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Diagnosis and distribution of Giardia parasites in dogs in Mosul city, Iraq

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Abstract--The current study aimed at diagnosing the Giardia parasite that parasitizes dogs, based on a number of morphological and standard characteristics of the trophozoites and the cysts, using different techniques for microscopic examination. The results of the current study showed that the total percentage in dogs amounted to 73.4%. With regard to the rate of infection with Giardia parasite using different techniques of positive samples for microscopic examination, the results showed significant differences. Whereas, the concentration-deposition and flotation technique with zinc sulfate recorded the highest infection rate of 44.5%, followed by the flotation technique–Sodium chloride and flotation technique - Sucrose at 25% and 19.4%, respectively. And the zinc sulfate technique by adding Lugol's iodine recorded the highest infection rate of 43.8%, and the direct technique by adding Lugol's iodine recorded the highest infection rate of 50%. The results showed that the intestinal mucosal scrapings stained with Giemsa stain recorded the highest infection rate for diagnosing Giardia parasite, with a value of 100%. The results showed that there were no significant differences between the age group of less than one year–More than a year. The male infection rate was 65.3%. And the rate of infection with Giardia cysts in dogs whose stools are of solid consistency recorded the highest rate of 68.1%, depending on the number of cysts presented (more than 10 cysts of Giardia means infection). With regard to the months of the year, the results showed an increase in the incidence of infection in the month of October, reaching 31.9%. The average length of the trophozoites was 1.05 ± 13.3 and width 0.31 ± 6.1 . The average length of the cysts was 0.49 ± 9.9 and width 0.33 ± 5.9 . There was no significant difference in the incidence of infection in the areas of both sides of Mosul city.

Keywords---diagnosis, distribution, Giardia parasites, dogs.

Introduction

Giardia: known as a single-celled parasite of anaerobic protozoa, containing many species with similar morphological characteristics. It colonizes and reproduces in the small intestine of many mammals, amphibians, birds and reptiles. It is responsible for giardiasis which causes diarrhea in both humans and other invertebrate (Ali and Israa,2017). The parasite spreads widely in areas that suffer from poor sanitation through stool and water contaminated with cysts of Giardia, so the disease is more endemic in third world countries and developing countries, especially areas that suffer from poor sanitation (Feng *et al.*,2021). . Eight species have been described, namely, Giardia intestinalis , G. lamblia , G. duodenalis , G. ardeae , G. agilis ,G. muris and G. psittaci. Several studies have been conducted to investigate the spread and distribution of Giardia in dogs in Iraq and the world, including a study conducted in Mosul city by (Hadi *et al.*,2014) They were able to diagnose both Giardia and Cryptosporidium parasites in stray dogs and cats, and they confirmed that the rate of infection of cats with Giardia was higher than that of dogs, at a rate of 52%, while in dogs it amounted to 26%.

As for the Arab world and the world, many studies have been conducted, including a study conducted in Egypt by (Ibrahim *et al.*,2016) Confirmed by collecting 395 stool samples from pet dogs and 145 feces samples from dog owners, using microscopy and flotation and sedimentation technique, confirmed that the prevalence rate of infectious parasites was observed in police dogs, by 43.3%, the prevalence of Giardia in dogs was 2.8% . They concluded that dogs have a role in transmitting many intestinal parasites. Gultekin *et al.* (2017) pointed out In a study conducted in Turkey, Eden, on the prevalence and distribution of Giardia and its molecular description, they demonstrated that the highest prevalence of Giardia in dogs less than three months old was 18.8%. The aim of the study was to diagnosis of Giardia parasite based on microscopy using different techniques. and study of the prevalence and distribution of Giardia in Mosul city / Iraq.

Materials and Methods

Study animals

The study was conducted on 98 dogs of different ages, ranging from less than a year–More than a year, of both sexes, included 20 domestic dogs and 78 stray dogs. It was collected from both sides of Mosul city (right and left) for the period from October 2021 to April 2022.

Samples

Samples were collected from dogs and placed in plastic containers with a capacity of 50 milliliters and were immersed in 10% formalin solution to preserve the cysts and trophozoites from decomposition and preserved at 4°C For the purpose of conducting laboratory tests. Intestinal scrapes were collected from the duodenum of dead dogs on glass slides and fixed with alcohol to stain them with gimsa stain for microscopic examination.

The solutions and stains used

Saturated zinc sulfate solution, Saturated sugar solution, Saturated salt solution were used. And the stains, Methylene blue stain, Giemsa stain and Lugol's iodine.

Microscopic techniques

- Direct method
- Concentration-deposition and flotation technique with zinc sulfate
- Flotation technique – Sugar solution
- Flotation technique – Sodium chloride
- Microscopic examination of mucosal scrapings

Result

It was found by examining 98 dogs of different ages and of both sexes, including 78 stray dogs and 20 pet dogs. Stool samples were collected from different areas on both sides of Mosul city for the period from October 2021 to April 2022. The results showed, through the use of different techniques, that dogs were infected with cysts and trophozoites of Giardia. In diagnosing the parasite, it relied on many important formal and standard characteristics, including: The active phase (trophozoite) is characterized by: the front end is wide and the rear is greatly attenuated, bilaterally symmetrical, has two nuclei, the length of the trophozoite is between 10–12 micrometers, 5-7 micrometers wide, relatively flat, containing a large concave sucking disc located on the ventral front side, having four pairs of flagella. The cystic phase is characterized by having two or four nuclei, shrunken cytoplasm, a pair of axons and two medium particles. Image (1, 2, 3, 4).

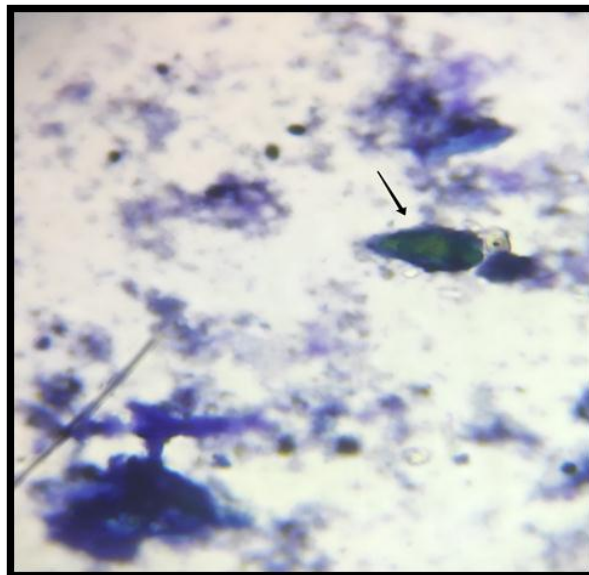


Image 1. Shows the trophozoite of Giardia parasite using Methylene blue stain, light microscope, 400X



Image 2. Shows the trophozoite of Giardia parasite by direct technique (A) nucleus, (B) flagella, (C) ventral disc, (D) basal bodies, light microscope, 400X

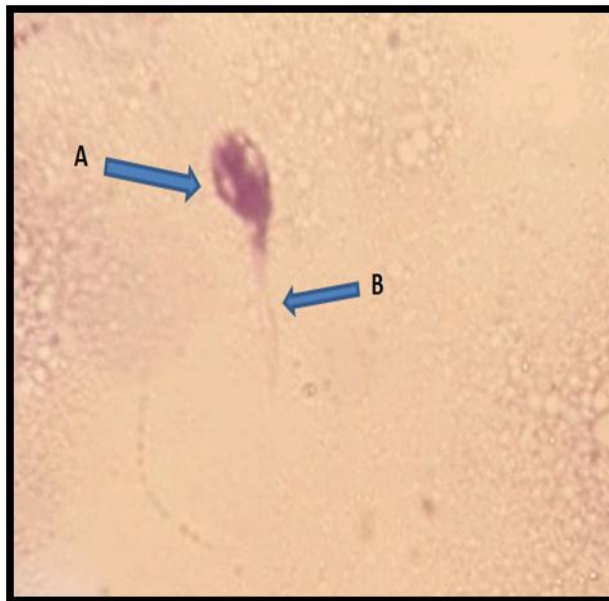
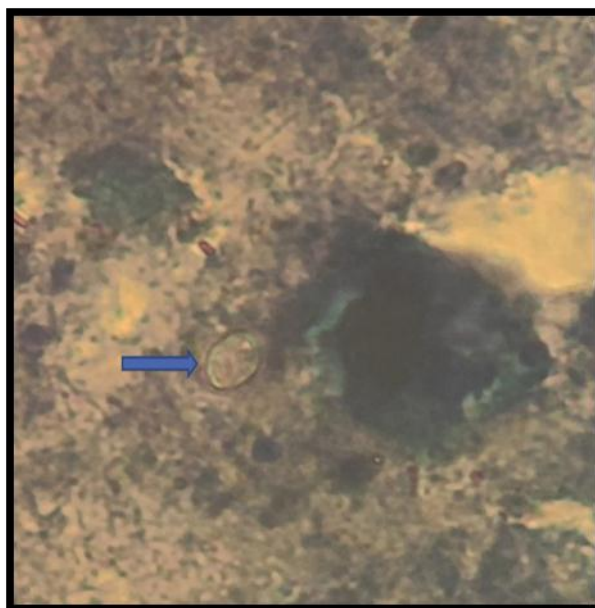


Image 3. Shows the active phase of Giardia parasite with Giemsa stain. (A) nucleus, (B) flagella, light microscope, 400X



Picture 4. Shows a cyst of Giardia parasite using methylene blue stain, light microscope, 400X

Table (1) indicates that there are significant differences in the rate of infection with Giardia parasite using laboratory techniques for microscopic examination. It was clear from the results that the concentration-deposition and straightening technique with zinc sulfate recorded the highest infection rate of 44.5%, followed by the flotation technique–Sodium chloride and flotation technique–sucrose ratio.419% and 25%, respectively, while the lowest percentage of infection with direct swab technique was 11.1%.

Table 1
The rate of infection with Giardia parasite using laboratory microscopic techniques for positive samples

No	Technique/Microscopy	The number of positive samples	%
1	Direct method	8	11.1 a
2	Concentration-deposition and flotation technique with zinc sulfate	32	44.5 b
3	Flotation technique - sugar solution	18	25 a
4	Flotation technique - Sodium chloride	14	19.4 a
	Total	72	

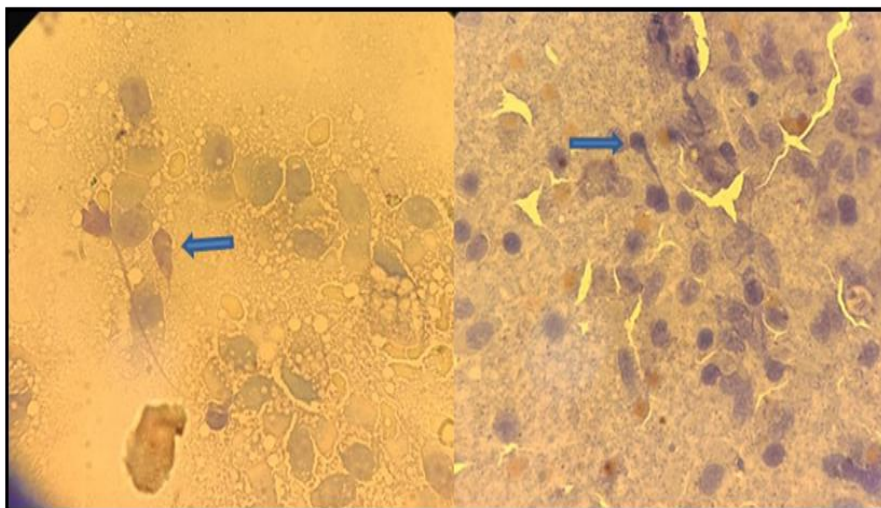
Table (2) indicates the presence of significant differences in the rate of infection with Giardia parasite in dogs using different stains for microscopy technique. The results showed a significant difference using zinc sulfate. The zinc sulfate

technique by adding Lugol's iodine recorded the highest rate of infection and diagnosis of cysts and trophozoites, which amounted to 43.8%. It was followed by zinc sulfate technology by adding the Methylene blue stain with a percentage of 37.5%. While the rate of infection and diagnosis with zinc sulfate technique by adding Giemsa stain reached 18.7%. The results showed that the direct technique with the addition of Lugol's iodine reached the highest rate of infection and diagnosis of 50%, followed by the technique of sodium chloride by adding Lugol's iodine, which also recorded a high rate of infection and diagnosis of 43.7%. With regard to the sucrose solution technique, the highest percentage of infection was reached by the sucrose technique by adding the Methylene blue stain, at a rate of 42.8%.

Table 2
The rate of infection with Giardia parasite in dogs using different stains for microscopy technique

No	Type of stain / microscopy	The total number of positive samples	Number of positive samples	%
1	Direct technique + Lugol's iodine	8	4	50 a
	Direct technique + Giemsa stain		3	37.5 a
	Direct technique + Methylene blue		1	12.5 a
2	Zinc sulfate + Lugol's iodine	32	14	42.8 a
	Zinc sulfate + Giemsa stain		6	21.4 b
	Zinc sulfate + Methylene blue		12	35.7 a
3	Sodium chloride + Lugol's iodine	16	7	43.7 a
	Sodium chloride + Giemsa stain		5	31.2 a
	Sodium chloride + Methylene blue		4	25 a
4	sugar solution + Lugol's iodine	14	5	35.7 a
	sugar solution + Giemsa stain		3	21.4 a
	sugar solution + Methylene blue		6	42.8 a

The results of the examination of intestinal mucosal scrapings stained with Giemsa stain showed the highest diagnosis rate of Giardia trophozoites, which reached 100%, Image (5).



Picture 5. Giardia trophozoites in intestinal scrapings stained with Giemsa stain, light microscope, 1000X

With regard to the age group, the table (3) indicates to the absence of significant differences in the rate of infection with Giardia parasite in dogs.

Table 3

The rate of infection with Giardia parasite in dogs by age group for positive samples by microscopic techniques

No	Age group	The number of positive samples	%
1	less than one year	43	59.7 a
2	more than a year	29	40.3 a
	Total		

While Table (4) indicates that there are significant differences in the rate of infection with Giardia parasite by sex, as the highest infection rate in males reached 65.3%, while the infection rate in females decreased to 34.7%.

Table 4

The rate of infection with Giardia parasite by sex for positive samples by microscopic techniques

sex	The number of positive samples	%
male	47	65.3 b
female	25	34.7 a
Total	72	

The results showed that there were significant differences in the rate of infection with cysts of the parasite Giardia in dogs whose stools were of solid consistency.

The highest rate of infection and diagnosis of Giardia parasite was recorded at 68.1%, Table (5)

Table 5
The rate of infection with cysts of Giardia in the stool of positive dogs by microscopic techniques

Stool texture	The number of positive samples	%
Soft stool texture	23	31.9 a
Hard stool texture	49	68.1 b
Total	72	

Figure (1) indicates that there are significant differences in the rate of infection with the Giardia parasite according to the months of the year. It was clear from the results that the highest percentage of infection with the Giardia parasite appeared in December, with an infection rate of 31.9%, followed by the infection rate in November and January, with an infection rate of 23.6% and 15.4 %, respectively, while the lowest rate of infection with Giardia parasite reached in the month of March, at a rate of 6.9%.

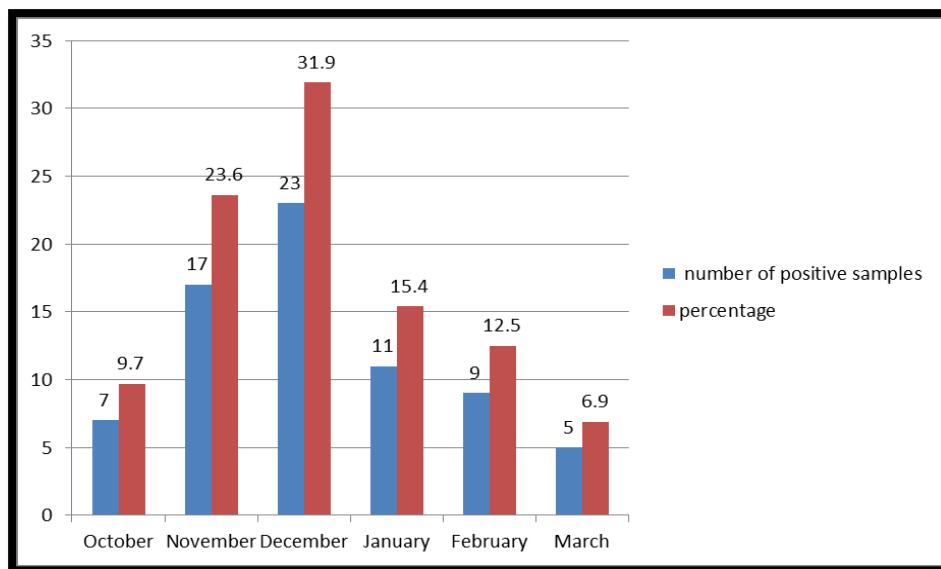


Figure 1. The rate of infection with Giardia parasite according to the months of the year for positive samples by microscopy

Table (6) shows the dimensions of the trophozoites and cysts in samples of dogs infected with the Giardia parasite. The average length of the trophozoite was $1.05 \pm 13.3 \mu\text{m}$ and a width of 0.33 ± 6.1 micrometers, and the average length of the bags reached 0.49 ± 9.9 micrometers and a width of $0.33 \pm 5.9 \mu\text{m}$.

Table 6

Dimensions of trophozoites and cysts in samples of dogs infected with Giardia parasite

Cysts width(μm)	Cysts length(μm)	Trophozoites width (μm)	Trophozoites length (μm)
5.9 \pm 0.33	9.9 \pm 0.49	6.1 \pm 0.31	13.3 \pm 1.05

The results showed that there were no significant differences in the rate of infection with Giardia parasite in dogs in the areas of both sides of the city of Mosul. Table (7).

Table 7

The rate of infection with Giardia parasite in dogs in the areas of both sides of Mosul city for positive samples by microscopy

Both sides of Mosul City	The number of infected dogs	%
The left side	41	56.9 a
the right side	31	43.1 a
Total	72	

Discussion

Giardia parasite is a unicellular anaerobic protozoan. Parasitizes the upper digestive tract, especially the duodenum, in mammals, amphibians, reptiles, and birds. The results of the current study showed that 72 dogs out of a total of 98 dogs were infected with a total infection rate of 73.4%. The results of the current study agreed with what was recorded by (Uiterwijk *et al.*, 2018) They recorded a close infection rate of 73.3% by diagnosing Giardia in four groups of dogs, which are domestic dogs, hunting dogs, shelter dogs and clinical dogs. While the current study relied on two types of dogs, they are stray and pet dogs. The results of the examination of different laboratory techniques for microscopic examination showed that the centrifugal concentration-deposition and flotation technique with zinc sulfate showed the highest diagnosis rate for Giardia cysts and trophozoites, which amounted to 44.4%, and this technique was adopted as a gold standard for other techniques, including direct wet technique and flotation technique–sodium chloride and flotation technique –sucrose . The results of the current study are in agreement with the findings of (Ibrahim *et al.*,2016) Through a study they conducted on three types of dogs, which are police, domestic and (pet) dogs, they confirmed that police dogs recorded the highest rate of infection with Giardia parasite using centrifugal technology–concentration-sedimentation and flotation with zinc sulfate.

The results of the study also agreed with what was reported by (Hassan,2021) as he was able to diagnose the cysts and trophozoites of Giardia parasite by centrifugal technique–Sedimentation and flotation. The reason is that the direct wet technique and the flotation technique– sodium chloride and sucrose flotation technique give false negative results due to the collapse and shrinkage of the parasite cysts and trophozoites by flotation and also decomposition by flotation

with sodium chloride, while the centrifugal technique of concentration-sedimentation and flotation is more sensitive because it maintains the survival of parasite cysts and trophozoites for a long time.

The results of the current study showed that by using the centrifugal-concentration-sedimentation and flotation technique and by adding coloring stains such as Methylene blue 1% and Lugol's iodine to the wet faecal smears, the highest diagnosis rate for Giardia parasite was recorded, 42.8% and 35.7% for both Lugol's iodine and Methylene blue, while the diagnosis rate reached by adding Giemsa stain to the wet faecal smears were 21.4%, meaning that there was a significant difference between the addition of Lugol's iodine and Methylene blue stain and Giemsa stain. The results of the current study are in agreement with the findings of (Rajurkar *et al.*, 2012) They confirmed that the addition of Lugol's iodine and Methylene blue stain to the wet faecal smears gave positive results. while (Hadi *et al.*, 2014) explaining that the addition of Giemsa stain to the fecal smears of Giardia parasite in both stray dogs and cats gave positive results and diagnostic rates of 30.76% and 18.75%, respectively. While the results of the current study did not show any significant difference using Lugol's iodine, Methylene blue stain and Giemsa stain in both the direct technique, the flotation technique - sodium chloride and the flotation technique- sucrose. The results of the current study showed that when adding Lugol's iodine, Methylene blue and Giemsa stain to intestinal mucosal scrapings, the highest diagnosis rate of giardia cysts and trophozoites was recorded, which amounted to 100%. The results of the current study agreed with what was recorded (Hadi *et al.*, 2014) who confirmed the diagnosis of trophozoites of the parasite Giardia by adding Giemsa stain to the intestinal scrapings.

With regard to age groups, the results of the current study showed that there was no significant difference between age groups less than one year–More than a year for dogs infected with Giardia parasite. The current study agreed with what was reported (Bouزيد *et al.*,2015) They confirmed that all age groups are infected with cysts of Giardia, while a study previously reported by (Naser,2017) The young age group is more susceptible to giardiasis than the older age group (more than a year–two years). The reason for the infection of young puppies may be due to the incompleteness of their immune system, in addition to the malnutrition of the puppies, especially after weaning from their mothers, which makes them more susceptible to infection with the parasite from old ages. The results of the current study did not agree with the study of (Bianciardi *et al.*, 2004) They confirmed the prevalence of infection in dogs of the age group of two years to more than three years, and indicated that the rate of infection with Giardia parasite reached 33.07% in the three-year-old groups and reached 7.4% in puppies.

The results of the current study showed that male dogs recorded the highest rate of infection of Giardia parasite, which amounted to 65.3%, while females recorded a lower rate, which reached 34.7%. The results of the current study are in agreement with the findings of (Swadi and Zenad,2019) They showed that males are more susceptible to infection with Giardia parasite than females, with a rate of 10.86%. With regard to the consistency of dog stool, the results of the current study showed that the percentage of infection with Giardia cysts from samples positive for microscopic examination was variable, as the percentage of the

presence of cysts of the parasite reached 68.1% for the consistency of hard stool, which is a high percentage compared with the percentage of the presence of cysts in the consistency of soft stool 31.9% . While a study presented by (Uiterwijk *et al.*, 2018) confirmed that the cysts of Giardia parasite in soft stool appeared in a high rate of presence in hunting dogs compared with domestic and clinical dogs.

The results of the current study showed the prevalence and rate of infection of the Giardia parasite in the month of December and the emergence of a significant difference with the rest of the months, as the infection rate increased in the month of December to 31.9%, while the infection decreased in the month of March to 6.9%. The reason may be due to changing weather conditions and lack of care and attention to stray dogs, which leads to their exposure to various types of intestinal parasites, including Giardia. The results of the current study agreed with what was reported (Swadi and Zenad,2019) They confirmed that the infection rate increased in October to 9%, which is a percentage close to what our results reached, as it was recorded at 9.7%, and it also agreed with (Bianciardi *et al.*, 2004) They confirmed the increase of infection in winter and its decline in spring and summer. The results showed that there was no significant difference in the rate of infection of Giardia on both sides of Mosul city.

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