

**MICROSCOPIC AND NESTED-PCR DETECTION OF *TOXOCARA CANIS* AND  
*TOXOCARA CATI* IN DOGS AND CATS FROM AL-QADISIYAH PROVINCE****\*Murtadha Nabeel Murtadha Al-Tameemi, Ali Bustan Muhsen Al-Waaly**

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Article Received: 17 February 2026

Article Revised: 07 March 2026

Article Published: 01 April 2026

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DOI: <https://doi.org/10.5281/zenodo.19330423>**How to cite this Article:** \*Murtadha Nabeel Murtadha Al-Tameemi, Ali Bustan Muhsen Al-Waaly (2026). Microscopic And Nested-Per Detection Of *Toxocara Canis* And *Toxocara Cati* In Dogs And Cats From Al-Qadisiyah Province. World Journal of Advance Healthcare Research, 10(4), 71–78.

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**ABSTRACT**

*Toxocara cati* and *Toxocara canis* are roundworms, nematodes belonging to the order Ascaridida. They are common intestinal parasites in pets, particularly cats and dogs, respectively. These parasites are significant because they are zoonotic diseases, meaning humans can be infected accidentally through the ingestion of parasite eggs, leading to various illnesses. This study investigated *T. cati* and *T. canis* in cats and dogs in Al-Qadisiyah Governorate, from December 2024 to September 2025. It involved microscopic examination of 200 fecal samples (100 from cats and 100 from dogs). The results showed an infection rate of (19%) in cats and (16%) in dogs. According to lifestyle, the infection rate was higher in stray cats and dogs than in domestic cats and dogs, the results in cats showed statistically significant difference ( $P= 0.045$ ), while in dogs there is no significant difference ( $P= 0.568$ ). According to age and living site, the infection rate in kittens and puppies, also rural areas higher than among adults and urban areas, and the results were statistically significant difference at ( $P \leq 0.05$ ), while the differences according to gender and months did not have a statistically significant at ( $P>0.05$ ). The positive samples were confirmed using molecular methods by the Nested PCR technique, which is considered more sensitivity and specificity. The results demonstrated the importance of studying the parasite due to its pathological and economic impact on domestic animals.

**KEYWORD:** *Toxocara cati*, *Toxocara Canis*, Cats, Dogs, PCR.**INTRODUCTION**

*Toxocara cati* and *Toxocara canis* are among common nematodes of felids and canids, causing toxocariasis (Natália *et al.*, 2020). The definitive hosts of *T. cati* and *T. canis* are cats and dogs respectively, both are have zoonotic importance. In the intestine of the definitive host, mature worms lay eggs. Unembryonated eggs are excreted with feces into the environment and be embryonated to be infective eggs, when ingested by human, the larval migration occur by larvae migrate to different viscera (Carvalho and Rocha 2011). Dogs are most often by ingesting mature eggs from the environment or by consuming paratenic hosts such as rodents or poultry. Vertical transmission also occurs in dogs from mother to puppies via the placenta, transplacental or through breast milk, transmammary during the puppy stage (Bowman, 2020). Cats are

infected by ingesting mature eggs or by consuming paratenic hosts like mice and birds and transmission through transmammary may also occur in kittens (Taylor *et al.*, 2016). Many adults may have no obvious symptoms (Bonilla-Aldana *et al.*, 2024), In severe infections or in puppies: you may notice a pot-bellied abdomen, poor growth, malnutrition, cachexia, a generally poor appearance, diarrhea, vomiting, sometimes coughing or respiratory symptoms due to larval migration through the lungs. In rare cases, severe intestinal obstruction or life-threatening complications may occur in puppies with high density infections (Bowman, 2009). Infected kittens may show slowed growth, loss of appetite, bloated abdomen, and general weakness (Wu, 2023). the diagnosis is mainly done by examination of eggs in feces under the light microscope (Okulewicz *et al.*, 2012), by serological tests, such as

Western blot and ELISA (Enzyme Linked Immunosorbent Assay) (Noordin *et al.*, 2020), or by molecular methods, as PCR (Polymerase Chain Reaction) technique (Khademvatan *et al.*, 2013). In recent years, there has been increased interest in toxocara in Iraq; however, research on this parasite remains limited, both in the past and present. Among recent Iraqi studies, (Alani and Kawan, 2024) study in Baghdad province, (Alhayani and Alshawi, 2024) study in Al-Anbar province, (Thamer *et al.*, 2022) study in Basrah, (Jarad, *et al.*, 2019) study in Al-Diwaniah province, (Rashid *et al.*, 2022) study in Sulaimani province and (Al-Daamy and Al-Khaled, 2025) study in Karbala. The study aimed to microscopic diagnosis of *Toxocara cati* and *Toxocara canis* eggs in fecal samples from cats and dogs in Al-Qadisiyah province, and to confirm the diagnosis of positive samples by molecular testing using Nested-PCR technique.

## MATERIALS AND METHODS

### Sample collecting

Two hundred fresh fecal samples were collected from stray and domestic cats and dogs (100 cats and 100 dogs). Samples were collected in plastic containers, and the date, sex, age, location, and whether stray or domestic were recorded. The samples are then sent to the Parasitology Laboratory at the College of Science, Al-Qadisiyah University, for microscopic detection. The samples were collected during the period from December 2024 to September 2025.

### Direct Smear

Using a wooden stick, a small amount of fecal sample was taken and placed on a glass slide, mixed with drops of distilled water, then the slide cover was placed and examined under a microscope at 40X and 100X to detect *Toxocara* eggs (Urgel *et al.*, 2019).

### Floation Method

Approximately 4 grams of fecal sample were placed in glass beaker and mixed well with a small amount of tap

water. The mixture was then filtered and collected in test tubes. The mixture was centrifuged at 1000 rpm for 3 minutes. The supernatant was then decanted and 10 ml of shehear sugar solution was added to the precipitate, mixing well. The mixture was centrifuged again at the same speed for 5 minutes. A drop was then taken from the surface of the tube by a pipette and placed on a glass slide, covered by glass cover slide and examined under a microscope at 40X and 100X (Urgel *et al.*, 2019).

### Molecular Study

The Nested PCR technique was performed for detection *T. cati* and *T. canis* from cats and dogs fecal samples. Primers based on 5.8S small subunit ribosomal RNA gene and internal transcribed spacer 2 region sequence according to (Pourshahbazi *et al.*, 2023) and Nested PCR primers were design using NCBI-Genbank and primer3 plus, table 1.

### Fecal DNA Extraction

Fecal DNA from fecal samples were extracted by using Presto™ Stool DNA Extraction Kit and done according to company instructions.

### Genomic DNA estimation

The extracted genomic DNA from stool samples was checked by using Nanodrop spectrophotometer (THERMO. USA), that check and measurement the purity of DNA through reading the absorbance in at (260 /280 nm).

### Nested-PCR

The Nested PCR master mix was prepared by using (Go taq Green PCR Master Kit) and this master mix done according to company instructions. The technique was conducted in two rounds, first round PCR Thermo-cycle and second round nested-PCR Thermo-cycle, As shown in the table 2. The Nested-PCR products was analyzed by agarose gel electrophoresis.

**Table 1: primers for *T. cati*.**

| Primers    | Sequence 5'-3' |                       | Product size | Reference and Genbank code        |
|------------|----------------|-----------------------|--------------|-----------------------------------|
| PCR        | F              | CTTCTGGTGCATTCTTTTCGC | 204bp        | Pourshahbazi <i>et al.</i> , 2023 |
|            | R              | CCAAGCAACAACAACTACGC  |              |                                   |
| Nested PCR | F              | AACGTGCATTTCGGTGAGCTA | 139bp        | KY003083.1                        |
|            | R              | GGAACACATACGCCAATGGC  |              |                                   |

### Primers for *T. canis*

| Primers    | Sequence 5'-3' |                      | Product size | Reference and Genbank code        |
|------------|----------------|----------------------|--------------|-----------------------------------|
| PCR        | F              | ATTAACGCGCAAGGTTGTGG | 260bp        | Pourshahbazi <i>et al.</i> , 2023 |
|            | R              | TGGCCATGCATTCTTCATTC |              |                                   |
| Nested PCR | F              | GTGAGCTATGCTGGTGTGGT | 206bp        | PX062270.1                        |
|            | R              | ATAACGCCACCTCCAACCTG |              |                                   |

**Table 2: PCR Thermo-cycler Conditions.**

| PCR step             | Temp. | Time    | Repeat   |
|----------------------|-------|---------|----------|
| Initial Denaturation | 95°C  | 5min.   | 1        |
| Denaturation         | 95 °C | 30sec.  | 35 cycle |
| Annealing            | 61 °C | 30sec   |          |
| Extension            | 72 °C | 2min.   |          |
| Final extension      | 72 °C | 5min.   | 1        |
| Hold                 | 4 °C  | Forever | -        |

**Nested PCR Thermo-cycler Conditions**

| Nested PCR step      | Temp. | Time    | repeat   |
|----------------------|-------|---------|----------|
| Initial Denaturation | 95°C  | 5min.   | 1        |
| Denaturation         | 95 °C | 30sec.  | 35 cycle |
| Annealing            | 60 °C | 30sec   |          |
| Extension            | 72 °C | 1min.   |          |
| Final extension      | 72 °C | 5min.   | 1        |
| Hold                 | 4 °C  | Forever | -        |

**Statistical analysis**

Chi-square test was used to study association between any two categorical variables. The level of significance was considered at P-value of less 0.05 and highly significant level at 0.01 or less (Daniel, 2018).

**RESULTS****Microscopic results**

*T. cati* and *T. canis* were detected by microscopic examination by identifying the parasite eggs, *T. cati* egg appears to have a shell with pits slightly spaced apart from each other, comparison of *T. canis* egg with a shell that has a regular pits that illustrated in the figure 1 and 2.

**Figure 1: *T. cati* egg under light microscopy (X40).****Figure 2: *T. canis* egg under light microscopy (X40).****Prevalence of *T. cati* and *T. canis* infection in cats and dogs**

The current study included 100 cats and 100 dogs for microscopic detection of *T. cati* and *T. canis*. The results showed that 19 (19.0%) of the cats were infected with *T. cati*, and 16 (16.0%) of the dogs were infected with *T. canis*, the difference was not statistically significant, as shown in Table 3.

**Prevalence of *T. cati* and *T. canis* infection according to Lifestyle of cats and dogs**

Depending on the lifestyle of cats and dogs shown in the table 4, the infection of *T. cati* in stray cats was 14(26.4%) higher than that of domestic cats, which was 5(10.6%), and the difference was significant ( $P= 0.045$ ). The infection of *T. canis* in stray dogs was 10(17.8%), was slightly higher than that of domestic dogs, which was 6(13.6%), but the difference was non-significant ( $P= 0.568$ ).

**Prevalence of *T. cati* and *T. canis* infection according to gender of cats and dogs**

As shown in the table 5, the prevalence of *T. cati* infection according to gender included 10(18.2) in male cats, that was slightly lower than 9(20%) female, the

difference was non-significant (P= 0.818). Also the prevalence of infection with *T. canis* included 8(15.1%) in male dogs was slightly lower than 8(17%) in female, also the difference was non-significant (P= 0.793).

**Prevalence of *T. cati* and *T. canis* infection according to age of cats and dogs**

The results showed that the infection according to age in table 6, *T. cati* infection included 6(10.2%) in Adults cats was lower than 13(31.7%) in Kittens, the difference was significant (P= 0.007). Also the prevalence of infection with *T. canis* in Adults 5(8.6%) was lower than 11(26.2%) in Poppies, also the difference was significant (P= 0.018).

**Prevalence of *T. cati* and *T. canis* infection according to living sites of cats and dogs**

As shown in the table 7, the prevalence of *T. cati* infection in cats according to living sites included 12(30%) in rural higher than 7(11.7%) in urban, the difference was significant (P= 0.022). Also the prevalence of *T. canis* infection in dogs was 12(27.9%) in rural higher than 4(7%) in urban, also the difference was significant (P= 0.005).

**Prevalence of *T. cati* and *T. canis* infection according to months of cats and dogs**

The results showed according to the months of study, the highest infection with *T. cati* in cats was 4(40%) in April, and the lowest infection 1(9%) in June, the difference was non-significant (P= 0.520). The highest infection with *T. canis* in dogs was 3(33.3%) in March, and the lowest infection was 1(9%) in July, also the difference was non-significant (P= 0.825) as shown in the table 8.

**Table 3: Prevalence of *T. cati* and *T. canis* infection in cats and dogs.**

| Host           | Samples No. | + ve | Percentage % |
|----------------|-------------|------|--------------|
| Cat            | 100         | 19   | 19           |
| Dog            | 100         | 16   | 16           |
| Total          | 200         | 35   | 17.5         |
| X <sup>2</sup> |             |      | 0.322        |
| P value        |             |      | 0.577        |

**Table 4: Prevalence of *T. cati* and *T. canis* infection of cats and dogs according to Lifestyle.**

| <i>T. cati</i> infection  |             |      |              |
|---------------------------|-------------|------|--------------|
| Lifestyle                 | Samples No. | + ve | Percentage % |
| Domestic                  | 47          | 5    | 10.6         |
| Stray                     | 53          | 14   | 26.4         |
| Total                     | 100         | 19   | 19           |
| X <sup>2</sup>            |             |      | 4.042        |
| P value                   |             |      | 0.045*       |
| <i>T. canis</i> infection |             |      |              |
| Lifestyle                 | Samples No. | + ve | Percentage % |
| Domestic                  | 44          | 6    | 13.6         |
| Stray                     | 56          | 10   | 17.9         |
| Total                     | 100         | 16   | 16           |

|                |          |
|----------------|----------|
| X <sup>2</sup> | 0.327    |
| P value        | 0.568 NS |

\*: Significant at P ≤ 0.05, NS: No Significant at P > 0.05.

**Table 5: Prevalence of *T. cati* and *T. canis* infection of cats and dogs according to gender.**

| <i>T. cati</i> infection  |             |      |              |
|---------------------------|-------------|------|--------------|
| Gender                    | Samples No. | + ve | Percentage % |
| Male                      | 55          | 10   | 18.2         |
| Female                    | 45          | 9    | 20.0         |
| Total                     | 100         | 19   | 19           |
| X <sup>2</sup>            |             |      | 0.053        |
| P value                   |             |      | 0.818 NS     |
| <i>T. canis</i> infection |             |      |              |
| Gender                    | Samples No. | + ve | Percentage % |
| Male                      | 53          | 8    | 15.1         |
| Female                    | 47          | 8    | 17.0         |
| Total                     | 100         | 16   | 16           |
| X <sup>2</sup>            |             |      | 0.069        |
| P value                   |             |      | 0.793 NS     |

\*: Significant at P ≤ 0.05, NS: No Significant at P > 0.05.

**Table 6: Prevalence of *T. cati* and *T. canis* infection of cats and dogs according to age.**

| <i>T. cati</i> infection  |             |      |              |
|---------------------------|-------------|------|--------------|
| Age                       | Samples No. | + ve | Percentage % |
| Adults                    | 59          | 6    | 10.2         |
| Kittens                   | 41          | 13   | 31.7         |
| Total                     | 100         | 19   | 19           |
| X <sup>2</sup>            |             |      | 7.291        |
| P value                   |             |      | 0.007*       |
| <i>T. canis</i> infection |             |      |              |
| Age                       | Samples No. | + ve | Percentage % |
| Adults                    | 58          | 5    | 8.6          |
| Poppies                   | 42          | 11   | 26.2         |
| Total                     | 100         | 16   | 16           |
| X <sup>2</sup>            |             |      | 5.595        |
| P value                   |             |      | 0.018*       |

\*: Significant at P ≤ 0.05, NS: No Significant at P > 0.05.

**Table 7: Prevalence of *T. cati* and *T. canis* infection of cats and dogs according to living sites.**

| <i>T. cati</i> infection  |             |      |              |
|---------------------------|-------------|------|--------------|
| Living sites              | Samples No. | + ve | Percentage % |
| Rural                     | 40          | 12   | 30.0         |
| Urban                     | 60          | 7    | 11.7         |
| Total                     | 100         | 19   | 19           |
| X <sup>2</sup>            |             |      | 5.241        |
| P value                   |             |      | 0.022*       |
| <i>T. canis</i> infection |             |      |              |
| Living sites              | Samples No. | + ve | Percentage % |
| Rural                     | 43          | 12   | 27.9         |
| Urban                     | 57          | 4    | 7.0          |
| Total                     | 100         | 16   | 16           |
| X <sup>2</sup>            |             |      | 7.958        |
| P value                   |             |      | 0.005 *      |

\*: Significant at P ≤ 0.05, NS: No Significant at P > 0.05.

**Table 8: Prevalence of *T. cati* and *T. canis* infection of cats and dogs according to months.**

| Month                | <i>T. cati</i> infection |      |      | <i>T. canis</i> infection |      |      |
|----------------------|--------------------------|------|------|---------------------------|------|------|
|                      | Samples No.              | + ve | %    | Samples No.               | + ve | %    |
| December             | 10                       | 1    | 10   | 9                         | 1    | 11.1 |
| January              | 9                        | 1    | 11.1 | 10                        | 1    | 10   |
| February             | 9                        | 1    | 11.1 | 9                         | 1    | 11.1 |
| March                | 10                       | 3    | 30   | 9                         | 3    | 33.3 |
| April                | 10                       | 4    | 40   | 10                        | 2    | 20   |
| May                  | 12                       | 3    | 25   | 12                        | 2    | 16.6 |
| June                 | 11                       | 1    | 9    | 10                        | 1    | 10   |
| July                 | 10                       | 1    | 10   | 11                        | 1    | 9    |
| August               | 10                       | 1    | 10   | 10                        | 1    | 10   |
| September            | 9                        | 3    | 33.3 | 10                        | 3    | 30   |
| <b>Total</b>         | 100                      | 19   | 19   | 100                       | 16   | 16   |
| <b>X<sup>2</sup></b> | 8.143                    |      |      | 5.108                     |      |      |
| <b>P value</b>       | 0.520 NS                 |      |      | 0.825 NS                  |      |      |

\*: Significant at  $P \leq 0.05$ , NS: No Significant at  $P > 0.05$ .

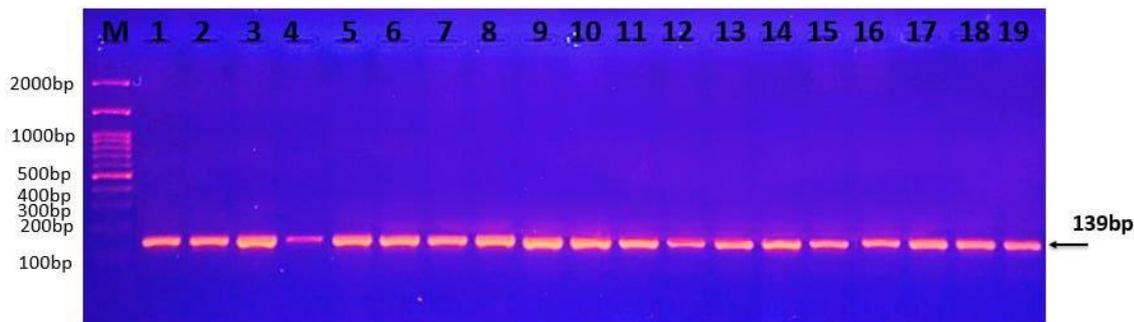
**Molecular results**

Microscopic diagnosis was confirmed by molecular methods using the Nested-PCR technique. Thirty-five fecal samples were tested (19 fecal samples positive for *T. cati* from cats and 16 fecal samples positive for *T. canis* from dog).

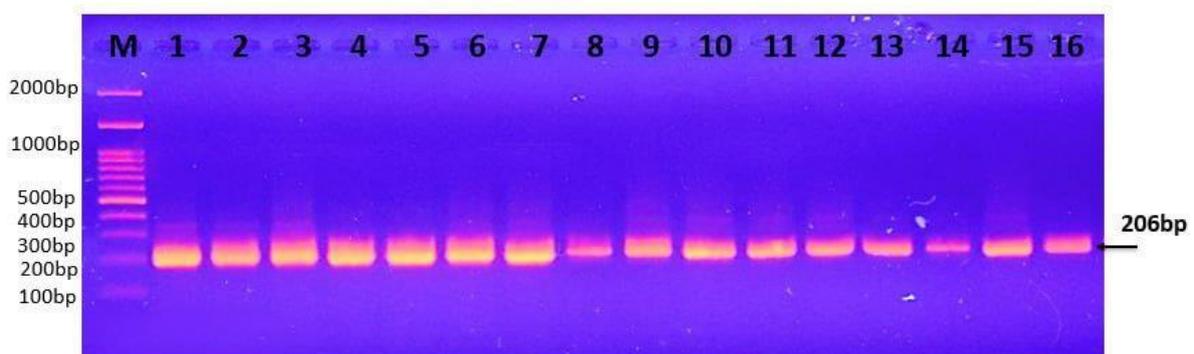
Positive results for *T. cati* appear within the molecular size of 139 bp partial area of ITS2 and 5.8 S rRNA, the

positive samples were from 1-19, and the molecular marker ranges from 100-2000 bp, that illustrated in the figure 3.

Positive results for *T. canis* appear within the molecular size of 206 bp partial area of ITS2 and 5.8 S rRNA, the positive samples were from 1-16, and the molecular marker ranges from 100-2000 bp, that illustrated in the figure 4.



**Figure 3: Agarose gel electrophoresis image for *T. cati*.**



**Figure 4: Agarose gel electrophoresis image for *T. canis*.**

## DISCUSSION

In the study, the infection rate in cats was 19%, which is slightly higher than the global average of 17%, but within the range of regional variation, not an exceptional difference. The rate in dogs was 16%, which is higher than the global average of 11.1% and close to or within the range of high regional estimates, the Eastern Mediterranean, Africa, and some other regions (Rostami *et al.*, 2019). The infection rate in cats was slightly lower than Alani and Kawan (2024) study (23%) in Baghdad province, and lower than Alhayani and Alshawi (2024) study (40%) in Al-Anbar province, and higher than Pourshahbazi *et al.*, (2023) study (16.52%) in Isfahan. The infection rate in dogs was higher than Thamer *et al.*, (2022) study (10.5%) in Basrah, and lower than Jarad, *et al.*, (2019) study (58%) in Al-Diwaniah province. This difference is attributed to local environmental and climatic factors, the prevalence of stray animals, and the inadequacy of periodic parasite removal programs (Bonilla-Aldana *et al.*, 2024). The infection was higher in stray cats (26.4%) and dogs (17.9%) than in domestic cats (10.6%) and dogs (13.6%), statistically in cats with Significant difference, but in dogs there is no Significant difference. According to lifestyle, the study agreed with Rashid *et al.*, (2022) study in Sulaimani province on stray and domestic cats and dogs. Owned animals often receive periodic deworming treatments or follow-up veterinary care, whereas stray animals often do not (Wickramasinghe *et al.*, 2021). The infection was higher in female cats (20%) and dogs (17%) than in male cats (18.2%) and dogs (15%), statistically there is no Significant difference. According to gender, the study agreed with Nijse *et al.*, (2016) study on dogs, and (Alani and Kawan 2024) study on cats. It is sometimes noted that males have capabilities that females possess, and sometimes noted that no clear gender difference is mentioned, no clear gender difference. (Rostami *et al.*, 2020). The infection in kittens (31.7%) and puppies (26.2%) was higher than in adult cats (10.2%) and dogs (8.6%), statistically, there is a significant difference. According to age, the study agreed with Al-Daamy and Al-Khaled (2025) study in Karbala on dogs, and (Alani and Kawan 2024) study on cats. kittens and Puppies often play on the ground, pick things up with their mouths, and are less careful, which exposes them more to infectious oocysts in the soil or contaminated environment (Bourgoin *et al.*, 2022). The infection in cats (30%) and dogs (27.9%) in rural was higher than in cats (11.7%) and dogs (7%) in urban, statistically, there is a significant difference. According to age, the study agreed with Pourshahbazi *et al.*, (2023) study on cats and dogs. In rural areas, animals often roam freely, unlike urban animals which may be more restricted. This increases the likelihood of them encountering polluted environments, eating paratenic hosts, or contaminated soil (Studzińska *et al.*, 2017). The highest infection with *T. cati* in cats was (40%) in April, and the lowest infection (9%) in June, while the highest infection with *T. canis* in dogs was (33.3%) in March, and the lowest infection was (9%) in July, statistically there is no

significant difference, the study agreed with Raissia *et al.*, (2020) in Tehran. The outer eggs of *Toxocara* require moderate humidity and not too high temperatures to mature and become infectious. Spring and autumn usually provide better warmth and humidity than winter or harsh summer (Avcioglu and Burgu 2007). The use of Nested-PCR in confirming the diagnosis increases sensitivity, which increase the ability to detect small amounts of the target DNA (Green and Sambrook 2019), Increase specificity, reducing the amplification of unwanted or untargeted fragments, is achieved by using two pairs of primers, the first is used for initial amplification, and the second is used “inside” the first product (Bello, 2023).

## CONCLUSIONS

The study results showed that cats were infected with *Toxocara cati*, and dogs with *Toxocara canis*. The infection rate was higher in stray animals than in domesticated ones, and also higher in young animals than adults. Infection was also more common in spring and autumn than in winter and summer. Gender did not have a statistically significant effect. Regarding the confirmation of molecular diagnosis, the Nested PCR technique demonstrated high diagnostic sensitivity, highlighting the importance of using this technique with low concentrations of genetic material to ensure accurate scientific results.

## ACKNOWLEDGMENT

The Researcher would like to thank the Department of biology, College of Science, Al-Qadisiyah University for facilitating scientific research.

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