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MICROSCOPIC AND MOLECULAR DIAGNOSIS OF *ISOSPORA* SPP PARASITES IN CATS AND DOGS

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ABSTRACT

Isospora species are protozoan parasites that commonly infect the intestinal tracts of dogs and cats, leading to symptoms such as diarrhea and abdominal discomfort. The purpose of the study was to detect *the parasites Isospora* spp. in cats and dogs (both domestic and stray) of various ages and sexes in Basrah Governorate. Using microscopic examination with the flotation method, the infection rate with *Isospora* spp. in dogs was 9.5% (2/21), and in cats, it was 10% (11/109). Molecular analysis using PCR was performed on positive samples to confirm the infection with *Isospora* spp. and to identify the species based on the host. The study found that *Isospora canis* infects dogs, while *Isospora rivolta* and *Isospora suis* infect cats. This is the first recorded case of *Isospora* infection in cats in Basrah.

Keywords: *Isospora*, Microscopic Molecular, Cats And Dogs

INTRODUCTION

Cyclospora and Cystoisospora (formerly Isospora) are protozoan coccidian parasites that can cause severe disease in immunocompromised cats, dogs, and humans. These parasites reproduce sexually in the digestive tracts of their hosts, eventually releasing oval oocysts into the feces (Suh *et al.*, 2015). Originally, Isospora spp., including Cryptosporidium spp., were classified under the Eimeriidae family due to their similar life cycle to that of *Eimeria spp.* (Levine, 1988). Isospora, Toxoplasma gondii, Eimeria spp., and Cryptosporidium spp. are obligate intracellular protozoan parasites within the class Apicomplexa, known as coccidia. The oocysts of these parasites vary in size, shape, life cycle patterns, and host specificity. For example, large (25–35 μm) spindle-shaped oocysts belong to the Isospora canis group, while medium-sized oocysts (17–23 μm) belong to the Cystoisospora ohioensis group (Lindsay *et al.*, 1997). Isospora spp. infections are common, especially in young animals. The genus Isospora is complex due to variations in oocyst morphology, host specificity, and intermediate stages (Barta *et al.*, 2005; Raza *et al.*, 2018). In humans, Cyclospora and Cystoisospora infections are often associated with immunocompromised individuals worldwide (Hechenbleikner *et*

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al., 2015). Cystoisospora canis, C. ohioensis, and C. burrowsi cause diarrhea in puppies under 6 months of age and in immunocompromised dogs (Buehl *et al.,* 2006). Parasite invasion and replication within enterocytes damage the small intestinal epithelium, leading to disruptions such as loss of the brush border, loss of membrane-bound digestive enzymes, and intestinal villous blunting and atrophy (Giangaspero *et al.,* 2019; Sun *et al.,* 1996). These changes decrease the small intestine's absorptive capacity, resulting in reduced uptake of water, nutrients, and electrolytes (Giangaspero *et al.,* 2019). Symptoms of infection include weight loss, anorexia, nausea, abdominal cramping, low-grade fever, and frequent, watery diarrhea. The stool may occasionally contain blood or mucus. More severe disease is typically seen in older individuals, neonates, and those with severely compromised immune systems, such as HIV/AIDS patients (Giangaspero *et al.,* 2019). Travelers from non-endemic areas are also at risk of severe infection. Prolonged diarrhea can lead to dehydration and malnutrition, and may rarely result in death, particularly in infants and those with other infections or comorbidities. Symptoms usually begin after a median incubation period of seven days following the ingestion of infectious oocysts, ranging from 2 to over 2 weeks, and may last for weeks to months without treatment (Fleming *et al.,* 1998; Herwaldt *et al.,* 1996). Some individuals experience mild symptoms, while others may have a single, self-limiting episode (Hoge *et al.,* 1995; Huang *et al.,* 1995). Isospora belli infections cause diarrhea, steatorrhea, headache, fever, malaise, abdominal discomfort, vomiting, dehydration, and weight loss, with feces typically not containing blood (Brandborg *et al.,* 1970; Liebman *et al.,* 1980). Severe cases may require hospitalization due to dehydration from fluid-like, secretory diarrhea, which can be associated with fever and weight loss (Bialek *et al.,* 2001). In dogs and cats, Isosporiasis can be diagnosed based on case history, clinical signs, and microscopic examination using direct smear and flotation techniques with Sheather's solution to detect Isospora spp. (Ljungström *et al.,* 2018). Due to the lack of extensive studies on Isospora spp. parasites and their potential threat to human and animal health. This research was conducted to diagnose the parasite both microscopically and molecularly in the Basrah province.

MATERIALS AND METHODS

Study Area and Sample Collection:

Field studies were conducted on 130 samples (109 from cats and 21 from dogs) collected from various areas in Basrah City between September 20, 2023, and April 15, 2024. Fecal samples were collected from cats and dogs of different sexes and ages. Each sample was placed in a separate plastic container with a lid. Information regarding the age, sex, and consistency of the feces was recorded. The samples were then transported in a cool box to the Parasitology Laboratory at the Faculty of Veterinary Medicine, Basrah University, for examination.

Laboratory Examination of Fecal Samples:

Fecal samples from all cats and dogs were examined daily using direct smear and flotation techniques with Sheather's solution to detect Isospora spp. (Ljungström *et al.,* 2018).

DNA Extraction and PCR Assay:

Genomic DNA was extracted from oocysts recovered from fecal samples of cats infected with **Isospora** spp. using the DNA Stool Mini Kit (QIAGEN, Germany). DNA integrity was assessed using 1% agarose gel electrophoresis with 0.05% ethidium bromide. PCR was performed to differentiate Isospora spp. using specific primers: (ITSF: 5′-CCGTTGCTCCTACCGATTGAGTG-3′) and (ITSR: 5′-

CCGTTGCTCCTACCGATTGAGTG-3′), which amplify the mitochondrial ITS gene (450 bp), following the method described by Madani *et al.* (2018). Five µl of the PCR amplification products were analyzed by 1.5% agarose gel electrophoresis, and the results were visualized using a UV transilluminator.

Data analysis

The gathered data was meticulously entered into a spreadsheet in Microsoft Excel 2010 and then analyzed using SPSS software (version 20) for Windows 10. Frequencies, proportions, and standard deviations (SD) for non-parametric quantitative variables were estimated. A P-value of less than 0.05 was indicative of statistical significance.

Ethical Approval

Ethical Considerations: Ethical approval for the study was obtained from the Faculty of Veterinary Medicine, on 30/37/2024.

RESULTS

Morphology of Isospora spp.:

The completely formed (sporulated) oocyst of the genus *Isospora* is spindle-shaped and contains two sporocysts, each with four sporozoites, as observed in the parasitological research (Figure 1). Additionally, *Isospora* sporulation was identified by the presence of two sporocysts through microscopic examination of stool smear slides (Figure 2).

In this study, 130 fecal samples (109 from cats and 21 from dogs) were randomly collected from various areas in Basrah City and examined microscopically. The infection rate among dogs was 9.5%, indicating the presence of *Isospora* spp. infections. Similarly, the infection rate in cats was 10%. These findings suggest that *Isospora* spp. infections can potentially affect humans as well.

The study identified one species of *Isospora* infecting dogs, *I. canis* (9.5%), and two species infecting cats, *I. rivolta* and *I. suis* (10%) (Table 2). Fecal samples that tested positive for *Isospora* spp. by microscopic examination were further analyzed using PCR to confirm the diagnosis (Figure 3). Additionally, all positive samples from cats and dogs were subjected to PCR testing, and seven samples were selected for sequencing to confirm the infection and identify the species.

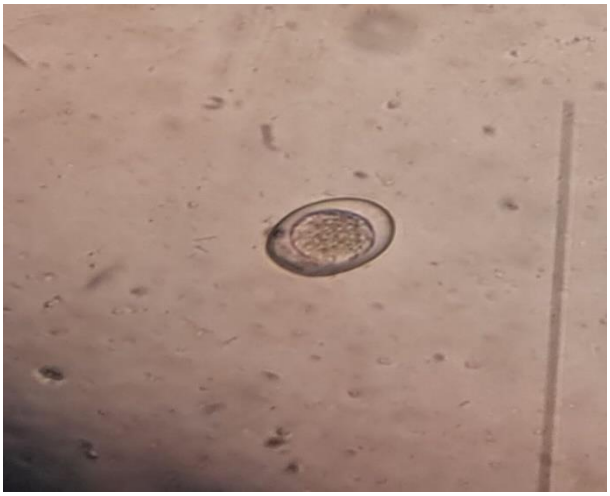


Figure 1: Oocyst of *Isospora* spp. isolated from infected cats by flotation technique (40x).

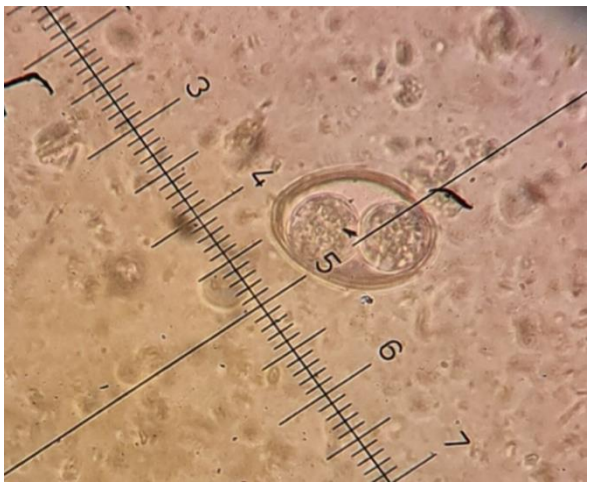


Figure 2: Mature (sporulated) oocyst of *Isospora* spp. isolated from infected cats by flotation method (40x).

Table 1: Microscopic diagnosis of isospora spp parasite in a fecal sample of dogs and cats species:

Host	No. of samples	No. of positive samples	Infection percentage
Dogs	21	2	9.5%
Cats	109	11	10%

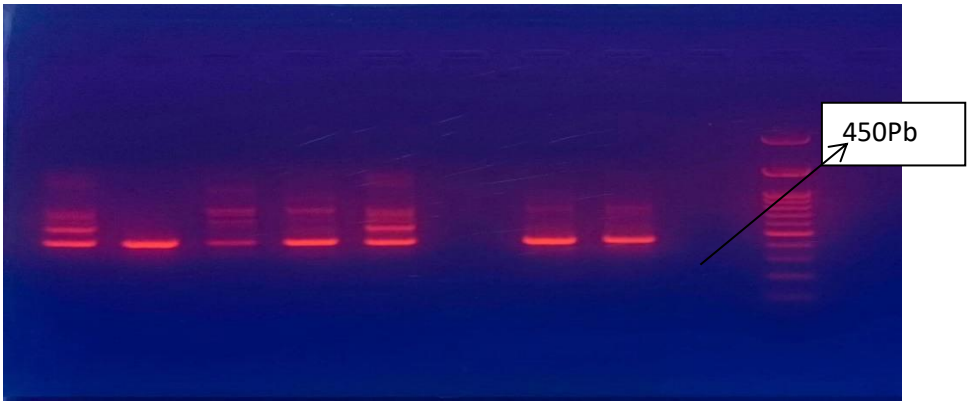
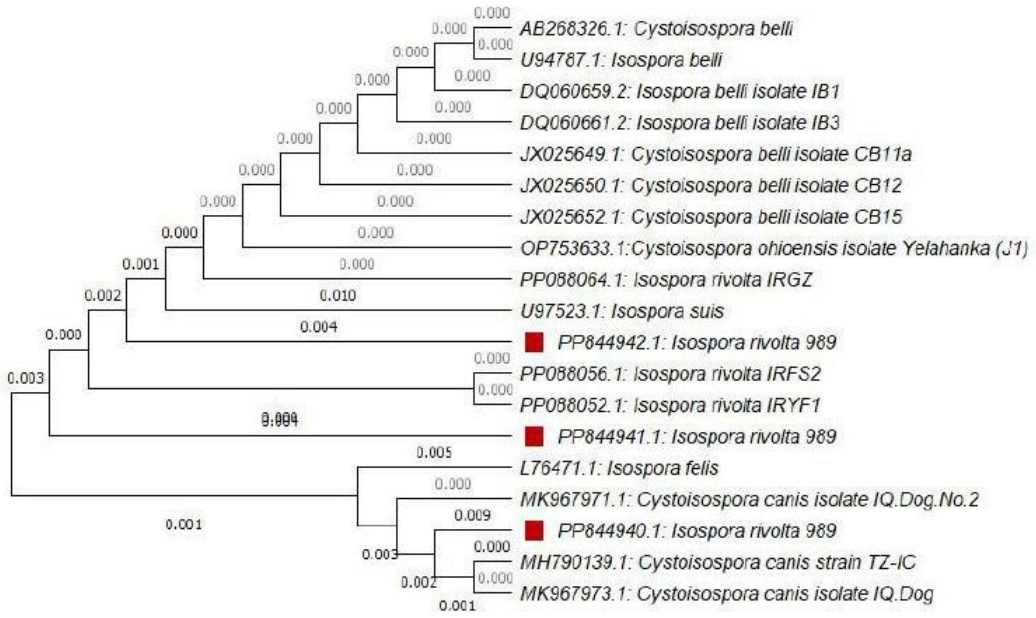


Figure 3: Agarose gel electrophoresis of PCR product

Table 2: Result of PCR that explains species of *isospora* parasite according to host:

Host	No.of sample	Positive. sample percentage	PCR percentage	Sequence
Cat	109	10%	10%	Isospora suis Isospora rivolta
Dog	21	9.5%	9.5%	Isospora canis



Figuer4: (Phylogenetic tree analysis)

DISCUSSION

Samples for this study were collected from various areas within Basrah Governorate between September 20, 2023, and April 15, 2024. A total of 130 fecal samples (109 from cats and 21 from dogs) were examined. The study identified infections with *Isospora* spp. in these animals. Cats are particularly susceptible to parasitic infections due to their tendency to roam, which increases their exposure to parasites that can affect their health and that of humans (Krecek *et al.,* 2010; Kalef and Al-Khayat, 2022).

In this study, 10% of cats (11 out of 109) tested positive for *Isospora* spp., a prevalence consistent with several earlier studies (Arai *et al.,* 1990; Mtambo *et al.,* 1991; Nash *et al.,* 1993; Sargent *et al.,* 1998; McReynolds *et al.,* 1999; Hill *et al.,* 2000; Spain *et al.,* 2001) and in agreement with Tzannes and German *et al.* (2008). The infection rate was notably higher in February, reaching 23.8%, which aligns with findings from Beigi *et al.* (2017), who reported *Isospora felis* at 38% and *Isospora rivolta* at 25% in Iran. In contrast, Arbabi and Hooshyar *et al.* (2009) recorded much lower rates in Kashan, Iran, with *both I. rivolta* and *I. felis* at 5.3%. These discrepancies may be attributed to environmental differences, variations in immune system development, and adaptation to colder climates in different regions.

For dogs, the study found a 9.5% infection rate with *Isospora canis*, consistent with Mitchell *et al.* (2007), who reported similar findings. Mitchell *et al.* (2007) also noted that *I. canis* is associated with diarrhea, which corresponds with our finding of a 23.8% rate of severe diarrhea in affected dogs. This is further supported by Houk and Lindsay *et al.* (2013), who confirmed the pathogenic nature of *I. canis.*

In terms of clinical symptoms in cats, the study observed severe diarrhea in 30%, cramping in 10%, vomiting in 30%, weight loss in 19%, and dehydration in 4.3% of infected animals. These findings differ from Jablonski and Wennogle *et al.* (2021), who did not report clinical signs in their study cats. This discrepancy may be due to the disease being in its incubation period, or the cats having some degree of immunological resistance to the infection.

Lastly, while Scorza *et al.* (2021) reported that *C. felis* DNA was detected by PCR in some samples where oocysts were not visible on fecal flotation, our study found that all samples were positive for *Isospora* spp. by flotation were also confirmed by PCR. This suggests that PCR is a reliable method for rapid diagnosis, as it amplifies DNA and may be more sensitive than fecal flotation in detecting infections.

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التشخيص المجهري والجزيئي لطفيليات ISOSPORA SPP في الكلاب والقطط

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أنواع متماثلة الأبواغ هي طفيليات أولية تصيب عادة القناة المعوية للكلاب والقطط، مما يؤدي إلى أعراض مثل الإسهال وألم في البطن. كان الغرض من الدراسة هو الكشف عن طفيليات Isospora spp. في القطط والكلاب (المنزلية والضالة) بمختلف أعمارها وأجناسها في محافظة البصرة. باستخدام الفحص المجهري بطريقة الطفو تم تحديد معدل الإصابة ببكتيريا Isospora spp. وفي الكلاب وجد ٩,٥% (٢/٢١)، وفي القطط ١٠% (١٠٩/١١). تم إجراء التحليل الجزيئي باستخدام PCR على العينات الإيجابية للتأكد من الإصابة بطفيليات Isospora spp. وتحديد الأنواع على أساس المضيف. ووجدت الدراسة أن Isospora canis يصيب الكلاب، في حين يصيب Isospora rivolta وIsospora Suis القطط. وهذه هي الحالة الأولى المسجلة للإصابة بمرض Isospora في القطط في مدينة البصرة.