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## Conventional Assessment of Intestinal Protozoa in Dogs from Mosul City

### Article Info.

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### Abstract

Intestinal protozoa are among the most important pathogens affecting dogs. Therefore, the present study was conducted to investigate the occurrence of intestinal protozoan infections in dogs through the examination of fecal samples. A total of 100 fecal samples were collected from dogs of different ages, sexes, health statuses, and management systems. Each sample was examined using several diagnostic approaches, including direct wet smear, flotation (concentration) technique, and culture methods. In addition, various staining techniques were employed to facilitate protozoan identification under a light microscope. The findings revealed that 59 samples (59%) were positive when all diagnostic methods were considered. The detection rates varied according to the diagnostic technique used, with positivity rates of 41% for the direct smear, 55% for the concentration method, and 59% for the culture method. Regarding staining procedures, differences in detection efficiency were also observed, with a positivity rate of 59% using Lugol's iodine stain, 56% using the modified acid-fast staining technique, and 39% by using Iron-Hematoxylin Staining technique. Out of the 100 examined fecal samples, 59 (59%) were infected with at least one intestinal protozoan species. Three protozoan species were identified: *Cystoisospora* spp. (42.37%), *Cryptosporidium* spp. (33.89%), whereas *Giardia* spp. (23.72%). A markedly higher infection rate was observed among stray dogs (83.72%). Females exhibited a higher prevalence of infection (70.68%) than males. Younger dogs were more frequently infected (76.31%) than older ones. Furthermore, dogs with diarrheic conditions demonstrated a higher prevalence of infection (73.91%) compared with clinically healthy ones, which showed a relatively lower infection rate.

**Key words:** intestinal protozoa, fecal, dogs, cyst, oocyst, , stain.

## Introduction

Intestinal protozoa are common parasitic organisms that infect dogs worldwide and constitute a significant concern in veterinary medicine due to their impact on animal health and their potential zoonotic importance. Dogs are susceptible to several species of intestinal protozoa, the most important of which include *Giardia spp.*, *Cystoisospora spp.*, and *Cryptosporidium spp.* The prevalence of these parasites varies according to age, environmental conditions, hygiene level, and management practices, with higher infection rates commonly observed in puppies, stray dogs, and animals housed in crowded conditions. (1)

Transmission of intestinal protozoa in dogs occurs mainly via the fecal–oral route through ingestion of infective cysts or oocysts present in contaminated food, water, soil, or fomites. Direct contact with infected animals also plays an important role in the spread of infection, particularly in kennels and shelters. Poor sanitation and environmental contamination contribute significantly to the persistence and dissemination of these parasites. (2)

Clinical signs associated with intestinal protozoan infections range from subclinical and asymptomatic cases to severe gastrointestinal disturbances. Infected dogs may exhibit diarrhea (acute or chronic), dehydration, weight loss, anorexia, abdominal discomfort, and poor growth, especially in young or immunocompromised animals. The pathogenic effects of these protozoa are primarily due to damage to the intestinal epithelium, malabsorption, inflammation, and disruption of normal gut function (3).

Diagnosis of intestinal protozoa in dogs is based on fecal examination using direct smears and concentration techniques for the detection of cysts and oocysts. Additional diagnostic methods include special staining techniques, immunological assays such as ELISA and rapid antigen tests, and molecular methods like polymerase chain reaction (PCR), which provide higher sensitivity and specificity and allow species and genotype identification. (4, 5)

## Material and Methods

**Animals:** The animals included 100 dogs, which differed in age, sex, type of husbandry and health status.

### Sample collection

A total of 100 fecal samples were collected in sterile plastic containers, properly labeled, and transported to the Parasitology Laboratory, Department of Microbiology, College of Veterinary Medicine, University of Mosul. All samples were stored at 4 °C until examination.

### Direct Smear Method

A direct wet smear was prepared by collecting a small amount of fecal material using a sterile applicator and thoroughly emulsifying it on a clean glass slide. A drop of Lugol's iodine solution

was then added to enhance the visualization of internal structures. The preparation was covered with a coverslip and examined under a light microscope for the detection of protozoan stages, following the procedure described by (6).

### **Concentration Method**

For the concentration method, approximately 2 g of fecal sample was homogenized with 20 mL of saturated sodium chloride (NaCl) solution to obtain a uniform suspension. The mixture was subsequently filtered through two layers of cotton gauze with a mesh size of 250  $\mu\text{m}$  and collected in a beaker. The filtrate was transferred into a 15 mL conical Falcon tube and centrifuged at  $600 \times g$  for 5 minutes. After centrifugation, additional flotation solution was gently added to form a convex meniscus, and a coverslip was placed on top. After allowing it to stand for 3–5 minutes, the coverslip was carefully removed, mounted on a glass slide, and examined microscopically as described in (7).

### **Culturing Method**

For *in vitro* cultivation, a pea-sized portion of each fecal sample was inoculated into sterile plastic culture tubes containing 3 mL of Jones medium supplemented with 10% heat-inactivated horse serum. The cultures were incubated in an upright position at 37 °C for a period of 48–72 hours before examination. Protozoan growth was assessed by examining a drop of the sediment under a light microscope at 100 $\times$  magnification. Positive cultures were maintained by subculturing every 3–4 days to preserve parasite viability. In cases where no growth was observed, the sediment was re-suspended in fresh culture medium and re-incubated at 37 °C for an additional 48 hours. The presence of characteristic vacuolar forms in the medium was considered indicative of a positive result (8).

### **Acid-Fast Staining Technique**

Following completion of the concentration procedure, smears were prepared from the sediment obtained from each sample and allowed to air-dry at room temperature. The slides were then fixed with methanol for approximately five minutes. Subsequently, a modified acid-fast staining method was applied to stain the prepared smears. Finally, all slides were examined under a light microscope for the detection of acid-fast protozoan stages (9,10).

### **Iron-Hematoxylin Staining Technique**

A thin smear was prepared from the fecal sediment preserved in diluted 10% formalin. The glass slide was allowed to air-dry, then passed through a series of ethyl alcohol concentrations (70%, 90%, and 100%) for 2 minutes at each step. The slide was subsequently immersed in iron-hematoxylin solution for 8–10 minutes, rinsed with running water, and differentiated in ferric ammonium sulfate for 5–10 seconds. After thorough washing with running water, the slide was

dehydrated again through ethyl alcohol at concentrations of 90% and 100% for 2 minutes. Finally, the alcohol was removed by placing the slide in xylene for 2 minutes (11).

### Statistical Analysis

The collected data were analyzed using IBM SPSS software (version 22). Variations in protozoan infection prevalence and their relationships with factors such as breeding conditions, sex, age, animal origin, and health status were examined using two-tailed Chi-square and Fisher's exact tests. Differences were considered statistically significant at  $P \leq 0.05$ .

### Results

Overall, 59 samples (59%) were found to be positive when all diagnostic techniques were considered. The detection rates varied according to the method used, reaching 41% with the direct smear, 55% with the concentration technique, and the highest rate (59%) with the culturing method (Table 1).

**Table 1. Total of infection rates in dogs infected with intestinal protozoa according to different diagnostic methods**

| N0 of samples | Direct smear method     | concentration method    | Culturing method        |
|---------------|-------------------------|-------------------------|-------------------------|
|               | No. of positive samples | No. of positive samples | No. of positive samples |
| 100           | 41(41%)                 | 55(55%)                 | 59(59%)                 |

The stains which can be used to detect the protozoa appear with different positivity rate 59% using Lugol's iodine stain, and 56% using ast Staining Method, and 39% using Iron-Hematoxylin Staining Technique (Table 2).

**Table 2. Total of infection rates in dogs infected with intestinal protozoa according to different strains.**

| No of samples | Lugol's iodine stain | Acid Fast stain | Iron-Hematoxylin Stain |
|---------------|----------------------|-----------------|------------------------|
| 100           | 59<br>(59%)          | 56<br>(56%)     | 39<br>(39%)            |

Out of the 100 fecal samples examined, 59 (59%) were positive for infection with one or more parasitic species. Three protozoan parasites were identified. *Cystoisospora* spp. showed the highest prevalence (42.3%) with an average size of oocyst measuring  $29 \times 38 \mu\text{m}$ .

This was followed by *Cryptosporidium* spp. prevalence of 33.89% with, while *Giardia* spp. was recorded at the lowest rate (23.72%). The morphological characteristics and distribution of these protozoa are illustrated in Figures 1–6, and the prevalence data are summarized in Table 3.

**Table 3. Infection rates in dogs infected with some intestinal protozoa.**

| Animals | No. of positive animals (%) | <i>Cystoisospora</i> spp. | <i>Cryptosporidium</i> spp. | <i>Giardia</i> spp. |
|---------|-----------------------------|---------------------------|-----------------------------|---------------------|
| 100     | 59 (59%)                    | 25 (42.37%)               | 20 (33.89%)                 | 14 (23.72%)         |

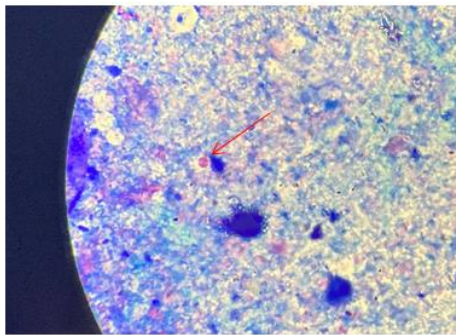


Figure 1. *Cryptosporidium* spp. oocyst using Acid Fast stain 100X

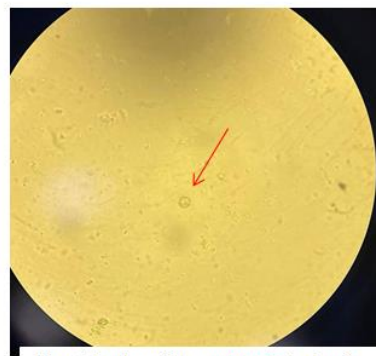


Figure 2. *Cryptosporidium* spp. oocyst using Lugol's iodine 40X



Figure 3. *Cystoisospora* spp. oocyst using Lugol's iodine 40X

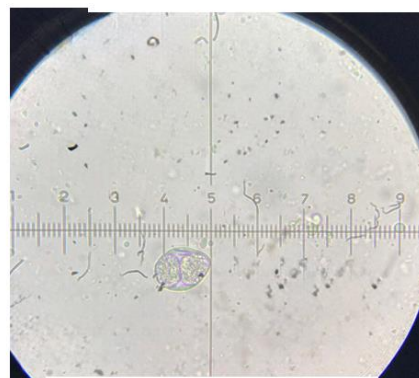
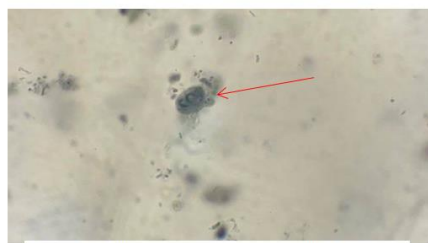


Figure 4. *Cystoisospora* spp. oocyst in 100X



The prevalence of parasitic infection was notably higher in stray dogs, reaching 83.72%. Female dogs exhibited a greater infection rate (70.68%) compared to males. Age-related differences were also observed, as dogs older than six months showed a lower prevalence (41.93%) than puppies younger than two months (58.62%), indicating a decline in infection rates with increasing age (76.31%). In addition, dogs with a healthy clinical appearance showed a lower prevalence of infection when compared with diarrheic animals, in which the infection rate reached 73.91%. These findings are presented in Table 4.

## Discussion

Parasitic diseases continue to pose a major health challenge for both animals and humans worldwide (12). Limited awareness and variability in perceptions regarding appropriate preventive and control measures are considered among the most significant obstacles to combating parasitic infections. Although dogs are commonly regarded as companion animals and integral members of households in many societies, they may act as important reservoirs of zoonotic pathogens that pose a potential risk to human health (13). A wide spectrum of parasitic infections affects both owned and stray dogs in Iraq. Clinical manifestations associated with protozoan infections in dogs include vomiting, dehydration, weight loss, anorexia, and severe diarrhea, in addition to the presence of mucus or oocysts in fecal samples.

The conventional microscopic examination conducted to detect intestinal protozoa in dogs revealed an overall infection rate of 59%, with 59 positive cases out of 100 examined animals. Some local studies reported an infection rate of 10% among dogs in Basrah Province, Iraq (14), which differed from this prevalence (14).

**Table 4. Relationship between the infection with intestinal protozoa and some factors related to dogs.**

| <b>Factors</b>                           | <b>No. tested cases</b> | <b>No. of Positive</b> | <b>(%)</b> |
|--|-------------------------|------------------------|------------|
| <b>Animals management</b>                |                         |                        |            |
| <b>Stray</b>                             | 43                      | 36                     | 83.72a     |
| <b>Companion</b>                         | 57                      | 23                     | 40.35b     |
| <b>Gender</b>                            |                         |                        |            |
| <b>Males</b>                             | 42                      | 18                     | 42.85a     |
| <b>Females</b>                           | 58                      | 41                     | 70.68 b    |
| <b>Age</b>                               |                         |                        |            |
| <b>Less than 2 months</b>                | 38                      | 29                     | 76.31a     |
| <b>More than 2 months –<br/>6 months</b> | 29                      | 17                     | 58.62b     |
| <b>More than 6 months</b>                | 31                      | 13                     | 41.93b     |
| <b>Clinical presentation</b>             |                         |                        |            |
| <b>Healthy appearance</b>                | 31                      | 8                      | 25.80a     |
| <b>Diarrhea</b>                          | 69                      | 51                     | 73.91b     |

**Different letters indicate a significant difference at the probability level of P<0.05**

The findings of the present study, using various conventional diagnostic methods such as fecal culture, concentration techniques, and direct fecal examination, confirm that culture techniques represent highly sensitive diagnostic tools for detecting intestinal protozoa in dogs. The highest diagnostic rate was recorded using the culture method (59%), indicating that this technique may serve as an effective alternative to traditional flotation methods for diagnosing most intestinal parasites in animals. This was followed by the concentration method (55%) and the direct examination method (41%). Furthermore, differences in diagnostic sensitivity among the applied methods may be attributed to variations in sample size, as the culture method demonstrated higher sensitivity compared with other techniques (15).

The present results are consistent with previous studies regarding the sensitivity of commonly used diagnostic methods for parasite detection. Several earlier investigations have demonstrated that culture-based techniques exhibit higher sensitivity and diagnostic accuracy in identifying parasitic infections, thereby supporting the findings of the current study. In contrast, the results of direct examination and concentration methods were relatively comparable.

However, their diagnostic sensitivity appeared to be influenced by parasite load and the components present in fecal samples. (16) across different studies (17,18) Microscopic examination has been shown to have lower performance when compared with alternative diagnostic approaches. In addition, its sensitivity may be influenced by the concentration steps used during sample preparation.

Examination of fecal samples using conventional techniques and specialized diagnostic stains adopted in this study—including Lugol's iodine stain, modified Ziehl–Neelsen stain, and iron–hematoxylin stain—resulted in the identification of several species of intestinal protozoa. Lugol's iodine stain demonstrated the highest diagnostic efficiency (59%), followed by the modified Ziehl–Neelsen stain (56%) and the iron–hematoxylin stain (39%). Diagnosis was based on established morphological and morphometric characteristics. (19)

These findings are partially consistent with previous studies, which have reported that Lugol's iodine stain is among the most commonly employed stains in routine fecal examinations due to its ability to clearly highlight internal structures of cysts and trophozoites, thereby enhancing detection rates, particularly in cases with a high parasite burden. (20)

Furthermore, several studies have indicated that the modified Ziehl–Neelsen stain demonstrates relatively high efficiency in the diagnosis of acid-fast intestinal protozoa such as *Cryptosporidium spp.*, while its effectiveness may be limited for other protozoan species. Such results may explain the comparatively high efficiency observed in the present study, depending on the predominant protozoan species detected in the samples examined. (21)

In contrast, the iron–hematoxylin stain showed lower diagnostic efficiency in the current study when compared with some reports that have highlighted its high sensitivity, especially when performed by experienced personnel. Iron–hematoxylin is considered a permanent staining technique that provides detailed morphological visualization of nuclear and cytoplasmic structures. The reduced efficiency observed in the present study may be attributed to the complexity of the staining procedure, variations in parasite load, sample quality, or technical factors related to fixation and staining duration (22)

Overall, these findings support the conclusions of previous literature that the diagnostic performance of conventional staining techniques for intestinal protozoa is influenced by multiple factors, including protozoan species, intensity of infection, sample preparation methods, and examiner expertise. The results further emphasize the importance of employing a combination of diagnostic and staining techniques to improve sensitivity and achieve more reliable diagnostic outcomes. (21)

In this study, sporulated oocysts of *Isospora spp.* were identified using conventional diagnostic methods based on recognized morphological and morphometric criteria. The oocysts appeared

oval to spherical in shape, and each sporulated oocyst was observed to contain two sporocysts. In addition, unsporulated oocysts were identified based on their morphological features, as sporocysts could not be distinguished within them, which is a known diagnostic characteristic of this developmental stage. These findings are consistent with those reported in previous studies (23; 11).

*Cryptosporidium* spp. were among the intestinal protozoa diagnosed in the present study. Oocysts of this parasite were observed through conventional microscopic examination using several diagnostic stains in feline fecal samples. The oocysts appeared spherical to oval in shape, with relatively small and large sizes, and exhibited variable coloration depending on the staining method used. When stained with the modified Ziehl–Neelsen technique, the oocysts appeared light red to dark pink against a blue background, which is consistent with the morphological description reported by (10).

Furthermore, partially transparent *Cryptosporidium* oocysts with stained borders ranging from yellow to brown were observed when fecal samples were stained with Lugol’s iodine. This method is considered rapid and cost-effective and is widely used for the preliminary detection of oocysts in fecal samples, in agreement with the findings reported by (20).

Among the protozoa diagnosed in the current study using conventional methods, specialized diagnostic stains, and morphological and morphometric criteria, *Giardia* spp. were identified in feline fecal samples. The cysts appeared oval to nearly spherical in shape and contained clearly visible nuclei when stained with Lugol’s iodine and iron–hematoxylin stains. Their dimensions fell within the accepted standard ranges for diagnostic identification. Loiola *et al.*,2024 (19)

The conventional microscopic examination revealed that the highest infection rate was recorded for *Isospora* spp. (42.37%), compared with other protozoa identified in the study, including *Cryptosporidium* spp. (33.89%) and *Giardia* spp. (23.72%). The higher prevalence of *Isospora* spp. may be attributed to its direct life cycle and short developmental period, which facilitate rapid transmission and increase the likelihood of spread among animals.( 23) Similar patterns have been reported elsewhere; for example, in southern Germany, the most frequently detected protozoa among 100 farm-reared puppies were *Cystoisospora* spp (67.1%), *Cryptosporidium parvum* (4.3%), and *Giardia* spp. (1.4%). In indoor puppies, the most common protozoa included *Giardia* spp., (6.6%), and *C. parvum* (3.3%). Differences in protozoan prevalence were associated with housing conditions, *showing* the highest infection rate among farm-reared dogs [24].

The study also demonstrated that the overall prevalence of intestinal protozoan infections in stray animals was 83.72%, which was significantly higher than that recorded in household animals (40.35%). Several studies have reported variable distributions of protozoan parasites depending on housing conditions. Among farm-reared puppies, *Cystoisospora* spp (67.1%), *Cryptosporidium parvum* (4.3%), and *Giardia* spp. (1.4%) were the most frequently detected protozoa. In contrast, indoor puppies showed lower but still notable infection rates with *Giardia* spp. (6.6%), with *C.*

*parvum* (3.3%) being the predominant species (25) , whereas higher infection rates in stray animals were attributed to the lack of adequate veterinary care and hygiene measures.

Additionally, female animals exhibited a significantly higher overall infection rate (70.68%) compared with males (42.85%). This increased prevalence among females may be related to hormonal changes associated with pregnancy, estrus, or lactation, which can affect immune system efficiency and increase susceptibility to infection. (26)

Higher overall infection rates with intestinal protozoa were recorded in younger age groups (76.31%) compared with older animals (41.93%), with a statistically significant difference. This finding may be explained by the immaturity of the immune system in younger animals. The relationship between age and gastrointestinal parasitism remains controversial, largely due to variations in age categorization, study design, geographical settings, and sample sizes across investigations. Nevertheless, the widespread occurrence of gastrointestinal parasites in both juvenile and adult dogs indicates susceptibility across all age groups (27)

Moreover, the results indicated that the overall prevalence of intestinal protozoan infections was significantly higher in animals suffering from diarrhea (73.91%) than in apparently healthy animals (25.80%). Reports indicate detection rates of 12% in symptomatic dogs in Chile (25) and 3% in England, while lower prevalence has been documented in asymptomatic dogs, including 6.3% in Spain, 5.6% in Australia (28), and values ranging from 0.2% to 9.7% in the United States. These findings support previous studies that have linked protozoan infections with the occurrence of diarrhea in infected animals which may be attributed to the ability of certain protozoa to cause damage to the intestinal mucosa (29,30)

## **Conclusion**

The findings of the present study confirm that dogs are commonly infected with intestinal protozoan parasites, with a notably high prevalence recorded in Nineveh Province.

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## **Competing Interests.**

The authors declare that there are no conflicts of interest associated with this manuscript.

## **Ethical approval**

The Scientific Committee of the College of Veterinary Medicine, University of Mosul, Mosul, Iraq, tested and approved. The official approval code UM.VET.2025.030.

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## تقييم الإصابة بالأوالي المعوية في الكلاب بمدينة الموصل بالطرق التقليدية

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### الخلاصة

تُعدّ الأوالي المعوية من أهم المسببات المرضية التي تصيب الكلاب. لذلك، أُجريت هذه الدراسة للتحري عن حدوث إصابات الأوالي المعوية في الكلاب من خلال فحص عينات البراز. جُمعت 100 عينة براز من كلاب مختلفة الأعمار، والجناس، والحالة الصحية، ونظم التربية. تم فحص كل عينة باستخدام عدة طرائق تشخيصية، شملت الفحص المباشر بالمسحة الرطبة، وطريقة الطفو (التركيز)، وطرائق الزرع. بالإضافة إلى ذلك، استُخدمت عدة تقنيات تصبغ لتسهيل تشخيص الأوالي تحت المجهر الضوئي. أظهرت النتائج أن 59 عينة (59%) كانت موجبة عند اعتماد جميع الطرائق التشخيصية. وقد اختلفت نسب الكشف باختلاف الطريقة المستخدمة، إذ بلغت نسبة الإيجابية 41% باستخدام الفحص المباشر، و55% بطريقة التركيز، و59% بطريقة الزرع. وفيما يخص طرائق التصبغ، لوحظ تباين في كفاءة الكشف، حيث بلغت نسبة الإيجابية 59% باستخدام صبغة اللوكل ايودين، و56% باستخدام تقنية الزيل نلسن المحورة و39% باستخدام صبغة الهيماتوكسيلين الحديد. من أصل 100 عينة براز مفحوصة، كانت 59 عينة (59%) مصابة بنوع واحد على الأقل من الأوالي المعوية. تم تحديد ثلاثة أنواع من الأوالي، اعلى نسبة إصابة سجلت بطفيلي متماتلة الابواغ (42.37%) تلاها طفيلي الابواغ الخبيثة 33.89% في حين سجل طفيلي الجيارديا اقل نسبة إصابة 23.72% ولوحظت نسبة إصابة أعلى بشكل ملحوظ في الكلاب السائبة (83.72%) مقارنة بالكلاب المنزلية. كما أظهرت الاناث نسبة إصابة أعلى (70.68%) مقارنة بالذكور. وكانت الكلاب الصغيرة العمر أكثر عرضة للإصابة (76.31%)، إضافة إلى ذلك، أظهرت الكلاب المصابة بالإسهال نسبة إصابة مرتفعة (73.91%) مقارنة بالكلاب السليمة سريريًا

الكلمات المفتاحية: الأوالي المعوية، البراز، الكلاب، الاكياس، اكياس البيض، صبغة.