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## First microscopic detection of *Cystoisospora* protozoan infecting dogs in Babylon province

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### Abstract

*Cystoisospora* species are protozoa parasites belonging to the phylum of Apicomplexa that live in the small intestine of hosts, such as dogs. *Cystoisospora* infection in dogs has not been investigated in Babylon province. Therefore, this study aimed to diagnose the disease by this protozoan macroscopically and microscopically in stray and domestic dogs using a direct smear, flotation methods based on Sheathers solution, and staining by modified Ziehl Neelsen stain. In the current study, a total of 150 fecal samples of dogs (different in sex and age) were collected from other areas of Babylon City during the period from December 2024 to February 2025. The results of macroscopic observation showed that the infected dogs exhibited various clinical symptoms, including dehydration, diarrhea, fever, weight loss, vomiting, and even death in extreme cases. These observations also noticed that watery diarrhea of positive samples 15/40 (37.5) recorded the highest rate among other consistency of fecal samples with significant differences ( $p \leq 0.05$ ). In addition, the microscopic results demonstrated that 26 out of 150 dogs (17.33%) were microscopically infected in both stray and domestic dogs. According to sex, no significant difference ( $p = 0.688$ ) was found in the infection, where it was 18.60% (16 out of 86) in males, while 15.62% (10 out of 64) in females. For the age groups, there was a significant difference ( $p \leq 0.01$ ), which has a high incidence of illness in canines less than 6 months 15/45 (33.33%) compared to other age groups. Current data also revealed that the infection rate in rural areas was slightly lower than in urban areas, where it recorded 11/75 (14.66%) and 15/75 (20%), respectively, without significant differences ( $P = 0.469$ ). Overall, these findings provide baseline data and valuable information to the owners and the veterinary community regarding this canine coccidian's presence and risk factors. It is suggested that this is the first study recording the infection of *Cystoisospora* in dogs living in Babylon province. Further molecular investigation has been carried out to confirm the presence of this protozoan and its species in dogs.

**Keywords:** *Cystoisospora* species, dogs, microscopic diagnosis

### Introduction

The protozoan parasites belonging to *Cystoisospora* (*Isoospora*) enter their hosts' intestines regularly. Worldwide, canids, felids, pigs, and people host *Cystoisospora* species, which can cause diarrhea (Frenkel, 1977; Lindsay *et al.*, 2011) <sup>[1, 2]</sup>. While adult dogs typically show no symptoms of cystoisosporiasis, the condition is more common in puppies because they are more likely to experience clinical signs. Damage to the small intestinal epithelium by parasite invasion and reproduction within enterocytes causes changes such as the loss of the brush border, digestive enzymes linked to cell membranes, and blunting and atrophy of the intestinal villous (Giangaspero *et al.*, 2019; Sun *et al.*, 1996) <sup>[3, 4]</sup>. These changes decrease the small intestine's absorptive capacity, resulting in reduced uptake of water, nutrients, and electrolytes (Giangaspero *et al.*, 2019) <sup>[3]</sup>. Diarrhea, ranging from watery to melena, vomiting, and tenesmus are all signs of protozoan infection. Another possible cause of death is severe dehydration (Garanayak *et al.*, 2017) <sup>[5]</sup>. The infection is particularly harmful to the health of pups about four months of age when their immune systems are still developing, and they do not inherit immunity to coccidia from their mothers. As a result, it is crucial to diagnose, treat, and prevent this infection (Unal and Gokpinar, 2020) <sup>[6]</sup>.

Among the many protozoan parasite genera found in the phylum Apicomplexa are the coccidia *Cystoisospora canis* and *Cystoisospora ohioensis*-complex, which includes *C. neorivolta*, *C. burrowsi*, and *C. ohioensis*. These species are known as monoxenous because of their solitary host requirement and global distribution (Dubey and Lindsay, 2019) <sup>[7]</sup>. The genus *Isoospora* is complex due to variations in oocyst morphology, host specificity, and intermediate stages (Barta *et al.*, 2005; Raza *et al.*, 2018) <sup>[9]</sup>.

A sporulated *Isoospora*-type oocyst contains two sporocysts with four sporozoites inside each sporocyst (Saari *et al.*, 2018) [8]. The sporulated oocysts resist common disinfectants and can live for months in nature, but cresols can inactivate coccidian oocysts (Deplazes *et al.*, 2016) [12]. Transmission is usually fecal-oral, and dogs become infected when they consume infective oocysts in the environment. It has also been found that paratenic hosts like rodents can become infected by ingesting oocysts, and the parasite will leave the gut and create monozyotic tissue cysts (MZT) in tissues like the lymph nodes, spleen and liver (Dubey, 1975; Mitchell *et al.*, 2009) [7, 11]. The oocysts released into the environment by infected dogs increase the likelihood of the disease spreading to additional canines (Unal and Gokpinar, 2020) [6]. According to Ilić *et al.* (2021) [13], dogs housed in kennels are also at risk of infection because of the lack of cleanliness in that setting.

The infection by *Cystoisospora* in dogs can be identified using a case history, clinical symptoms, and microscopic investigation using the direct smear and flotation technique with Sheather's solution. According to the literature illustrated by (Marhoon and Al-maeahi 2020) [15], the occurrence of *Cystoisospora* in dogs was reported in many countries, such as 22.0% in Ukraine (Suvorov and Melnychuk, 2023) [16], 13.4% in Morocco (Houda Idrissi *et al.*, 2022) [33], 7.6% in Korea (You-Jeong Lee *et al.*, 2024) [17]. In addition, studies conducted in Basrah, Iraq, where *Cystoisospora* was detected in cats and dogs (both domestic and stray) of various ages and sexes, where it was in dogs 9.5% (Entedhar. K. Jassim *et al.*, 2024) [18]. Also, in Baghdad, a study revealed the infection rate of *Cystoisospora spp* was 14.17% (Khalaf *et al.*, 2015) [26]. In the Northern part of Iraq, a study was conducted on the infection rate of *Cystoisospora spp*, which was 21.51% (Wahab, 2022) [25]. According to currently available data, the infection by *Cystoisospora* species in dogs in Babylon province was not previously studied. Thus, the current study aims to detect *Cystoisospora spp* in dogs microscopically and understand its prevalence in this city.

## Material and Methods

### Collection of fecal samples

150 fecal samples (75 strays, 75 domestic) are collected from dogs of different sexes and ages from various areas in Babylon province between October 2024 and January 2025. Each sample was placed in a separate plastic container with a lid. The consistency of collected fecal samples was macroscopically examined and recorded. These samples were then transported in a cool box to the Parasitology Laboratory at the College of Veterinary Medicine at Al-Qasim Green University for the microscopic examination using the traditional diagnosis methods (direct smear, flotation, and staining).

### Laboratory examination of fecal samples

We promptly collected at least 5 g of excrement, put it in a plastic container, and kept it at 4°C. During feces collection, some animals exhibited clinical indications such as diarrhea. After collecting the samples, they were analyzed under a microscope using a direct smear. To facilitate sporulation and identification, the samples were placed in an incubator at 38 degrees Celsius with 2.5% potassium dichromate (Alhayali and Al-Amery. 2019 [20]; Al-Amery and Al-Amery, 2020) [19]. Lastly, the samples were analyzed using

the sheather sugar flotation method to identify the oocysts, and they were stored at 4°C for subsequent examination.

### Direct smear

A drop of the fecal solution diluted in water was taken, a cover slide was placed on it, and it was examined directly under the microscope, using microscopic lenses (40X), gradually adjusted from the lowest power to the highest (Ljungström *et al.*, 2018) [21].

### Flotation method.

According to (Ljungström *et al.*, 2018) [21], the feces was taken, diluted with water, and placed in a test tube at a rate of a quarter of the total tube volume. Then, the tube is filled with the sugar flotation solution until the liquid reaches the highest point in the tube and becomes convex, and then a cover slide is placed on the orifice. The convex shape is necessary for the stick oocysts to the cover slide after floating. After 30 minutes, the cover slide was removed, placed directly on the slide, and examined under a microscope.

### Staining by modified Ziehl Nielsen stain.

The Modified Ziehl-Neelsen staining method is widely used for detecting acid-fast organisms, including parasites like *Cryptosporidium* and *Isoospora* (Garcia, 2016) [22]. The process was started by placing one or two drops of the specimen onto a glass slide, and the slide was allowed to air dry. Care was taken to ensure the smear was not too thick so the wet material could still be seen before it dried completely. Two smears were prepared to ensure reliable results. After drying, the slide was placed on a heating block at 70°C for five minutes to fix the specimen correctly. Once the slide was dry, it was placed on a staining rack and flooded with carbon fuchsin stain. Using an alcohol lamp or Bunsen burner, the slide was gently heated until the stain began to steam, ensuring it was not allowed to boil. Heating was stopped as soon as steaming started, and the stain was left to work on the specimen for five minutes. If the slide dried during this time, more stain was added without further heating. After the staining period, the slide was rinsed thoroughly with water to remove excess stain and then drained. Next, the slide was decolorized using 1-3% sulfuric acid for 30 seconds, with the understanding that thicker smears might require a longer decolorization time. The slide was then rinsed again with water and drained. In the next step, the slide was flooded with methylene blue stain for one minute to counterstain the background. After the minute passed, the slide was rinsed with water, drained, and left to air dry. Finally, the slide was examined under the microscope using the low or medium power objectives. For a more detailed observation of the internal morphology, the oil immersion objective (100×) was used (Garcia, 2016) [22].

### Statistical analysis

Statistical analysis of the current study was carried out using the chi-square test. The value of  $p \leq 0.05$  was considered to be statistically significant.

## Results

### Macroscopic examination

During the collection of fecal samples, consistency was recorded for each sample. According to consistency, the observation revealed that the highest infection rate of

*Cystoisospora* in dogs was in cases with watery diarrhea 15/40 (37.5%) compared with other consistency features of fecal samples (Steatorrhea 2/15 (13.33%). Solid feces 1/60 (1.66%), and semisolid feces 8/35(22, 85%) noticed notable variations among them at a significance level of ( $p \leq 0.05$ ), as displayed in Table 1.

**Table 1:** Infection rate of *Cystoisospora spp.* in dogs according to stool sample consistency

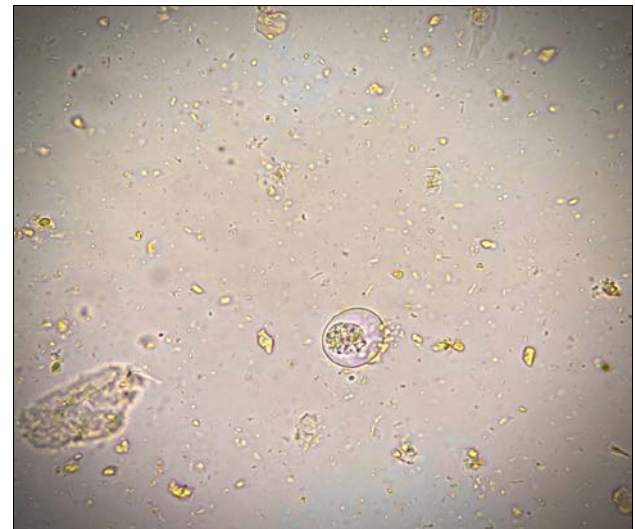
Consistency	Examined samples	Infected samples	Infection Rate%
Steatorrhea	15	2	13.33%
Watery diarrhea	40	15	37.5%
Solid feces	60	1	1.66%
Semisolid feces	35	8	22.85%
Total	150	26	17.33%
Chi-square ( $\chi^2$ )	15.806		
p-value	0.001**		

\*\* refer to significant difference at ( $p \leq 0.05$ ).

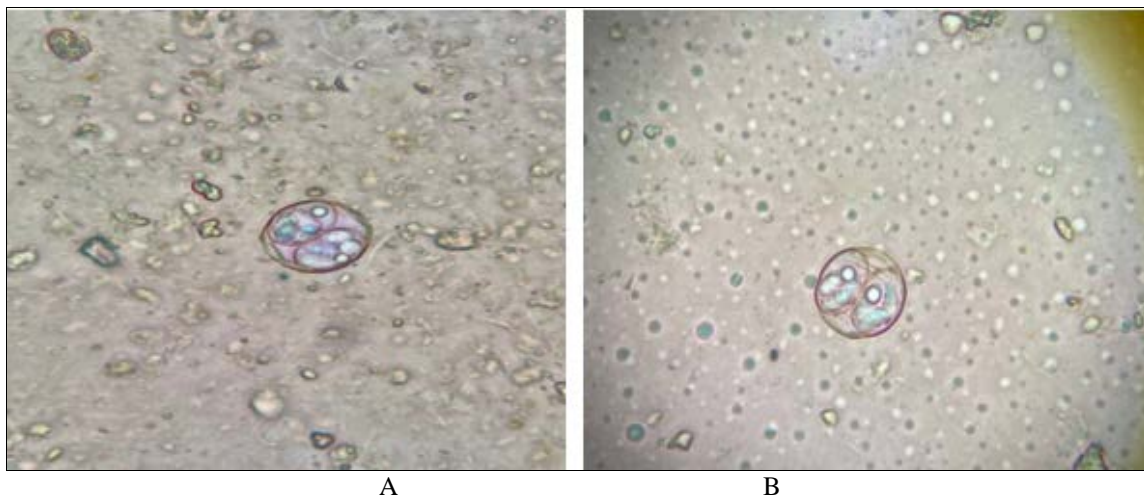
**Microscopic examination**

The microscopic examination of fecal samples revealed that 26 out of 150 (17.33%) dogs were positive for *Cystoisospora* species. The results of fecal examination using direct smear and floating method showed an

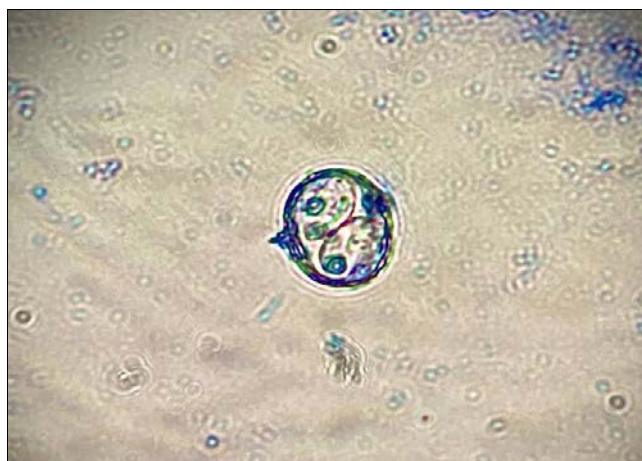
unsporulated oocyst (Figure 1) and a completely mature (sporulated) oocyst of the parasite, which consists of a slightly oval-shaped body with two sporocysts, each of which contains four sporozoites (Figure 2) (Figure 3).



**Fig 1:** Unsporulated oocyst of *Cystoisospora*. isolated from the infected dog by floatation method (40X).



**Fig 2:** A. Sporulated oocyst to *Isospora spp.* Isolated from the infected dog by direct smear (40X). B. Sporulated oocyst to *Isospora spp.* Isolated from infected dog by floatation Method (40X).



**Fig 3:** Sporulated oocyst to *Isospora spp.* isolated from an infected dog, stained by a modified Ziehl Nielsen stain

Dogs' infection rates varied by sex and were 16/86 (18.60%) in males and 10/64 (15.62%) in females without significant differences, as shown in (Table 2).

**Table 2:** infection rate of *Cystoisospora spp.* in dogs according to the sex of dogs

Sex	Examined sample	Infected samples	Infection rate
Male	86	16	18.60%
Female	64	10	15.62%
Total	150	26	17.33%
Chi-square ( $\chi^2$ )	0.161		
p-value	0.688 (N.S)		

**N.S: non- significant:** Regarding age groups, current data revealed that young puppies in age group  $\leq 6$  months exhibited the highest infection rate (33.33%) 15/45 with significant differences ( $p \leq 0.01$ ) as shown in (Table 3.).

**Table 3:** Infection rate of *Cystoisospora spp.* in dog according to age groups

Age	Examined sample	Infected samples	Infection rate
Less than 6 months	45	15	33.33%
6-12 months	60	8	13.33%
1-2 years	20	2	10%
More than 2 year	25	1	4%
Total	150	26	17.33%
Chi-square ( $\chi^2$ )	8.503		
p-value	0.037*		

\* refer to significant difference at ( $p \leq 0.01$ ).

Although there were no statistically significant differences between the study's rural and urban regions, (Table 4) shows that the infection rate was no significant differences, where the higher infection rate was in the urban area 15/75 (20%) compared to the rural area 11/75 (14.66%)

**Table 4:** Infection rate of *Cystoisospora spp.* in dog according to the area of study

Areas	Examined sample	Infected samples	Infection rate
Urban	75	15	20%
Rural	75	11	14.66%
Total	150	26	17.33%
Chi-square ( $\chi^2$ )	8.503		
p-value	0.469 (N.S)		

## Discussion

Although the prevalence of *Cystoisospora* species in Iraq tends to be minor or absent in some parts of Iraq, the current study recorded it in stray and domestic dogs living in Babylon province. The initial investigation of fecal samples revealed that most positive cases were shown in dogs suffering from watery diarrhea. According to a previous study (Mitchell *et al.*, 2007) [32], found that the stage of the structural and substructural survey of the parasites that infect human *Cystoisospora* when immunity is low, as well as those that infect dogs, *Cystoisospora* that they have similar to the extra-intestinal stage that causes watery diarrhea and weight loss. This explanation might agree with the current observations which indicated that the highest infection rates were infected dogs with watery diarrhea. Furthermore, the macroscopic observations were in consistent with the results of a study in morocco which recorded the higher infection rate of *Cystoisospora spp* in watery diarrhea samples (Idrissi *et al.*, 2022) [33]. This is further supported by Houk and Lindsay *et al.* (2013) [36], who confirmed the pathogenic nature of *Cystoisospora spp.* Regarding the microscopic examination, the infection rate of *Cystoisospora* species was recorded in dogs with a percentage (17.33%). This rate could be considered a significant observation in dogs in terms of recording it for the first time in this area, giving future warning about its prevalence in the country. Previously, a study in Basra, Iraq conducted the infection rate of *Cystoisospora spp* in dog by microscopic examination as indicated 9.5% (Jassim *et al.*, 2024) [18]. Another study done by (Thamer *et al.*, 2022) [24] in Basrah revealed the infection rate of *Cystoisospora spp* was 11.7%. In Baghdad, Iraq the prevalence of *Isospora spp.* was (14.17%) in dogs (Khalaf *et al.*, 2015) [26]. these local studies recorded less infection rates compared with the current findings. While, a recent study in the northern part

of Iraq investigating different gastrointestinal parasites in 269 stray dogs found that the infection rate of *Cystoisospora spp* was (21.51%) (Wahab, 2022) [25], this rate was closely related and in agreement with the results of this study. The reason for the difference infection rates of *Cystoisospora spp.* in dogs might be ascribed into the study areas, study sessions, numbers of examined animals, and techniques. In addition, the availability of a favorable environment for oocysts to persist for extended durations (Arsalan *et al.*, 2006) [27].

In other countries, the current finding was in line with research conducted in Iran (Bahrami *et al.*, 2011) [28] and Spain (Ortuño and Castellà, 2011) [29], which also discovered that 15.17% and 16.4% of dogs, respectively, had the prevalence of *Isospora spp.* However, that ran counter to what Coggins (1998) [30] found in the United States, where 5.2% of dogs tested positive for isospora infection. His disagrees with the result of this study because the focus of our research revolved around one type of endoparasite that infected dog's environmental differences their adaptation to the cold climates and constant humidity found in those countries. The lack of veterinary supervision and some merchants exploit cats commercially without providing means of controlling them, such as vaccines and treatments, the lack of veterinary supervision and some merchants exploit cats commercially without providing them, such as vaccines and treatments. In Nigeria, a study showed that 21% (n = 44) out of 203 of the fecal samples were positive for *Cystoisospora* oocysts, which agreed with the results of the present study (Eze *et al.*, 2019) [37].

Regarding the age groups, this study revealed the higher infection rate of this study according to age groups was in the young dog under six month. This agrees with a study In serbia who found The parasites detected microscopically by flotation test Twenty percent in puppies younger than one year old and 4.9% in dogs older than one year old were found to be *Cystoisospora spp* (Ilić *et al.*, 2021) [13]. In addition, a study in Ukraine found that Infants and young children less than six months old were the most impacted (32.0%) (Suvorov and Melnychuk, 2023) [16], which agreed with the current results.

The results of infection rates based on the area of study showed that the infection rate was high in urban areas compared with rural areas. In the northwest area of Mexico City, The study by Trasviña-Muñoz *et al.* (2017) [35] aimed to determine the prevalence and distribution of intestinal parasites in stray dogs across urban and rural areas of Mexicali County in northwest Mexico. Over one year, 380 stray dogs were examined, the infection rate of *Cystoisospora spp* in urban areas was higher (6.5%) than rural areas (0%).this agrees with this study's results as the infection rate in urban areas was higher than in rural areas.

The study was conducted In Prague City, Czech. Dubná *et al.* (2007) [34] investigated the prevalence of intestinal parasites in dogs from urban, rural, and shelter environments in the Czech Republic. The infection rate of *Cystoisospora spp* in rural areas (8.0%) is higher than in urban areas (2.4%). This result disagrees with the results of this study, which showed that urban areas were higher. I disagree with the study by Dubná *et al.* (2007) [34] because dogs in rural areas of Babil Province tend to have less contact with each other compared to those in densely populated urban areas. In rural Babil, dogs seem to live in relative isolation due to the greater distance between homes

and the vast spaces separating them. Additionally, household dogs are usually kept in designated areas outside the house, which reduces direct contact with other dogs. In contrast, urban areas have a higher number of stray dogs, increasing the chances of parasite transmission through direct contact or exposure to shared sources of infection, such as contaminated food or water. These environmental and social factors may lead to different patterns of parasite prevalence than those observed in the Czech Republic, emphasizing the need for localized studies.

### Conclusion

The current study highlighted the detection and importance of *Cystoisospora* spp infections among dogs in Babylon province. The total infection rate was 17.33% of *Cystoisospora* species, where watery diarrhea, dehydration, vomiting, and even death in some cases were the main clinical symptom. The results showed that males had the highest contamination rate compared to females. In addition, infected dogs under six months showed a high infection rate among other age groups. It was also found that the infection was higher in urban areas than rural; this is probably due to the increase in the number of stray dogs in urban areas more than in rural areas and little interest in combating stray dogs. Thus, there is no doubt that the current study added significant knowledge about Babylon City as a hotspot for this protozoan. Further molecular research would be helpful in confirming and understanding the epidemiology of this parasite, whether in Babylon province or other cities in Iraq.

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### Competing Interests

The authors declare that they have no competing interests.

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