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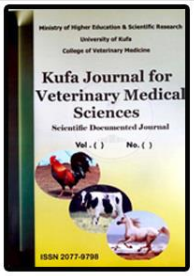

Phylogenic study of Genotypeing *Giardia duodenalis* from Cattle in Wasit province

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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 duodinalis*** **,genotypes ,cattle, Molecular diagnosis, phylogenic study.**

دراسة وراثية لانماط الجيارديا في الابقار في محافظة واسط

زينب علوان مكاوي اثمار خضير عباس

فرع الطفيليات / كلية الطب البيطري / جامعة بغداد

الخلاصة:

هدفت الدراسة الحالية للتحقق من طفيلي الجيارديا في الابقار في بعض مناطق مختلفة من محافظة واسط باستخدام الدراسة الجزيئية والتحقق من الأنماط الجينية في طفيلي الجيارديا ،حيث تم جمع 100 عينة من برازالابقار ، أظهرت النتيجة أن معدل الإصابة كان 83 ٪ (100)..حيث تم استخراج الحمض النووي من العينات الموجبة والبالغة 100 عينة من الابقار ثم بعد تضخيمها باستخدام الجين ثلاثي الفوسفاتيزوميراز الخاص للنمطين A and B .

كانت نتيجة الاصابة 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 and7). 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 duodinalis* 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
Giardia duodenalis	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*Giardia duodnalis*		No. of Samples examined	No. of positive	Percentage (%)
Assemblage A	100	69	69	
Assemblage B		45	45	

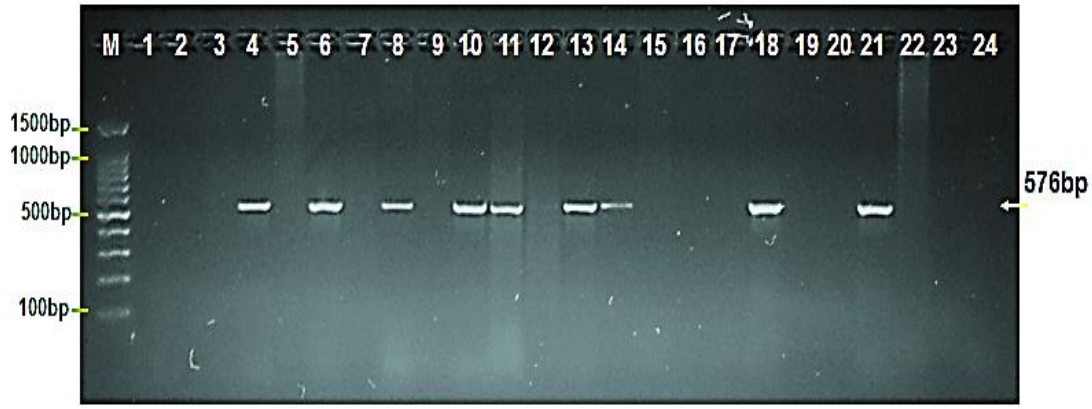


Figure (1): Agarose gel electrophoresis image that showed PCR product analysis for triosephosphate isomerase gene (TPIA) gene in *Giardia duodinalis* genotype (A) isolates. M (Marker ladder 1500-100bp). Lane (4,6,8,10,11,13,14,18,21) positive Giardia *duodinalis* genotype A at 576bp PCR product size.

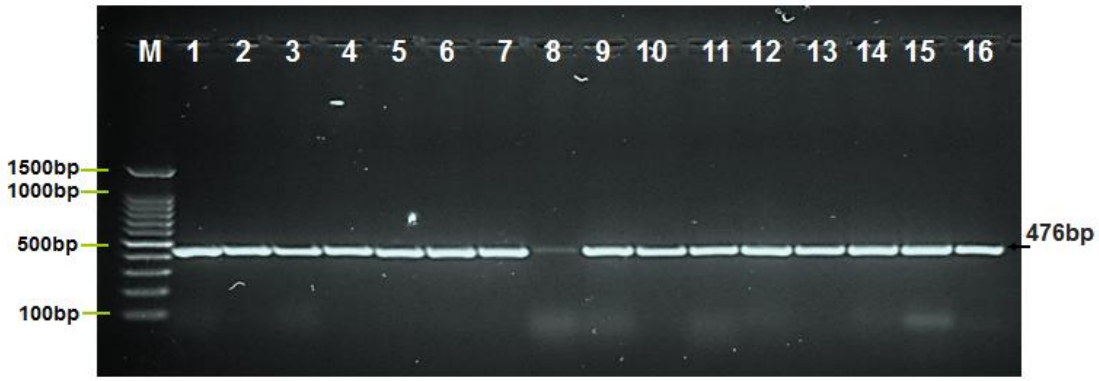


Figure (2): Agarose gel electrophoresis image showed Nested PCR product analysis for triosephosphate isomerase gene (TPIA) gene in *Giardia duodinalis* genotype (A) isolates. M (Marker ladder 1500-100bp). Lane (1-16) some positive for genotype A at 476bp .

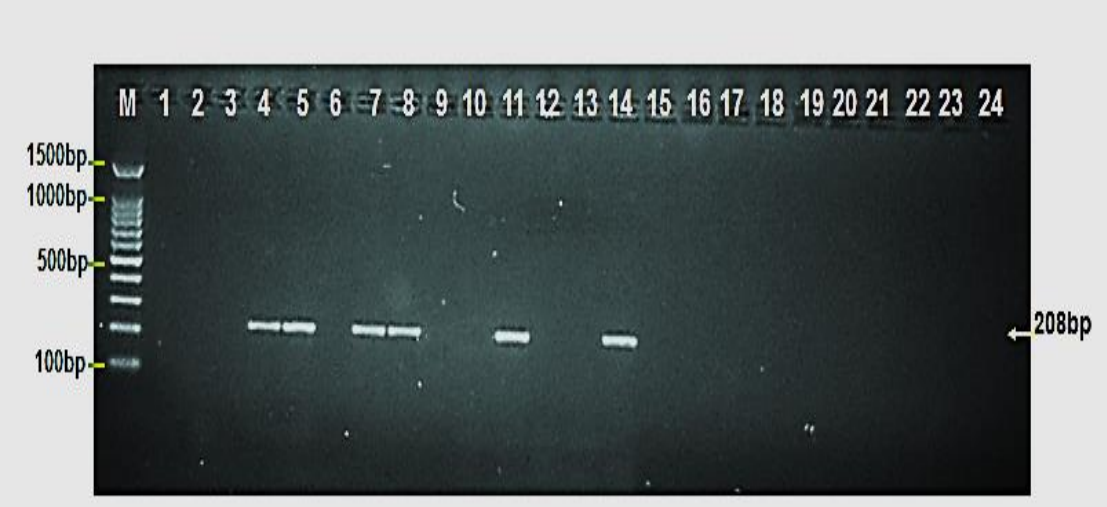
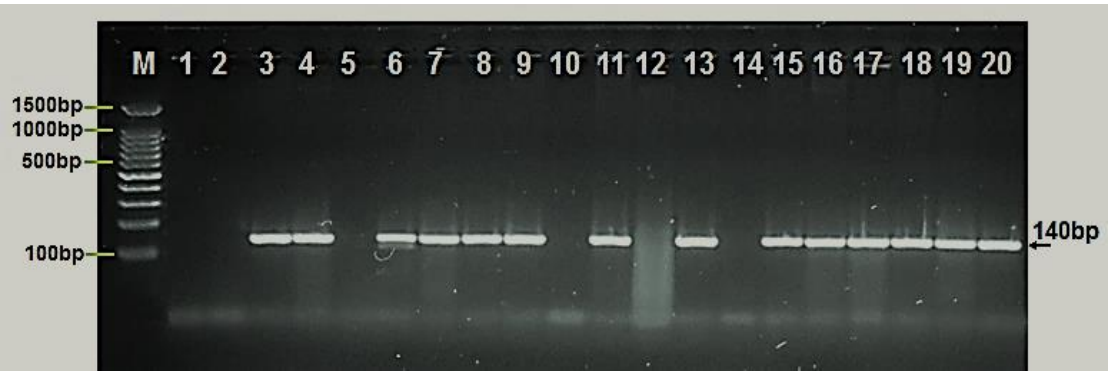


Figure (3): Agarose gel electrophoresis image that showed PCR product analysis for triosephosphate isomerase gene (TPIA) gene in Giardia *duodinalis* genotype (B) isolates. M (Marker ladder 1500-100bp). Lane (4,5,7,8,11,14) positive Giardia *duodinalis* genotype B at 208bp PCR product size.



Figure(4): Agarose gel electrophoresis image showed Nested PCR product analysis for triosephosphate isomerase gene (TPIA) gene in *Giardia duodinalis* 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 duodinalis* genotype B at 140bp .

2-Result of sequence analysis of TPI gene of *G.duodinalis* 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.duodinalis* alignment compared with references for *G duodinalis* which previous recorded in NCBI genbank. Five *G duodinalis* of genotype A isolate IQS (No.1, No.2, No.3, No.4, No.5) were showed closed related to Ncbi blast *G duodinalis* 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 duodinalis* genotype B isolate (No.6, No.7, No.8, No.9, No.10) were showed closed related to Ncbi blast *G duodinalis* 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 triosephosphateisomerase, 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.1isolates and NCBI-BLAST submitted *Giardia duodinalis* isolates:

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

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

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

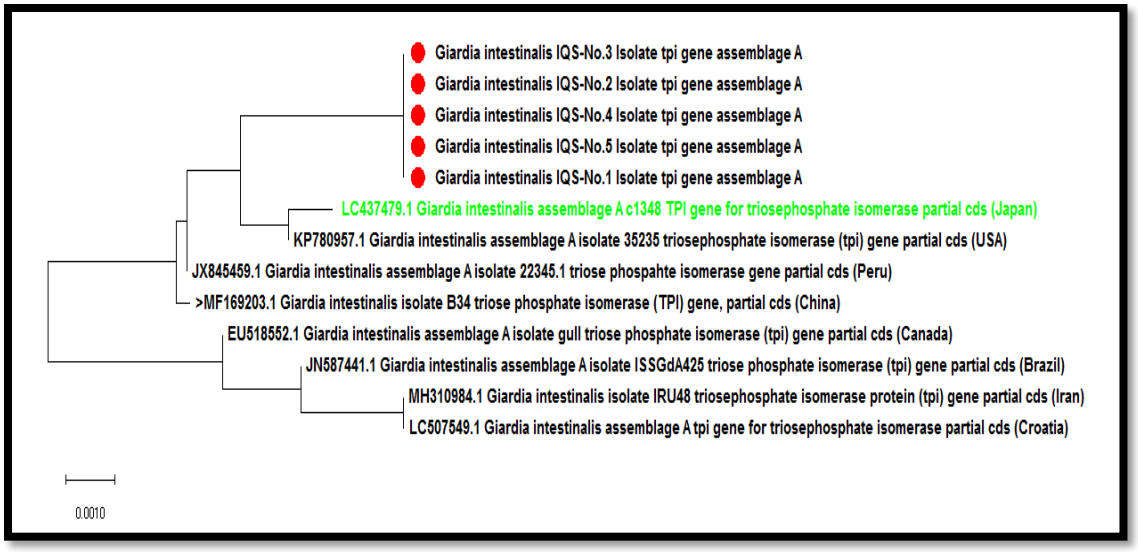


Figure (5): Phylogenetic tree analysis based on triose phosphate isomerase (tpi) gene partial sequence in local *Giardia duodinalis* IQS-No.1-No.5 that used for genetic relationship analysis . The phylogenetic tree was constructed using Unweighted Pair Group method with Arithmetic Mean (UPGMA tree) in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

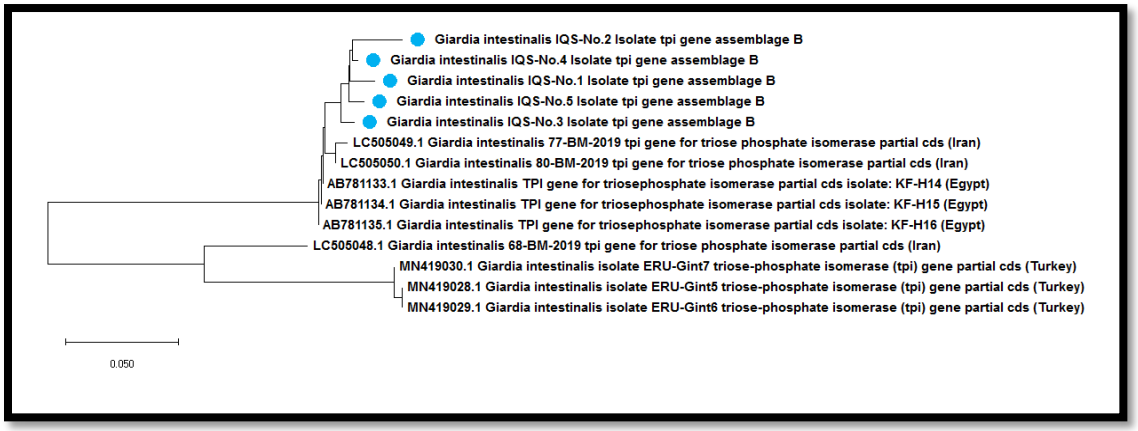


Figure (6): Phylogenetic tree analysis based on triose phosphate isomerase (tpi) gene partial sequence in local *G. duodenalis* IQS-No.1-No.5 that used for genetic relationship analysis . The phylogenetic tree was constructed using Unweighted Pair Group method with Arithmetic Mean (UPGMA tree) in (MEGA 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 infectionwas (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).

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