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Molecular identification of *Giradia intestinalis* in human and cats in Dohuk city, Kurdistan Region-Iraq

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A B S T R A C T

The goal of the study to determine the prevalence of *Giardia* infection in 200 samples (100 at breeders' stool and 100 cats faecal samples) that were collected between the beginning of January 2024 until end of April 2024, from different reigns of Dohuk City, Iraq. For the purpose of confirming the presence of *Giaria intestinlis* parasite, both conventional and molecular diagnosis were used. The results of microscopic examination showed that the infection rate in humans was 45/100 (45%), showed higher rate of infection in females (45.45. %) compared to males (44.44%). The rate infection between age groups, with a rate (46%) at 5-10 years old and (44%) at 11–20-year-old. The microscopically results showed the rate of infection at *G.intestinalis* in cats was55/100 (55%). Showed a significant difference between sexes, and the infection rate was higher in males was 70% compared to females was 40%. The Prevalence of *G.intestinalis* related to age was (72%) and(38%) in Kittens and adult age groups respectively . In this study, PCR assays with primer specific 18 S r RNA have been applied for the detection of *Giardia* isolates in cats breeders' stool and cats faecal. After being sequenced, ten positive PCR results were contributed to the Genebank database. Phytogenic analysis revealed that five of the PCR results (PP486374.1, PP486375.1, PP486376.1, PP486377.1, PP486378.1) were associated with isolates of *G. intestinalis* from humans, while the remaining isolates (PP486379.1, PP486380.1, PP486381.1, PP486382.1, PP486383.1) were associated with isolates of *G. intestinalis* from cats. In conclusion: The molecular study found a 97.68% relationship between *Giardia intestinalis* isolates from humans (PP486376.1, PP486378.1) and cats (PP486379.1, PP486380.1), as per the phylogenetic tree.

Keywords: Giardiasis, human protozoa, Phytogenic analysis, cats.

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INTRODUCTION

Giardiasis is caused by *Giardia intestinalis* infection, which affected humans and a variety of other animals, such as dogs and cats (Dixon, 2021). One of the most serious illnesses that can lead to intestinal infections in both human and animals is giardiasis (Fantinatti *et al.,* 2022). Human zoonotic illnesses like giardiasis can be transmitted by stray and domestic cats, posing a concern for public health (Veyna-Salazar *et al.,* 2023). In newborn animals, toddlers, and immunocompromised patients, the clinical manifestations of giardiasis are fever, stomach pain, weight loss, and diarrhea (Al Mosawy *et al.,* 2020). Through the fecal-oral route, animals and humans can contract the parasite by consuming the mature cysts in contaminated water or food (AL-Yasary and Faraj, 2021). Early identification is essential for diseases control in both humans and animals and it may be help to stop the disease's transmission via hosts (Idan and Al-Hasnawy, 2023). Cysts of *Giardia* in feces can be diagnosed by using microscopic examination. But this, can be challenge to identify this parasite because the cysts are occasionally excreted or may be confused with other organisms (Piekara-Stępińska *et al.,* 2021; Kuthyar e*t al.,* 2022). The diagnosis of *G. intetinalis* infections by molecular techniques are more precise and helpful (Alhayali *et al.,* 2020; Alali and Alkhaled, 2023; Abdulzahra and Abdullah, 2023). In Dohuk, Iraq, does not yet have any epidemiological or molecular data pertaining to *G. intestinalis* infections in humans or domestic cats. Thus, the purpose of this study is to identify *G. intestinalis* in humans and domestic cats by using microscopic and molecular tests.

MATERIALS AND METHODS

Ethical approve

The initiative was authorized and funded by the college of veterinary medicine's local committee on animal care. No. 910 of the University of Baghdad, issued January 2, 2023.

Samples collection

One hundred samples were selected from cats breeders and 100 from cats were collected randomly of different age and sexes, from several regions of Dohuk city, Iraq during the period from the beginning of January 2023 to end of April 2024.Then the samples were transported in refrigerator bag to the parasitology laboratory which belongs to the college of veterinary Medicine-University of

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Baghdad. Then, these samples were divided into two parts; the first part of the sample was kept cool at 4oC for the laboratory tests, and another part in deep freeze under -20oC for DNA extraction.

Microscopic examination

2 mg of each fecal sample examined by direct smear preparation, using iodine stain, for the detection and of parasite cyst, as described by (Hamza and Hameed, 2020; Jebur and Abbas, 2021). Examination of smears was made by olympus light microscope at low power (10×) and subsequently at higher power (40×) magnification.

DNA Extraction

genomic DNA was extracted from 100 microscopically positive sample (45cat breeders stool samples and 55 cat feces)., by using AccuPrep® G- spin DNA extraction kit (INTRON biotechnology, Korea), and done according to company instructions.

Genomic DNA Estimation

The concentration of DNA was estimated using a Nanodrop spectrophotometer (THERMO, South Korea), at 260/280 nanometres of absorbance, the purity was determined. Prior to PCR analysis, the collected DNA was stored in a deep freezer. 1.5% agarose gel was used to assess the integrity and quality of the DNA.

Polymerase Chain Reaction (PCR)

The NCBI gene-Bank data was used to perform a PCR technique for the direct detection of *G. intestinalis* using the *18SrRNA* gene primers designs (Table1,2).

Table 1: The primers that used for human *18SrRNA* gene

Gene	Sequence	Primer sequence	Tm	GC%	Size of product (bp)
18SrRNA	F	5'- CCCCAAGGACACAAGCCAT - 3'	59.92	57.89	307
gene	R	5'- GCTGCCGTCCTTGGATGT - 3’	60.05	61.11	

Table 2: The primers that used for cat *18SrRNA* gene

Gene	Sequence	Primer sequence	Tm	GC%	Size of product (bp)
18SrRNA gene	F	5'- CTCTCCCCCAGGACGAAGC - 3'	61.73	68.42	260
	R	5'- CGAACCCTGATTCTCCGCC - 3’	60.52	63.16	

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Sequencing and phylogenetic analysis

Utilizing an INTRON kit and a Macrogen analyzer, the PCR products were purified. They were then processed by terminator cycle sequencing and the BLAST database (http://blast.ncbi.nlm.nih.gov), modified with Mega 6.

PCR Master Mix Preparation

The AccuPower PCR PreMix Kit was used to implement a PCR Master Mix as in (Table3), and then transferred into a BioRad, USA thermocycler.

Table 3: Reaction components of PCR.

PCR Master mix	volume
Master Mix or GoTaq® Green Master Mix	12.5µl
Forward primer	10 picomols/µl (1 µl)
Reverse primer	10 picomols/µl (1 µl)
DNA	1.5µl
Distill water	9µl
Component	25µL (Final volume)

PCR Thermo Cycler Conditions

The PCR thermocycler was performed using a conventional PCR thermocycler system, as shown in (Table 4).

Table 4: The optimum conditions thermocycler system

No.	Phase	Tm (°C)	Time	No. of cycle
1-	Initial Denaturation	95°C	5 min	1 cycle
2-	Denaturation -2	95°C	45sec	35 cycles
3-	Annealing	58°C	45sec	
4-	Extension-1	72°C	1min	
5-	Extension -2	72°C	5 min.	1 cycle
6-	Hold		For ever	

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Statistical analysis

The data was analyzed using Chi-square tests for significance using the Statistical Analysis System (SAS -2012) (Cary,2012).

Microscopic examination

In Human:

Out of 100 cat breeders stool samples examined for *G. intestinalis* 45% (45/100) were positive by microscopic examination. The prevalence in females was (45.45. %), while in males was (44.44%), without significant difference (Figure,1) (Table,4).

RESULTS AND DISCUSSION

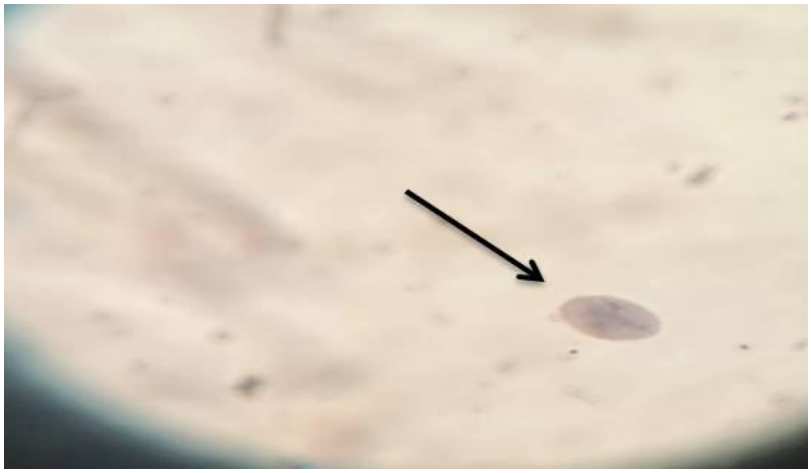

Figure 1: Cyst of *Giardia spp*. from handlers stool sample (Black arrow) by direct smear ((100X).

Table 4. Rate of *Giardia intestinalis*, infection in human based on gender.

Gender	No. Samples examined	No. of infected	%
Males	45	20	44.44
Females	55	25	45.45
Total	100	45	45
Chi-Square (X^2)	-	-	0.0539NS

NS: Non-Significant (P >0.05)

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Infection rate of *G.intestinalis* showed insignificant relation among age groups of cats breeders however, the rate infection was recorded a percentage 46% (23/50) at 5-10 years old and 44% (22/50) at 11-20 years old (Table, 5).

Table 5. Rate of *Giardia intestinalis* infection in human based on age groups.

age	No. Samples examined	No. of infected	%
5-10	50	23	46
11-20	50	22	44
Total	100	45	45
Chi-Square (X^2)	-	-	0.52 NS

NS: Non-Significant (P >0.05)

The current study revealed 45% rate of *G. intestinalis* infection in cat breeders according to the microscopic examination, equalled to another previous studies conducted in Iraq which was done (Al-Difaie,2016) in Al-Qadisiyah province and (Aboody *et al.*,2020) in Thi-Qar province as they have been recorded the infection rate 54%% and 47.5%% respectively. Our results also agreed with (Hijjawi *et al.*, 2018) in Jordan, in which it recorded42%%, but Other studies in others countries, also recorded different results, The lower rate of infection recorded by (Avendaño *et al.*, 2019), in Colombia and Zambia by (Tembo *et al*., 2020), in which it recorded total percentage of positive result were 9.9% and10% respectively.The variation in prevalence of Giardia related to Numerous factors, such as population's variation , age, gender, personal hygiene, drinking or using untreated water, contacting with suspected animal and poor economic status of the families may play a key role in the high result of the present study which agreed with (El Ayis *et al.*, 2023).*G. duodenalis* infection rates were similarly found in males and females (44.44% vs. 45.45%). The result of present study showed an agreement with previous studies in the Egyptian provinces of El-Dakahlia, El-Gharbia, and Damietta (Naguib *et al*., 2018). In contrast, other studies have found that males were more susceptible to infection in previse studies conducted, Algeria (Rebih *et al*., 2020), Saudi Arabia (Shalaby *et al.*, 2011) and Yemen (Al-Mekhlafi, 2017). Infection rate of giardiosis showed insignificant relation among age groups of cat breeders. Showed that giardiosis infections were more frequent in aged 5- 10 years than in other

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age group. This result is consistent with the studies in Egypt (Ismail *et al.*, 2016; Elhadad *et al.*, 2021). Young children might be more exposed to infections because of their poor personal hygiene practices, and undeveloped immune system compared to adults (Bauhofer *et al*., 2021; Gebru *et al*., 2023).

In cats:

Significant differences were recorded between female and male cats at (P<0.05), 70(35/50%) and 40(20/50%) respectively (Table, 6).

Table 6: Rate of *Giardia intestinalis* infection in cat according to sex.

Sex	No.samples examined	No. animal infected	%
Male	50	20	40
Female	50	35	70
Total	100	55	55
Chi-Square (X^2)	-	-	5*

* (P<0.05).

The study results found that *G. intestinalis* can be infected in all ages of cats, with higher rate infection showed in kittens was (72%) than the adult cats was (38%), with significant differences (P<0.05) (Table,7).

Table 7: Rate of *Giardia intestinalis* infection cats according to age groups.

Gender	No. samples examined	No. animal infected	%
Kitten <6 months	50	36	72
Adults >6 months	50	19	38
Total	100	55	55
Chi-Square (X^2)	-	-	6.28*

* (P<0.05).

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In the present study, the infection rate of *G. duodenalis* in cats was 55% (55/100) which is highly agreement with the results of Al Mosawy *et al*., (2020) in Wasit province, Iraq. This study found a higher infection rate was (70%) in female cats, consistent with previous findings in Iraq (Al-Mosawy *et al*.,2020) and Mexico(Núñez *et al.,* 2021) possibly due to decreased immune defence mechanisms in female cats. The highest rates infection in cats were found in the young age group than old age groups. These results agree with Hadi *et al*. (2014), Idan and Al-Hasnawy (2023). The higher infection rate in young animals due to the immune system not will developed that may be increase the susceptibility to *Giardia* infection (AL-Kuraishi, 2004; Fadhil *et al*., 2021).

Genomic DNA Estimation

Following the extraction of DNA from 100 microscopically positive sample (45cat breeders stool samples and 55 cat feces). The Nanodrop spectrophotometer (THERMO, South Korea) was used to verify the results. The DNA concentration ranged from 5 to 50 ng/µl, and the absorbance at 260 and 280 nm indicated that the DNA was pure (1.6–1.7).

PCR analysis

Following a 1.5% agarose gel electrophoresis analysis of the PCR, the sample was stained with ethidium bromide stain using a voltage of 70 volts and 65 AM for one hours. As seen in (Figure,2,3), the positive DNA bands measured (307 bp and 260bp).

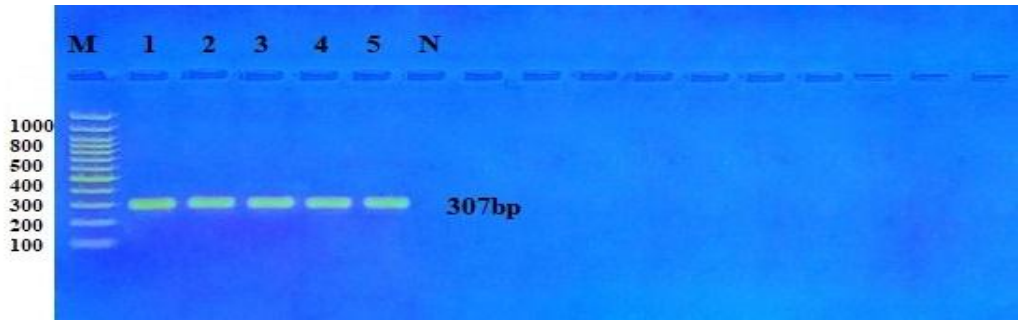

Figure 2: The products of PCR by 1,5% agarose. 1:30 hours. M: DNA marker ladder (100). 1-5 positive result (human): 307bp and N=negative control.

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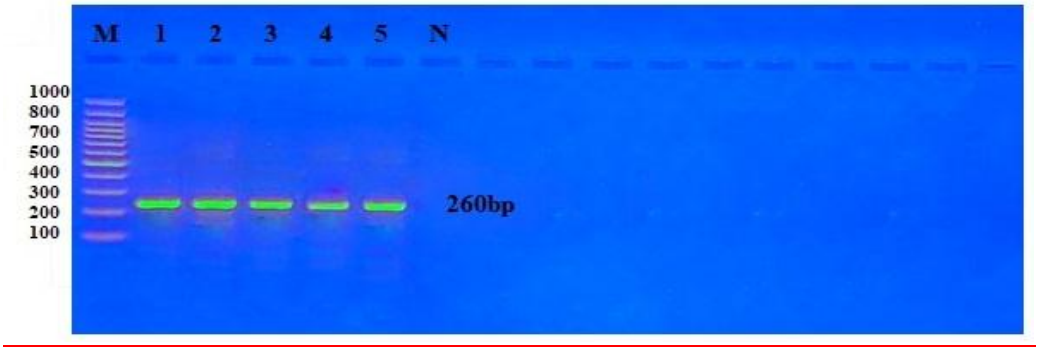

Figure 3: The products of PCR by 1,5% agarose. for 1:30 hours. M: DNA marker ladder (100). 1-5 positive result (cat): 260bp and N=negative control.

The ten PCR products were sequined and deposited in GeneBank under the specified accession numbers; PP486374.1, PP486375.1, PP486376.1, PP486377.1, PP486378.1, PP486379.1, PP486380.1, PP486381.1, PP486382.1, PP486383.1). The sequencing results indicated that five PCR products were detected (PP486374.1, PP486375.1, PP486376.1, PP486377.1, PP486378.1) belongs to human *Giardia intestinalis* isolates, Iraqi isolates demonstrated a high degree of 99% identity among all other isolates (Turkey, Canada, Kenya, USA, Australia, Netherlands and China). as in (Figure 4). while (PP486379.1, PP486380.1, PP486381.1, PP486382.1, PP486383.1) belongs to domestic cats *Giardia intestinalis* isolates, Iraqi isolates demonstrated a high degree of 99% identity among all other isolates (Chile, Japan, Honduras, Uganda, Saudi Arabia, Brazil, Egypt, Canada, Argentina, Kenya, USA, Turkey and Spain) as in (Figure5). The study found a 97.68% relationship between *G. intestinalis* isolates from human (PP486376.1, PP486378.1) and domestic cats (PP486379.1, PP486380.1), as per the phylogenetic tree (figure 6).

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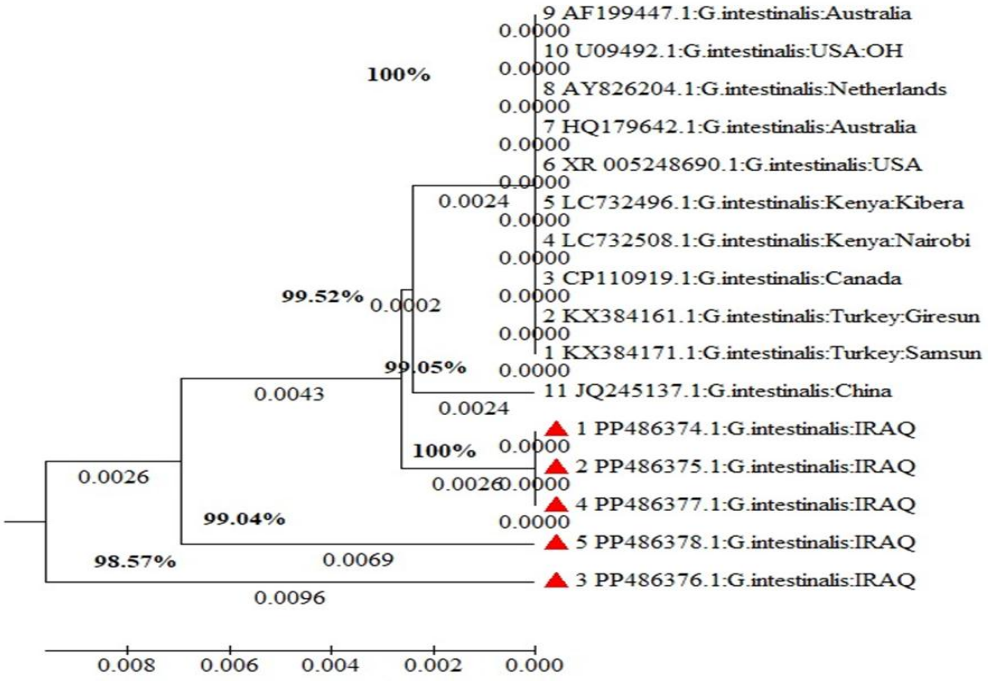

Figure 4: The phylogenetic tree. Red spot for the *G.intestinalis* strain isolate from human.

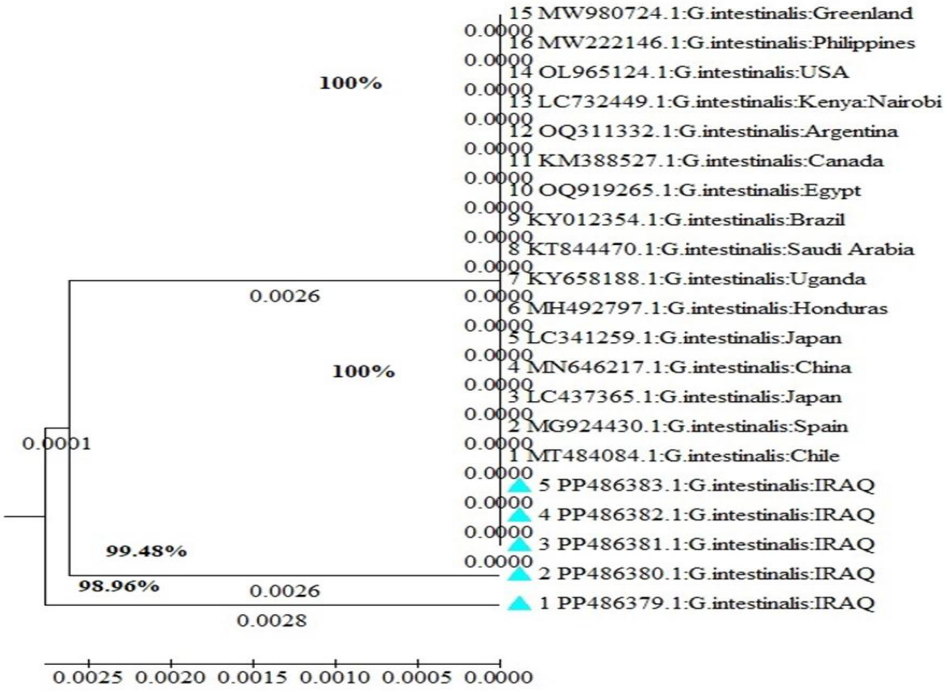

Figure 5: The phylogenetic tree. Green spot: *G.intestinalis* strain isolate from cats.

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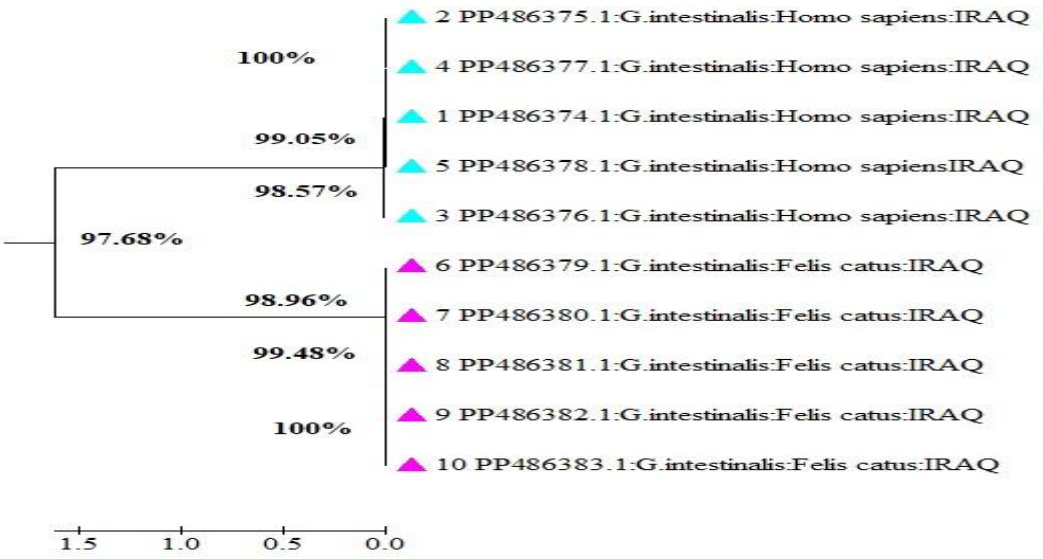

Figure 6: The phylogenetic tree. Green spot: *G.intestinalis* strain isolated from human, Purple spot: *G. intestinalis* strain isolated from cats.

In this study, PCR assays with primer specific *18 S r RNA* have been applied for the detection of Giardia isolates in human stool and cats faecal. It showed that molecular weight of *Giardia intestinalis* isolated from the human and cat was 307bp and 260 bp respectively. According to the phylogenetic tree (between the Iraqi genotype), the *G. intestinalis* isolated from the human and cats, showed the relationship between them. The *Giardia* which was isolated from (PP486376.1, PP486378.1) in human matches with *Giardia intestinalis* isolated from cats Accession No: PP486379.1, PP486380.1 respectively with an identical percentage 99%. Numerous domestic cats and live in cities, and walking animals in restricted areas that can overlap with stray animal habitats is a typical practice (Li *et al.*, 2024; Al-Khazraji and Mohsin,2024). *Giardia intestinalis* infection may be encouraged by the high levels of fecal pollution in these places, especially during the spring

(Tangtrongsup *et al.*, 2020; Alali and Alkhaled, 2023). Giardiasis and other illnesses that can infect humans are significantly more common as a result of all of these factors (Bouzid *et al.*, 2015; Mansour and Hasso,2021; Hameed and AbdAlkhazraji,2024)

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CONFLICT OF INTEREST

Authors declared that there is no conflict of interests

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