Al-Qadisiyah J. Vet. Med. Sci. 2025; 24 (3): 10.29079/qjvms.2025.164969.1115
The 1st National and International Scientific Conference November 26 / 2025
ISSN P: 1818-5746
E: 2313-4429
qjvms.qu@edu.iq

The Rate and Identification of Isolates of *Toxocara canis* in Iraqi Stray Dogs

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Submitted: September 24, 2025
Revised: November 05, 2025
Accepted: November 05, 2025

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Abstract Using molecular and microscopic techniques, we assessed the prevalence of Toxocara canis in 270 stray dogs in Southern Iraq between January and June 2025. Season, age, and gender effects on prevalence were noted. Using the fecal flotation approach, we discovered that almost 23% of the men and women were infected, with 23.7% and 22.8% of the cases being identical. In February, when the temperature was about 26°C and the humidity was about 30%, the prevalence reached its highest point at 40%. Compared to adults (15.8%), the incidence was greater ($P < 0.05$) in younger dogs (34.9%). According to PCR, the identity of T. canis 3 and 4 was 99%, while the prevalence was 58% for 10 isolates that were identical to those obtained in Iran (accession no. KF577855.1). 99% similarity between the 10 isolates' Internal Transcribed Spacer (ITS1) regions and reference sequences of T. canis from China (accession no. JF837169.1) was found in the results.

Keywords: Identification, Toxocara canis, Dogs, PCR, Iraq.

Introduction Dogs can transmit diseases to humans, especially to elderly, children and immuno-compromised persons (1, 2). *Toxocara* (T.) canis is the common roundworm of dogs and can infect humans by fecal-oral transmission to produce toxocariasis (3). Dogs can be infected by ingestion of infective embryonated eggs with second stage larve or by acquisition of larvae in uterus (prenatal), ingestion of infective larval with paratenic hosts such as rodents or transmission of infective larvae by mother's milk (4,5). In the right conditions, toxocara eggs can spread through unembryonated excrement and remain infectious in soil for years (4). *T. canis* is distributed worldwide, particularly in the tropics, where humidity and temperature favour survival of eggs in soil (6).

Diagnosis of helminths in dogs is made by flotation and sedimentation of eggs or larvae because of simplicity and low cost (7). However, PCR using ITS1 and ITS2 segments of rDNA can identify *Toxocara* species precisely (8). A molecular method based on the polymerase chain reaction (PCR) may offer trustworthy markers for a more precise identification of Toxocara species (8). We report prevalence and species of *Toxacara canis* isolated from feces of 270 dogs located at Al-Diwaniya, Iraq.

Material and Methods

Ethical approval

The project was approved (3944 in 23/9/2025) by the Committee for Research Ethics at the College of Veterinary Medicine, University of AL-Qadisiyah, Iraq.

Fresh feces from 270 stray dogs located at Al-Diwaniya, Iraq were placed in sterile plastic containers and labeled by date, age and gender and subjected for examination by flotation as described by (9). *Toxocara canis* isolates the 5.8S ribosomal RNA gene, internal transcribed spacer 1, GenBank: KF577856.1, was used as a primer (Bioneer Company, Korea), as shown in Table 1.

Table (1): PCR product size and primer sequence.

Gene	Primer	Sequence		PCR product size
ITS 5.8S rRNA	*T. canis*	F	3-CTCACCTAGCTATTGCCCGG-5	516bp
		R	5-CCTTGGCAAGGTACGCTGTA-3	

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Following the manufacturer's instructions, 100 fecal samples were used to extract genomic DNA using the AccuPrep® stool DNA Extraction Kit, Bioneer, Korea. At an absorbance of 260–280 nm, the DNA's purity and concentration were verified using a Nanodrop spectrophotometer (THERMO, USA). Table 2 below lists the conditions for the PCR Thermocycler.

Table (2): Conditions for the A thermocycler for PCR process.

PCR step	Temp.	Time	Repeat
First Denaturation	95 °C	5min	1
Denatured	95 °C	30sec.	30 cycle
Annealing	58 °C	30sec.	
Extending	72 °C	60sec.	
Last extension	72 °C	5min.	1
Hold	4 °C	-	-

Agarose gel electrophoresis was used to evaluate the PCR results. Ten PCR positive products of local *Toxocara canis* isolates were sent to The Bioneer Company, Korea to confirm local *Toxocara canis* and for Analysis of phylogenetic trees between local *Toxocara canis* NCBI-Blast and isolates as our own submission of isolates in NCBI-GenBank.

Results

By light microscopy, we found *T. cani* in 63 of 270 fecal samples equivalent to an infection rate of 23.3%. There was no difference in prevalence between males (23.7) and females (22.8%). However younger dogs had higher (P<0.04) incidence (34.9%) compared with adults (15.8%). (Figure-1, Tables 2).

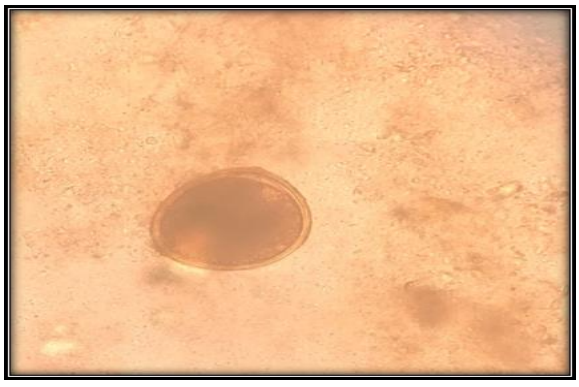

Figure (1): Eggs of T. canis found using light microscopy(40X).

Table (3): Incidence of *T. canis* by age and sex in stray dogs determined by microscopy.

Risk factor	Categories	Tested	Positive	Prevalence	Chi-square	P-value
Sex	Male	143	34	23.7	0.794	0.373
	Female	127	29	22.8		
Age	Young	106	37	34.9	3.842	0.049
	Adult	164	26	15.8		

By PCR, 58% fecal samples from 100 canines tested positive for the ribosomal RNA gene known as internal transcribed spacer 1 (ITS1). DNA sequences were initially aligned withClustalW (MEGA 6.0, multiple alignment analysis tools) for the alignment of several sequences. We discovered 99-100% homolog for sequence of *T. canis* with reference sequences. A phylogenetic tree was constructed with MEGA 6.0 based on local Toxocara canis isolates' partial ITS1 gene sequence (No.1- No.10) utilized for the study of genetic relationships. *Toxocara canis* isolates 1 to 10 were closely related to NCBI-Blast isolated in Iran (KF577855.1). Another Chinese isolate was found and shown in the phylogenetic tree (Figures 2 , 3, 4and Table-4).

Figure (2): Agarose gel electrophoresis picture of the *T. canis* PCR internal spacer that has been transcribed1 (ITS1) ribosomal RNA gene from DNA taken from stray dogs' excrement. The PCR product size of 516 bp indicates that *Toxocara canis* is present in the ladder (2000-100 bp) and other lanes.

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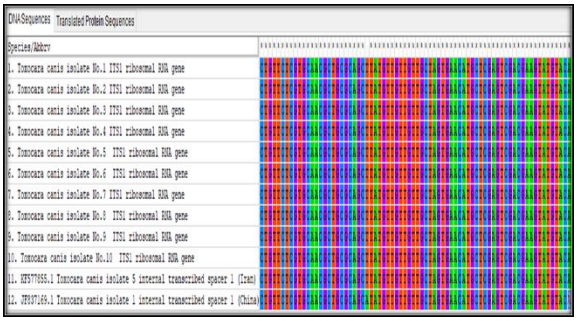

Figure (3): Using MEGA 6.0, multiple sequence alignment study of the partial ribosomal RNA and internal transcribed spacer 1 gene in NCBI-Genbank isolates and local Toxocara canis isolates using ClustalW alignment analysis. The similarity of the multiple alignment analysis (*).

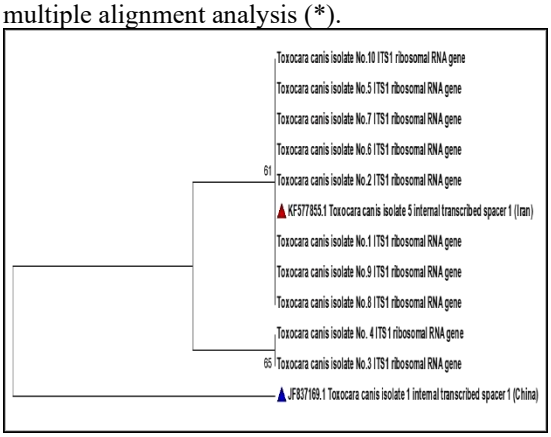

Figure (4): Analysis of phylogenetic trees using the incomplete ITS1 gene sequence in local isolates of *Toxocara canis* (No. 1–No. 10) utilized to analyze genetic relationships. The evolutionary distances were computed using the phylogenetic tree UPGMA algorithm (MEGA 6.0 version).

Table (4): The identity (%) of the homology sequence of NCBI-BLAST between local *Toxocara canis* and NCBI-BLAST submitted *Toxocara canis* Isolates.

Local *T. canis* isolate No.	Genbank Accession number	NCBI-BLAST Homology Sequence identity	
		NCBI Blast *T. canis* Iran isolate (%)	NCBI Blast *T.canis* China isolate (%)
No.1	MF663782	100	99
No.2	MF663783	100	99
No.3	MH213148	99	99
No.4	MH213149	99	99
No.5	MH213150	100	99

Discussion

By microscopy of feces, we found prevalence for *T canis* of 31.9% in Al-Diwaniya in Southern Iraq. This was higher than (10) who reported 21.4% for Diyala and (11) 8.20% for Baghdad, Iraq and for Nigeria (6.3%), Malaysia (11.9%) and Egypt (5.38%) (12, 13, 14). Others found prevalence of 67.5% in Baghdad from 120 canine feces ((15) or 65.5% in 90 strays and 42.4% in domestic dogs (16) These variations in prevalence are probably due to differences in helminth infestations between locations, climatic factors, Veterinary care and public awareness (1).

We found no differences in prevalence between males and females confirming findings of (15, 17and 18). However, we did find a higher incidence in younger dogs as reported by (16 ,19, 20,21) *T. canis* are found more in younger dogs which are mostly transmitted through the placenta (22) and transmammary passage (23 ,24). It should be noted that others found no difference by age (25). Also we found no differences between months, with possible higher infections during February. Cold may prolong survival of eggs in soil with temperature and humidity favouring development of infective larvae (25). Study by(27) found lower prevalence in summer caused by high temperatures which prevented survival of parasites. Study by (25) reported higher rates in Basrah during winter and (15) in Baghdad during February and March.

Molecular techniques are being increasingly used to diagnose parasites at different life-cycle stages (28). We isolated DNA from dogs feces to detect *T. canis* ITS1 gene to support our microscopic and serological methods by conventional PCR technique. ITS1 was found between the nuclear ribosomal DNA's 18S, 5.8S, and 28S coding areas, which are employed for species-level diagnostics (28). For other parasitic helminths, ITS sequences have been shown to be helpful molecular markers that can function as a reliable genetic marker for identification (29). Using this method we found 58% 100 dogs' feces from Al-Diwaniya were contaminated. Therefore, it seems that the PCR assay is a sensitive, specific, and trustworthy technique for determining *T. canis*. *Toxocara canis* differentiation using molecular techniques like PCR is sensitive enough to identify Toxocara spp. eggs in feces and detect low parasite levels. Thus, PCR-based methods have offered helpful substitutes for precise *Toxocara* species identification and separation (30).

The evolutionary relationships of an organism can be learned through phylogenetic analyses based on variations in DNA sequences. The molecular characterisation of Toxocara species has not been

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extensively studied, however (31) reported using internal transcribed spacer (ITS)-2 sequences to differentiate nematodes of *T. canis, T. cati,* and *T. leonina*. BLAST 644 in the current investigation

Conflict of interest

There is no conflict of interest in this study as stated by the authors.

Acknowledgment

Not applicable.

Funding source

This research had no specific fund; however, it was self-funded by the authors.

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