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Prevalence and Molecular Studies of *Isospora Spp*. in House and Stray Cats at Baghdad Province

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Abstract

The purpose of this study was to look at the prevalence of the intestinal protozoa *Isospora* spp. in cats in Baghdad from November 1st to April 30th, 2018 in terms of sex, age group (Young and Adult), and months. The current study's findings indicated that both sexes were infected with Isospora spp (with non-significant differences). In domestic cats, 3(10.3%) of 29 males were found diseased, whereas 5(5%) of 31 females were afflicted (16.1 percent). In addition, infection rates in stray cats were 8% in 25 males and 14.2% in 35 females. There were no significant differences (P> 0.05) in infection rates between young and adult house cats and stray cats. In house cats, the greatest rate of infection with *Isospora* spp. (20%) was reported in March and April, with a significant difference (P0.05), whereas in stray cats, the highest rate of infection (20%) was recorded in December, with a significant difference (P0.05). In this investigation, one PCR primer was utilized to identify *Isospora* utilizing the 18S rDNA ribosomal gene. The increased PCR readings might be related to the discovery of residual DNA from previous infections.

Keywords: Prevalence, Molecular, Protozoa, *Isospora* spp. in cats

INTRODUCTION

Cyclospora and Cystoisospora (previously Isospora) are protozoan coccidian parasites that have long been recognized as possible infections in cats, producing severe morbidity in immunocompromised hosts. They reproduce sexually in cats' gastrointestinal systems, culminating in the release of oval oocytes into excrement. Suh et al. (2015) Isospora spp. is found all over the world and appears to be host specific; infections are frequently communal, mostly in young animals. Because of many clarifications concerning oocyte form, host specificity, and intermediary phases, the genus *Isospora* is quite complicated (Barta *et al*., 2005; Raza *et al*., 2018). Furthermore, various investigations in Baghdad have demonstrated the incidence and distribution of coccidian (sporozoa: Eimeriidae) in animals (Duszynski and Upton, 2001). *Isospora* species that infect animals include, *Isospora condylurae Isospora brevicauda, Isospora neurotrichi Isospora lamoillensis, Isospora cristatae,* and *Isospora palustris* (Greene, 2013; Garanayak *et al.,* 2017). *Isospora* is a huge genus with several species and a wide range of host creatures, including humans, monkeys, pigs, dogs, and felines (Daugschies *et al*., 2007; Lappin, 2010). Cats are the major hosts of the feline protozoan parasites *Isospora felis* and *Isospora rivolta,* which are both ubiquitous globally with little clinical implications. At various points of their life, all cats exhibit symptoms of

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infection (Wang *et al*., 2012). Oocysts, on the other hand, are very resistant to harsh environmental conditions, remaining alive in freezing temperatures and chemical materials (antiseptics); therefore, cages, food utensils, and other apparatuses should be disinfected by steam cleaning or immersion in boiling water or a 10% ammonia solution (Dubey, 2009). Furthermore, the primary means of control is to promote adequate cleanliness, with rapid removal of feces prior to oocyst sporulation. To kill oocysts that contaminate surfaces, steam cleaning can be employed (Daugschies *et al.*, 2000). In cats, *Isospora* spp. infection is acquired by ingestion of sporulated oocysts found in the environment or tissues of other infected vertebrate intermediate hosts. If a cat consumes sporulated oocysts carried by paratenic animals such as flies, cockroaches, or beetles, infection might ensue (Tzannes *et al*., 2008). When a cat consumes an infective oocyst or a rat with bradyzoites in its tissues, the zoites assault the intestinal cells and develop into the schizont stage, however when a rodent consumes an infective oocyst, the sporozoites enter tiny intestine cells and encyst as bradyzoites (Lloyd and Smith, 2000). On the other hand, various epidemiological studies have showed that the infection rate of *Isospora felis* and *Isospora rivolta* in China was 16.94% and 11.38%, respectively, based on flotation technique and direct smear test using a microscope (Beigi *et al*., 2017). In India, the prevalence of the intestinal parasite *Isospora* spp in cats was 7%. (Mitchell *et al.,* 2007). Furthermore, the incidence of infection with *Isospora* spp. in stray cats in Kirkuk and Alkwait, Iraq. was 10.41 percent and 7.3 percent, respectively, based on microscopic inspection the rate of infection was infected with *Isospora felis* by microscopic (Bialek *et al.,* 2002; Shehab *et al.,* 2017). *Isospora* spp. infection was diagnosed at a high rate of 27 percent in Brazil (Lorenzini *et al.,* 2007). In Germany, 30.4 percent of the investigated 22.8 percent of the cats were infected with endoparasites, with *Isospora* accounting for 6%. (Barutzki and Schaper, 2010).The objective of the present study was to determine the prevalence rate and the influence of seasonal variation, age and gender on the prevalence of *Isospora* spp. Also, using the molecular diagnostic PCR technique to detection and diagnosis of *Isospors spp.* in the cats.

MATERIAL AND METHODS

STUDY AREA AND SAMPLE COLLECTION

Field study were carried out on 120 cats (60 stray cats and 60 house cats) from different areas in Baghdad city, during the period from 1/November 2017 till 30 April 2018. Fecal samples were collected from cats with different sexes about (54 males and 66 female) and different age. Samples were applied in plastic container separately with a lid and the data pertaining to the age, gender and feces consistency were recorded and transported in cool box to the laboratory of parasitology in Veterinary Medicine, Baghdad University for examination. This study was regarding the sex, age, and months during a period.

LABORATORY EXAMINATION OF FAECAL SAMPLES

Fecal samples collected of all cats were examined daily using the direct smear and floatation technique with sheather's solution to detect *Isospors spp.* (Ljungström *et al.*, 2018).

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DNA EXTRACTION AND PCR ASSAY

The DNA Stool Mini Kit /QIAGEN / Germany was used to separate genomic DNA from oocysts recovered from fecal samples collected from cats infected with *Isospors* spp. DNA integrity was determined using 1 percent agarose gel electrophoresis with 0.05 percent ethidium bromide. The PCR experiment was carried out to differentiate *Isospors* spp. by using specific primers COIf (5′ - GTTCATTAGTATGGCACATCA-3′) and COIr (5′ - CCAAGATAATACGAAATGGAA-3′) that amplified the mitochondrial COI gene (300 bp) similar to the technique described by (Madani *et al.*, 2018). Five µl of amplification samples were placed immediately into a 1.5 percent agarose gel electrophoresis, and the results were observed using a UV transilluminator.

RESULTS

The parasitological investigation revealed that the completely developed (sporulated) oocyst of the genus *Isospora* is a spindle-shaped body with two sporocysts each containing four sporozoites. They are 20-33 micrometers long and 10-19 micrometers broad, as seen in the figure (1). Furthermore, microscopic inspection of a stool smear slide revealed *Isospora* sporulation as two sporocysts, as shown in figure (2). In this study, however, 120 feces samples were obtained at random (60 from house cats and 60 from feral cats) from various areas in Baghdad city and inspected microscopically. According to the findings of an epidemiological investigation, only 8 of the 60 houses investigated. According to the results of an epidemiological investigation, only 8 out of 60 house cats were found to have *Isospora* spp. infections, which corresponds to a 13.3% infection incidence. The current investigation also discovered that stray cats had an infection rate of 11.6 (7/60) for *Isospora* spp. The results of this study show that Isospora spp. infections can affect people of any sex (non-significant differences). In addition, whereas 16.1% of 31 female house cats had the infection, 3 (10.3 percent) of the 29 males did. In contrast, stray cat infection rates were 8.2% in 25 men and 14.2% in 35 women, as shown in table (1). Additionally, the research period revealed no significant differences (P> 0.05) in infection rates between juvenile and adult house and feral cats (2). Additionally, a summary of the distribution of *Isospora* spp. infection in relation to study months is provided in table (3). In the current study, it was discovered that house cats had an *Isospora* spp. infection rate of 20% in March and April, with a significant difference (P 0.05). In contrast, the chart demonstrates that the month of December has the greatest rate of infection (20%) in stray cats (3). Between the total rate of infection during the months in house and stray cats, there was no significant variance (P> 0.05). The 15 fecal samples that tested positive for *Isospora* spp. by microscopic inspection, on the other hand, were investigated using PCR method to confirm the diagnosis. As shown in Figure(3), the PCR results showed that the mitochondrial COI gene's 300 bp long fragment could be amplified by a particular pair of primers (COIf, COIr). Additionally, 10 out of 100 cats tested positive for *Isospora* spp. according to the PCR results.

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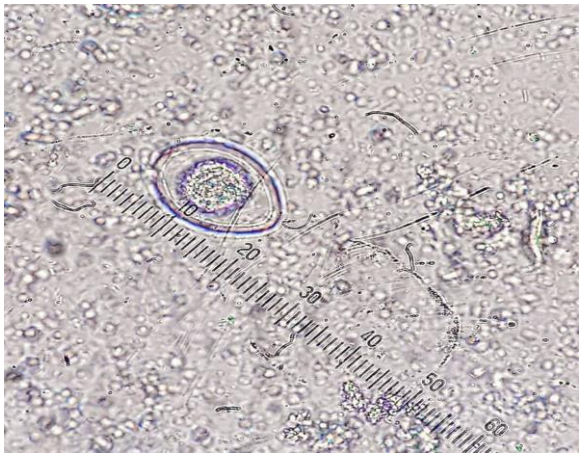

Figure 1: Cyst of *Isospora* spp. isolated from infected cats by Flotation technique (10x).

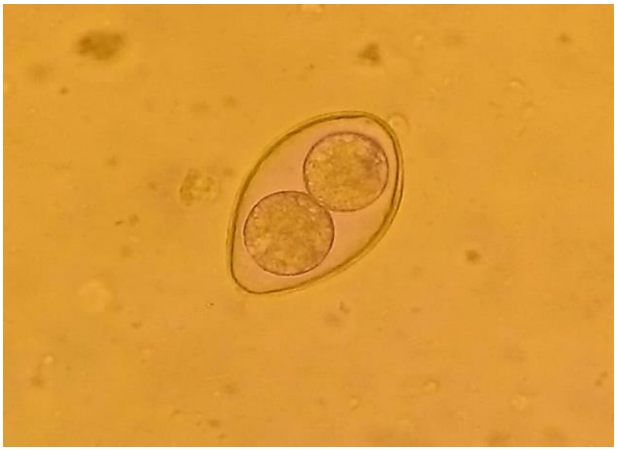

Figure 2: mature (sporulated) oocyst of *Isospora* spp. isolated from infected cats by direct smear method (40x).

Table 1: Shows the percentage of Infection House and Stray Cats with *Isospora* spp. regarding to sex.

Sex	House			Stray		
	No. Examined	No. of infection	%	No. Examined	No. of infection	%
Male	29	3	10.3	25	2	8
Female	31	5	16.1	35	5	14.2
Total	60	8	13.3	60	7	11.6

Table 2: Shows the percentage of Infection House and Stray Cats of *Isospora* spp. according to age.

Age	House			Stray		
	No. Examined	No. of infection	%	No. Examined	No. of infection	%
Young	35	5	14	32	4	12.5
Adult	25	3	12	28	3	10.7
Total	60	8	13.3	60	7	11.6

Table 3: Shows the percentage of Infection House and Stray Cats of *Isospora* spp. according to the months

Duration (month)	House			stray		
	No. of Examined Animals	No. of Infection	%	No. of Examined Animals	No. of infection	%
November/2017	10	1	10	10	1	10
December	10	1	10	10	2	20
January/2018	10	1	10	10	1	10
February	10	1	10	10	1	10

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March	10	2	20	10	1	10
April	10	2	20	10	1	10
Total	60	8	13.3	60	7	11.6

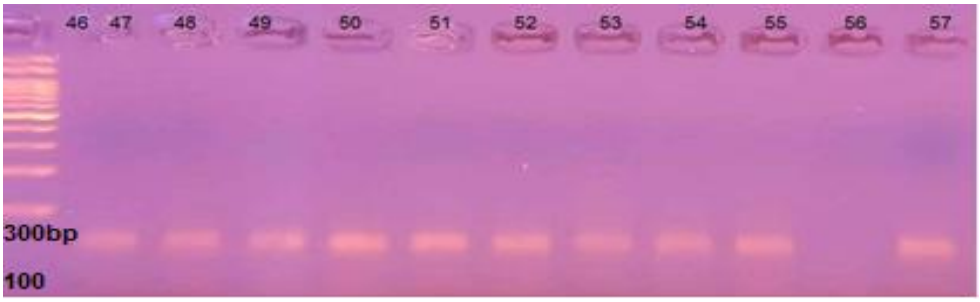

Figure 3: Agarose gel electrophoresis of PCR product of the mitochondrial COI gene of *Isospors spp*. from fecal samples in Lanes 64,47,48,49,50,51,52,53,54,55 and 57 positive samples (300bp). Lanes 56 were control lanes.

DISCUSSION

Cats are particularly vulnerable to parasite diseases due to their proclivity to wander around. Cats carry a wide range of parasites that can be damaging to their health as well as the health of humans around (Krecek *et al*., 2010; Kalef and Al-khayat, 2022). Cats may potentially act as a reservoir for harmful infections to humans (Bowman and Lucio-Forster, 2010). Intestinal protozoa infection was common, with a high overall infection incidence and no statistically significant variations across age groups. Several studies in adjacent countries found different findings, with Bahrami *et al*., (2011) in Iran recording 16.66 percent and Khalafalla, (2011) in Egypt recording 12 percent. Furthermore, the rate of infection with intestinal protozoa in male cats was 22.22 percent (12/54) which differs from the 35.82 % reported by Arbabi and Hooshyar (2009) in Iran. According to Bahrami *et al*. (2011), the infection rate in Iran is 14.28 percent. While Japan had the lowest rate at 5.22 percent (Ito *et al*., 2017). In this study, however, the infection rate in females was 36.36 percent (24/66) compared to 43.47 percent in Arbabi and Hooshyar, (2009) and 21.26 percent in Khalafalla, (2009). Additionally, Bahrami *et al*. (2011) discovered a 25% infection incidence in Iran. While Japan had the lowest rate (3.37 percent) (Ito *et al*., 2017). The explanation for this disparity is attributed to climate characteristics that ensure the development and survival of pre-parasitic stages, resulting in increased availability, as well as geographical location, animal ownership status, and diagnostic techniques, which are responsible for the wide range of endoparasite prevalence (Mundim.*et al*., 2007; Grandi *et al*., 2017).

On the other hand, polymerase chain reaction (PCR) has lately become the most popular and sensitive method for detecting and distinguishing *Isospors* spp. in infected cats (Madani *et al*., 2018). The PCR results indicated that particular primers (COIf, COIr) effectively amplified the mitochondrial COI gene with a 300bp-long fragment to *Isospora* spp. Furthermore, PCR findings indicated that 12 (80%) of the 15 cats tested positive for *Isospora* spp. This result was anticipated (Madani *et al*., 2018). The application of this

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approach in the identification and differentiation of *Isospora* spp. in the current investigation is based on prior findings that demonstrated that identifying parasite species simply based on morphological traits might provide imprecise results owing to substantial variances.within and between species. As a result, molecular techniques such as PCR have been used (Hongliang *et al*., 2011).

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CONFLICT OF INTEREST

Authors declare no competing interests in this article.

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