Sumer Journal for Pure Science (ISSN: 2790-7031)
2nd International Scientific Conference on Pure and Medical Sciences/University of Sumer 2024

Prevalence and Molecular Characterization of *Giardia duodenalis* in Cats in Babylon Province, Iraq

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Abstract

Giardia is a genus of the highest prevalent flagellated protozoa in human and other mammals. The study aimed to detect prevalence microscopically and genetic diversity of *Giardia duodenalis* isolates from cats and identify the risk of zoonotic transmission in Babylon province, Iraq. A total number of one hundred fecal samples (36 male, 64 female) were collected from cats of different ages. Microscopic method used for determining the ratio of *Giardia duodenalis* infection in cats. Molecular techniques; Nested-PCR used to determine the types of *Giardia* assemblages in cats, targeting triose phosphate isomerase gene (tpi) on the positive samples. The total infection of *Giardia duodenalis* was 24% by using conventional microscopic method in cats. Based on the gender In the relation to gender there was significant difference between female (31.25%) and male (11.11%). The highest infection rate was recorded at age < 2 years old (33.33%) and the lowest at age > 4 years old (16.66%). The highest (70.83%) of cat positive samples were allocated to assemblages F, followed by (33.33%) assemblage A and the lowest assemblages B (29.16%) with significant difference (P≤0.05). The detection of *Giardia duodenalis* assemblage A and B in cats indicated that cats pose a threat to human health by zoonotic transmission.

Keywords: Cats, *Giardia duodenalis*, Tbi gene, Prevalence, Zoonotic.

الانتشار والتوصيف الجزيئي لطفيلي *Giardia duodenalis* في القطط في محافظة بابل، العراق

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الخلاصة

الجيارديا (الاسم العلمي: *Giardia*) هو جنس من الأوليات السوطية الأعلى انتشارًا في الإنسان والثدييات الأخرى. هدفت الدراسة إلى الكشف عن مدى انتشار عزلات *Giardia duodenalis* مجهريا والتنوع الوراثي في القطط وتحديد خطر انتقال الأمراض المشتركة الحيوانية المنشأ في محافظة بابل، العراق. تم جمع مائة عينة براز (36 ذكر، 64 أنثى) من قطط بأعمار مختلفة. الطريقة المجهرية المستخدمة لتحديد نسبة الإصابة ب *Giardia duodenalis* في القطط. التقنيات الجزيئية تم استخدام تقنية Nested-PCR لتحديد أنواع الجيارديا في القطط، حيث تم استهداف جين ايزوميراز ثلاثي الفوسفات (tpi) على العينات الموجبة. بلغت نسبة الإصابة بطفيلي الجيارديا 24% بالطريقة المجهرية التقليدية في القطط. فيما يتعلق بالجنس كان هناك فرق كبير بين الإناث (31.25%) والذكور (11.11%). تم تسجيل أعلى معدل إصابة في عمر أقل من سنتين (33.33%) وأدنى معدل في عمر أكثر من 4 سنوات (16.66%). اظهرت النتائج أعلى نسبة اصابة (70.83٪) من عينات القطط الموجبة للجين نوع F assemblages، تليها (33.33٪) الجين نوع A assemblages وأقل نسبة (29.16%) B assemblages مع وجود فرق معنوي (P ≥0.05). يشير تشخيص مجموعة A وB *Giardia duodenalis* في القطط إلى أن القطط تشكل خطراً على صحة الإنسان عن طريق انتقال الأمراض المشتركة الحيوانية المنشأ.

1. Introduction

Giardia is an enteric flagellated parasite in vertebrates including humans and other mammal, amphibian, and bird species [1, 2]. The parasite has a worldwide spreading and is recognized as a very important causal agent of diarrhea in humans, cats, and other host species [3, 4]. The communication of *Giardia* is by the fecal-oral route, also concluded in polluted food and water, immediately from animals to human and may be from person to person connection [5, 6]. There were 8 assemblages (genotypes) of *G. duodenalis*; A to H assemblages, the two main potentially zoonotic assemblages are A and B that present in humans and animals; other such as (assemblages: C and D detected in canine, E in livestock, F in feline, G in rodents and H in marine animals) are more specific [7-10].

The most common method is the microscopic detection of *Giardia* cyst in fecal samples from various hosts. Serological diagnostics such as immunofluorescence antibody test, enzyme-linked immunosorbent assay, polymerase chain reaction technique is highly specific and sensitive in comparative with microscopy and serological procedures [11, 12].

The study aimed to detect prevalence microscopically and genetic diversity of *Giardia duodenalis* isolates in cats and identify the risk of zoonotic transmission of parasite to human in Babylon province, Iraq.

2. Materials and methods

2.1 Ethical approval

All procedures was obtained from a guidance of Research, Publication and Ethics of the College of Veterinary Medicine, University of Baghdad, in compliance with the ethical principles of animal welfare

2.2 Samples collection

A total number of one hundred fecal samples (36 male, 64 female) were randomly collected from different ages of domesticated cats in Babylon province during the period from September 2022 to September 2023. Fecal samples collected in a clean plastic container and were tightly closed, given sequential numbers, age, sex, date of sampling also included protective measure was taken such as wearing disposable gloves. The samples were transported in cool box to parasitology laboratory and divided into two parts for traditional examination and DNA extraction, in Technical Institute of Babylon, Al-Furat Al-Awsat Technical University. Microscopic method used for determining the prevalence of *Giardia duodenalis* in cats. Nested PCR technique targeting (tpi) gene was used to identify *Giardia duodenalis* genotype (assemblage) in cats as per the method used by [13].

2.3. DNA extraction

DNA was extracted using the faecal lysis procedure and Proteinase K in accordance with the company's (DNA extraction kit for stools by Bioneer) instructions. After that, the extracted gDNA was evaluated using a Nano-drop spectrophotometer and stored in a refrigerator at -20C until it was employed in PCR amplification [14].

2.4 Molecular technique

Nested PCR pathway used for detection *Giardia duodenalis* assemblage A, B and F based triose phosphate isomerase (tpi) specific gene from cat's fecal samples. Using primers that were donated by (Bioneer company) and were specifically designed for genotyping *Giardia duodenalis* A and B, this approach was completed in accordance with the method reported by [15], assemblage F designed by gene bank accession number (LC341570) for *Giardia duodenalis* triose phosphate isomerase gene, as in following table (Table-1).

Table 1- Giardia duodenalis gene sequences

Nested PCR	Primers		Sequences	Amplicons
First rounds	**Tpi A**	F	5'- CGAGACAAGTGTTGAGATG -3'	**576 bp**
		R	5'- GGTCAAGAGCTTACAACACG -3'	
	Tpi B	F	5'- GTTGCTCCCTCCTTTGTGC -3'	**208 bp**
		R	5'- CTCTGCTCATTGGTCTCGC -3'	
	Tpi F	F	5'- AACGGCTCGCTCGACTTTAT-3'	**471bp**
		R	5'- GGGCTCGTAGGCAATAACGA-3'	
Second rounds	**nTpi A**	F	5'- CCAAGAAGGCTAAGCGTGC -3'	**476 bp**
		R	5'- GGTCAAGAGCTTACAACACG -3'	
	nTpi B	F	5'- GCACAGAACGTGTATCTGG -3'	**140 bp**
		R	5'- CTCTGCTCATTGGTCTCGC -3'	
	nTpi F	F	5'- GGCCATTGCTGCCCACAAG-3'	**401bp**
		R	5'- TCTTCGACTCTCCAAGCTCC-3'	

Tris-HCl (pH 9.0), dNTPs 250 mM, and Taq DNA polymerase 1U The freeze-dried pellet found in the PCR premix container contains 10 mM, 1.5 mM MgCl2, 30 mM KCl, stabiliser, and tracking dye. According to the instructions of kit's, a total volume of pure genomic DNA 20 µl, 1.5 µl, 10 pmole from forward primer, and 1.5 µl from reverse primer were added to create the master mix of the PCR. De-ionized PCR water was then added to the 20 µl PCR premix tube, and Exispin vortex used for centrifugation of the mixture. There were 30 cycles, at 95 °C initial denaturation for 30 sec, at 52 °C annealing for 30 sec, at 72 °C extension for 1 min, and ultimately at 72 °C the final extension for 7 min. employing agarose gel electrophoresis 1.5%, Ethidium bromide staining, and transilluminator visualization (UV) to examine the PCR data.

2.5 Statistical Analysis

Computerized statistical analyses were performed by using SPSS version 31, and Chi-square test was used to estimate the variables [16].

3. Results

The prevalence of *Giardia duodenalis* by traditional microscopic method. The total infection rate of *Giardia duodenalis* in cats by microscopic method was 24% (Table -2).

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Table 2- The infection rate of *Giardia duodenalis* in cats by microscopic method

Host	No. of samples examined	No. of Positive samples	Percentage %
Cat	100	24	24

3.1. Infection Rate Giardia duodenalis according to gender:

There was significant (P<0.05) difference of *Giardia duodenalis* in cats between males (11.11%) and females (31.25%) (Table -3).

Table 3- *Giardia duodenalis* infection rate in cats according to gender.

Gender	No. of examined samples	No. of positive samples	Percentage (%)	X^2
Males	**36**	**4**	**11.11**	
Females	**64**	**20**	**31.25**	**7.69**

***P<0.05**

3.2. Infection Rate of Giardia duodenalis According to Age:

A higher infection rate (33.33%) of *Giardia duodenalis* in age ≤ 2 years old followed by age 2-4 years old (21.73%) and the lowest infection rate in the age ≥ 4 years old (16.66%), with significant (P≤0.05) deference (Table -4).

Table 4- Infection rate of *Giardia duodenalis* according in cats to age.

Age/years	No. of examined samples	No. of positive Samples	Percentage (%)	X^2
≤ 2	**30**	**10**	**33.33**	
2-4	**46**	**10**	**21.73**	**7.69**
≥ 4	**24**	**4**	**16.66**	

***P≤0.05**

3.3 Microscopic examination

The cysts examination of *Giardia duodenalis* in cats fecal samples appear oval in shape with four nuclei at 40X examined by wet smear (Fig.1)

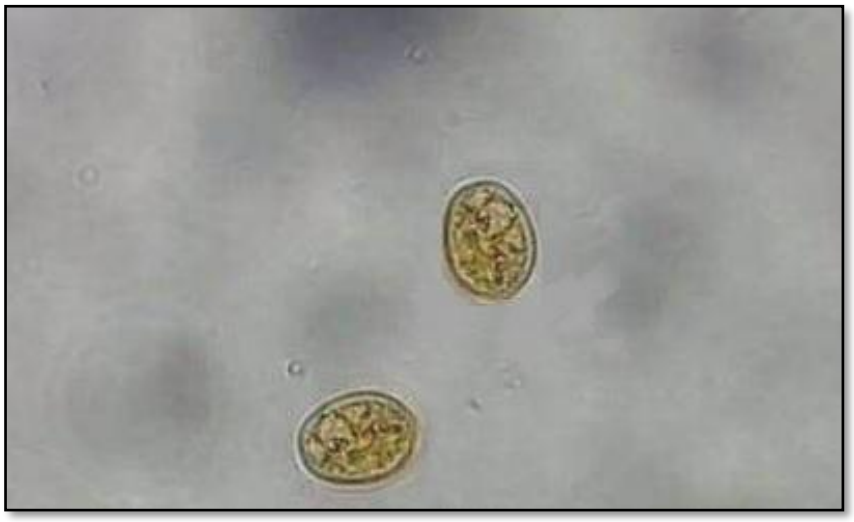

Figure-1 *G. duodenalis* cyst in cat's fecal sample, oval in shape with four nuclei (40X).

3.4 Molecular distribution of Giardia duodenalis assemblages in cats:

Three assemblages (genotypes) of *Giardia duodenalis* were detected in cats; showed that highest rate was *G. duodenalis* assemblage F 70.83% (Fig.-2), followed by *G. duodenalis* assemblage A 33.33% (Fig.-3) and the lowest *G. duodenalis* assemblage B 29.16% (Fig.-4) (Table-5).

Table 5- Distribution of *Giardia* assemblages in cats.

No. of microscopic +ve examined samples	Assemblages	No. of Ass. Detected	Percentage (%)
24	Ass. A	8	33.33
	Ass. B	7	29.16
	Ass. F	17	70.83

***P≤0.05**

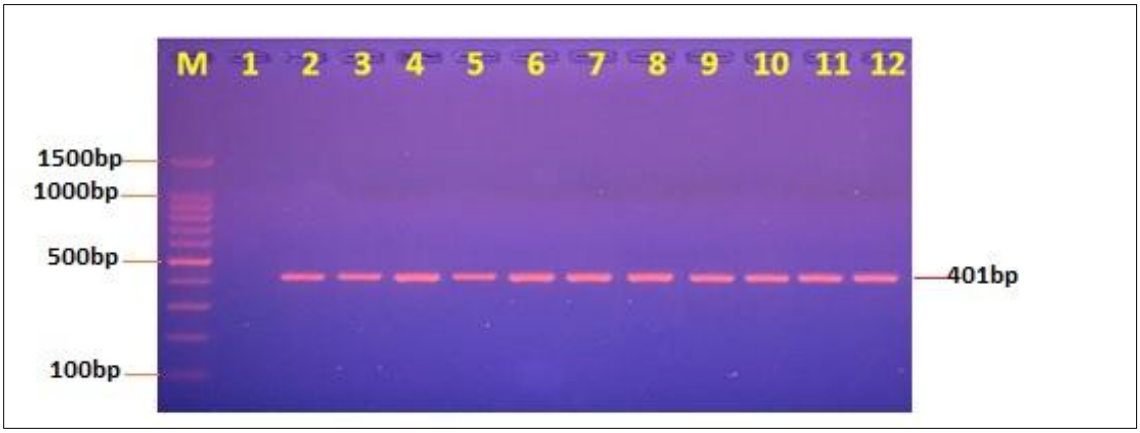

Figure-2 Image from an agarose gel electrophoresis showing the analysis of the Tpi gene by nPCR in fecal samples from *Giardia duodenalis* assemblage F. (M) Marker rung (1500-100bp). Lane (1-12) 401-bp nPCR product for the *Giardia duodenalis* assemblage F.

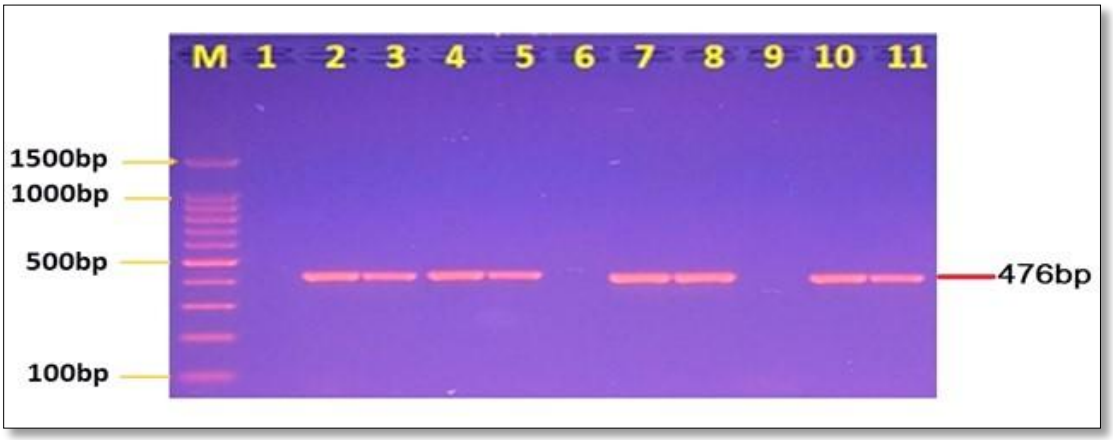

Figure-3 Image from an agarose gel electrophoresis showing the analysis of the Tpi gene by nPCR in fecal samples from *Giardia duodenalis* assemblage A. Marker ladder (M) (1500-100bp). Lane (1-11) nPCR product for *Giardia duodenalis* assemblage A at 476 bp.

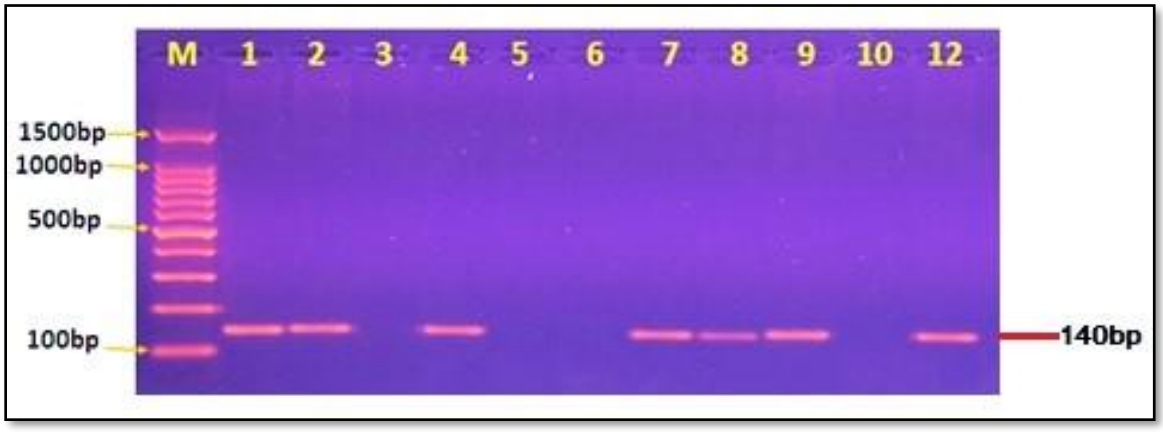

Figure-4 Image from an agarose gel electrophoresis showing the analysis of the Tpi gene by nPCR in fecal samples from *Giardia duodenalis* assemblage B. (M) Marker rung (1500-100bp). Lane (1-12) 140-bp nPCR product for the *Giardia duodenalis* assemblage B.

4. Discussion

The results were in agreement with [17, 18] whom recorded 25% in cats of Brazil and Mexico respectively. In cats [19] in Baghdad province showed lower prevalence was 10%. [20] Reported the infection rate of *G. duodenalis* in cats was 16%. While [21] in Iran was showed a higher percentage (50%) in cat. The differences between the infection rate of *G. duodenalis* in our study the infection rate of other studies, due to difference in number of collected samples, environmental condition, difference in diagnostic methods and the age of host also play an important role.

The microscopic examination showed that female cat's infection rate was higher than male, which in agreement with [22] in Colorado showed the rate in female cat was 40.7%, [23] reported the infection in female cats was higher than male. It is clear that the females showed higher prevalence rate of infection compared with males as a result of stress factors faced the females particularly during pregnancy and lactation**.**

The prevalence rate of infection in young cats showed numerically higher than adult. [24] showed the higher infection rate in young cat was 33.33%. These differences in the prevalence rates of *Giardia duodenalis* infection was due to the difference in area of samples collected different managemental regimes, season of samples collection and the etiological conditions as recorded by the previous study.

The prevalence rate in young cat higher than adult. These results were in agreement with other previous studies that reported the highest rate of infection appeared at ages of ≤ 2 years then the infection decline in adults due to acquired immunity of these animals [25, 26].

4.1. Molecular distribution of gene for cats

This study showed that *G. duodenalis* assemblage F was recorded (70.83%) in total (24) cat positive samples, it was in agreement with other study in Germany which recorded rate 75% [27].

G. duodenalis assemblage A in cat 33.33%, which in agreement with [28] who showed that the ration of *G. duodenalis* assemblage A was 35%. While in China [2] founded the higher the ration of *G. duodenalis* assemblage A in cat was 80%.

G. duodenalis assemblage B was detected 29.16% in cats positive samples. Which in agreement with study in china that recorded 28.5% [2]. These variations in the results which depend on the difference of techniques and detected genes.

5. Conclusion

The detection of *G. duodenalis* assemblage A and B cats indicated that cats play an important source of zoonotic infection and can pose a risk and threat human health, in addition to the species-specific assemblage F.

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