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# Molecular detection of *Giardia duodenalis* in human and dogs

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## Abstract

Study was used routine diagnosis & molecular detection of *Giardia duodenalis* in human and dog, 100 samples were taken from humans and 100 from dog. where 7% infected samples were found in humans and 13% infected samples were found in dog after examining it under a microscope, the flotation method was also use and stain with iodine (2-5%) lugols solution and Giemsa stain and use PCR maker genes *TPI* and SSU-*rRna* and sequencing genetic analysis. Concluded PCR is better technique for detecting giardia from lab culture.

## I. Introduction

Giardia is a genus of anaerobic flagellated protozoan parasites of the phylum Metamonada that colonise and reproduce in the small intestines of several vertebrates, causing the disease giardiasis. Their life cycle alternates between a swimming trophozoite and an infective, resistant cyst. Giardia was first described by the Dutch microscopist Antonie van Leeuwenhoek in 1681 (waddood Al-mofti, 2005; Raof, 2011; Erlandsen & Meyer 2013; Makawi and Al-Zubaidi, 2017). The genus is named after French zoologist Alfred Mathieu Giard (Adam, 2001).

*Giardia duodenalis*, also known as *Giardia intestinalis* and *Giardia lamblia*, is a flagellated parasitic microorganism of the genus Giardia that colonizes the small intestine, causing a diarrheal condition known as giardiasis (Basima, 2005; Ibrahim, 2012; Hadi & Faraj, 2016; kraft *et al.*, 2017). The parasite attaches to the epithelium by a ventral adhesive disc or sucker, and reproduces via binary fission. Giardia infection (giardiasis) can cause a variety of intestinal symptom which include (diarrheal, gas, foul-smelling, greasy poop that can float, stomach cramps or pain, dehydrations) (Kawan, 2004; AL-Khayat, 2015; Rumsey & Waseem, 2018). Chief pathways of human infection include ingestion of untreated drinking water (which is the most common method of transmission for this parasite), food, and soil contaminated with human feces, as well as ingestion of sewage, a phenomenon particularly common in many developing countries (Dixon, 2021). PCR assay based on the small subunit ribosomal RNA gene of Giardia for the specific detection of DNA in stool samples and thereafter compared the results with microscopy and antigen detection (Heyworth, 2016).

## II. Material and method

100 samples were taken from humans and 100 from dog. Using a catching device can prevent contamination of the stool by water and dirt. Another way to collect a stool sample is to loosely place plastic wrap over the seat of the toilet. Then place the stool sample in a clean, sealable container before taking it to the lab. Fecal flotation with centrifugation is used primarily to detect Giardia cysts in solid or semisolid stool. Approximately 2 g of feces is mixed with a solution of zinc sulfate and centrifuged for 3 to 5 minutes at 1,500 rpm to 2,000 rpm. As with the direct smear method, Lugol's iodine may be added to aid identification.

**Giemsa stain**

Giemsa stain is an easy to use permanent stain for routine clinical laboratory use. In this staining, flagella and nuclei are reddish pink stain, and cytoplasm stains grey-blue.

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**Lugol's Iodine**

500ml with 4 oz. dropper bottle. 2.1% Iodine Stain. 500ml, strong iodine tends to coagulate the fecal particles and to destroy the refractile nature of the organism.

**Flotation**

Fecal flotation with centrifugation is used primarily to detect Giardia cysts in solid or semisolid stool. Approximately 2 g of feces is mixed with a solution of zinc sulfate and centrifuged for 3 to 5 minutes at 1,500 rpm to 2,000 rpm. As with the direct smear method, Lugol's iodine may be added to aid identification.

**PCR technique**

**The primers preparation**

The primers were used *TPI* with size product (bp) 530 bp as in table (1) and *18s RNA* in (table: 2) the size product (bp) were 500 bp.

**Table (1): The sequence of primers that used this study.**

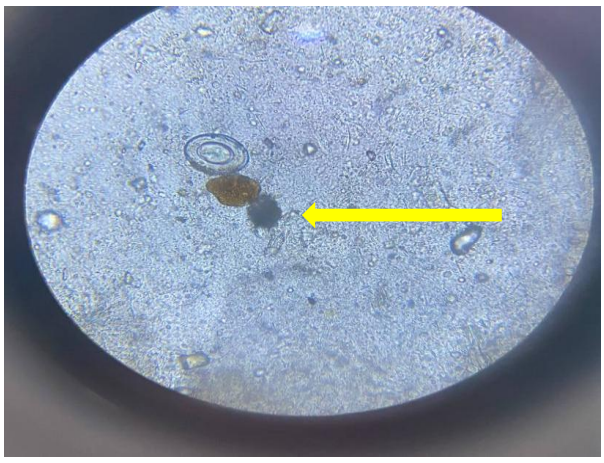
| Primer | Sequence | Primer sequence | Size of Product (bp) |
|---|---|---|---|
| ***TPI*** | F | **5′- CCCTTCATCGGIGGTAACTT -3′** | **530 bp** |
| | R | **5′ GTGGCCACCACICCCGTGCC -3′** | |

**Table (2): The sequence of primers that used this study.**

| Primer | Sequence | Primer sequence | Size of Product (bp |
|---|---|---|---|
| ***18s RNA*** | **F** | **5'- TTGGATGTAGAGATACATTC- 3'** | **500 bp** |
| | **R** | **5'- CACTATTGGAGCTGGAATTAC- 3'** | |

## III. Results

According to primary diagnosis of *Giardia duodenalis* cysts isolated from fecal fecal samples showed oval to ellipsoid with cyst that depended on (direct examination) by using light microscopic, giemsa stain (figure:1).



**Figure(1)*: Giardia duodenalis*** **cysts isolated from fecal sample from human by Giemsa stain under light microscope x40**

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and flotation method. 7 (7%) human samples out of 100 isolations and 13 (13%) dogs samples out of 100 samples was diagnosed as giardia.as in table (3),

**Table (3): Result of primary isolates for human and dogs**

| Samples source | No. of samples | Positive samples (%) |
|---|---|---|
| Human | 100 | 7 (7%) |
| Dogs | 100 | 13 (13%) |
| Total | 200 | 20 (10%) |

In human appeared the percentage in different ages that infected with *Giardia duodenalis* were in children (1-9) years (20%) that the highest than other age groups table (4).

**Table (4): Human in different age's percentage that infected with *Giardia duodenalis***

| Age/ years | Number of cases | Positive cases | percentage |
|---|---|---|---|
| 1-9 | 25 | 5 | **20%** |
| 10-19 | 25 | 1 | 4% |
| 20-29 | 25 | 1 | 4% |
| 30-39 | 25 | 0 | 0% |
| Total | 100 | 7 | 7% |

In dogs percentage that infected with *Giardia duodenalis* in different ages increased 3-12 months (38.23%) than other age groups from (1 to 12) years that showed 0% as in table (5).

**Table (5): dogs in different age's percentage that infected with *Giardia duodenalis***

| Age | Number of cases | Positive cases | percentage |
|---|---|---|---|
| 3-12m | 34 | 13 | **38.23%** |
| 1-5yrs | 33 | 0 | 0% |
| 6-12yrs | 33 | 0 | 0% |
| Dog | 100 | 13 | 13% |

**- Nested PCR**

Table (6) Results of nested PCR and compared with other routine examination of *Giardia duodenalis*, percentage were 13% in dog and 7% in human resemble to infection percent using direct examination by light microscope with Giemsa stain and without stain and also the same result when using floatation (table 6).

There is no difference in the diagnosis between PCR and diagnosis laboratory due to the accuracy of the work in the laboratory, especially microscopic diagnosis.

**Table (6) Results of nested PCR and compared with other routine examination of *Giardia duodenalis***

| Number of samples | Number of positive in microscopic | Number of positive on flotation | Number of positive on Giemsa stain | Nested PCR% |
|---|---|---|---|---|
| Dog 100 | 13 | 13 | 13 | 13 |
| Human 100 | 7 | 7 | 7 | 7 |

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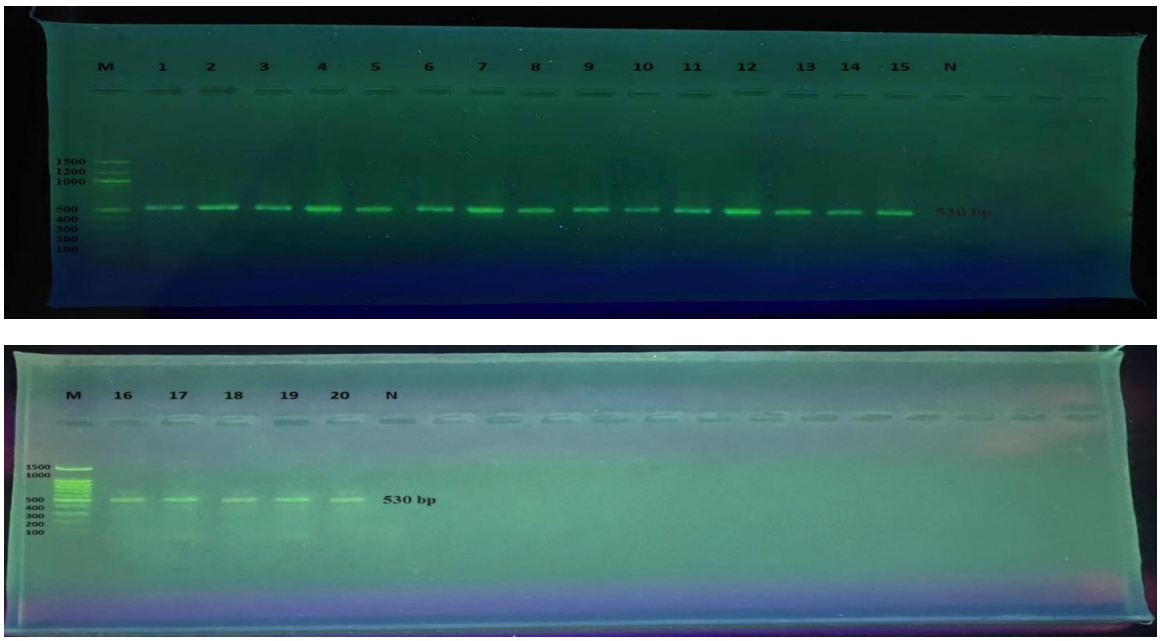


**Figure (2): PCR product the band size 530 . The product was electrophoresis on 1.5% agarose. 1x TAE buffer for 1:30 hours. M: DNA ladder (100), *Tpi* gene (1-13) Dog and (14-20 )Human,volt(70)**

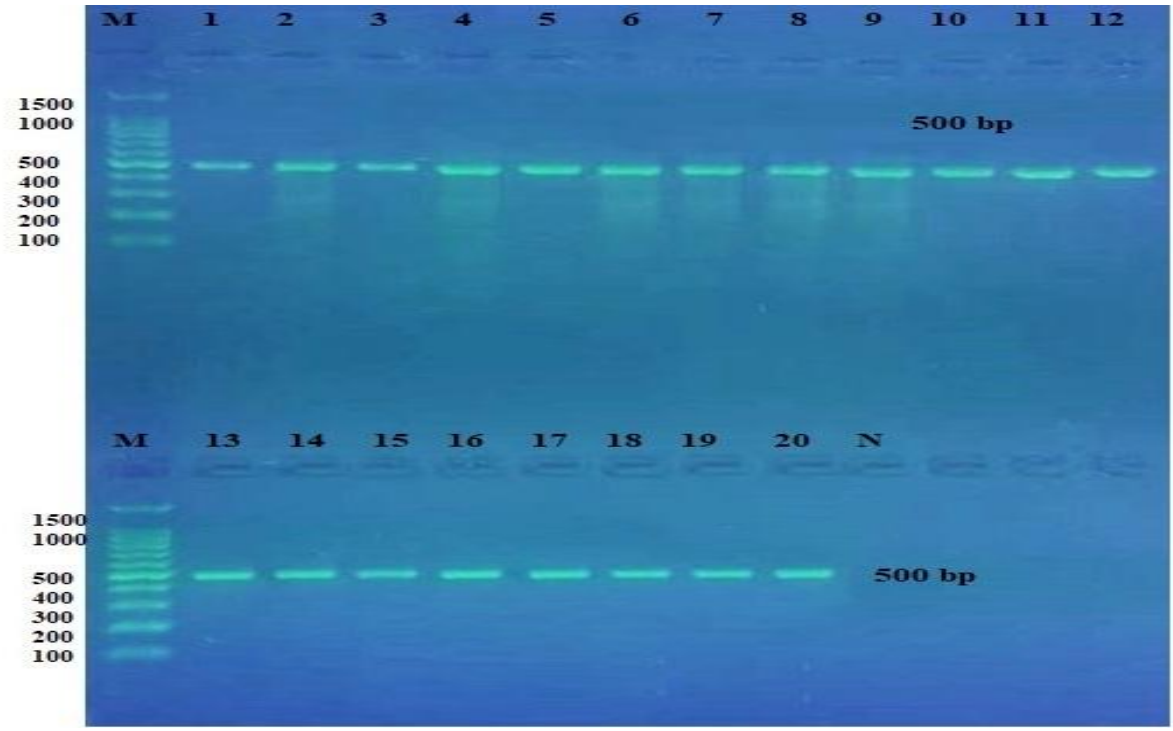


**Figure (3) PCR product the band size 500 . The product was electrophoresis on 1.5% agarose. 1x TAE buffer for 1:30 hours. M: DNA ladder (100). 18s RNA gene,Dog(1-13)and (14-20)Human,volt(70)**

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**Results of sequencing and genetic analysis**

Has been used to successfully genotype isolates of *G. duodenalis* from 20 isolates faeces, This fragment was also amplified from the following faecal isolates.

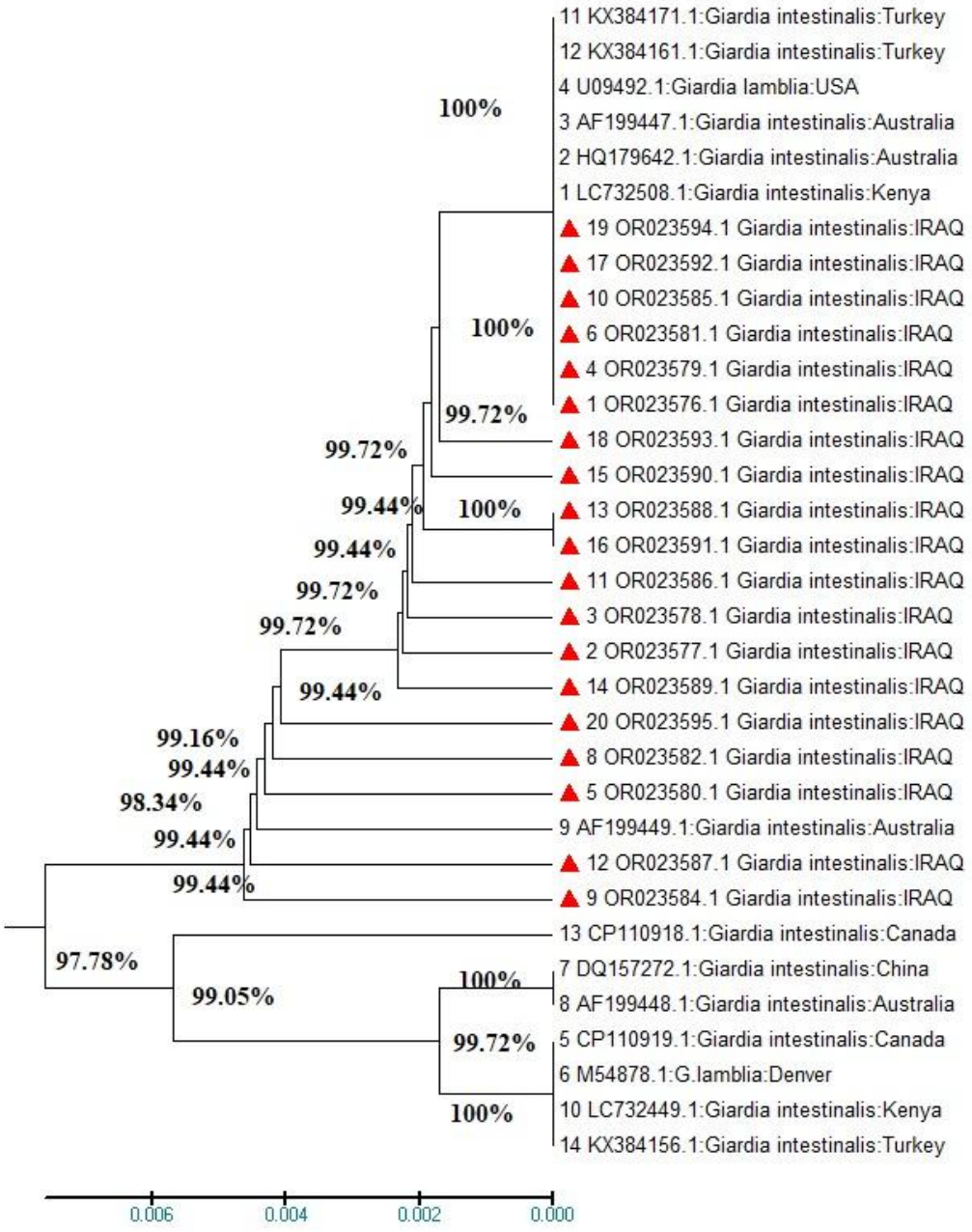


**Figure (4):** the phylogenetic tree of giardia

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## IV. Discussion

In our study, the detection rate of *G. duodenalis* in pet dogs was higher than reported in Brazil (6.9%) (Chiebao *et al.*, 2020), and Poland (6.0%) (Piekara-Stępińska *et al.*, 2021). However, it is lower than the prevalence of rural dogs in Argentina (44.4%) (Kuthyar *et al*., 2022), and Italy (15%) (Berrilli *et al.*, 2004). The detection rate of G. *duodenalis* in human was lower than the detection rate of *G. duodenalis* was 32.3% (469/1452) in Hainan, China (Chen e*t al.*, 2019), and 181/318 (56.9%) in Egypt (Yu *et al.*, 2019). The incidence of dogs is more than that of humans because the human population had a low prevalence of Giardia infection and that agreement with (Palmer *et al.*, 2008). The detection rate of *G. duodenalis* in children higher than in other ages because the children more exposure to infection as a result of neglecting prevention and that agreement with (Robertson and Thompson, 2002) he says Risk factors for infection in humans include geophagia (especially in children), and concluded with (Ghieth *et al.*, 2016) isolated (13.9%) from children attended Cairo University Pediatrics Hospitals. DNA from all 20 isolates faeces, cyst and oocyst was subjected to the *TPI*, melting peaks with a T m of 90.63–91.74 °C (sd 1.0–1.2) were generated exclusively from DNA recovered from *G. duodenalis*, Nested PCR for the TPI gene was the most variable of the targets employed. I conclude with (Amar *et al.*, 2003) was found *TPI* gene in 20 isolates from faeces. (Gómez-Muñoz *et al.*, 2012) found 89.2%of *TPI* gene by nested PCR in isolates from sheep I accepted with that. While I agreement with (Puebla *et al.*, 2014) DNA from 90 of 103 (87.4%) samples was successfully amplified by PCR–*tpi.* I conclude with (Read *et al.*, 2004) used to successfully genotype isolates of G. duodenalis. I agreement with (Ebner et al., 2015) used sequencing and genetic analysis of giardia from isolates. While I conclude with (Monis *et al*., 1996) analysis and DNA sequence analysis of giardia. Because it was discovered that GenBank isolates with the accession numbers KX384171.1, KX384156.1 and UO9492.1 which was deposited from Turkey and the United States of America matching is get 99 percent, it was determined that the observations made through the phylogenetic tree regarding my isolate were confirmed by other neighboring sequences.

## V. Conclusion

1- There are no different results when use PCR technique and classical examination of *Giardia duodenalis.*
2- The most susceptible group to infection in humans is from 1 to 9 years old
3- The most susceptible group to infection in dogs is from 3 to 12 months

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