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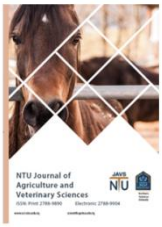

# Traditional investigation of intestinal protozoa in cats in Mosul city

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**Article Informations**

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**A B S T R A C T**

The major pathogens which infect cats is intestinal protozoa, so this study was applied to detect these protozoa in cats through collection of fresh fecal samples from 100 cats of various ages, sexes, management (stray or companion), sources and health conditions were gathered, placed in a sterile container and examined by direct smears, flotation method and culture methods were applied to detect the protozoa then different types of staining methods were used to investigate the protozoa by using light microscope. The results showed a total 58 (58%) were positive using all the diagnostic techniques, 46% direct smear, 52% using concentration method and 58% using culturing method , the stains which can be used to detected the protozoa appear different positivity rate 52% using Lugol's iodine stain, 50% using Safranin methylene blue staining, and 58% using Acid Fast staining method and Malachite green staining. Out of 100 feces samples, 58 were found to be infected with at least one parasite (58%). Among protozoon species, three protozoal species were detected including *Isospora spp.* (39.7%), *Cryptosporidium spp.* (32.7%), and lowest rate of protozoa detected was *Giardia spp.* (27.8%), The infection rate of stray cats (90%), female cats showed higher prevalence of infection (64.4%) compare with male, older cats which showed less exposure to infections than younger cats (71%). The native animals recorded (72.9%). Healthy appearance animals showed low prevalence of infection compared with diarrheic animals (66.7%).

## Introduction

Stray cats are reservoir hosts for many parasitic diseases in medical and veterinary importance [1]. The variety of intestinal parasites differs depending on the type of cat population (domestic cats, cats in shelters, or stray cats), geographic location, and availability of proper veterinary care [2]. Due to the lack of parasite treatment and a greater reliance on wild animals as prey, feral cats are more likely than domestic cats to suffer from parasitic infections [3]. As unicellular microorganisms, protozoa are members of the kingdom Protista, which also contains a variety of other unicellular microorganisims, Giardia spp., Isospora spp., and Cryptosporidium spp. are the three protozoa most frequently found in cats [4]. According to Tan et al. [5], cats are a significant host for Toxoplasma gondii and can shed millions of oocysts after ingesting a small number of tissue cysts. Several coccidia species infect cats, including other protozoa like *Isospora spp.* [6]. Protozoa commonly infect cats, colonizing their large intestine and causing uncontrollable, prolonged diarrhea [7]. The fecal-oral pathway is how the parasite spreads by sharing litter bins, tainted food and water; and bowls can all be potential sources of infection. Thankfully, humans cannot contract *Tritrichomonas fetus* from cats [8]. Conventional qualitative tests, such as the direct technique and fecal flotation, are straightforward and inexpensive methods that are frequently employed in clinical settings to make a diagnosis, the culture technique is rarely employed in routine small animal clinical practice, despite the fact that it enables a quantitative diagnosis of parasite disorders [9]. Culture has emerged more lately is employed in the qualitative and quantitative diagnosis of cat parasite infections caused by protozoa. They need skilled workers and a centrifuge-equipped lab, which are not available in most veterinary clinics [10]. Different investigations have been carried out to assess the sensitivity and specificity of these diagnostic techniques, and the results indicate that intestinal protozoa can be successfully detected and diagnosed using these devices [11], Comparing the diagnostic performance of the various copromicroscopic techniques mentioned above with the conventional and gold standard methods for the diagnosis of intestinal parasites in cats that were naturally infected was the goal of the current investigation [12].

## Materials and methods

### Ethical approve

The scientific board of the College of Veterinary Medicine at Mosul University in Mosul, Iraq, approved this study; the approval number for this study is UM.VET.2023.037.

### Animals

This study examined 100 cats of various ages, sexes, management (stray or companion), sources, and health conditions.

### Samples

Fresh fecal samples were gathered, placed in sterile containers, and added 2.5% potassium dichromate solution or preservation, and then sent to the parasitology lab at Mosul University, Department of Microbiology, College of Veterinary Medicine.

### Parasitological examination

#### *Direct smear*

Directly applied wet smear by taking a tiny quantity of feces with a pin stick, mixed them well on a glass slide, and added a coverslip. On a glass slide, a drop of Lugol's iodine stain was mixed and then examined by light microscope according to [13].

#### *Concentration method*

In order to create a homogenous fecal suspension, 2 g of feces were mixed with 20 mL of saturated NaCl solution. After passing through two layers of cotton gauze with a mesh size of 250 µm, the mixture was gathered in a beaker. After that, the fecal liquid was put into a 15 mL conical-bottomed Falcon tube and centrifuged for five minutes at 600× g. Following centrifugation, a coverslip was placed on top of the meniscus created by adding the same floating solution to the tube's top. The coverslip was put on a slide and examined under a microscope after three to five minutes [14].

#### *Culturing method*

A pea-sized quantity of each fecal sample was put into a sterile plastic culture tube with snap-caps that contained 3 ml of Jones medium enhanced with 10% heat-activated horse serum as part of an in vitro cultivation procedure [15]. Prior to testing, each sample was incubated vertically for 48 to 72 hours at 37 °C. To find protozoa, a drop of the sediment was inspected at 100 x magnification. Following isolation, the parasites were kept alive by subculturing them every three to four days. The sediment would be further re-suspended in a new culture medium and kept for an additional 48 hours at 37 °C incubation if no growth was seen (Observing the vacuolar shape in the medium would confirm positive results) [16].

#### *Acid Fast staining method*

After the concentration technique was completed, smears were made from the pellets of every sample, and the slides were left to dry at room temperature for five minutes after the methanol fixation. A modified acid-fast staining technique was then used to stain each sample. At last, every slide was examined using a light microscope [17].

***Safranin methylene blue staining (SMB)***

The staining procedure was used in the current study in accordance with method using a stick, thin smears of the fecal debris were applied to the glass slide. The smears were then temporarily cemented over a flame and allowed to air dry. Once more, the smears were fixed for three to five minutes in acid alcohol. After that, the smears were gently heated until steam formed and submerged in a 1% safranin solution for one minute. Following a water wash, the smears were counterstained for 30 seconds using 1% methylene blue. The streaks were then once more cleaned with water, let to air dry, and inspected [18].

***Malachite green staining***

With minor adjustments, the malachite green staining process was carried out in accordance with the methodology. On a glass slide, a drop of 5% malachite green stain and a drop of fecal sediment were combined, spread equally, and allowed to air dry. The smears were analyzed using oil immersion (100X) in all of the aforementioned methods [19].

## Results

In total, 58 (58%) were positive using all the diagnostic techniques,46% using direct smear, 52% using concentration method and 58% using culturing method (Table 1).

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

| No of samples | Direct smear No of positive samples | concentration method No of positive samples | Culturing method no of positive samples |
|---|---|---|---|
| 100 | 46(46%) | 52(52%) | 58(58%) |

The stains which can be used to detect the protozoa appear different positivity rate 52% using Lugol's iodine stain, 50% using Safranin methylene blue staining (SMB) and 58% using both Acid Fast staining method and Malachite green staining (Table 2).

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

| No of samples | Lugol's iodine stain | Acid Fast staining method | Safranin methylene blue staining (SMB) | Malachite green staining |
|---|---|---|---|---|
| 100 | 52 (52%) | 58 (58%) | 50 (50%) | 58 (58%) |

Out of 100 feces samples, 58 were found to be infected with at least one parasite (58%). Among protozoon species, three protozoal species were detected including, highest rate of protozoa detected was *Isospora spp*. (39.6%) with the average size of these cysts was 28 X39 um, followed by *Cryptosporidium spp*. (32.7%), and lowest rate of protozoa detected was *Giardia spp.* (27.8%) were identified. (Figures 1,2,3,4,5,6,7) ( Table 3).

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

| Animals | No. of positive animals (%) | *Isospora spp.* | *Cryptosporidium spp.* | *Giardia spp.* |
|---|---|---|---|---|
| 100 | 58 (58%) | 23 (39.6%) | 19 (32.7%) | 16 (27.8%) |

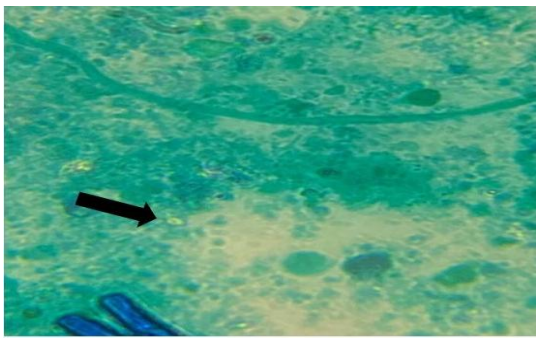

**Figure 1.** *Cryptosporidium spp.* in Malachite green 100X.

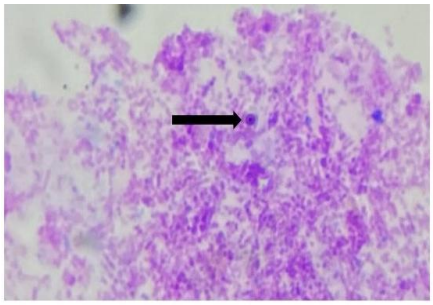

**Figure 2.** *Cryptosporidium spp.* oocyst Safranin methylene blue staining 100X.

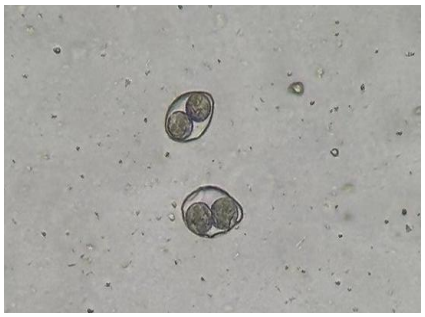

**Figure 3.** *Isospra spp.* sporulated oocyst using culturing method 100X.

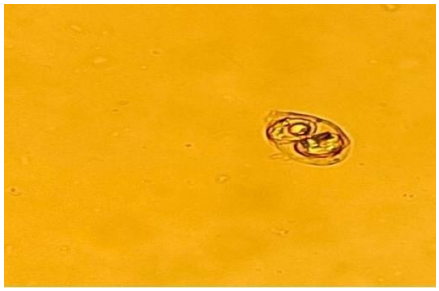

**Figure 4.** *Isospra spp.* oocyst using Lugol's iodine 40X.

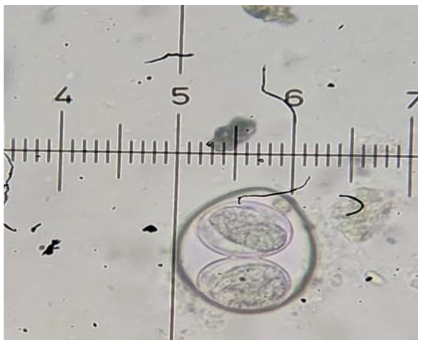

**Figure 5.** *Isospra spp.* sporulated oocyst (25X30um).

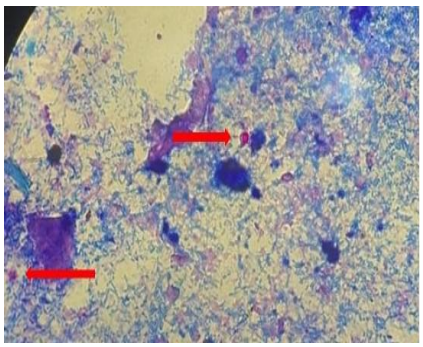

**Figure 6.** *Cryptosporidium spp.* oocyst Acid Fast staining method 100X.

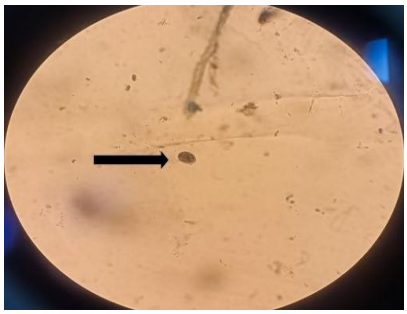

**Figure 7.** *Giardia spp.* cyst in Logul's iodine 40X.

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

| Factors | No. tested cases | No. of Positive | (%) |
|---|---|---|---|
| Animals management | | | |
| Stray | 42 | 36 | 90 |
| companion | 58 | 22 | 37.9 |
| Gender | | | |
| Males | 41 | 20 | 48.7 |
| Females | 59 | 38 | 64.4 |
| Age | | | |
| Less than 2 months | 39 | 28 | 71.7 |
| More than 2 months – 6 months | 27 | 17 | 62.9 |
| More than 6 months | 34 | 13 | 38.2 |
| Breed | | | |
| Native | 48 | 35 | 72.9 |
| Imported | 52 | 23 | 44.2 |
| Clinical presentation | | | |
| Healthy appearance | 28 | 10 | 35.7 |
| Diarrhea | 72 | 48 | 66.7 |

The infection rate of stray cats (90%), female cats showed highest prevalence of infection (64.4%) compared with male cats, cats older than 6 months (38.2) showed less exposure to infections than cats less than 2 months (71.7%) the prevalence of infection decrease with development the age of animals, according to breed of animals the native animals (72.9%) recorded high prevalence of infection , the healthy appearance animals showed low prevalence of infection compared with diarrheic animals(66.7%) (Table 4).

## Discussion

The world's animal and human populations are still severely burdened by parasitic illnesses [20]. The lack of understanding and differing views of their duty to react correctly to this circumstance, however, is one of the main issues in the control of parasite infections. Although their owners frequently view cats as members of the family, they can act as vital hosts for zoonotic diseases that impact people [21]. Both domestic and stray cats are among the many kinds of parasite infections that affect local Iraqi animals. Cats with protozoal infections displayed a number of symptoms, including vomiting, dehydration, weight loss, appetite loss, severe diarrhea, and the presence of mucus or oocysts in their feces. Findings in the current study validate the existence of sensitive technologies for diagnosing feline protozoa and suggest that they could be a viable substitute for traditional flotation for the majority of intestinal parasites in companion animals. Due to variations in the size of the examined samples [22], the culture technique's higher sensitivity when compared to the other methods for all found parasites is consistent with the former methods known sensitivity. Previous research has demonstrated that the culture approach has a better sensitivity and accuracy than other methods in the identification of parasitic infections, which is consistent with the current findings. Prior research has suggested that culture methods are more effective at diagnosing problems. In the current study, the outcomes from direct smear and concentration smear were comparable. However, the parasite components in the stool samples affected the sensitivity of these techniques [23]. Geographical locations, local soil and climate conditions, cat population types, and seasons can all affect the incidence of intestinal parasites. Geographical regions may have different intestinal parasite prevalences. Taking into account the regional conditions, this study found a distinct trend wherein Isospora infection was more prevalent than other protozoa. *Isospora felis* (67.1%), *I. rivolta* (48.6%), *C. parvum* (4.3%), and Giardia (1.4%) were the most common protozoa infections in 100 farm-raised kittens in southern

Germany. In indoor kittens, the most common protozoa infections were *I. felis* (46.6%), *I. rivolta* (33.3%), Giardia (6.6%), and *C. parvum* (3.3%). There were differences between the conditions in which cats were kept indoors and on farms, and the highest protozoa rate in the former was associated with *I. felis* (67.1%) [24]. The data gathered by other researchers in other nations is consistent with the incidence of Isospora spp. identified in this investigation. As a result, Isospora was found in 12% of stool samples from cats displaying clinical symptoms in Chile [25] and 3% in England [26]. According to reports, the prevalence of *Isospora spp.* infection in cats without clinical symptoms is 6.3% in Spain [27], 5.6% in Australia [28] and 0.2% to 9.7% in the USA [29, 30]. Protozoa have been detected in cat feces at high rates around the world, including 42% in Iran and 41.39% in China [31]. In cat feces, our investigation revealed that Isospora spp. had the highest prevalence of any protozoa (39.6%), which is in line with findings from a prior study carried out in China (41%) [32]. Furthermore, a study that was carried out in North America, Alaska, and Hawaii discovered that between 3 and 36 percent of cats have Isospora spp. Cats with Isospora infections typically show no symptoms. However, a cat infected with Isospora spp. may experience diarrhea, vomiting, and appetite loss in some situations, such as in a crowded setting. Isospora in cats, however, cannot infect people. This is due to the fact that every species of Isospora is host-specific [33]. According to another study, 103 cats that were captured from Meshkin Shahr had the following coccidia infections: *Isospora spp.* 6/103 (5.8%). Among 100 farm-raised kittens in southern Germany, the most common protozoa infections were *I. felis* (67.1%), *I. rivolta* (48.6%), Toxoplasma/Hammondia (17.1%), *C. parvum* (4.3%), and Giardia (1.4%). Of indoor kittens, *I. felis* (46.6%), *I. rivolta* (33.3%), Giardia (6.6%), and *C. parvum* (3.3%) were the most common protozoa infections. There were differences between the conditions under which cats were kept indoors and on farms, and the highest protozoa rate was associated with *I. felis* (67.1%) in the former [24]. Aboriginal groups in Western Australia's west Kimberley region have recorded prevalence rates of *T. gondii* (18.2%), *I. felis* (15.1%), and *G. duodenalis* (17.0%) [34]. Despite the fact that none had diarrhea or other clinical symptoms, a survey of sales at pet retailers in Atlanta revealed that 34% of them had Giardia [35]. On the other hand, several investigations have found modest levels of protozoa infection [36, 37], and these results are in line with our study. According to a study, the United States had an overall fecal prevalence of Giardia species (0.58%) and coccidia (1.4%) [37]. As a result, Isospora was found in 12% of stool samples from cats displaying clinical symptoms in Chile [25] and 3% in England. According to reports, the prevalence of *Isospora spp.* infection in cats without clinical symptoms is 6.3% in Spain [27], 5.6% in Australia [28] and 0.2% to 9.7% in the USA [29]. Adult cats had a higher prevalence than young cats, although this difference was not statistically significant (P = 0.115). Better-controlled studies are needed because the association between gastrointestinal illness and age has been debated [38, 39]. However, each study was based on a different age classification, study region, and sample size. Whatever the argument, the fact that gastrointestinal parasites are common in both young and adult cats suggests that they are susceptible to infection at every stage of life [40]. The incidence did not significantly differ between male and female cats. Similar findings for various regions have been reported in numerous earlier research [41, 39]. Infections in general and the particular parasite species *T. cati* and *T. leonina* in particular were far more common in stray cats than in companion cats. Prior research on stray cats revealed that, in comparison to house cats, the frequency of gastrointestinal parasites was extremely high (>85%) [42, 43]. *Giardia spp.* are the third most prevalent protozoa found in cat feces. The prevalence of *Giardia spp.* in this study was 27.8%, although environmental factors may affect this. According to a prior study conducted in Germany, 60 cat fecal samples had *Giardia spp.* infections [44]. Due to variations in cat sex, age, breed, and symptoms, other research revealed that the prevalence of Giardia in cat feces varied globally [45]. According to another study that used ELISA to identify *Giardia spp.* in Romanian domestic cats' feces, 28% of the samples had Giardia spp. antigens [46]. Different detection techniques may be the cause of the global heterogeneity in parasitic protozoan detection rates across various investigations [47]. Microscopy is known to perform badly when compared to other detection techniques. Furthermore, the sensitivity of the technique might be impacted by the concentration steps of the preparations, and microscopy is not as uniform as the other procedures.

## Conclusion.

Cats are afflicted with intestinal protozoa, and Nineveh province has a higher prevalence of parasites.

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**Competing Interests**
The authors declare that there is no conflict of interest in the manuscript.

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