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Comprehensive Study of *Toxocara cati* Infection in Stray Cats in Al-Anbar Province: Molecular Detection and Investigation Vision

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A B S T R A C T

Cats are important hosts for different zoonotic including *Toxocara*, a group of parasitic roundworms known as ascarid nematodes. This study aimed to analyse the presence of *T. cati* in stray cats in Al-Anbar province. Iraq This is first study of this parasite in Al-Anbar province, which include the prevalence of *T. cati* in stray cats, as well as molecular study. This study was conducted on 50 stray cats, were examined clinically, the signs were recorded for each animal, as well as the collection of faecal samples to detect the eggs of parasite under microscope, and molecular examinations. Blood samples also examined to know the effect of parasite on blood cells, especially the eosinophils. Our results recognized the prevalence of infection was 48% when the molecular method was used, and 40% when the microscopy method was used. Clinically, the infected cats with signs were 4 (8%) from the total sick animals, as well as the same percentage appeared in haematological examination (the eosinophilia). However, in Al-Anbar cities, T. cati is highly prevalent among stray cats. In order to eradicate the T. cati parasite from the region's stray cat population, efficient methods for doing so need to be devised, and public education on animal and human health should also be stressed.

Keywords: stray cats, *Toxocara cati*, *ITS2* gene, Al-Anbar

INTRODUCTION

Toxocara cati is a parasitic roundworm that commonly infects cats worldwide. It belongs to the family Ascarididae, and it is one of the most common intestinal parasites in cats (1). Kittens are most at risk, although cats of any age may contract this parasite. Infectious eggs are spread through consumption and can be detected in cat faeces or contaminated soil (2). Afterwards, it can grow up to 10 cm in length, and females can produce hundreds of thousands of eggs per day. In addition to cats, this pathogen can also infect other animals such as dogs, foxes, and wild felids (3).

Moreover, the infection in cats can cause a range of symptoms, including vomiting, diarrhea, and poor growth in kittens. In severe cases, it can lead to intestinal obstruction or rupture (4). Humans can also be infected with *T. cati* accidentally by ingesting the infective eggs, which can lead to a condition known as toxocariasis (5, 6). This can cause a range of symptoms, including fever, cough, abdominal pain, and even blindness in severe cases (7, 8). Furthermore, children are under high risk of infection due to their tendency to play in dirty conditions, and put their hands in their mouths (9). More importantly, there are several methods to diagnose this pathogen, for instance, faecal flotation method, that is a commonly microscopic

detection method for detection Toxocara cati eggs in the faeces of infected feline. This technique involves mixing a faecal sample with a solution of higher specific gravity than the eggs, such as zinc sulphate or sodium chloride, and then centrifuging the mixture (10-12). The prevalence of Toxocara infection in reservoir species like dogs and cats can be estimated using molecular assays (13,14), so a novel diagnostic approaches whose results can be compared to those of conventional tests, such as normal stool inspection.

Overall, the prevalence of endoparasites of cats in Europe has been found to vary between 20% and 40% (15). Also, several studies reported that stray or free-ranging outdoor cats have a higher frequency of parasites than indoor kept cats (16, 17). In Greece stray cats were 8.8 times more likely to be infected with Toxocariasis when compared to owned cats. Similarly, the infection of *T. cati* was 2.7 times higher for cats living outdoors in comparison to those staying indoors (18).

Furthermore, there are some studies conducted in Iraq related to the epidemiology of this disease, for example study that led by Hassan and Barzinji (19) investigated the prevalence of this parasite in stray dogs and cats in the north of Iraq, and they have found the infection rate of *T. cati* among stray cats 39.58%. Also, coprological detection of Toxocariasis in domicile of another recent research by (20) on this parasite in Kurdistan region of Iraq, found the infection rate among strays was 47.62%, which about four times that of indoor cats at 5.5%. Furthermore, a comparative study, of some intestinal parasites in faecal samples of domestic and stray cats in Baghdad city, found low infection rate (1.65%) and the infestation reported only in October and February (21). There is no comprehensive study in Al-Anbar province to determine the prevalence and detection of this disease by using molecular assay in stray cats, therefore we have designed this study by using clinic, microscopic, haematology, and molecular methods.

MATERIALS AND METHODS

Ethical Approval

This study was approved by the College of Veterinary Medicine at the University of Baghdad, and the author has validated that all necessary procedures were followed. The first author, Alhayani," obtained the blood samples from the animal in accordance with the aforementioned ethical criteria**.**

Study Area, Sampling, and Designing

Study Area and Sampling

The study was conducted in Al-Anbar, the western province of Iraq, with the sampling period extending from October 2022 to January 2023. A total of fifty faecal and blood samples were collected from stray cats across various cities, namely Al-Ramadi, Al-Fallujah, Heet, Haditha, Al-Qaim, Rawa, and Ana. These samples were classified according to the cats' age, gender, and the region from which they were collected. In addition, detailed records were maintained for any clinical or physical signs observed in the cats.

Parasitological Examination Methodology

For the parasitological examination, the standard flotation method was performed. This involved collecting a small sample (3-5 g) of fresh feces from each cat using a clean container or plastic bag. The feces were then mixed with a flotation solution, like a saturated salt solution, in a cup or tube. The mixture was strained to remove debris, creating a liquid suspension that was transferred to a centrifuge tube and centrifuged at high speed. This process separated the parasite eggs, allowing them to float to the surface of the liquid. The top layer of the liquid containing the eggs was collected with a pipette or syringe, placed on a microscope slide, covered with a coverslip, and examined under a microscope for egg identification and counting (22-24).

Haematological Examination Procedure

The haematological examination involved collecting a small blood sample from the cat's cephalic vein using a sterile needle and syringe. The blood was then spread into a thin layer on a slide, left to dry, and stained with Giemsa stain to differentiate between types of white blood cells. The stained smear was examined under a microscope, and the percentage of each white blood cell (WBC) type was calculated based on the total count (25).

Molecular Assays and DNA Extraction

Molecular assays for genomic DNA isolation from fecal samples utilized the Presto™ Stool DNA Extraction Kit (Geneaid Biotech Ltd) which uses advanced DNA extraction buffer and spin column technology. For PCR amplification and identification of *T. cati*, the ITS2 region of ribosomal DNA was targeted (26). Fifty DNA samples underwent conventional PCR using specific oligonucleotide primers designed for the ITS2 region gene of *T. cati*. The sequences of these primers for the ITS2 region of the *T. cati* 5.8S rRNA gene and internal transcribed spacer 2 are detailed in Table 1.

Table 1. KT873462.1 *T. cati* 5.8S ribosomal RNA gene and internal transcribed spacer 2

Primer	Sequence 5'-3'	Band size
Forward	TGGTGCATTCTTTCGCAACG	232 bp
Reverse	GCCGATGACGTTACCTCCAA	

The PCR process included collecting information after amplification procedures. A 10 µL sample of the amplified product was placed on a 1.5% agarose gel with 3 µL/100 mL ethidium bromide and subjected to an electric field of 80 V for 1 hour. The setup also included a DNA size marker

and a loading buffer. Positive results were determined by matching the number of base pairs in the DNA bands of the sample with that of the target product. The bands were visualized using a UV transilluminator and captured with a digital camera, as documented in Tables 2 and 3.

Table 2. PCR Reaction mixture

Volume	Components
5 µL	DNA
1.5 µL	Forward primer
1.5 µL	Reverse primer
12.5 µL	Master Mix
4.5 µL	Free nuclease water

Table 3. Steps of the PCR condition and thermo cycle. Amplification of the ribosomal ITS-2 gene of *T. cati* is under specific condition of the PCR thermos cycles, and is accompanied by the following stages

Steps	Temperature °C	Time	Cycle
Initial denaturation	94	10 min	40
Denaturation	94	1 min	
Annealing	58	20 sec	
Extension	72	24 sec	
Final extension	72	10 min	

Statistical Analysis

Statistical analysis was conducted by SPSS (version 25) for analysis data, by using the Chi-Square Test (McNemar Test) to evaluate the correlation between all parameters in this study (27). Significant Results (at levels p 0.05) were suggested (28).

Results and Discussion

The prevalence of T. cati infection was also analyzed in terms of geographic region and feline gender (Table 4).

Table 4. Descriptive data of this study

Variable	Sample	
Age	**Number**	**%**
< 1 year	28	56
> 1 year	22	44
Gender		
Female	32	64
Male	18	36
Region		
Al-Fallujah (east)	16	32
Al-Qaim (west)	2	4
Al-Ramadi (middle)	21	42
Ana (west)	3	6
Haditha (west)	3	6
Heet (west)	2	4
Rawa (west)	3	6

Our clinical outcomes indicated variance in their different signs, diarrhea which was the most common sign that recorded. The rest of the signs were uneven, such as general weakness, vomiting, conjunctivitis, and some neurological symptoms. This is in agreement with previous studies (29, 30), when they mentioned that forty-eight (53.3%) out of 90 cats that infected with T. cati appeared with digestive symptoms. Also, in kittens and young cats, diarrhea is the most frequent digestive sign, either as a unique sign or in combination with other digestive signs (vomiting and inappetence) (31, 32)

Microscopical analysis revealed that 20 of 50 samples of stray cat faeces in the Al-Anbar area tested positive for the presence of *T. cati* worm eggs. This represents 40%, of the total 50 *T. cati* eggs, which are characterized by being round, jagged, and having strong walls, when viewed under a microscope at 40× magnification (Figure 1).

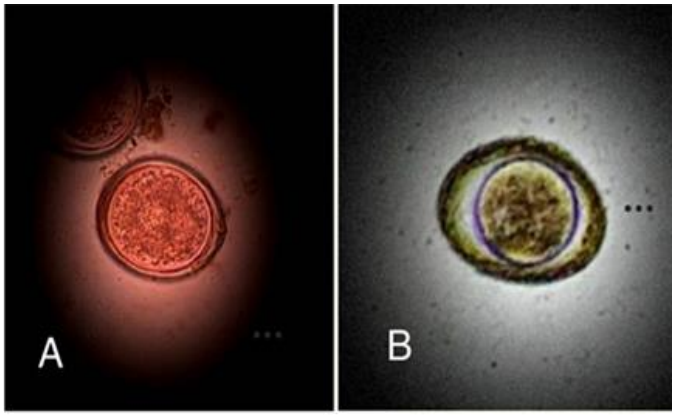

Figure 1. *T. cati* eggs detected under microscopic examination with 40× magnification using two Flotation methods, **(A)** Centrifugal Flotation method, **(B)** Simple test tube Flotation method

Furthermore, the clinical signs in cats that infected with *T. cati* were also low (8%) in compared to the prevalence rate (40%) that found through microscopic identification of eggs in the faeces of stray cats. So, cats with clinical signs of infection (4/50) had a significantly higher prevalence of parasite eggs in their faeces (P = 0.028; Table 5).

Table 5. Evaluation of the clinical signs in relation to *Toxocara* spp. egg presence

	Microscopic eggs detection			
Clinical Signs	**Negative**	**Positive**	**Total**	**P-value**
Normal	44	2	46	0.028
Abnormal	2	2	4	
Total	46	4	50	

This result is agreed with (33) study, when he found a similarly high rate of infection (40%) in stray cats in the city of Mosul. Also, our findings are in line with those of another study on stray cats in northern Iraq with an infection rate 47.62% (20).

These findings of egg percentage appeared with 12% found in males and 28% in females. So, the prevalence of infection did not differ significantly between male and female cats (Table 6, P = 0.33) (34).

Table 6. Prevalence of *T. cati* in microscopically according to sex of stray cats

Clinical Signs	Microscopic eggs detection			P-value
	Negative	Positive	Total	
Female	18	14	32	0.330
Male	12	6	18	
Total	30	20	50	

Later, this study declared 4 of the infected cats had elevated eosinophil counts. So, leukocytosis and eosinophilia were found to be significantly elevated based on the haematological parameters measured in this investigation (Figure 2). Microscopic examination of faeces for parasite eggs revealed a statistically significant (P = 0.021) correlation between the prevalence rate and the percentage rise in eosinophils in the blood (Table 7).

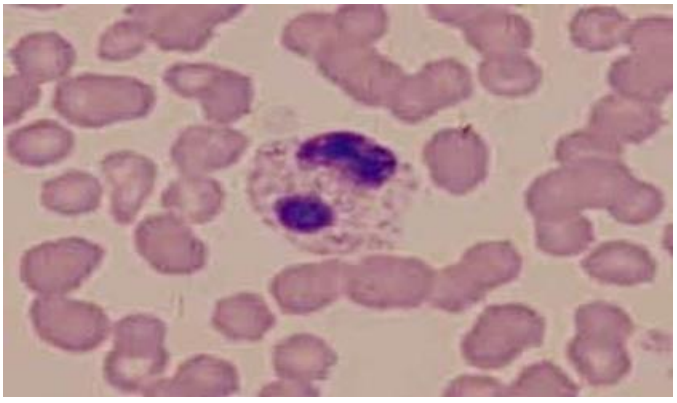

Figure 2. Eosinophil of cats (differential WBC counts.

The same findings, when authors also detected leukocytosis in their animal studies (33, 35). Likewise, leukocytosis and eosinophilia may appear early on in a Toxocara infection (36). It likely that an immune response to the parasite or their toxins, that was stimulated by the unique antigenic structure of the Toxocara larva and bone marrow.

In the molecular side, the amplifying of target gene (ITS2 gene with 232 base pairs) of this parasite from stool samples, revealed a parasite found in 48% of cases (24 of 50) (Figure 3). When compared to more traditional assays, it appears that using PCR technology to detect Toxocara eggs in faecal samples is a very accurate method. This result is resembled to research by (37) when they found T. cati ribosomal DNA in the faeces of infected cats, with a prevalence of infection was 47.0% in stray cats.

Furthermore, only 8%, or 4 cats of the infected animals, developed clinical symptoms, and 8% (or 4 of the infected cats) had elevated eosinophil counts. Clinically, ill cats have an increased eosinophil percentage, which correlates significantly with the prevalence rate of PCR results (P = 0.00). Moreover, all samples that passed the microscopic test also passed the 232 bp amplicon stage of PCR, while only four of the samples that failed the microscopic test passed this step of PCR (8%). Also, there was no statistically significant difference between the results obtained using the molecular method (infection rate, 48%) and those obtained using the microscopic technique (infection rate, 40%) (P = 0.125) (Table 8).

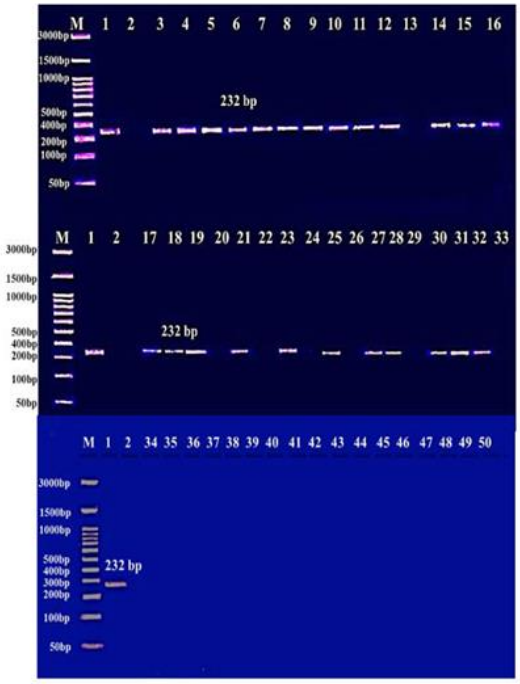

Figure 3. PCR amplification results of internal transcribed spacer 2 region of *T. cati* isolated from stray cats. Agarose gel picture appears the PCR product bands with molecular weight of 232 bp. (M) refers to (3000 bp) DNA ladder, (1) positive control. (2) Negative control. (3-50) PCR results of faeces samples

PCR and clinical signs				
	Clinical signs			
PCR	**Abnormal**	**Normal**	**Total**	**P-value**
Positive	4	20	24	0.000
Negative	0	26	26	
Total	4	46	50	
PCR and Eosinophils results				
	Eosinophils results			
PCR	**Positive**	**Negative**	**Total**	
Positive	4	20	24	0.000
Negative	0	26	26	
Total	4	46	50	
PCR and Microscopic eggs detection				
	Microscopic eggs detection			
PCR	**Positive**	**Negative**	**Total**	**P-value**
Positive	20	4	24	0.125
Negative	0	26	26	
Total	20	30	50	

Similar results were found by (38) in southwestern Iran's, using microscopy and molecular techniques to detect and identify soil contamination by Toxocara eggs, by Using a microscope and PCR which indicated Toxocara eggs in 30.4% and 33.8% of soil samples, respectively.

Toxocara spp. eggs can be distinguished from the eggs of other closely related and/or physically similar adult

helminths by using PCR-based approaches that utilise the ITS1 and ITS2 sections of rDNA (39, 40).

In the present investigation, four faecal samples from cats tested positive in the PCR assay but were negative when examined under the microscope. All the data from microscopical process passed the PCR threshold of 232 base pairs.

Similarity, T. cati was found in all 36 of the copro-microscopically positive faeces samples from cats in a comparable investigation (41). It likely that molecular techniques based on ribosomal and mitochondrial markers have been developed for the precise detection and diagnosis of several Ascarididae species, particularly in their larval and egg phases, which can be tough to identify morphologically (42,43).

The sensitivity and specificity of the PCR are so high that they have extensive consequences for the detection of inviable eggs. However, it could be able to detect infected patients or eggs that have already begun to develop. Microscopic testing was negative for *T. cati* in all the cats tested, but the PCR results were positive (44, 45).

Furthermore, eggs of this parasite may not be shed normally due to a combination of factors, including an extended period in which they are not embryonated and intermittent shedding in patent infections (46,47). Because of these factors, we think that faecal microscopic testing should be used repeatedly in feral cats to confirm an intestinal *T. cati* infection. In addition, if a complete parasitological examination is not possible (for instance, because of insufficient faeces), PCR may be suggested as an alternative (48-50).

The present investigation confirms that the limits of microscopic inspection of eggs for the identification of the infection caused by *T. cati*, which can be overcome by using molecular technologies. Furthermore, Egg measuring can be a valuable and effective tool of indicating the Toxocara species in routine diagnosis, whenever there are grounds for doubt, regarding the infection status and no molecular tools are available. By using a genetic technique with ITS-2 rDNA as a genetic marker, this study represents the first molecular demonstration of Toxocara cati eggs in the faeces of stray cats in Al-Anbar province and in Iraq also. As a result, it is essential to educate the public on both animal and human health and to develop effective programmes for identifying and eradicating the T. cati parasite from the region's stray cat population.

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CONFLICT OF INTEREST

The authors declare no conflict of interest.

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عدوى *Toxocara cati* في القطط الضالة في محافظة الأنبار: الكشف الجزيئي ورؤية استقصائية

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الخلاصة

تعد القطط مضيفًا مهمًا للطفيليات الحيوانية المنشأ المختلفة التي يمكن أن تكون خطرة على صحة الإنسان. إحداها هي توكسوكارا، والتي تشير إلى مجموعة من الديدان الأسطوانية الطفيلية. تهدف هذه الدراسة إلى تحليل وجود التوكسوكارا كاتي في القطط الضالة في محافظة الأنبار. هذه هي الدراسة الأولى لهذا الطفيل في محافظة الانبار، وتشمل انتشار التوكسوكارا كاتي في القطط الضالة، وكذلك الدراسة الجزيئية الأولى. أجريت هذه الدراسة على ٥٠ قط متشرد، وتم فحص الحيوانات سريرياً، وتم تسجيل العلامات لكل حيوان، وكذلك جمع عينات البراز للكشف عن بيض الطفيل تحت المجهر. كما تم فحص عينات الدم لمعرفة تأثير الطفيل على خلايا الدم وخاصة الحمضات. أدركت نتائجنا أن انتشار العدوى كان ٤٨٪ عند استخدام الطريقة الجزيئية، و٤٠٪ عند استخدام طريقة الفحص المجهري. سريريا كانت القطط المصابة بالعلامات ٤ (٨٪) من اجمالي القطط المصابة وبنفس النسبة ظهرت في فحص الدم (فرط الحمضات). ومع ذلك، هناك انتشار كبير لـ *T. cati* بين القطط الضالة في مدينة الأنبار. لذلك، يجب تطوير إجراءات فعالة لتحديد وإزالة هذا الطفيلي من مجموعة القطط الضالة في المنطقة، كما يجب إعطاء الأولوية للتثقيف العام حول صحة الحيوان والبشر.

الكلمات المفاحية: القطط الضالة، توكسوكارا كاتي، الجين *ITS2*، الانبار