Molecular Analysis of "E" Genotyping to Giardiasis In Ruminants In Karbala Province

التحليل الجزيئي للتنميط الجيني (أي) للجياردیا في المجترات في محافظة كربلاء المقدسة

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البحث مستل

Abstract

The current study included examination of 540 fecal samples of animals (180 cattle, 180 sheep and 180 goats) from the slaughter house at Karbala province. The animals suffered from diarrhea within the period from October 2013 to the end of June 2014,

The current study showed the rate of total infection of Giardiasis was (277 of 540 samples). They were examined by smear method and using light microscope trophozoites and cyst phases of parasite.

Molecular results showed in finding three assemblage genotype (A, B, E) and found mixed infection with genotype A & B in (cattle, sheep and goat), there is no significant difference at ($P \leq 0.05$) to infection by assemblage genotype E. while the assemblage A there is found significant difference foundation.

The foundation of genotype B no significant difference between three animals. Foundation of assemblage A & B together have significant difference between goats and cattle & sheep at $P \leq 0.05$) while in cattle we found significant difference with genotype (E, A, B, A & B).

In sheep and goats they have significant difference between the three assemblage genotype (E, A, B) and A & B together according to the infection by Giardiasis in animals in Karbala.

المستخلص .

الدراسة تضمنت فحص (540) عينة براز للحيوانات (الأبقار 180 ، الأغنام 180 ، الماعز 180) والتي كانت تعاني من إسهال شديد في المجزرة الواقعة في محافظة كربلاء المقدسة خلال الفقرة من تشرين الأول 2013 ولغاية حزيران عام 2014.

الدراسة الحالية أظهرت نتائج مدى الإصابة بالجياردیا وكانت النسب (277 عينة مصابة من أصل 540 عينة). تم إجراء الفحص المجهري بطريقة الفحص المباشر باستخدام المجهر الضوئي للكشف عن وجود الطور الخضري والكيسي للطفيلي.

أظهرت نتائج الدراسة الحالية في الجانب الجزيئي وجود أنواع جينية مختلفة وهي (A, B & E) بالإضافة إلى وجود إصابة مختلطة بالجين (A & B).

كما أظهرت الدراسة أنه لا يوجد إختلاف في المستوى المعنوي بالنسبة لإصابة الحيوانات (الأبقار، الأغنام، الماعز) بالجين (E)، بينما يوجد إختلاف في المستوى المعنوي بالنسبة للجين (A). أما بالنسبة للجين (B) فلا يوجد أي إختلاف معنوي بين الأنواع الثلاثة من الحيوانات.

أما بالنسبة للإصابة المختلطة بالجينين (A& B) معاً فهنالك إختلاف في المستوى المعنوي بين الماعز والأبقار والأغنام عند ($P \leq 0.05$)، في الأبقار وُجد إختلاف في المستوى المعنوي في الجينات (E, A, B, A&B) في الأغنام والماعز وُجد هنالك إختلاف في المستوى المعنوي بين الجينات الثلاث (E, A, B) والإصابة المختلطة بالجينين (A&B) معاً.

Introduction:

Giardia lamblia(Syn: *Giardia duodenalis, Giardia intestinalis*) is the etiological agent of giardiasis, a gastro intestinal infection of humans, companion animals, livestock and wild life ,Symptoms of a *Giardia* infection range from a symptomatic to sever diarrhea as well as chronic disease. (1) *Giardia* has a simple life cycle comprising rapidly multiplying, non-invasive trophozoite on the mucosal surface of the intestine, and production of environmentally resist at cysts that are shed with host faces (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),(4).

Giardiasis it has worldwide distribution, it is traditionally considered an epidemic and zoonosis disease between the human and animals (5).

Ruminants which infected with *Giardia* are mostly asymptomatic, but subclinical signs such as reduction in growth rate, impairment in feed conversion efficiency and persistent diarrhea are observed occasionally (6).

Giardia is common protozoan parasites that infect domestic and wild animals and humans, generally cause diarrhea. (7)

There are several major genotypes , Recently, genetic analysis using Polymerase Chain Reaction (PCR) characterized isolates of *Giardia* directly from feces, allowing the identification of a comprehensive range of genotypes from human and animals. (8).

More recently, a number of additional assemblages (genotype) have been proposed for *Giardia* isolates from a variety of mammals. These isolates are morphologically identical to human *Giardia lamblia*but sequences of their protein – coding regions differ. (9).

The species *Giardia duodenalis* has assigned even assemblages from A to H. Assembage A and B have been identified to infect humans and other mammalian hosts (10). Although Assemblage C infects only dogs, Assemblage F infects only cats, and Assamblage D infects both dogs and cats, Assemblage E infects cattle, sheep and goats and pigs, and Assemblage G infects, rats. Recently, Assemblage H infecting marine vertebrates, has been reported (11) .

Some of these assemblages can be classified even further into subtypes like for example (A-I, A-II, A-III, A-IV) each assemblage is capable of infecting certain species, and some assemblage are more commonly seen than others (1); (12);. This study was aimed to:

1. Detection of Giardiasis randomly from (cattle, sheep, goat) which have diarrhea
2. DNA Extraction for each sample which infected by *Giardia* by PCR.
3. Detection of genotyping assemblage in (cattle, sheep, goat) by multiplex – PCR.

Materials and methods

Total of 540 fecal samples are collected from animals (180 cattle, 180 sheep and 180 goats) which suffered from diarrhea in the house slaughter in Karbala province during the period from October – 2013 to the end of June – 2014 .

These samples were collected in the sterile plastic containers and stored in the large containers containing ice bags, then transported to the parasitology laboratory to perform the examination directly by Microscopicaly and PCR .

stools collected on non-consecutive days. The direct smear of fecal samples as soon as possible after being passed (13).

Genomic DNA from feces samples were extracted by using AccuPrep

Results

The method was used to detect the *Giardiatrophozoite* or cyst, the direct smear by using normal saline (10 %) and lougal' iodine. This diagnostic features was seen the cyst and trophozoite, thetrophozoite as a tennis or badminton racket with longitudinally splitpear, two and two sides (Table -1, 2)..

Table -1- Prevalence of Giardiasis in ruminants according to animal spp.

Animal	No. of examination	Positive	Negative	Percentage
Cattle	180	94	86	17.4%
Sheep	180	89	91	16.4%
Goat	180	94	86	17.4%
Total	540	277	263	

Table – 2 – Results of PCR technique.

*Giardialambliag*enotypes	Number of tested samples	No. of positive samples	Percentage (%)
Cattle	35	16	45.7%
Sheep	35	14	40%
Goat	30	16	53.3%

There were non-significant differences at $P<0.05$ between animals (Cattle, Sheep and Goat) according to animals species.

Cattle: out of 16 fecal samples positive, by using PCR technique assembly (E) 7(43.75%) assemblage (A) 3(18.75%) assemblage (B) 4(25%) and assemblage (A and B) 2(12.25%) (Table -3).

Table – 3 – Genotype of Giardiasis in cattle .

Giardialamblia genotypes	Cattle	Percentage (%)
Assemblage E	7	(43.75%)
Assemblage A	3	(18.75%)
Assemblage B	4	(25%)
Assemblage A and B	2	(12.5%)
Total number	16	(100%)

- Sheep: out of 14 samples positive, assemblage (E) 4(28.57%), assemblage (A) 4(28.57%), assemblage (B) 5(35.7%) and assemblage (A and B) (7.15%)(Table -4).

Table – 4 – Genotype of Giardiasis in sheep .

Giardialamblia genotypes	Sheep	Percentage (%)
Assemblage E	4	(28.57%)
Assemblage A	4	(28.57%)
Assemblage B	5	(35.71%)
Assemblage A and B	1	(7/ 15%)
Total number	14	(100%)

Goat: out of 16 samples positive, assemblage (E) 6(37.5%) assemblage (A) 2 (12.5%), assemblage (B) 4(25%) and assemblage (A and B) (25%) (Table -5).

Table – 5 – Genotype of Giardiasis in goats .

Giardialamblia genotypes	Goat	Percentage (%)
Assemblage E	6	(37.5%)
Assemblage A	2	(12.5%)
Assemblage B	4	(25%)
Assemblage A and B	4	(25%)
Total number	16	(100%)

Table – 6 –Differenses in Genotype of Giardiasis in animals of study .

***Giardialamblia* 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 =Horizental reading of statistic .
- The capital letters =Vetical reading of statistic

Discussion

Genotype E has been found largely in cloven-hoofed domestic mammals (cattle, water buffaloes, sheep, goats and pigs), assemblage E was reported in cats and humans (14).

In our study the results of Giardiasis according to the genotypes by Molecular techniques show there is no significant difference to assemblage E and B between (cattle, sheep and goats). While we found significant differences at ($P \leq 0.05$) between the animal with assemblage genotype (A and A & B) respectively.

In present study, assemblages A, B in cattle (18.8 %) (25 %), this agree with (15) no found *G.duodenalis* assemblage A and B in farm animals and prevalence determination in pre-weaned, post weaned and 1 to 2 years old dairy cattle from the United States revealed that 15%, 7% and 3% respectively (16).

In Italy, *G.duodenalis* assemblage A and B were isolate from 16 and 5 out of 24 calves respectively .Assemblage A and B have also been isolated from dairy calves in New Zealand (17).

Ideally, genotyping should be performed at the single-cyst, as this will allow the differentiation between mixed infections and the occurrence of recombinants, because there is a possibility of genetic exchanges between isolates of assemblage A or even between isolates of assemblages A and B .The use of real-time PCR appears to be promising in reaching this technically demanding level of sensitivity and specificity. A real-time PCR assay targeting the bg gene was developed (18).

It could differenctiate assemblages A and B of *G.duodenalis* with sensitivity of detection an equivalent of one cyst of *G.duodenalis* (19).

In view of frequency of human pathogenic *G.* genotypes, this puplic health risk of giardiasis from domestic animals appears to be small. This is case at least for cattle in North America, Australia and Europe, where *G.duodenalis* assemblage E predominate (20).

The human-pathogenic assemblages (A and occasionally B) in cattle may have to compete with the more common assemblage E. This study agree with assemblages above, and disagree in Argentine who found these assemblages not found in animals and (21) in Japan, who found that genotype assemblage B has no zoonotic risk between cattle and human. (22), 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.

The difference in the distribution of assemblage E and A was repeated for cattle on 14 dairy farms in the eastern U.S. involving nearly 2,000 cattle from birth to adulthood reported that assemblage E was found in 34% of pre weaned calves, 45% of post weaned calves, 33% of heifers and 25% of cows, whereas assemblage A was detected in 6%, 7%, 3% and 2% of animals respectively (23).

In Maryland, a longitudinal study of 30 calves from birth to 24 months of age on a dairy farm, assemblage E was detected in 61%, 25% and 6% of pre weaned calves, post weaned calves and heifers respectively (24).

Some studies reported that assemblage A was less commonly found in beef than in dairy cattle (25).

In studies with reasonable sample sizes assemblage E was the most common genotype identified in preweaned lambs (36/52) lambs in one study and (74/75) lambs in another, juvenile and adult sheep (33/46) (26) .

In Brazil, because goats farms are generally small and most farm workers live inside or near the property, close contact with these animals could represent a risk for zoonotic transmission of this parasite. (27)

The occurrence of infections with mixed assemblages in humans and animals is common. Mixed infection was also found in cat, cattle, goat, sheep, pig and wild life specimens mixed infection involving assemblages A to E is specially common (8).

The use of real-time PCR appears to be promising in reaching this technically demanding level of sensitivity and specificity. A real-time PCR assay targeting the bg gene was developed (19).

Assemblages C, D, E, F and G have strong host specificities and narrow hostranges. (20).

The variable 5′ and 3′ ends of the SSU r RNA genelocus can be used for identifying the relatively closely related assemblages, whereas the more conserved regions would provide sufficient information only for the differentiation of G. species. Thus, when the SSU r RNA.

Gene locus is used for assemblage differentiation primer selection should be careful because the products by some primer sets are too small to differentiate all *G.duodenalis* assemblage (29). One reason for targeting small fragments of the SSU r RNA gene is the difficulty in the PCR amplification of the Locus, the use of dimethyl sulfoxide or special PCR buffers designed for GC-rich targets is frequently needed for efficient PCR amplification of the target.

The tpi, gdh and bg loci are also common genotyping markers. Because most primers amplify 40% to 60% of the gdh gene and 60% of the tpi and bg genes, these loci have been used for both genotyping and subtyping (30).

A large number of *G.duodenalis* genotyping studies in ruminants reported a higher occurrence of genotype E in these animals with genotypes A and B being less frequent (31).

Even though zoonotic genotypes were not observed in the studies population, the genotype E has also been detected in humans living in close contact with livestock, suggesting a potential for zoonotic transmission under certain circumstance (14).

The result of our study finding of the same genotype in humans and animals is itself conclusive evidence that zoonotic transmission has taken place. A better assessment of zoonotic transmission can only come from studies that examine the dynamics of *Giardia* transmission between humans and animals living in the same house hold or localized focus of endemicity.

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