Use of Molecular Method to Detect Giardiasis in Different Animal in Al-Qadissiya Province – Iraq

Ghaidaa Abass Jasim[1], May Naji Alkhanaq[2], Musafer H. Al_Ardi[3], Hanaa Salih Abd Ali Alrammah[4]

[1]Prof. College of Veterinary Medicine/University of AL-Qadisiyah, [2]Assist Prof. College of Biology, University of Wasit, [3]Ministry of Education General Director for Education _Al_Qadisiyah, [4]Lecturer College of Veterinary Medicine/University of Baghdad, Veterinary Public Health Department, Zoonotic Diseases Unit

Abstract

Giardiasis infection main causative agent of humans, companion animals, livestock and wild life is *G. lamblia*. Thus, a total of 300 fecal samples of cattle, sheep, goats suffered from diarrhea in slaughtering house in AL-Diwanyha province were collected. The multiplex PCR technique applied for *G. lamblia* detection as well as genotyping. The research findings revealed that 154 positive cases, their prevalence percentage were 35, 35 and 30 respectively. *Giardia lamblia* E, A, B and AB assemblages genotypes showed significant differences, indicating a potential for zoonotic spread under definite situations where humans living in close contact with livestock, therefore, More awareness urgently needed to eliminate these infections.

Key Words : *Giardiasis, cattle, sheep, goats, multiplex PCR*

Introduction

Giardia lamblia (Syn; *Giardia duodenalis, Giardia intestinalis*) referred to be the causative microorganism for giardiasis, the gastro-intestinal humans infection accompanied with their owned animals, cattle and wild life. *Giardia* infection signs vary from a typical to sever diarrhea leading to chronic disease [(1)]. Its life cycle is simple involving rapid multiplications, then, trophozoite (non-invasive) on intestinal mucosal surface ending by producing environmentally resist cysts which were shedding via host feces [(2)]. The infections cysts are excreted in large numbers in feces of infected host and they contaminate drinking water hand swimming pool and food [(3)].

Giardiasis has worldwide distribution [(4)] , it is usually considered a zoonosis epidemic disease between the human and animals [(5)]. Mostly infected ruminants appeared asymptomatic; however, subclinical symptoms appeared as growth rate declining, feed alteration efficiency impairment, while sporadic persistent diarrhea might be observed [(6)].

The seven assemblages (A-G) have been documented for parasitic characteristic genes of *Giardia* based on studying the preserved genetic loci. Thus, the identified assemblages for human and verity of mammals hosts were A and B [(7)]. Therefore, A and B genotypes reported the possible highest zoonotic risk to public health[(8)] due to the wide spread of the zoonotic disease between human and animal and for the economic importance.

The *Giardia* zoonotic potential would be increasingly obvious by molecular technique [(9)] . In addition, PCR assays used to detect and identified the *G. deuodenalis* genotyping from clinical and environmental samples [(10)].

(14) The SSU r RNA marker genes were normally used for targeting different application to differentiate species and assemblage of *G.* [(11)]. Phylogenetic analysis data from the small – sub unit (SSU) r RNA gene, glutamate dehydrogenase (gdh) (housekeeping genes), B-giard (bg), elongation factor I alpha (elfa), triosephonphate isomerase (tpi) confirmed the assemblage A and B genetic uniqueness that studied to certified better *G. lamblia* isolates identification and comparisons [(12,13)]. A study confirmed the main human isolates divided into Polish (A) type and Belgian (B) [(14)].

While the strong host specificities were C, D, E, F and G assemblages, however, the narrow host ranges were C, D assemblages that mostly found in dogs, Foxes and coyotes and seals [15] .On another hand, sheep, cattle, goats, water buffaloes, and pigs essentially have E assemblage [16] . Rodents and cats generally have assemblages F and G [17] .

Furthermore, *Giardia* infection is believed to be associated with economic losses due to the occurrence onset of diarrhea, deprived growth or death of farm animals [18,19]. The zoonotic potential of *G. duodenalis* has been increasingly clear due to molecular genotyping techniques to its isolates [9] .

The study aims to detect the genotype and sequence of *Giardia intestinalis* in different animals.

Materials and Methods

Fecal samples collection

Total of 300 fecal samples are collected from animals (100 cattle, 100 sheep and 100 goats) which suffered from diarrhea in the house slaughter in AL-Diwanyha province during the period from October – 2014 to the end of June – 2015 and from age range of 1≤ 1 year.

These samples were collected in the sterile plastic containers and stored in the large containers containing ice bags, then transported to the parasitology laboratory in Al-Qadissiya University to perform the examination.

Macroscopic examination.

The direct smear method (wet mount method) by [20] were performed. Then, slides were examined under 40 x and 100 x powers.

Polymerase chain reaction (PCR)

The multiplex PCR technique was performed for detecting and genotyping of *G.* lamblia in feces samples that collected from suspected cases of cattle, sheep, and goat respectively. This method was carried out according to [21] as following steps:

A- Genomic DNA Extraction

A total of 200 mg of feces samples to extracted the enomic DNA according to the supplier instructions using AccuPrep® stool DNA Extraction Kit, Bioneer. Korea.

Primers

The PCR primers used for *G. lamblia* assemblage A, assemblage B genotyping were designed by [21] . While, the primers for *G. lamblia* assemblage E was designed in this study by NCBI-Genbank (*Giardia intestinalis* isolate 109 assemblage E, triosephosphate isomerase (TPI) gene, partial cds, GenBank code: AY228645.1) and primer 3 plus design. All primers were provided from Bioneer company, Korea is shown in Table 1.

Table1. Primers

Primer		Sequence	PCR product size (bp)
Assemblage E	F	CCGATTCCGTAGACGTTGTT	252
	R	GAGCACGCTTAGCCTTCTTG	
Assemblage A	F	CGAGACAAGTGTTGAGATG	576
	R	GGTCAAGAGCTTACAACACG	
Assemblage B	F	GTTGCTCCCTCCTTTGTGC	208
	R	CTCTGCTCATTGGTCTCGC	

B- Genomic DNA Profile

The purity of extracted genomic DNA from feces samples (2 µl) were checked and measured at 260 /280 nm absorbance using Nanodrop spectrophotometer (THERMO. USA).

C- PCR Master Mix Preparation

PCR master mix was prepared and applied for detection and genotyping of *Giardia lamblia* based TPI gene primers by AccuPower PCR PreMix Kit according to supplier instructions.

D- Multiplex PCR Master Mix Preparation

Multiplex PCR master mix was prepared and applied for *Giardia lamblia* A, B and E type based TPI gene primers detection and genotyping using AccuPower PCR PreMix Kit according to the company instructions.

E- PCR – thermo-cycler conditions and analysis

Convention-PCR-thermo-cycler system were applied according to the supplier company, then, PCR products were analyzed by 1.5% agarose-gel electrophoresis in 1x TBE, 3 µl of Ethidium bromide stain were added, 5 µl of 100bp ladder were run for 60 minutes on 100 volt, then visualized by UV trans-illuminator according to the supplier instructions.

Statistical Analysis

All data of this study were statistically analyzed by using Chi-square test [22].

Results

Giardiasis according to the macroscopic examination and PCR technique

From the total of 300 fecal samples the results show154 positive cases. When compare with animal species (cattle, sheep and goat) there are non-significant differences at $p < 0.05$ in infection with *Giardia* in Table 2.

A total of 35, 35 and 30 of cattle, sheep and goats fecal samples were respectively examined by PCR technique. Thus, they all were positive in microscopic examination and their prevalence percentage were 35, 35 and 30 respectively is shown in Table 2.

Table 2. *Giardia* infection according to the macroscopic examination and PCR technique

Animal	Macroscopic examination			Polymerase chain reaction		
	No. of examination	Positive	Percentage %	No. of examination	Positive	Percentage %
Cattle	100	56	18.6	35	35	100
Sheep	100	44	14.6	35	35	100
Goat	100	54	18	30	30	100
Total	300	154	51.2	100	100	100

There were no-significant differences at $P<0.05$ between animals (Cattle, Sheep and Goat) according to animals species

The Prevalence *of Giardia lamblia* PCR detecting and genotyping

In Cattle, PCR technique, the positive findings were shown in Table 3 as assemblage (E) 7 (45.7%), assemblage (A) 9 (25.7%), assemblage (B) 12 (34.2%) and assemblage (A and B) 8 (22.8%) out of 35 fecal samples positive were shown in Table 3 – 4. While the agarose gel electrophoresis of assemblage A and E PCR products *of Giardia lamblia* were shown in Figure 1 a,b . Phylogenetic tree analysis based on small subunit ribosomal RNA gene partial sequence that used for zoonotic relationship analysis of *Giardia intestinalis* animals isolates .

Table 3. The prevalence of *Giardia lamblia* genotypes in cattle, sheep and goats.

Giardia lamblia genotypes	cattle		sheep		goats	
	No.	Percentage (%)	No.	Percentage (%)	No.	Percentage (%)
Assemblage E	16	45.7	14	40	14	(40 %)
Assemblage A	9	25.7	8	22.8	4	(11.4%)
Assemblage B	12	34.2	8	22.8	6	(17.1%)
Assemblage A and B	8	22.8	5	14.2	6	(17.1%)
Total number	35		35		30	(100%)

While in Sheep the positive PCR results were assemblage (E) 14 (40%), assemblage (A) 8 (22.8%), assemblage (B) 8 (22.8%), and assemblage (A and B) 5 (14.2%) out of 35 samples were shown in Table 3.

In Goats, positive PCR result is shown in Table 3 were assemblage (E) 14 (40 %) assemblage (A) 4 (11.4%), assemblage (B) 6 (17.1%) and assemblage (A and B) 6 (17.1%) out of 16 samples is shown in Table 3.

Table 4. There are significant differences at p<0.05 between animals species in genotyping of *Giardia lamblia* in infected and non-infected animals.

Giardia lamblia genotype	Cattle	Sheep	Goats
Assemblage E	7 (43.75%)Aa	4 (28.57%)Aa	6 (37.5%)Aa
Assemblage A	3 (18.75%)ab	4 (28.57%)Aa	2 (12.5%)Bb
Assemblage B	4 (25%)Ba	5 (35.71%)Aa	4 (25%)Ca
Assemblage A & B	2 (12.5%)Ca	1 (7.15%)Ba	4 (25%)Cb
Total numbers	16 (100%)	14 (100%)	16 (100%)

The small letters refers to the horizontal statistical reading whereas the capital letters refers to the vertical statistical reading, while the similar letters refers to the non-significant differences whereas the different letters refers to the significant differences at ($P \leq 0.05$). There were significant difference between the genotype (E, A, B and AB) with type of animals at ($P \leq 0.05$).

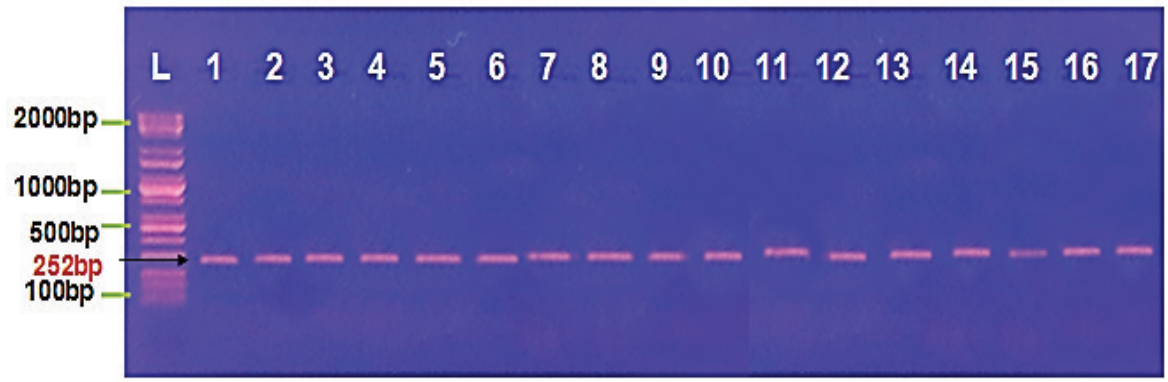

Figure 1.a Agarose gel electrophoresis of assemblage E PCR product analysis of TPI gene *of Giardia lamblia* genotyping. Shows L: Ladder 2000 - 100 bp, lane (1-7), (8-11) and (12-17) positive Assemblage E in cattle, sheep and goat respectively at 252 bp PCR product size.

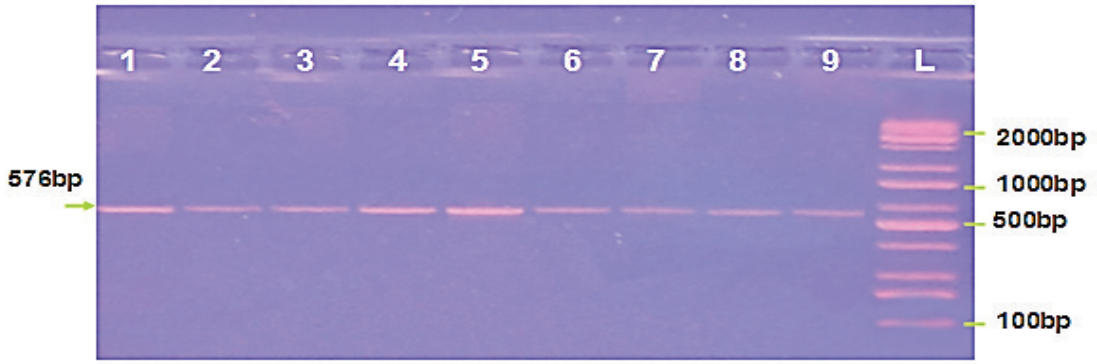

Figure1.b Agarose gel electrophoresis of assemblage A PCR product analysis of TPI gene *of Giardia lamblia* genotyping. Shows L: Ladder 2000 - 100 bp, lane (1-3), (4-7) and (8-9) positive Assemblage A in cattle, sheep and goat respectively at 576 bp PCR product size.

Discussion

The Prevalence *of* Giardiasis in (Cattle, sheep and goat) PCR detecting and genotyping

Six species *of Giardia* are accepted by most researchers, among them *G. agilis*, *G. ardeae*, *G. muris*, *G. microti* and *G. psittaci* infected various animals, whereas *G. lamblia.* Assemblage E was found mainly in domestic mammals (cloven-hoofed) v [16], also assemblage E was reported in cats [23,24] and human [25]. Assemblage A and B were mainly believed to be only associated with human infection [26], however, *G. duodenalis* unusual assemblages C, D, E and F genotypes in humans [27,28]. Assemblage H was identified from *Giardia* cysts isolated in the U.S.A seals, gray seals, harbor seals and a gull [29]. Thus, the new assemblage (H) was only supported by the gdh sequence analysis, but not tpi [30] , and the gdh sequence of the new genotype ID placed outside the *G. duodenalis* cluster in phylogenetic analysis [31] . However, the current study Giardiasis results according to the genotypes by molecular techniques show there is no significant difference to assemblage E and B among cattle, sheep and goats, while it shows significant differences at $P \leq 0.05$ between the animal with assemblage genotype A and AB respectively.

G. duodenalis infects human and many mammals [32] .The species names *G. (duodenalis*, *intestinalis* and *lamblia*) have been used in literatures referring to the same organism [8]. *G. duodenalis* and *G. intestinalis* are both used in identical frequency to refer to the most

mammals' *G.* species that infect human, compared to animals and cattle [33,34] .While, *G. lamblia* is the main species in human according to the medical field [34]. However, *G. duodenalis* is the only species reported in human and neomerous mammals, including cattle, pets that considered a multi-species complex [35]. On the other hand, it found significant differences between the genotype (E, A, B, A & B) with type of animals at ($P \leq 0.05$). In sheep three genotype, E, A and B can be found, while in cattle and goats, all genotypes are found that can be revealed the cattle, sheep and goats are a good source for human giardiasis.

The zoonotic assemblages A & B

A, B and AB were present in cattle, sheep and goat while the host-adapted assemblages E is capable of establishing infection, the PCR methodology and primers employed have been used, results from current study shows that 43.75 % in cattle, 28.57 % in sheep and 37.5 % in goat of *Giardia* isolate that assemblage E appears to be limited to artiodactyles in alpacas, pigs, goats, sheep and in addition to livestock [36] , It has been suggested that infected cattle pose a minimal threat to public health [6,37]. Nevertheless, [38–40] demonstrate the potential of infected cattle to be the source of infections in humans infection, thus these findings agreed with the current study.

The technically challenging level of sensitivity and specificity of real-time PCR assay by targeting the bg gene was developed to detect a single- cyst genotyping [41,42].The genotyping *G. duodenalis* assemblage E for livestock in Europe, North America, Australia were predominate [43–45]. The current study, assemblages A, B in cattle (18.8 %), (25 %), while it agreed with [38] not *G. duodenalis* assemblage A and B in farm animals determination in pre-weane d, post weaned, 1 to 2 years old dairy cattle from the USA showed that 15%, 7% and 3% respectively. In contrast, In Italy, Out of 24, the *G. duodenalis* assemblage A and B appeared in 16 and 5 calves, respectively[46] . Assemblage A and B have also been isolated from dairy cattle in New Zealand [40].

This study agrees with assemblages above, and disagrees with [47] in Japan, who found that genotype assemblage B has no zoonotic risk between cattle and human, and with [48], who found that the evidence indicate that cattle are most commonly infected with non-zoonotic livestock genotype of *G. duodenalis* which limits their role as reservoirs of giardiasis in human in central and western United States.

In Maryland, assemblage E genotyping isolates were identified in 61%, 25% and 6% in pre/ post-weaned calves as well as heifers, respectively in a longitudinal study of 30 calves from birth to 24 months of age on a dairy farm [45].

In sheep [49] in Western Australia found that the prevalence of positive *Giardia* using PCR was 44% respectively, the majority of isolates genotyped are not commonly found in human [50] . In contrast, assemblage B is rarely found in sheep [16]. While goats, in one study assemblage E was (39/39) [24]. In Iran, prevalence rates was 15.9% of *G. duodenalis* by PCR in Ahvaz [51] have no zoonotic risk of giardiasis in sheep and goat, however it may play a role on enteric disorder. Furthermore, genotypes A, B and E have been only reported in goats [52]. *G. duodenalis* worldwide prevalence in goats differs from <10% up to 40% according to animal age, geographical distribution and diagnostic technique [53]. The role of goats in the zoonotic transmission of *G. duodenalis* has been controversial due to non-zoonotic genotype E, but is more common than the zoonotic genotype A and B [54]. However [55,56] reported that zoonotic genotype A infected goats in Belgium and [52] in (Côte d′ Ivoire) reported that genotypes A and B infected goats in Malaysia. Thus, the current study results are suggesting that goats could be a possible reservoir of zoonotic infection. In Brazil, due to goats small farms and most its workers live inside or near the farm, thus, the close contact with infected animals could be a risk for zoonotic *G.* spread [57]. The current study revealed it occurrence in cattle, sheep and goat. The C, D, E, F and G assemblages have robust host specificity within narrow host ranges [15] . However, the current study has demonstrated the prevalence of *Giardia lamblia* E, A, B and AB assemblages genotypes suggesting a source for zoonotic spread.

The SSU r RNA gene-locus could be used to identify the moderately close related assemblages, whereas the more conserved regions would provide sufficient information only to differentiate *G.* species. Thus, when the SSU r RNA gene locus used to assemblage diversity primers selection should be cautious due to

some primer sets are too small to differentiate all *G. duodenalis* assemblages' genotyping products [58,59]. An enormous number of *G. duodenalis* genotyping studies in ruminants reported a higher occurrence of genotype E compared to genotypes A and B were less frequent [60]. Even though, zoonotic genotypes were not observed in the studies population, the genotype E has also been detected in humans who closely lived with livestock, that suggested a future spread for zoonotic G. under certain occurrences [25], these facts were agreed with the current study findings and conclusion, thus, more awareness and studied are urgently needed to eliminate these possible infections.

Conflict of Interest: This is to certify that I Dr. May Naji Alkhanaq the author of the Use of molecular method to detect giardiasis in different animal in Al-Qadissiya province – Iraq. Certify that there is no conflict of interest regarding this manuscript. (NIL)

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