" Kerbala Journal of Veterinary Medical Sciences, Vol. 1, Supplement I, 2025 "
"Proceedings of the 6th Scientific Conference, College of Veterinary Medicine, University of Karbala"

Morphological and molecular diagnosis of Giardia duodenalis and prevalence in domestic dogs in Kerbala - Iraq

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Received: 8/5/2025 **Accepted:**25/5/2025 **Published:** 3/7/2025

***Abstract*— Giardia is organism that commonly causes Diarrhea in dogs, cats, and domestic animals. This condition is known as giardiasis. The finding of current research, the morphological and molecular diagnosis of Giardia duodenalis is being determined, along with the age, gender, and months influence on the occurrence of Giardia disease prevalence in dogs.**

The current study used traditional methods to identify and detect Giardia in household dogs in the Karbala district of Iraq. Molecular diagnostics confirmed this using the polymerase chain reaction (PCR) method and primer beta giardin gene (511 bp). In 2024, this study has been done from beginning of Jan. to Jun. One hundred and fifty fecal samples from dogs of both genders, both adults and puppies, were gathered.

the results revealed trophoziote and cyst of Giardia were examined under a microscope.

***Keywords* — Giardia, dogs, diarehhea, PCR.**

INTRODUCTION

Overall Giardia infection rate in dogs was 52% (69/150) according to the results of the traditional techniques. Compared to older dogs, The high of infection prevalence has been recorded 40% (30/75), younger Puppies had a higher incidence of 52% (39/75). The infection rates for males and females were 48% (36/75) and 44% (33/75). A greater infection rate of 60% (15/25) in April and a lower infection rate of 28% (6/25) in February were among the different infection rates that were documented during the research period. Out of 150 dog fecal samples obtained at random in Karbala city, the PCR overall infection rates in DNA samples revealed 55% (55/100).

We concluded Giardia is more common in female dogs and young pups, and molecular diagnostics is thought to be an accurate form of diagnosis. However, there is a chance of mistake even with the traditional use of microscopic examination.

The worldwide parasite protozoan flagellate Giardia duodenalis causes waterborne diarrheal disease in vertebrates host and in humans (1). The single-celled parasite Giardia is a type of protozoa that includes several species with similar morphologies. In many mammals, birds, reptiles and amphibians, it colonizes and multiplies in the small intestine. It is in charge of giardiasis, which causes invertebrate diarrhea (2). Both domestic and wild animals can contract the common protozoan Giardia duodenalis (3). Reinfection occurs often until environmental infectious cysts are eliminated and animals with giardiasis, whether symptomatic or asymptomatic, can infect other people and animals (4).The infection is more common in poor and third-world countries, particularly in places with inadequate sanitation, as the parasite spreads extensively through feces and water tainted with Giardia cysts (5). Growth retardation, hidden infections, different signs, like a weight loss and persistent diarrhea are caused by the non-invasive small intestine bacterium *G. duodenalis* Given these diverse symptoms and the host and parasite variances, it is extremely difficult to comprehend the processes underlying this vast range of disorders (6). During the development of Giardia consists of two phase, The trophozoites are pear-shaped and range in length from 15 to 20 m. They feature four pairs of flagellae. Another feature of these cysts is a broad front disk that by which attach to the wall of intestine. Cysts are spherical to ovoid shap and have four nuclei without flagella. (6,7). In the lab, fecal microscopic analysis, a variety of immunological-based assays, and molecular methods can all be used to diagnose giardiasis. When it comes to identifying *G. duodenali*s infections, PCR is more accurate and practical than fecal microscopy and enzyme-linked immunosorbent test (8). Due to the possibility of an outbreak and the resulting financial losses, Giardia duodenalis is important for public health and

has economic ramifications for the nation. Thus, the purpose of this study was to use molecular and microscopic methods to identify and quantify the incidence of *G. duodenalis* in domesticated dogs in the region of Karbala.

MATERIALS AND METHODS

Sample gathering. Present study has been conducted during 2024 from Jan. to Jun. In different regions across Kerbala, 150 fecal samples (10 g) were taken from household dogs of all ages and genders. Laboratory tests were performed to detect the Trophozoit and cystic phases while collecting sample data, including date, age and sex. To identify Giardia duodenalis, the samples were kept in 5-milliliter tubes with 2% potassium dichromate and 70% ethanol as a preservative. (9).

The samples were taken to the University of Kerbala's College of Veterinary Medicine's Parasitological Laboratory. Each fecal sample was split into two sections, one of which was used for microscope investigation by use saline and iodine stain and the other had been stored at -20°C until molecular investigation (PCR). A droplet of the material was put onto a glass slide and the coverslip using the wet mount method of direct saline or iodine stainingand Giardia duodenalis Trophozoit and cysts were examined under a 40X microscope. The color, texture, and mucous material of each sample were inspected macroscopically. The size, shape, and contents of the cysts were used to determine the parasites. (10).

DNA Extractions

According to the inventor's instructions, DNA extraction kit (ADDBIO, South Korea) was used to extract the DNA of 100 fecales specimens. Approximately 50 mg of fecal material was lysed using 200 µl of lysis buffer and 20 µl of proteinase k (20 mg/ml).The resultant lysate was purified using a spin column, then washed several times before being eluted with the provided elution buffer. In order to assess absorbance at (260/280 nm), the recovered genomic DNA was put through a Nanodrop spectrophotometer, in order to assess its purity. The extracted DNA was kept in a deep freezer until it was subjected to further PCR analysis.

Primers of PCR.

To create primer stocks that have a 100 Pico mole/µl ultimate concentration, the primers were lyophilized and then dissolved in clean water. Throughout the whole PCR experiment, they were kept at 0.5 Pico mole/20 µl a concentration at -20°C until they were used. All of these primers were provided by Macrogen/Korea.

Polymerase chain reaction (PCR)

To directly identify Giardia duodenalis, the PCR technique was utilized to amplify the beta giardin gene (Table 1). DNA amplification of the 511 bp beta giardin gene (F.5-GAACGAACGAGATCGAGGTCCG-3) and (R.5' CTCGACGAGCTTCGTGTT-3) (11). The PCR components were then put into a heat cycler to amplify the desired gene at the following temperature (table. 2).

Table 1: Explain the Mixture of PCR reaction

PCR Master mix	Volume
Master mix	10µl
Forward primers1 (10pmol)	1µl
Reverse primers1 (10pmol)	1µl
DNA template	3 µl
PCR water	5µl
Total volume	20µl

Table 2: Show the conditions of thermocycler for PCR

PCR step	Temp.	Time	repeat
Initial denaturation	94°C	5min.	1
Denaturation	94°C	30 sec.	35cycle
Annealing	60 °C	30 sec.	
Extension	72°C	60 sec	
Final extension	72°C	5min	1

The PCR result was examined using agarose gel electrophoresis: After dissolving of powder agarose (1 gm) in TBE buffer, it was microwave at 95 °C about (2 minutes). After cooling, the molten agarose was combined with seven microliters of gel dye stain, put onto a gel tray, and left to set for fifteen minutes. After removing the comb, placed the gel tray in an electrophoresis tank filled with TBE buffer. Five microliters of PCR products were added to each well, and the electric current was set at 100 volts and 80 AM for 30 minutes. The PCR findings were visualized using the gel documentation system. The gel tray should be put in an electrophoresis tank with TBE buffer once the comb has been removed. Each well received five microliters of PCR products, and for 30 minutes, the electric current was set to 100 volts and 80 AM. The gel documentation system was used to visualize the PCR results.

Statistical Analysis:

Additionally, influence of various elements on the research percentages have been determined by used the - SAS (12) program. The chi-square test was used in this study to compare percentages (0.05 and 0.01 likelihood) in a meaningful way.

RESULT & DISCUSSION

Microscopic analysis showed that the trophozoite was pear-shaped, with four pairs of flagellae and a pair of nuclei. Additionally, they have a large anterior disc that sticks to the intestinal wall (Figure 1). ovoid cyst with four nuclei but no flagella (Figure 2). high frequency of Giardia duodenalis in dogs, with a 46% overall infection rate (69/150).

Molecular findings using specific primer sequences of the 511 bp (beta giardin gene), the current study showed that the amplification zone for the PCR assay (Figure 3). To find out the prevalence of Giardia duodenalis, samples were sent for molecular analysis in line with traditional PCR examination. 100 samples were randomly selected to be studied for PCR from the 150 fecal sample. 55% (55/100) tested positive for infection, according to the dog DNA samples.

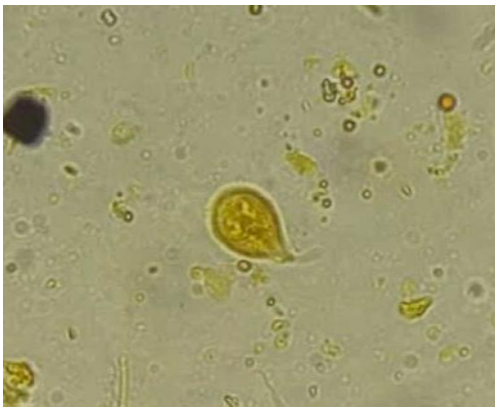

Figure 1. Morphology appearance of Giardia duodenalis trophozoite by using iodine method

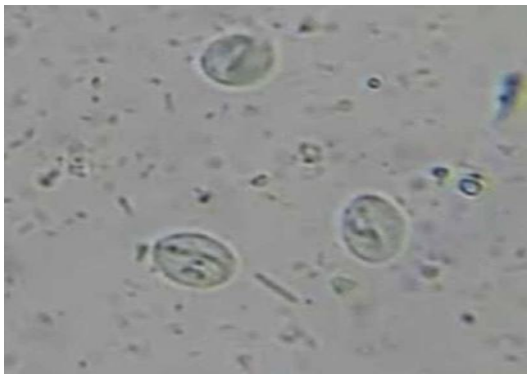

Figure 2. Morphology appearance of Giardia duodenalis cyst by using direct saline method

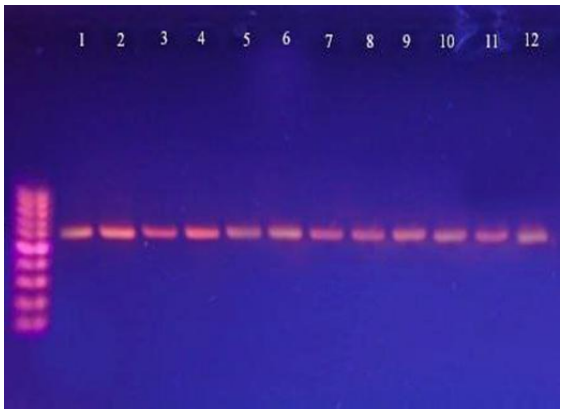

Figure 3. Show the gel electrophoresis of PCR product of (beta giardin gene), Amplicon size about 511 bp. using 1.5% agarose gel at 6volt /cm for 1 hour

Prevalence of Giardia duodenalis in dogs

Based on the age of the animals, the prevalence of Giardia duodenalis was as follows: the adults dogs have a lower infection proportion 40% (30/75), while young pups had a higher infection rate of 52% (39/75) (Table.3). where Giardia duodenalis is found. The rate of infection varied by sex, with the highest number of significant cases (48%) occurring in males and the lowest percentage (44%) occurring in females (36/75) (Table 4). Giardia duodenalis prevalence in dogs by month: the higher infection rates was 60% (15/25) in April, while the lowest percent was 28% (6/25) in February (Table.5).

Table 3. Prevalence of Giardia duodenalis in dogs related to age.

Age stage	Total number	Positive	Percentage (%)
Young's	75	39	52
Adults	75	30	40
Total	150	69	46%
Chi-Square (χ^2)	---	---	9.163 **
** (P≤0.01).			

Table 4. The overall Giardia duodenalis infection rates for each dog gender

Sex	Total number	Positive	Percentage (%)
Females	75	33	44
Males	75	36	48
Total	1500	69	46%
Chi-Square (χ^2)	---	---	8.663 **
**** (P≤0.01).**			

Table 5. Occurrence of Giardia duodenalis in dogs through months.

Months	Number of samples	Positive	Percentage (%)
January	25	8	32
February	25	6	24
March	25	11	44
April	25	17	68
May	25	15	60
John	25	12	48
Total	150	69	46
Chi-Square (χ^2)	---	---	14.882 **
**** (P≤0.01).**			

In Iraq the frequency of *G. duodenalis* varies among research based on geographic locations, habitats of local animal, animal populations, and period fluctuations during the year, the disease is extremely prevalent due to filthy circumstances and suitable weather. Interestingly, compared to pets, stray dogs had a greater infection risk of *G. duodenalis* (13). In present study the morphology of Trophozoit and cyst of Giardia detected microscopically, that revealed trophozoite pear-shaped, with pair of nucleus and four pairs of flagellae. A large front disk that attaches to the intestinal wall is another characteristic of them, while Cyst oval shape and contain four nuclei but no flagella agree with finding of (14). Infection rate of Giardia in dogs through Kerbala provenance by using conventional methods was 46 % (69/150) and by molecular

method (PCR) 55% (55/100), agree with study conducted in Basrah that revealed infection rate was (40%) (2). Also in Korea the incidence of giardiasis in dogs was 33.2% demonstrated by (15). Incidence of disease giardiasis in dogs in Turkey, was 26.79% reported by (16). And in Poland that investigated the infection rate in dogs was 21.1% (17). Disagree with previous studies in Iraq, study has been done in Erbil Province that reported low prevalence rate of the disease in stray dogs (10.46%) (18). While in Babylon Province (10) have been recorded the prevalence rate was 14.67%. also disagree with study conducted in Iran that has been reported incidence of Giardia duodenalis in dogs (2.48%) (19). Also in Egypt the giardiasis was 8.5% reported by (20). Disagree with study performed In Brazil the occurrence giardiasis in dogs was 5.6% reported by (21). This variation in prevalence rate in studies due to difference in the number of samples, study area, age of animals, dog's life style, climate, and period of study (17,22). The current study's findings show that the prevalence of giardiasis in dogs varies by animal age, with young pups having a higher infections rates of 52% and older dogs having a lower infection rate of 40% agree with another study that recorded higher infection rate in young dogs was 59.7% and the lower infection rate in adult dogs it was 40.3 % (23) . Also in Iran (19) found that the infection rate was lower in older dogs (1.25%) and greater in young puppies (5.74%). Another study reported high infection rate in young dogs than adult dogs, 14% and 1.3 respectively investigated by (21). The greater infection rate in young animals relative to older animals may be caused by the animals' differing immunological condition (24). The immaturity of the immune systems of early pups may be the source of infection, in addition to malnutrition, particularly after weaning off of their mother, which increases their susceptibility to parasite infection as they become older., present study's results are consistent with the research conducted by (25). According to gender, the present study investigate The higher infection rates among males than females (48% (36/75) and 44% (33/75), respectively, are consistent with earlier Iraqi research, in Mosul city the prevalence of infection related to sex of dogs in males was 65.3% and in females was 34.7% (23). Agree with study in Babylon reported infection rate in males higher than females 22.22% and 7.69%, by (10). Due to their greater activity over wider regions, male dogs have been found to be more relevant disease carriers than females, putting them at higher risk, according to (13, 26). According to the period of study the higher infection rate was 60% (15/25) identified during April, with February having the loweer 28% (6/25) prevalence. It might be because of shifting weather patterns and a lack of care and attention for stray dogs, which exposes them to intestinal parasites like Giardia. The current study's findings concurred with the reports (27). This variation in prevalence throughout the months might be caused by insect activity, and rain contributes to the dissemination cyst of Giardia (28).

CONCLUSION

Although the standard method for identifying Giardia is microscopic examination, there is a considerable possibility that these species may be misidentified. The molecular diagnostics method is thought to be a precise diagnostic method. Females and young canines are more susceptible to Giardia than are males and adults.

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