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Molecular Study to Detect Genotyping of *Giardia lamblia* from Human and Cattle Feces in Al-Qadisiya Governorate, Iraq

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Abstract

The present study is designed to diagnose the giardiasis from cattle and patients with diarrhea arrivals to Maternity and Childhood Teaching Hospital and General Education Hospital in Al-Qadisiya Governorate by using direct wet smear method as well as knowledge of the rate of prevalence of giardiasis in Al- Diwaniyah province, and study the effect of age, sex and nature of residence in the proportions of infection and investigate the genotypes of *Giardia lamblia* from human and animal feces ,100 samples were collected (50 stool samples of human and 50 feces samples of cattle).

In human, the result showed that the infection rate was 54% (27). The age group of 2-4 years showed the highest rate of infection (40.7%), while children aged 8-10 years showed the lowest rate of infection (3.7%). The results showed that 16 (59.2%) out of 27 and 11 (47.8%) out of 23 of males and females were positive respectively, so the rate of females cases were higher than males , with non-significant differences at p > 0.05 . It was found that the majority of cases (66.6%) were from rural areas.

DNA was extracted from positive samples (25 samples of human and 25 samples of cattle) then after amplified using primers for triosephosphateisomerase (*tpi*) gene that specific for genotyping A and B, the result showed that 17(68%) were genotype B and 3(12%) were genotype A, while 5(20%) were both genotype A and B.

However in cattle, the result showed that the infection rate was 70% (35). The age group of <6 month showed the highest rate of infection was (45.7%), while calves aged 6month-1year showed the lowest rate of infection was (22.8%). The results showed the number of positive cases according to the sex were 14 (40%) and 21(60%) in male and female respectively that showed higher positively of females when compared with males with significant difference at p<0.05.

The result of distribution of genotypes of *Giardia lamblia* in cattle showed that 16(64%) were genotype B and 5(20%) were genotype A while 4(16%) were both genotype A and B.

Key words: Giardia – genotypes- PCR- Al-Qadisiya -Iraq

Introduction

Clinical reports demonstrate close interactions between Giardiasis and diarrhoeal disease in almost all vertebrates, including humans and cattle.

In developing countries in Asia, Africa and Latin America, approximately 200 million people have symptomatic giardiasis[1,2] specially among young aged people in poor communities, with a prevalence percentage of infection ranged between (10-50%)in developing countries[3,4]. Based on morphology, six species of *Giardia* genus are considered valid, one of them is *Giardia intestinalis* (syn. *G. lamblia* or *G. duodenalis*), in a wide range of mammals, inclusive humans, livestock, and companion animals[7].*Giardia duodenalis*, a flagellate enteric protozoan, is the most common intestinal parasite that frequently reported in the world [5,6].

Molecular studies of *G. intestinalis* show that at least eight major genotypes or assemblages have been comprises [8], a number of these assemblages seem to have restricted host ranges [9and10]. Only two of these assemblages, A and B, have genotypes that have been isolated from humans [8]. All of studies have corroborative the division of human isolates into two major groups called genotype A (or Polish) and genotype B (or Belgian) [11]. Studies on cattle mention that *G. lamblia* have some isolates belong to genotype E and others to genotype A, but only genotype A can cause infection in human [12].

Generally, Giardia cysts that ingested through contaminated food or water, is the most common way of infection transmission, as well as person to person transmissions may happen directly by faecal-oral contact among family members [13] children in day care centers and schools [14], and by sexual practices of adults [15,16] .The cysts have an essential role in Giardiasis, since they instantly infectious once as passed out meantime faeces, moreover, cysts have the ability to remain infectious for few months as they can acclimatize to or resist critical environmental conditions [14].

Much has been written concerning the chronic infection of Giardia during childhood, that mainly due to protein-energy malnutrition, vitamin A deficiency, anemia , mineral deficiency (particularly iron and zinc), poor cognitive and educational level [17−18]. In addition, there are several socioeconomic factors that have been identified as important risk factors associated with Giardia infection, such as illiteracy, lack of adequate sanitation and water treatment systems, indigence and poor hygienic [19, 20].

2.Materials and methods
2.1.Samples collections

Fifty samples of human stool were collected from Al-Diwanyia hospital (Maternity and Childhood Teaching Hospital and General Education Hospital) and 50 animals fecal samples (Cattle) were collected from different Fileds in Al-Diwanyia province during the period of December 2013 to May 2014 and placed in sterile container, then transported to laboratory and stored in refrigerator until microscpic and genomic DNA extraction step.

2.2.The direct smear method using lougal'siodin

Aaccording to [21], the method was done as follows:-

1-A drop of lougal'siodin solution was placed on a glass slide. 2-Small amount of fecal sample of cattle and stool sample of human (about pin head in size) was put on lougal'siodin drop and mixed thoroughly using wooden stick.3-Cover slip was applied with forceps or fingers. 4-examination of slide under (40X) and(100X) powers.

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Genomic DNA Preparation

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

Nested PCR amplification

Nested PCR assay was performed for detection and genotyping of *Giardia lamblia* from human and animal. The PCR assay was carried out according to [22] using primers for triosephosphateisomerase (*tpi*) gene that specific for genotyping A and B and these primers were provided by (Bioneer company . Korea) (table-1). The PCR products were examined by electrophoresis in a 1.5% agarose gel, stained with ethidium bromide, and visualized under UV transilluminator.

Results

1.Giardiasis according to microscopical examination

Prevalence of giardiasis in human according to direct smear, 27(54%) out of 50 stool samples were positive while in cattle 35(70%)out of 50 stool samples were positive.Table (2)

2.Prevalence of giardiasis in cattle and human according to sex

Human: The results of the present study showed that 16 (59.2%) out of 27 and 11 (47.8%) out of 23 of males and females were positive respectively, so the rate of females cases were higher than males , with non-significant differences at $p > 0.05$.Table (3)

Cattle: The study included 22 males and 28 females , so the number of positive cases according to the sex were 14 (40%) and 21(60%) in male and female respectively, that showed higher positively of females when compared with males with significant difference at $p < 0.05$. Table (4)

3.Prevalence of giardiasis according to age groups of cattle and human.

Human: The results of the present study showed the high percentage (40.7%) was recorded in age group (2-4 years)while the lowest percentage infect (3.7%) in the age group (8-10 years) with significant difference at $p < 0.05$.Table (5)

Cattle: The infection given highest percentage (45.7%) in calves whose age <6 month when compared with other groups while the lowest percentage infect 22.8% in the age group (6month_1year)with significant difference at $p < 0.05$.Table (6)

4. Distribution of genotypes A ,B ,A and B

The tpi gene was amplified from 25 of the analyzed 50 human fecal samples through humans isolates, 17(68%) were genotype B and 3(12%) were genotype A while 5(20%) were both genotype A and B, as shown in Table (7), Figure (1A) ,Figure (1B). however in cattle 16(64%) were genotype B and 5(20%) were genotype A while 4(16%) were both genotype A and B, as shown in Table (8)), Figure (2A) ,Figure (2B).

Discussion

Giardia lamblia is one of the most wide spread parasites in intestine of humans and animals causing giardiasis [23]. Giardiasis is traditionally considered an epidemic and zoonosis disease between human and animals (farm animals, dogs, cats, birds and rodents), it affects

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all age groups [24]. Domestic animals have important source of giardiasis for humans infection [25]. Giardiasis in animals have reived increased attention in recent years, particularly because Giardia infection do cause disease in people [26].

1.The prevalence of giardiasis in human according to traditional methods.

In the current study, the prevalence rate of giardiasis in human according to lougal'siodin(direct smear) was 54%. This result was line with the results of [27] in Al-Najaf and [28] in Al-Kufa when they pointed to that of infection with giardiasis which was 50.9% and 84.7% respectively. The rate of giardiasis in the present study was highest than results which was reported by [29] in Baghdad and in Sulaimaniya [30] and in Vietnam[31], when they recorded rates of infection 11.9%, 3.3% and 3% respectively. The differences in the prevalence rates of giardiasis in human could be attributed to several factors such as socio-economic education , sanitation and number of samples , differences in studies design and techniques were used , in addition to climate and water supply sources, food and community health, hygiene ,and the presence of domesticated and wild animals in addition to economic levels and were classified as having a role in the likelihood of exposure to parasite infection [32].

The current study recorded high prevalence of infection (59.2%) among males , while in females the rate was lower 47.8 % by Lougal'siodin, but there is non-significant difference at p>0.050.05, and these result were supported by results of study of [33] in north of Baghdad and [34] in Turkey. This result was disagreed with result of [35] in Sulaimaniya, when they recorded high rate of infection in females. Men are more exposed to giardiasis due to spending most of their time out the house and may be drink the water that may be contaminated of *Giardia* cysts, therefore they would be more exposed to infection.

The present study showed that the persons with age 2-4 years were more effective to giardiasis, where the rate of infection was 40.7% by Logul'iodinand found significant difference at p<0.05 between age groups. This result was in accordance with result of [36].There are other many studies which gave results with higher rate of infection when compared with the result of the current study like [37]in New Zealand which showed that higher ratesof giardiasis were observed in (25-44 year) age group. Also disagree with results that recorded by [38] in Baghdad , which reported the highest rate of intestinal protozoa (43.62%) in adults (21-40 years) age group. The high prevalence rates of giardiasis in children may be due to that the children prisoners move and spend most of their time outside home and forced to eat from street vendors.

2. The prevalence of giardiasis in cattle according to traditional methods:-

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In the present study , the prevalence rates of giardiasis in cattle according to lougal'siodin were 70% . There are many other studies which gave results with lower rate of infection compared with the present results like [39] and (40), which were 5.45% and 10.27% respectively. These differences between the prevalence of giardiasis in the present study and prevalence of other studies in the other regions and countries, may be related to many factors including environmental changes, a number of samples were collected , study season , laboratory methods which were used in diagnosis, all these factors affect the rate of infection of *Giardia* [41].

The current study showed that most infections of giardiasis were recorded in young animals, with a significant differences between age group at p>0.05, where the highest rate (45.7%) was seen among animals aged (<6 months) and (22.8) in animals aged (6 months -1 year), and (31.4)in age group (≥1yea) by Lougal'siodin method. These are consistent with the results that was reported by (42)in Baghdad when they recorded the highest prevalence of *Giardia* in cattle which was in group of animals aged(< 6 month). In other place in East Azerbaijan-Iran it recorded high rate in calves aged less two months[43]. This result may be attributed to the susceptibility of calves to infection when they were exposed to the *Giardia* cysts which excreted from chronic infected mothers due to the decrease in the level of acquired immunity, this notion is supported by [44] which mentioned that infection in new calves if they stayed with their mothers for three days and direct contact with contaminated mothers parasite cysts, did not observe any effect of colostrums in decrease or prevent occurrence of *Giardia* infection.

The present study showed that the prevalence of giardiasis in males was 40% and in females was 60% by lougal's iodine method with significant differences at p<0.05 in the prevalence of giardiasis among both sex groups. But majority of studies indicate that there is no significant difference between both sexes like [45] in Baghdad and [46] in Thi-Qar**.** Female are more exposed to giardiasis due to the low immunity during pregnancy.

3. Distribution of genotypes A , B , A and B in humans .

Assemblages A and B have the broadest host specificity, having been found to cause infection in humans and many other different mammals [47]. In the present study, the detection of the genotype of human by Nested PCR amplification, (68%) were genotype B and (12%) were genotype A while (20%) were both genotype A and B, assemblage B showed higher prevalence among the other assemblages. The results of present study agrees with another study carried out on human in England (48) which found that assemblage B were 64%, assemblage A(27%), and the rest was a mixture of B and A assemblages.   In Canada [49]

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found nine genotype B isolates, three genotype A isolates and three mixed (A+B genotypes). Other studies[50] were amplified showed higher prevalence in genotype were B (93.02%) while genotype AII were (6.98%). In India, the proportion of assemblage A and B infections in people were 39% and 61%, respectively [51].

4.Distribution of genotypes A , B , A and B in animals.

In the present study, the detection of the genotype of animals was by Nested PCR amplification, (20%) were genotype A and (64%) were genotype B while (16%) were both genotype A and B, assemblage B showed higher prevalence among the other assemblages. The current study agrees with [52]. Commonly, three assemblages have been detected in cattle, assemblages A, B, and E, furthermore E most frequently reported followed by assemblage A . A recent study in dairy calves in New Zealand found only assemblages A and B [53]. This study disagrees with [54]which found higher levels of assemblage A in calves than have typically been reported previously. Another study have found that calves have primarily assemblage E with generally low levels of assemblage A[55]. A recent longitudinal study of adult cows reported 57% of the positive isolates were assemblage E and 43% were assemblage A [56].

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Table (1) The primers

Primer	Sequence		Amplicon
TPIA-FI	F	CGAGACAAGTGTTGAGATG	576 bp
	R	GGTCAAGAGCTTACAACACG	
TPIB-FI	F	GTTGCTCCCTCCTTTGTGC	208 bp
	R	CTCTGCTCATTGGTCTCGC	
TPIA-FII	F	CCAAGAAGGCTAAGCGTGC	476bp
	R	GGTCAAGAGCTTACAACACG	
TPIB-FII	F	GCACAGAACGTGTATCTGG	140bp
	R	CTCTGCTCATTGGTCTCGC	

Minvielle, M.C.et al.(2008)

Table (2) The prevalence of infection with *Giardia* according to direct smear.

Host	No.of sample	No.of positive	%
Human	50	27	54
Cattle a	50	35	70

Table (3)The prevalence of infection with *Giardia* according to the sex in human

Sex	Total No.	No. of positive	%
males	27	**16**	59.2a
females	23	11	47.8a

Table (4) The prevalence of infection with *Giardia* according to the sex in cattle

Sex	Total No.	No. of positive	%
males a	22	**14**	40a
females a	28	21	60b

Table(5) Prevalence of infection with *Giardia* according to the age groups in human

Age groups	No.of Sample	No.of positive	%	
Less of 2 year	9	6	a	22.2
2-4	20	11	b	40.7
4-6	8	4	ac	14.8
6-8	4	2	cd	7.4
8-10	3	1	d	3.7
10-12	6	3	cda	11.1

Table (6) Prevalence of infection with *Giardia* according to the age groups in Cattle

Age groups	No.Sample	No.of positive	%	
<6 month	20	16	a	45.7
6month_1year	12	8	b	22.8
≥1year	28	11	ab	31.4

A note

*Similar letters indicate no significant difference at the level of probability of 0.05using test $X2$.

*Different letters indicate the existence of a significant difference at the level of probability of 0.05using test $X2$.

Table (7) The results of PCR technique for detection *of genotyping in Giardia* of human

Sample

Giardia lamblia genotypes	*human*	*Percentage (%)*
Assemblage A	*3*	*12*
Assemblage B	*17*	*68*
Assemblage A and B	*5*	*20*
Total number	*25*	*(100%)*

Table(8) The results of PCR technique for detection of genotyping in Giardia of cattle

Sample

Giardia lamblia genotypes	Cattle	Percentage (%)
Assemblage A	5	20
Assemblage B	16	64
Assemblage A and B	4	16
Total number	25	(100%)

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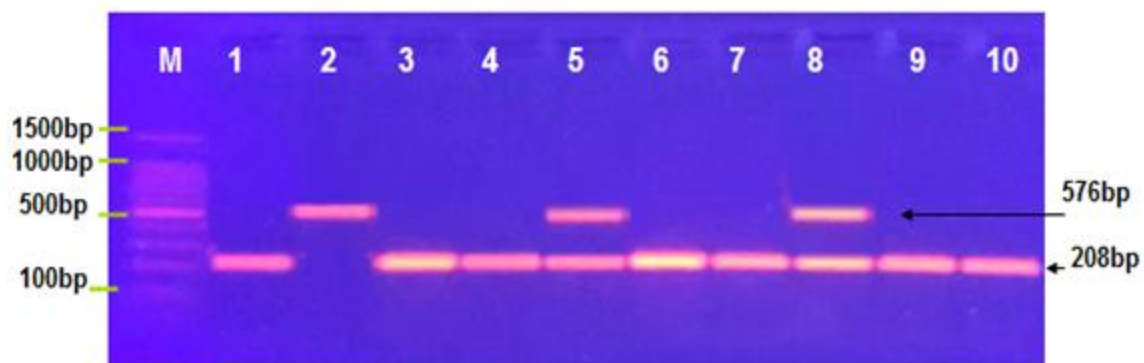Ibn Al-Haitham J. for Pure & Appl. Sci. | Vol. 29 (3) 2016

Figure (1A): Agarose gel electrophoresis image that shows PCR product analysis of *Giardia lamblia* genotype A and B in DNA from human stool samples where, Lane (M) DNA marker is (1500-100bp), Lane (1,3,4,6,7,9,and 10) are positive as Genotype B at PCR product size 208bp, Lane (2) is positive as Genotype A at PCR product size 576bp, Lane (5 and 8) are positive as Genotype A and B.
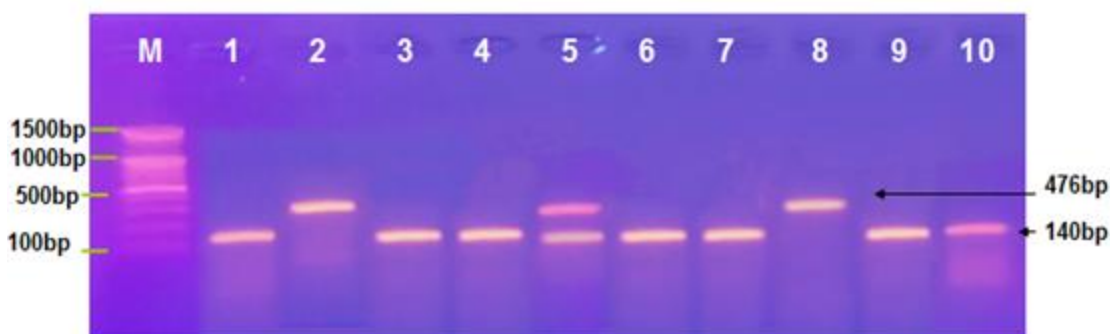

Figure (1B): Agarose gel electrophoresis image that shows nested PCR product analysis of *Giardia lamblia* genotype A and B in DNA from human stool samples where, Lane (M) DNA marker is (1500-100bp), Lane (1,3,4,6,7,9,and 10) positive as Genotype B at PCR product size 140bp, Lane (2 and 8) are positive as Genotype A at PCR product size 476bp, Lane (5) is positive as Genotype A and B.
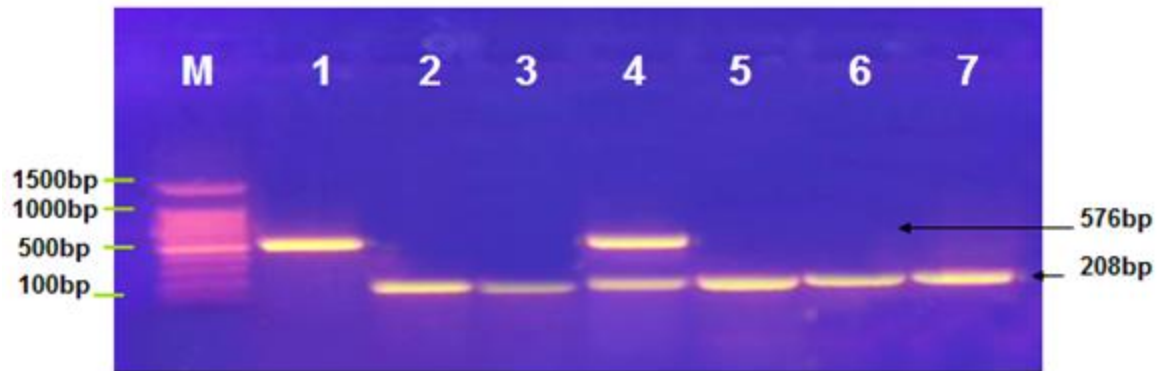
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Figure (2 A) Agarose gel electrophoresis image that shows PCR product analysis of *Giardia lamblia* **genotype A and B in DNA from animal stool samples where, Lane (M) DNA marker is (1500-100bp), Lane (1) is positive as Genotype A at PCR product size 576bp, Lane (2,3,5.6, and 7) are positive as Genotype B at PCR product size 208bp, Lane (4)is positive as Genotype A and B.**
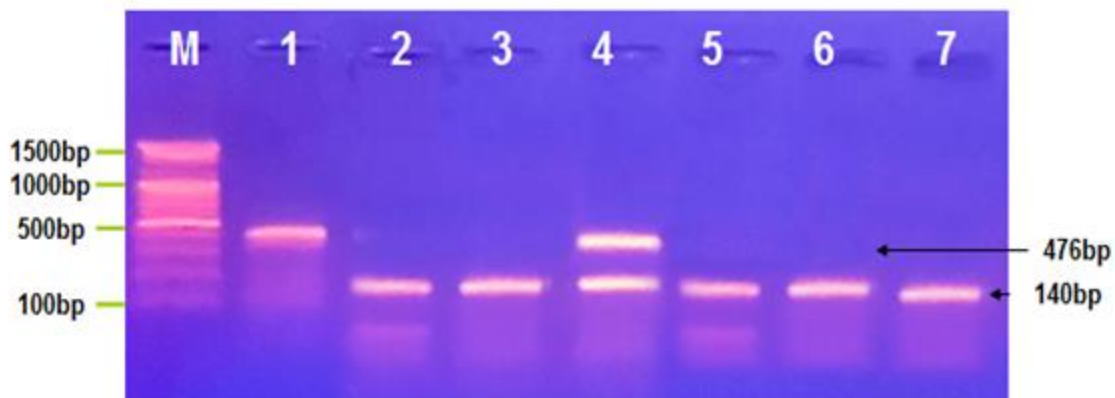

Figure (2B) Agarose gel electrophoresis image that shows nested PCR product analysis of *Giardia lamblia* **genotype A and B in DNA from animal stool samples where, Lane (M) DNA marker is (1500-100bp), Lane (1) is positive as Genotype A at PCR product size 476bp, Lane (2,3,5.6, and 7) are positive as Genotype B at PCR product size 140bp, Lane (4) is positive as Genotype A and B.**

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دراسة جزيئية للكشف عن التنميط الجيني لطفيلي الجيارديا لامبليا في عينات الانسان والابقار في محافظة القادسية، العراق

رنا صالح الدفاعي

الطب البيطري/ جامعة القادسية

الخلاصة

تهدف الدراسة الى تشخيص طفيلي الجيارديا في الابقار(والمرضى الذين يعانون من الاسهال الوافدين الى مستشفى النسائية والاطفال التعليمي ومستشفى التعليم العام في محافظة القادسية باستعمال طريقة المسحة الرطبة المباشرة ، معرفة معدل انتشار مرض الجيارديا في محافظة الديوانية ودراسة تأثير كل من العمر والجنس وطبيعة الإقامة في نسب الإصابة والتحقيق من التنميط الجيني لطفيلي الجيارديا لامبليا في براز الإنسان والحيوان، اذ جمعت 100 عينة (50 عينة من براز الإنسان و50 عينة براز من الابقار) خلال المدة من ديسمبر 2013 إلى مايو 2014.

أظهرت النتائج أن معدل الإصابة في الانسان كانت 54٪ (27)، وأظهرت الفئة العمرية من 2-4 سنوات أعلى معدل للإصابة (40.7٪)، في حين أظهر الأطفال الذين تتراوح أعمارهم بين 8-10 عاما أدنى معدل الإصابة (3.7 ٪). كما أظهرت النتائج أن 16 (59.2٪) من أصل 27 و 11 (47.8٪) من أصل 23 من الذكور والإناث كانت إيجابية على التوالي، وسجلت الاناث نسبة اصابة أعلى من الذكور، وأن معظم الحالات الموجبة (66.6٪) كانت من المناطق الريفية.

أستخلص الدنا من العينات الموجبة (25 عينة للإنسان و 25 عينة للأبقار)باستعمال بادئات لجين (tpi) المحدد للأنماط الجينية A,B   اذ اظهرت النتائج لعينات المرضى وجود 17 (68٪) النمط الجيني B و 3 (12٪) النمط الجيني Aبينما سجلت خمسة حالات (20٪) احتوائها على حد سواء النمط الوراثي Aو B.

أظهرت نتائج الاصابة بطفيلي Giardia lamblia في الابقار أن معدل الإصابة كان 70٪ (35)، وأظهرت الفئة العمرية الاقل من ستة أشهر أعلى معدل للإصابة (45.7٪)، في حين أظهرت العجول التي تتراوح اعمارها بين 6 اشهر الى سنة واحدة أقل معدل الإصابة (22.8٪). كما أظهرت النتائج أن عدد الحالات الإيجابية وفقا للجنس 14 (40٪) و 21 (60٪) في الذكور والإناث على التوالي.

أظهرت نتائج توزيع مورثات الجيارديا لامبليا في الابقار أن 16 (64٪) من النمط الجيني B و 5 (20٪) من النمط الجيني Aفي حين كانت 4 (16٪) تحتوي على حد سواء النمط الوراثي Aو B.