Genotyping Detection of Giardia Lamblia from Human and Animal Feces Samples In Wasit Province, Iraq

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Abstract

Background: *Giardia lamblia*, also defined as duodenalis or intestinalis, is a zoonotic enteroparasite that has a short life cycle and two active trophozoite and cystic types, both of these parasites can be identified in the stool of hosts and can be detected microscopically**Methods:** using microscopic examination of samples stained with iodine firstly, while the second one included determining the genotypes of the parasite-positive samples using the Polymers Chain Reaction (PCR) technique, and using Nested PCR to detect genotypes A, B, E and F in human and animals.**Results:** Genotypes A, B, E, F were detected in human samples using the molecular technique Nested PCR, recorded three genotypes in human samples A,B,E,F 7/15,8/15,0/15,0/15 , results showed are 7/15, 3/9, 3/9, 0/9 with 46.66%, 33.33%, 33.33%, for genotype A, B, E while F no sample was recorded, the results of the statistical analysis showed a significant differences between the genotypes in human samples P>0.05. In Nested PCR, three genotypes A, B, E were recorded in animal 3/9, 3/9, 3/9, 0/9 33.33%, 66.66%, 50%, 0%, respectively. While no positive sample was recorded for The F genotype.**Conclusions:** Giardiasis in human, men having a higher then female, children higher than adults, urban higher than rural.In Nested PCR human have genotypes A, B while animal A, B, E.

Keywords: AR- (Androgen Receptor), bg-(β-giarden), UV-(Ultraviolet).

1. Introduction

Ium parvum and rotavirus, humans are susceptible to the disease giardiasis, whichGiardiasis, a condition characterized by diarrhea, is brought on by its proliferation in an extracellular space found in the small intestine of vertebrate hosts (Cernikova *et al.*,2021). *G. lamblia* can infect all mammals. Human infections may be asymptomatic or accompanied by malabsorption, abdominal pain, bloating, diarrhea, weight loss, and fatigue (Rumsey & Waseem, 2018). Clinical manifestations of infection with the parasite *G. lamblia* vary; symptoms may be absent until they appear. Fatty diarrhea, weight loss, abdominal pain, vomiting, malnutrition, and nausea are some of the most prominent symptoms of this parasite infection (Hasan, *et al.*, 2021).

G. lamblia transfers via fecal-oral channel via direct or indirect intake of infectious cysts; the period of incubation varies from 9 to 15 days following cyst ingestion. Infection symptoms ranged from no symptoms to sudden watery diarrhea, weight loss, nausea, and epigastric discomfort (Hooshyar *et al.*, 2019).

G. lamblia is the third most prevalent cause of diarrhea worldwide, with over 300 million recorded cases each year, behind only Cryptosporid has a prevalence of 2%–3% in developed countries and more to 30% in poor and developing countries. It was previously listed as one of the WHO's neglected disorders and is strongly associated with both poverty and poor water quality (Cernikova *et al.*, 2021).

Molecular tools have demonstrated that *G. lamblia* is a species complex comprising at least eight assemblages A–H, among which assemblages B and A are major groups, they found in both humans and animals, while six groups C to H, may had host-specific and mostly not infect humans (Ryan *et al.*,2013).

Aims of the study:Find out the prevalence of G. Lamblia by using microscopically examination and Molecular technique in human and animal (Sheep, Caws) by using nested PCR in Wasit province Iraq, Identify some genotypes of G. lamblia A, B, E and F,Find out zoonotic transmission of the G. Lamblia between human and animals.

2. Materials and Methods

Study Area

A Total of 96 stool sample were collected 48 samples taken from diarrheal human patient and 48 samples from animal during the period from the beginning of October 2020 till the end of April 2021 for detect of G. lambila and determining genotypes of this parasite in Al- Kut province.

Collection of Samples

DNA Test Optical microscopy analysis of materials within thirty minutes of collection using direct wet swab and volunteering method to search for traphopzoite and cyst stage of the parasite *G. Lamblia*, and samples having positive microscopy were saved without adding preservatives at -20 ° C until the start of the process of extracting the DNA

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from them and subjecting to traditional PCR technology nested PCR in the research laboratory (Roveri, et al., 2020) .

Direct smear method

It's fast and easy method to notice by traphopzoites and cysts in fecal samples, On a transparent slide, a drop of normal saline was placed and mixed with a small amount from fecal samples and iodine stain by wood stick and then covered by cover slip and examine by X40 (Kaewjai, *et al.*, 2020)

Stool DNA Extraction

Using the PrestoTM Stool DNA Extraction Kit from Geneaid, Taiwan, parasitic DNA was extracted from stool samples, With the use of a Nanodrop spectrophotometer (THERMO, USA), the isolated stool DNA was examined for integrity and purity by reading the absorbance at (260/280 nm),for DNA investigation.

Nested PCR technique

Nested PCR for detection based on 18S ribosomal RNA gene and genotyping of Giardia intestinalis detection genotyping A, B, E, and F based on triosephosphate isomerase gene tpi. **Nested PCR primer *Giardia lamblia* assemblage (A, B, E, and F)**

Primers		Sequences (5'-3')	Amplicon	Reference or code of Gen Bank
Giardia lamblia 18Sr RNA gene PCR	F-	CAAGGACGAAGCCATGCATG	683bp	DQ157272.1
	R-	ATTGACAGAGGCGGTCTTGG		
Giardia lamblia 18Sr RNA gene nested PCR	F-	CTCAGGACGACGGTTGCAC	590bp	DQ157272.1
	R-	GGCGGGATCATCCTGTTTCA		
Giardia lamblia assemblage A TPI gene PCR	F-	CGAGACAAGTGTTGAGATG	576bp	Minvielle et al., 2008
	R-	GGTCAAGAGCTTACAACACG		
Giardia lamblia assemblage A TPI gene nested PCR	F-	CCAAGAAGGCTAAGCGTGC	476bp	Minvielle et al., 2008
	R-	GGTCAAGAGCTTACAACACG		
Giardia lamblia assemblage B TPI gene PCR	F-	GTTGCTCCCTCCTTTGTGC	208bp	Minvielle et al., 2008
	R-	CTCTGCTCATTGGTCTCGC		
Giardia lamblia assemblage B TPI gene nested PCR	F-	GCACAGAACGTGTATCTGG	140bp	Minvielle et al., 2008
	R-	CTCTGCTCATTGGTCTCGC		
Giardia lamblia assemblage E TPI gene PCR	F-	AAGTGTAACGGCTCGCTTGA	438bp	KJ363376.1
	R-	AGATTCCCCGAGCTCTTTGC		
Giardia lamblia assemblage E TPI gene nested PCR	F-	GACGTTGTTGTTGCCCCTTC	209bp	KJ363376.1
	R-	TCGGTCTCCCCCATGATTCT		
Giardia lamblia assemblage F TPI gene PCR	F-	AACGGCTCGCTCGACTTTAT	471bp	LC341570.1
	R-	GGGCTCGTAGGCAATAACGA		
Giardia lamblia assemblage F TPI gene nested PCR	F-	GGCCATTGCTGCCCACAAG	401bp	LC341570.1
	R-	TCTTCGACTCTCCAAGCTCC		

residency	Total samples	urban		Rural		X2	P value
		Total No.	Positive	Total	Positive		
Sheep	24	3	1(25) %	20	2(10) %	0.686*	0.408
Cattle	24	6	2(40) %	19	4(21.05) %	0.758*	0.384
Total	48	9	3(33.33) %	39	6(15.38) %	1.54*	0.214
X2	0.016*			0.914*			
P value	0.898			0.339			
The Prevalence rate of G.lamblia in human and animal by using PCR technique showed increase in number positive in human 15/48 based while in the animal 9/48, 18.75% no significant different P<0.05 between human							

species	Total samples	Positive samples	Percentage
human	**48**	**15**	**25%**
animals	48	9	18.75%
X2	2*		
P value	0.157		
The Prevalence rate of G.lamblia in human and animals by using nested PCR Technique			

Species	Total samples	Genotype			
		A	B	E	F
Human	15	7(46.66) %	8(53.33) %	0(0) %	0(0) %
Animals	9	3(33.33) %	3(33.33) %	3(33.33) %	0(0) %
X^2	5.73*				
P value	0.057				

The Prevalence rate of G.lamblia genotype A in human and animal by using nested PCR Technique showed no significant different association (P <0.05) between human and animal infection where result shows genotype A were in human 7/15 with percentage 46.66% this result were higher than genotype A in animal were 3/9 with percentage 33.33%. Prevalence rate of G.lamblia genotype A in human and animal by using nested PCR.

Species	Total samples	Genotype
		A
Human	15	7(46.66)%
Animals	9	3(33.33)%
Total	24	10(79.99)%
The Prevalence rate of *G.lamblia* genotype B in human and animal by using nested PCR		

Species	Total samples	Genotype
		B
Human	15	8(53.33)%
Animals	9	3(33.33)%
Total	24	11(86.66)%
The Prevalence rate of *G.lamblia* genotype E in human and animal by using nested PCR.		

Species	Total samples	genotype
		E
Human	15	0(0)%
Animals	9	3(33.33)%
Total	24	3(33.33)%
The Prevalence rate of G.lamblia genotype F in human and animal by using nested PCR.		

Species	Total samples	Genotype
		F
Human	15	0(0)%
Animals	9	0(0)%
Total	24	0(0)%
Genotyping of *G.lamblia* isolates from cattle and sheep		

Species	Total samples	genotype		
		A	B	E
Sheep	3	1(33.33) %	0(0) %	2(66.66) %
Cattle	6	2(33.33) %	3(50) %	1(16.66) %
Total	9	3(33.33) %	3(33.33) %	3(33.33) %
X^2	3*			
P value	0.223			

Nested PCR and Nested PCR product analysis by Agarose gel electrophoresis.

Analysis of statistics:

For statistical analysis, a Chi square test (X2) was applied to assess the independence of the variables, with the IBM Statistical Package for Social Sciences (SPSS) Statistics software, version 27. Values less than or equal to 0.05 were considered statistically significant (Purwanto *et al.*,2021.)

3. RESULTS

prevalence of *G. lamblia* in sheep and cattle by microscopic examination test according to residence

4. Discussions

Higher rates of infection in livestock may result from using native pastures, crop and fallow land, agricultural products, and waste materials near homes and villages for feeding. Inadequate grazing management of natural pastures frequently results in overgrazing and unsustainable animal production because it increases soil erosion and loses cover vegetation(Bettencourt et al., 2015). Additionally, the parasites that were discovered in a variety of water sources, particularly those that were found in rural areas and demonstrated a higher prevalence of contamination, may be related to poor sanitation and personal hygiene, marshy lifestyles and environments, contaminated water supplies, a lack of filtration, rotten water distribution pipes, and the warm climate, (Jar Allah, 2016). Equal results of PCR Technique in cattle and sheep by microscopic examination where include result PCR 3/24 in sheep and 6/24 in cattle and same result microscopic examination which were 3/24 in sheep and also 6/24 in cattle, the accuracy of the microscopic examination and the similarity of its results with the PCR may be due to several factors the most important of which is the correct collection of samples based on the not random collection and examination directly, and the use of the correct technique(Buonfrate et al., 2018).(The findings of the present study were in conflict with those of the Algerian study showed that a total of 355 fecal samples, Genotyping was done to the 30 Giardia positive samples targeting the beta-giardin gene by applying PCR/RFLP assay, Assemblage A was 70% more prevalent than assemblage B 30%(Rebih, 2020). Using molecular studies and verifying the genotype of G.lamblia, the current study found results that contradicted those of a previous study conducted in Wasit province, a total

of 100 bovine feces samples were collected, and the results showed an infection rate of 83%. DNA was extracted from the 100 positive samples from the cattle, and the special TPI gene was amplified to determine the genotypes A and B; the results showed that type A infection occurred in 69% of cases, and genotype B infection occurred in 45% of cases Also difference with molecular study in Argentina where showed genotype B was mainly detected in cattle and sheep where the identification of Giardia genetic genotype was limited in the studies evaluated and demonstrated that assemblage B was primarily found in animals while genotypes A and B were discovered in humans, it is likely that immunologically developed cattle can resist genotype A infection while host-adapted genotype E can establish infection. competition between genotypes in a host in favor of the host-adapted assemblage has also been proposed. (Rivero, et al., 2020).

5. Conclusions

Based on the nested PCR technique, humans have two genotypes A and B without E and F, but animals have three genotypes A, B, and E without F in cattle, and sheep have two genotypes A and E without B and F and genotype B is greater than genotype A in animals.The genotype B is the highest in human samples, while the three genotypes A, B, E were same percentage in animals without genotype F.

6. Conflict of interst

The authors affirm that they do not have any competing interests.

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