 were showed closed related to NCBI-BLAST *G. duodenalis* 77-BM-2019, Iran isolate genotype: assemblage B (LC505049.1) at total genetic changes (0.0020%).

Discussion

The prevalence of *G. duodenalis* assemblage A was high in the present study, Moreover (17, 18 , 19), who observed infection with *Giardia* was widely distributed among farms animal, as *G. duodenalis* assemblage A was identified in 78% of the dairy calve and 57% of the beef calve farm and similarly wide distribution of *G. duodenalis* assemblage A among cattle farms with other research reported in the United States . The wide distribution of *G. duodenalis* assemblage A on these farms suggests that *G. duodenalis* assemblage A is probably more widespread in calves than other assemblage has been assumed, although not diagnosed by commonly used PCR and sequencing protocols.

This study recorded high infection rate and similar with (20) who recorded high prevalence assemblage A of *G. duodenalis* especially in the dairy calves. And disagreement with (21) who recorded low infection rate of *G duodinalis* was (20%) of genotype A and high infection was (64%) of genotype B. The occurrence of Assemblage A and B of *G. duodenalis* have been reported in Thailand, China, and the Philippines. (22). In this study population of assemblage A was identified and similar to the previous studies from Korea, Japan, Egypt, and Brazil. (23).

The wide distribution of *G. duodenalis* assemblage A in cattle suggests that probably is uncertain whether or due to repeated infection of susceptible calves and reflects the ability of assemblage A isolates to persist and spread among calves (20). However, even a low prevalence of assemblage A or B isolates could pose a significant public health risk, since infected animals tend to excrete a large number of cysts (24).

References

1-Cacci, S.,M., Thompson, R.C., Lauchlin, J., Smith, H.V., (2005). Unravelling

Cryptosporidium and *Giardia* epidemiology. Trends Parasitology. 21,430–437.

2-Lalle M, Pozio G and Capelli F. (2005).Genetic heterogeneity at the giardin locus among human and animal isolates of *Giardia duodenalis* and identification of potentially, zoonotic subgeno types. International Journal parasitology 35: 207-213.

3-O' Handley RM, Olson ME and Fraser D, (2000). Prevalence and genotypic characterization of *Giardia* in dairy calves from Western Australia and Western Canada. Veterinary parasitology 90: 193-200.

4-Hunter, P.R and Thompson, R.C. (2005). The zoonotic transmission of *Giardia* and *Cryptosporidium*. International Journal for parasitology (35);1181-1190.

5-WHO, (1995).The World Health Report, Fighting Disease Fostering Development, World Health Organization, Geneva, p. 38.

6-Olson M, O'Handley R, Ralston B, McAllister T, Thompson R. (2004).Update on *Cryptosporidium* and *Giardia* infections in cattle. Trends in Parasitology. 20:151-198.

7-Thompson R, Monis P.(2012). *Giardia* – from genome to proteome. Advances in Parasitology. 78:57-95.

8-Gow S, and Waldner C. (2006). An examination of the prevalence of and risk factors for shedding of *Cryptosporidium* spp. and *Giardia* spp. in cows and calves from western Canadian cow calpherds. Veterinary Parasitology. 137:50-61.

9- Hamnes, I.S.; Gjerde, B., and Robertson, L.(2006). Prevalence of *Giardia* and *Cryptosporidium* in diary calves in three areas of Norway. Veterinary. Parasitology., 140:204-216.

10-Graczyk, T.K. Grimes B.H. Knight, R. Da Silva, A.J. Pieniazek, N.J. Veal, D.A. (2003). Detection of *Cryptosporidium parvum* and *Giardia lamblia* carried by synanthropic flies by combined fluorescent in situ hybridization and a monoclonal antibody. American Journal of Tropical Medicine and Hygiene. **68**:228-232.

11-Rendtorff, R.C. (1954). The experimental transmission of human intestinal protozoan parasites. II. *Giardia lamblia* cysts given in capsules. American Journal of Hygiene.**59**:209-220.

12-Faubert, G.M.(1996). The immune response to *Giardia*. Parasitology Today. **12**:140-145.

13-Thompson RCA, Hopkins RA, and Homan WL.(2000). Nomenclature and genetic groupings of *Giardia* infecting mammals. Parasitology Today 16: 210–218.

14- Monis, P.,T. and Thompson, R.,C.,A.(2003). *Cryptosporidium* and *Giardia* zoonoses: Fact or fiction Infect Genetic Evolution 3: 233-244.

15-Berrilli F, Di Cave D, De Liberato C, Franco A, Scaramozzino P, Orecchia P (2004). Genotype characterisation of *Giardia duodenalis* isolates from domestic and farm animals by SSUrRNA gene sequencing. Veterinary Parasitology. **122**:193-199.

16-Heyworth, M.,F.(2016). *Giardia duodenalis* genetic assemblages and hosts. Parasite;**23**:13.

17-Trout, J.M., Santin, M., Greiner, E.C. and Fayer, R. (2004). Prevalence of *Giardia duodenalis* genotypes in the pre- weaned dairy calves . Veterinary. Parasitology. 124, 179–186

18-Trout, J.M., Santin, M., Greiner, E.C. and Fayer, R. (2005). Prevalence and genotypes of *Giardia duodenalis* in post weaned dairy calves . Veterinary. Parasitology. 130, 177–183.

19-Trout, J.M., Santin, M., Greiner, E.C. and Fayer, R. (2006). Prevalence and genotypes of *Giardia duodenalis* in 1–2 year old dairy cattle. Veterinary. Parasitology. 140, 217–222.

20-Geurden, T. Geldhof , P. Levecke, B. Martens, C. Berkvens D. Casaert, S. Vercruyss, J. Claerebout, E. (2008).Mixed *Giardia duodenalis* assemblage A and E infections in calves. International Journal for Parasitology 38 ;259–264.

21-Al-Difaie, R.,S. (2016). Molecular Study to Detect Genotyping of *Giardia lamblia* from Human and Cattle Feces in Al-Qadisiya Governorate, Iraq. *Ibn Al-Haitham J. for Pure & Application. Sciences . Volum. 29 (3); 1-13.*

22-Yason, J.,A. and Rivera, W.,L., (2007). Genotyping of *Giardia duodenalis* isolates among residents of slum area in Manila, Philippines. Parasitology Res 101: 681–687.

23-Volota~o, A.,C., Costa-Macedo, L.,M., Haddad, F.,S., Branda,~o., A., Peralta, J.,M., Fernandes, O. (2007). Genotyping of *Giardia duodenalis* from human and animal samples from Brazil using b-giardin gene: a phylogenetic analysis. Acta Tropical 102: 10–19.

24-Geurden, T. (2007). *Cryptosporidium* and *Giardia* in calves in Belgium. Doc. Phy., Thesis. Faculty veterinary medicine. University of Ghent.