Traditional detection of *cryptosporidium* spp. in domestic dogs and cats in Baghdad city, Iraq

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Abstract

The aim of this study to investigate the prevalence of *Cryptosporidium* spp. in domestic dogs and cats in Baghdad city during the period of 2/1/2023 to 30/5/2023, by using microscopic examination, 80 fecal samples collected from dogs (40 samples) and (40 samples) from cats. The results revealed that the total rate of *Cryptosporidium* infection was 17.5% and 27.5% in dogs and cats respectively by microscopic examination without significant differences ($p \geq 0.05$). The results showed that male and female (dogs) recorded 20.8% (5/24) and 12.5% (2/16) while in the cats recorded in male and female was 21.4% (3/14) and 30.76% (8/26) respectively rate of infection with *Cryptosporidium* spp. significant differences ($p \geq 0.05$).

Keywords: *Cryptosporidium*, Dogs, Cats, Modified Ziehl-Neelsen

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Introduction

Invasive intracellular (extra cytoplasmic), single-celled parasites known as *Cryptosporidium* species infect both people and animals through the gastrointestinal tract. According to Checkley *et al.* (2015) and Efstratiou *et al.* (2017), *Cryptosporidium* parasites are transmitted mainly through contact with contaminated water (for example, in drinking water or swimming pools) and occasionally through contaminated foodit is a significant parasite that causes mild to severe profuse watery diarrhea in a range of animals and human**.**

As one of the main causes of neonatal diarrhea, cryptosporidiosis is a common zoonotic disease (Amphixenosis) that results in weight loss, growth retardation, morbidity, and death in severe cases. It is caused by the enteric pathogenic parasite *Cryptosporidium* spp. and is widespread in humans and a wide variety of animals (Van dooren and Striepen, 2013; Ryan *et al.,* 2016). The oral route of feces, oocyst-contaminated food and drink, contact with infected animals, and unintentionally contaminated laboratory environments are the main ways that *Cryptosporidium* is transmitted (Rousseau *et al*., 2018; Karimi *et al*., 2023).

Dogs and cats can be important in the zoonotic spread of cryptosporidiosis because of their frequent interaction with their owners; nevertheless, there haven't been any proven cases of zoonotic transmission in Iraq as of yet. Worldwide reports of *Cryptosporidium parvum* from dogs and cats have been made, but at lower rates than those of *C. canis* in dogs and *C. felis* in cats (Souza, *et al*., 2023). The modified Ziehl-Neelsen staining method is primarily used to identify *Cryptosporidium* parasites based on their morphology and oocyst sizes (Kar *et al*., 2014).

Aims of the study

Using conventional diagnostic techniques identify *Cryptosporidium* spp. from dogs and cats kept as pets in various parts of Baghdad and investigate the impact of sex on parasite prevalence.

Materials and methods

Samples collectio

Fecal samples (5-10) gr were collected from 40 pre, post-weaned dogs and cats (40 dogs and 40 cats) of different sex from different regions of Baghdad city, during the period of 2/1/2023 to 30/5/2023.

Fecal samples were extracted straight from the rectum, placed in a sterile plastic container, sealed firmly, and labeled with sequential numbers, the date of the sample, the sex, and any precautions taken, including donning disposable gloves. The samples were separated into two halves for a typical inspection at the College of Veterinary Medicine, University of Al-Qasim Green, and transferred in a refrigerated box to the parasitology laboratory.

Modified ziehl neelsen

It is also helpful to confirm the presence of oocysts of *Isospora belli* and *Cyclospora cayetanensis*. The modified Ziehl-Neelsen stain for faecal smears has already been established for coccidian protozoa, in particular, oocysts of *Cryptosporidium species* by Flotation Methods (Görkem *et al.*, 2022).

Microscopic examination

Microscopic examinations were carried as following:

Flotation methods

According to Chermette and Boufassa (1988), Sheather's and Zinc Sulphate solutions were employed to study *Cryptosporidium* oocysts by using Sheather's solutions method.

Staining method

Smears were produced and stained using Modified Ziehl-Neelsen (mZN) stains to analyze *Cryptosporidium* oocysts, as described by Aboed and Faraj (2017).

Statistical analysi

The Chi-squared (X2) test was performed to compare the findings. Differences were judged statistically significant at $p \leq 0.05$ (Petrie and Watson, 2006).

Results

A total of 80 fecal samples (40 dogs and 40 cats) were examined using traditional methods (floatation and staining with modified Ziehl-Neelsen (mZN) stain) to detect the prevalence of *Cryptosporidium* spp. and revealed the overall infection rate of *Cryptosporidium* spp. in dogs in Baghdad province. The results indicated that 17.5% (7/40) were positive for *Cryptosporidium* oocysts. In cats, the infection rate was 27.5% (11/40) (Table 1).

Table 1: Total infection rate of *Cryptosporidium* spp. in dogs and cats.

Host	Samples	Positive	Infection Rate %
Dogs	40	7	17.5
Cats	40	11	27.5

Prevalence rate of Cryptosporidium spp. *infection in relation to sex*

The findings indicated that male and female (dogs) recorded 20.8% (5/24) and 12.5% (2/16), while in cats recorded 21.4 (3/14) and 30.76 (8/26) correspondingly rate of infection with *Cryptosporidium* spp. with no significant differences ($p \geq 0.05$) (Tables 2 and 3).

Table 2: Prevalence of *Cryptosporidium* infection in dogs

Sex	Samples	Positive	
		No.	%
Male	24	5	20.8
Female	16	2	12.5
Total	40	7	17.5

Significant differences at a level of $p \geq 0.05$, X2= 1.34

Table 3: Prevalence of *Cryptosporidium* infection in cats.

Sex	Samples	Positive	
		No.	%
Male	14	3	21.4
Female	26	8	30.76
Total	40	11	27.5

Significant differences at a level of $p \geq 0.05$, X2= 8.12

Cryptosporidium spp. oocysts

In the Modified Ziehl Neelsen stain, the oocysts of *Cryptosporidium* spp. showed as round densely stained pink to red bodies with a visible halo around the oocyst, on a dark blue background of the methylene blue stain (Fig. 1).

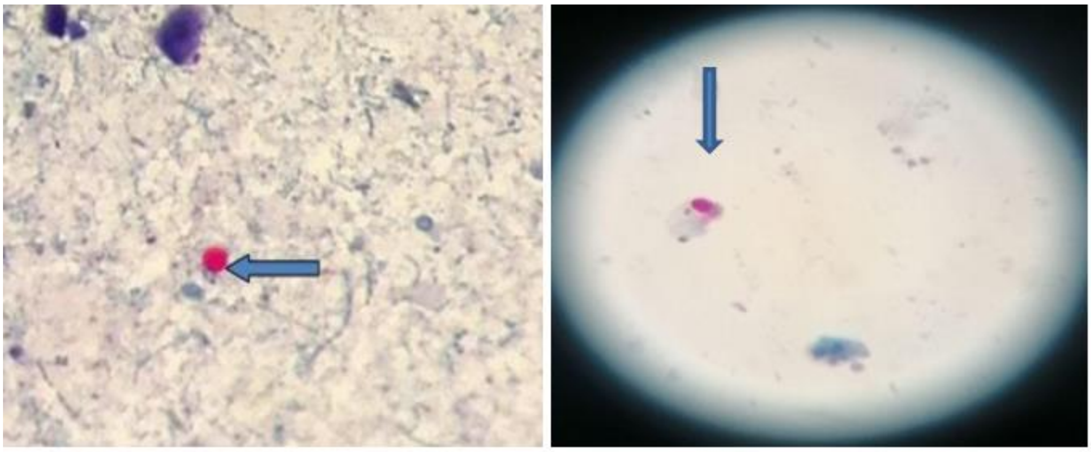

Figure 1: Oocysts of *Cryptosporidium*.

Discussion

Over the years, zoonotic transmission of various species of *Cryptosporidium* spp. and the role of animals as areservoir for human infection have been important issues in medical and veterinary practices (Alali and Alkhaled, 2023). Pets provide several important human benefits but are also associated with health hazards. Besides the risk of bites, scratches, and allergies as common health hazards, cats may harbor various zoonotic parasitic infections. Thus, close contact with these pets is considered a risk factor (Kankya *et al*., 2023). Nearly 300 publications related to *Cryptosporidium* spp. in dogs and cats have been published. Most of these studies were epidemiological surveys of Cryptosporidiosis around the world (Beaver and Jung, 1985; de Waal *et al*., 2022). In early studies, microscope-based morphological methods and antigen detection assays were commonly used to detect *Cryptosporidium* oocytes. The use of traditional tools has led to the identification of several zoonotic *Cryptosporidium* spp.in dogs and cats. *Cryptosporidium canis* and *Cryptosporidium felis* are dominant species causing canine and feline Cryptosporidiosis, respectively. Some *Cryptosporidium* parvum infections have also been identified in both groups of animals. On the other hand, dogs and cats in animal shelters commonly come into contact with adopters and visitors. Although veterinarians regularly impose the disinfection process and exo-parasite chemical deworming program before the animals are admitted, the infection risk of endoparasites still exists. Moreover, once *Cryptosporidium* oocytes are transmitted in animal shelters, the horizontal transmission may spread dramatically because of oral–fecal infection within companion animals, owing to the narrow space and high-contact environment. Thus, to reduce the risk of zoonotic disease occurrence, fecal samples of pet animals should be routinely submitted for parasitic diagnostic tests, and owners should be informed about the public health issues related to pet fecal pollution. In local animal shelters, expansion of the disinfection checkpoints to maintain the biosecurity of the animal shelters is warranted. The identification of *C. canis*, *C. felis*, and *C. parvum* and *C.Andersoni* in pets and owners suggests the possible occurrence of the zoonotic transmission of *Cryptosporidium* spp. between humans and pets (Alali and Alkhaled, 2023).

However, in this investigation, we found out that the prevalence rate of *Cryptosporidium* spp. in dogs and cats in Iraq municipality is still unclear, and this study attempts to provide an early assessment of its spread.

Conclusions

The prevalence of Cryptosoridium spp. in domactic dogs and cats in Baghdad city, by using microscopic examination (flotation and staining) was 17.5% and 27.5% in dogs and cats, respectively. significant differences ($p \geq 0.05$) between male and female in both dogs and cats.

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