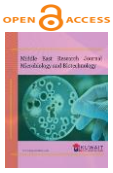

Middle East Research Journal of Microbiology and Biotechnology
ISSN: **2789-8644 (Print & Open Access)**
Frequency: Bi-Monthly
DOI: https://doi.org/10.36348/merjmb.2026.v06i03.003

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Immunological and Molecular Detection of *Toxocara canis* in Dogs and Owners in Babylon Province

Ayat Falah Hassan[1]*, Nawras Abdul-Bari Al-Kaabii[1]
[1]Department of Parasitology, College of Veterinary Medicine, Al-Qasim Green University, Babylon 51013, Iraq

Abstract: In endemic areas, the zoonotic helminth illness canine toxocariasis, which is caused by *Toxocara canis*, has a major impact on both public and canine health. This study's objectives were to assess immunological and molecular diagnoses of *Toxocara canis*, ascertain the disease's prevalence in dogs and their owners in Babylon Province, and assess the impact of sex, age, and geography on infection rates. A total of 150 samples were analyzed; 75 canine fecal samples were designated for molecular diagnosis and the PCR method showed a 54.67% prevalence rate. The ELISA method was used to analyze 75 dog owners' blood samples for the immunological evaluation. demonstrating a 17.33% zoonotic seroprevalence rate. The highest infection and exposure rates were found in the Al-Kifl district, indicating the influence of environmental contamination and husbandry techniques. Infection frequency varied by location. The established stability of this zoonotic agent in the area was supported by phylogenetic and sequencing investigations of three local isolates (PZ507135, PZ507134, and PZ507136), which revealed a high genetic identity (99.4% to 100%) with international isolates from several global lineages. These results highlight the necessity of integrated control measures, such as genetic surveillance, public health education, and stray dog management, to lessen the effects of zoonotic toxocariasis.

Keywords: Toxocariasis, Molecula, PCR, ELISA, Seroprevalence, Zoonosis, Iraq.

Research Paper

***Corresponding Author:**
Ayat Falah Hassan
Department of Parasitology, College of Veterinary Medicine, Al-Qasim Green University, Babylon 51013, Iraq

How to cite this paper:
Ayat Falah Hassan & Nawras Abdul-Bari Al-Kaabii; "Immunological and Molecular Detection of *Toxocara canis* in Dogs and Owners in Babylon Province" Middle East Res J. Microbiol Biotechnol., 2026 Jul-Aug 6(3): 166-172.

Article History:
| Submit: 25.06.2026 |
| Accepted: 29.07.2026 |
| Published: 01.08.2026 |

INTRODUCTION

Worldwide, toxocariasis is a common and often ignored zoonotic helminthic infection that has a significant negative impact on both animal welfare and population health (Saravanan *et al*., 2016; Rostami *et al*., 2020). The ascarid nematodes *Toxocara canis* and *Toxocara cati*, which live in the small intestines of dogs and cats, are the source of this illness (Chen *et al*., 2018; Rashid *et al*., 2022). Millions of non-embryonated eggs are released by mature female worms through their feces (Sowemimo, 2012; Ma *et al*., 2019). These eggs grow into the infectious stage (L3) in a matter of weeks, contaminating public soils and the environment (Mircean *et al*., 2011; Fakhri *et al*., 2018). *T. canis* is primarily found in dogs (Othman *et al*., 2021; Rashid *et al*., 2022). According to Carlin and Tyungu (2020), the transmission can occur by vertical transmission channels, paratenic hosts, or ingestion of embryonated eggs. The most effective routes of transmission in newborn puppies are transplacental and transmammary (Garces *et al*., 2020), which results in severe gastrointestinal symptoms and emaciation (Carlin and Tyungu, 2020; Rashid *et al*., 2022). On the other hand, somatic larval migration and encystment in tissues result from adult dogs developing an age-associated resistance (Magnaval *et al*., 2001; Garces *et al*., 2020). Due to geophagia, inadequate cleanliness, or tainted food and water, humans might unintentionally become paratenic hosts (Strube *et al*., 2013; Chen *et al*., 2018). Visceral Larva Migrans (VLM), Ocular Larva Migrans (OLM), and Neurotoxocariasis (NLM) are inflammatory reactions caused by ingested larvae that migrate through systemic circulation into various organs (Despommier, 2003; Fillaux and Magnaval, 2013; Saravanan *et al*., 2016; Chen *et al*., 2018). Due to their intimate soil contact and behavioral tendencies, children between the ages of 5 and 15 and occupational handlers are particularly sensitive (Sowemimo, 2012; Saravanan *et al*., 2016; Rostami *et al*., 2020; Al-Kappany *et al*., 2023). Regular anthelmintic programs must be strictly started for puppies and dams in order to lower zoonotic hazards (Carlin and Tyungu, 2020). Conventional microscopic evaluation often lacks sensitivity due to intermittent egg shedding, as generally established by Borecka and Gawor (2008). For epidemiological surveillance, it is therefore essential in the current study to use accurate

instruments like PCR that targets the ITS2 gene and Enzyme-Linked Immunosorbent Assay (ELISA) that targets TES antigens (Borecka and Gawor, 2008; Saravanan *et al*., 2016). Therefore, the primary goals of this study were to measure the molecular prevalence of *T. canis* in dogs using PCR, assess the immunological seroprevalence of toxocariasis among dog owners using ELISA, assess the impact of age, sex, and geography, and use phylogenetic analysis to compare local isolates with global lineages.

MATERIALS AND METHODS

Study Area

Between 2025 and 2026, 150 samples total—75 canine fecal samples and 75 human blood samples were gathered from the Babylon Governorate in Iraq for the current cross-sectional investigation. *Toxocara canis* infection and exposure rates were examined in a number of the province's districts, including Al-Hilla center, Kotha, Al-Kifl, Al-Qassim, and Al-Hashmia. Fresh fecal samples were taken from dogs of all ages and sexes, put in sterile plastic containers with tight-fitting lids, and sent right away to the lab in a cold box. At the same time, dog owners' median cubital veins were used to take blood samples, which were then put in EDTA tubes and kept in ideal cooling conditions. The Parasitology Laboratory at Al-Qasim Green University's College of Veterinary Medicine received all of the collected specimens. for further immunological and molecular examination. To guarantee accurate species-level identification and prevent geographic bias, positive samples from the canine specimens that were verified by molecular technique (PCR) targeting the ITS2 gene were then chosen for sequencing and phylogenetic analysis.

Statistical Analysis

Involves converting data into a computerized database structure. A request was made for professional statistical help. Computerized statistical tests utilizing In SPSS version 17, variables were analyzed using Yat's Chisquare test.

Molecular Detection

For the purpose of extracting genomic DNA, 75 canine fecal samples—38 male and 37 female—were processed. Following extraction, polymerase chain reaction (PCR) was carried out in accordance with Borecka and Gawor's (2008) established procedure. The internal transcribed spacer 2 region (ITS2 gene) was the target of PCR technology, and the amplified products were seen using agarose gel electrophoresis. Three (3) representative samples from the PCR products of the positive samples were shipped via DHL to Macrogen in South Korea so that Sanger sequencing technology could be used to sequence the DNA. The produced sequences were submitted to the National Center for Biotechnology Information (NCBI) for official accession numbers (PZ507134, PZ507135, and PZ507136) after being trimmed to remove noisy signals from the raw sequence data.

Primer

The primers used in this study were designed to amplify the internal transcribed spacer 2 (ITS2) region of the ribosomal RNA (rRNA) gene of Toxocara canis. The primers were supplied by Scientific Researcher Co. Ltd., Iraq. The forward primer (Tcan1 F) had the sequence 5′-AGTATGATGGGCGCGCCAAT-3′, while the reverse primer (NC2 R) had the sequence 5′-TAGTTTCTTTTCCTCCGCT-3′. These primers amplified a 298 bp fragment with an annealing temperature of 55°C, as described by Borecka and Gawor (2008).

Phylogenetic Tree

The phylogenetic tree helps to know how close or distant species are from each other on the genetic level, understanding evolutionary relationships and supports a more perfect classification of organisms based on evolutionary history, not just physical similarities (Fahmi & Al-Musawi, 2026; Rashid *et al*., 2022; Rostami *et al*., 2020). In the present study, the sequence alignments were performed using Clustal W, and the phylogram was constructed using the Molecular Evolutionary Genetics Analysis version 10 (MEGA X) software. To confirm the genetic proximity and evaluate the evolutionary positioning, the NCBI BLASTn server was utilized to compare the obtained sequences of the local *Toxocara canis* isolates (PZ507134, PZ507135, and PZ507136) with reference sequences available in the GenBank database.

Immunological Detection

A total of 75 human blood samples—40 male and 35 female—were randomly drawn from dog owners in different Babylon Province districts. In order to prepare serum, the blood samples were taken from the median cubital vein, put into special tubes, and sent to the Parasitology Laboratory under controlled refrigeration conditions. The separated serum was labeled and kept at -20 °C after centrifugation at 3,000 rpm for ten minutes. To find particular IgG antibodies in the human serum, an Enzyme-Linked Immunosorbent Assay (ELISA) that targets *Toxocara* Excretory-Secretory (TES) proteins was used. An ELISA microplate reader was used to assess the optical density (OD) values in order to distinguish between positive and negative cases. Thirteen samples (17.3%) of the entire population under investigation had positive *Toxocara canis* antibody tests.

RESULT

Molecular Result

Total 75 dog fecal samples were examined using a molecular examination (PCR) and the result was 41 infected samples.

Table 1: Infection rate of *Toxocara canis* in dogs determined by molecular examination (PCR)

Examined samples	Infected samples	Infected Samples (%)
75	41	54.67

According to the study, 54.67% of the 75 fecal samples from the handlers' dogs had *Toxocara canis* infections. The results of the current investigation revealed that the overall prevalence among the fecal samples examined using the PCR technique was 54.67% (41 out of 75).

Table 2: Infection rate of *Toxocara canis* in dogs determined by the geographical areas (PCR molecular examination)

Area	No. of examined samples	Positive samples	Percentage (%)
Al-hilla	15	7	46.67
Kotha	15	8	53.33
Al-kifl	15	10	66.67
Al-qasim	15	9	60
Al-Hashmia	15	7	46.67
Total	75	41	54.67
Calculated X^2	1.615		
Calculated P	0.806		

According to the study, 54.67% of fecal samples from various geographical locations had a *Toxocara canis* infection. Al-Kifl had the greatest infection rate at 66.67% (10 out of 15), followed by Al-qasim at 60% (9 out of 15) and Kotha at 53.33% (8 out of 15). Al-hilla and Al-Hashmia had the lowest infection rates at 46.67% (7 out of 15 each). According to the results of the current investigation, the overall prevalence among the samples examined using the PCR technique was 54.67% (41 out of 75). With a calculated Chi-square (X^2) of 1.615 and a P-value of 0.806 ($P > 0.05$), statistical analysis revealed that there was no significant difference between the areas under study.

Table 3: Infection rate of *Toxocara canis* in dogs determined by the sex (PCR molecular examination)

Sex	No. of examined samples	Positive samples	Percentage (%)
Male	38	19	50
Female	37	22	59.46
Total	75	41	54.67
Calculated X^2	0.677		
Calculated P	0.411		

According to the study, 54.67% of fecal samples by sex had a *Toxocara canis* infection. Females had a greater infection rate (59.46%, 22 out of 37) than men (50%, 19 out of 38). According to the results of the current investigation, the overall prevalence among the samples examined using the PCR technique was 54.67% (41 out of 75). With a calculated Chi-square (X^2) of 0.677 and a P-value of 0.411 ($P > 0.05$), statistical analysis revealed no significant difference between the sexes.

Table 4: Infection rate of *Toxocara canis* in dogs by PCR According to host

Host	No. of examined samples	Positive samples	Percentage (%)
puppy	35	24	68.57
Adult	40	17	42.50
Total	75	41	54.67
Calculated X^2	5.143		
Calculated P	0.023		

According to the study, the overall *Toxocara canis* infection rate for fecal samples based on host age was 54.67%. Puppies had a greater infection rate (68.57%, 24 out of 35) than adults (42.50%, 17 out of 40). According to the results of the current investigation, the overall prevalence among the samples examined using the PCR technique was 54.67% (41 out of 75). According to statistical analysis, there was a significant difference in host ages, with a computed Chi-square (X^2) of 5.143 and a P-value of 0.023 ($P < 0.05$).

Conventional PCR

DNA extraction were preformed for 75 dogs fecal sample, including microscopically positive sample (18) for purpose of confirmation. The result showed that PCR amplification of the gene ITS2, roughly 298 bp in length was positive for 41/75 sample (figure 4-10).

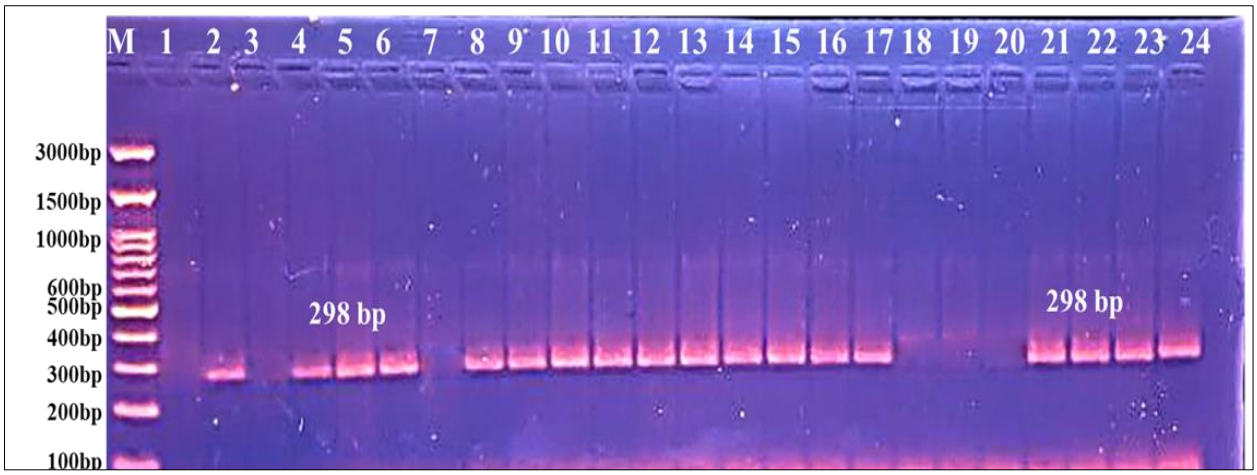

Figure 1: It shows the PCR amplification results of *Toxocara canis* ITS2 gene of isolated from dog fecal. Agarose gel picture appears the PCR product bands with molecular weight of 298 bp. (M) refers to (3000 bp) DNA ladder, (1) Negative control. (2-24) some of PCR results of fecal sample

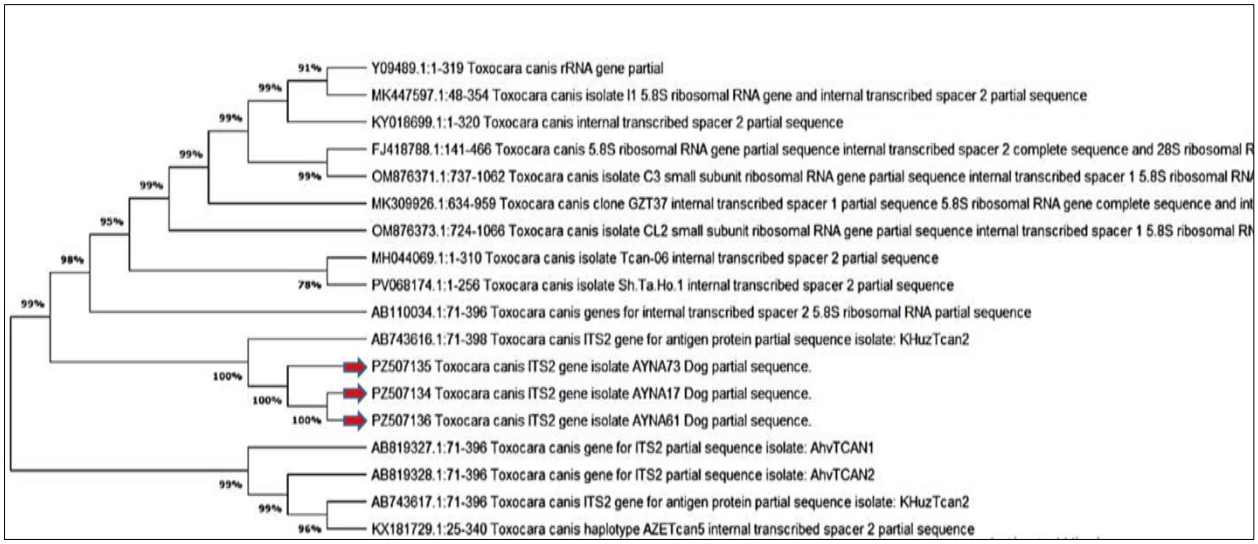

Figure 2: Phylogenetic analysis of *Toxocara canis* sequences, including PZ507135, PZ507134 and PZ507136 isolates from the Babylon Governorate and surrounding regions in Iraq, showing relationships with global reference strains

Immunological Result

75 human blood samples were collected from dog owners from both sexes and different age groups, and following immunological examinated by ELISA, the result was 13infected samples.

Table 5: Total Prevalence of infection with *Toxocara canis* in human in Babylon Provence

Examined samples	Infected samples	
	No.	%
75 blood samples	13	17.33%

The study showed the total rate of infection with *Toxocara canis* for 75 blood samples was 17.3%, collected from 75 of the handlers dogs. The current study's findings showed that among the blood samples analyzed using the ELISA technique the total prevalence was 17.33% (13 out of 75).

Table 6: The prevalence of infection with *Toxocara canis* in human in Babylon Provence according to area by immunological examination (ELISA)

Area	No. of examined samples	Positive samples	Percentage (%)
Al-Hilla	15	2	13.33
Kotha	15	1	6.67
Al-Kifl	15	4	26.67

Area	No. of examined samples	Positive samples	Percentage (%)
Al-qasim	15	3	20
Al-Hashmia	15	3	20
Total	75	13	17.33
Calculated X2	1.861		
Calculated P	0.7613		

The prevalence of the parasite in this study was highest in Al-Kifl at 26.67% (4 out of 15), followed by Al-qasim and Al-Hashmia at 20.00% (3 out of 15 for each), and Al-Hilla at 13.33% (2 out of 15), while Kotha exhibited the lowest infection rate at 6.67% (1 out of 15). The results of the present study exhibited an overall prevalence of 17.33% (13 out of 75). The statistical analysis reveals that there was no significant difference in the prevalence of the parasite between different areas (X^2= 1.861, P = 0.7613).

Table 7: The prevalence of infection with *Toxocara canis* in human in Babylon Provence according to sex by immunological examination (ELISA)

Sex	No. of examined samples	Positive samples	Percentage (%)
Male	40	8	20
Female	35	5	14.2
Total	75	13	17.33
Calculated X2	0.441		
Calculated P	0.5066		

In this study, males had a higher prevalence of the parasite (20.00%, 8 out of 40), but females had a lower infection rate (14.29%, 5 out of 35). According to the current study's findings, the overall prevalence was 17.33% (13 out of 75). The statistical analysis shows that the prevalence of the parasite did not differ significantly between the sexes (X^2 = 0.441, P = 0.5066).

Table 8: The prevalence of infection with *Toxocara canis* in human in Babylon Provence according to age by immunological examination (ELISA)

Age	No. of examined samples	Positive samples	Percentage (%)
5-15	13	5	38.46
16-26	19	2	10.53
27-37	16	3	18.75
38-48	13	1	7.69
49-59	14	2	14.29
Total	75	13	17.33
Calculated X^2	5.621		
Calculated P	0.2293		

In this study, the 5–15 age group had the highest parasite prevalence (38.46%, 5 out of 13), followed by the 27–37 age group (18.75%, 3 out of 16), the 49–59 age group (14.29%, 2 out of 14), and the 16–26 age group (10.53%, 2 out of 19). The 38–48 age group had the lowest infection rate (7.69%, 1 out of 13). According to the current study's findings, the overall prevalence was 17.33% (13 out of 75). The statistical analysis shows that the prevalence of the parasite did not significantly change between age groups (X^2 = 5.621, P = 0.2293).

DISCUSSION

Molecular study by amplifying the ribosomal ITS2 gene area, the current study demonstrated a high overall molecular prevalence of *Toxocara canis* infection among dogs in Babylon Province, reaching 54.67% (Borecka & Gawor, 2008). This high prevalence of infection indicates a crucial degree of environmental contamination with infectious eggs throughout the areas under study (Sowemimo, 2012). The Al-Kifl district had the highest rate of molecular positivity (66.67%), followed by Al-Qasim (60%) and Kotha (53.33%), while Al-Hilla center and Al-Hashmia had the lowest rates (46.67%). According to statistics, there was no significant difference between these geographical variations (P = 0.806), suggesting that the parasite's distribution and risk of exposure are comparatively uniform across the province (Rostami *et al*., 2020). According to Mircean *et al*., (2011), the high proportion in Al-Kifl and Al-Qasim can be explained by the predominantly rural and agricultural terrain of these areas, where careless guard dog management and husbandry techniques enhance interaction with contaminated soils. In terms of the definitive host's sex, female dogs had a greater infection rate (59.46%) than male dogs (50%). Both sexes are quite vulnerable to the parasite, nevertheless, as this difference was not statistically significant (P = 0.411) (Rashid *et al*., 2022). The physiological changes that occur during pregnancy

and breastfeeding, which frequently trigger the reactivation of arrested somatic larvae and result in active intestinal infections, can be epidemiologically connected to the slightly higher rate in females (Garces *et al*., 2020). On the other hand, when examining the infection rates according to the host's age, a highly significant statistical difference (P = 0.023) was found. Adult dogs had a much lower infection rate of 42.50%, but puppies had an exceptionally high rate of 68.57%. Given that puppies are extremely susceptible to vertical transmission from infected dams via trans-placental and trans-mammary channels, this observation is consistent with the traditional transmission pathways of *T. canis* (Garces *et al*., 2020).Additionally, while adult dogs develop an age-associated resistance that suppresses the development of adult intestinal worms, causing ingested larvae to undergo somatic migration and encystment in tissues instead, puppies' immature immune systems allow adult worms to fully develop in their small intestines (Carlin & Tyungu, 2020). The sequencing and phylogenetic tree analysis of three local isolates (designated NCBI accession numbers PZ507134, PZ507135, and PZ507136), which focused on the roughly 298 bp product of the ITS2 gene region, further confirmed the accuracy of the molecular surveillance (Borecka & Gawor, 2008). The greatest likelihood approach was used to create the phylogenetic tree showed that these local isolates have a remarkable genetic identity with international reference strains from several worldwide lineages, such as Iran, Egypt, Sri Lanka, Australia, China, India, and Malaysia, ranging from 99.4% to 100% (Rostami *et al*., 2020). This exceptionally low sequence divergence validates the ITS2 gene's high evolutionary stability and conservation in *T. canis,* confirming its validity as a diagnostic marker and showing that the zoonotic strains circulating in Babylon Province are genetically identical to well-established global lineages (Rashid *et al*., 2022). Immunological study by Enzyme-Linked Immunosorbent Assay (ELISA) targeting *Toxocara* Excretory-Secretory (TES) proteins was used to assess the zoonotic transmission of the parasite to the human population. The results showed an overall seroprevalence of 17.33% among dog owners in Babylon Province. According to Saravanan *et al.,* (2016), this seropositivity indicates an active or past exposure to migrating *Toxocara* larvae within the human cohort, which may result in severe symptoms such ocular or visceral larva migrans. Geographically, human seroprevalence closely resembled the molecular patterns seen in dogs, with the Al-Kifl district having the greatest exposure rates (26.67%). Al-Qasim and Al-Hashmia at 20%, Al-Hilla center at 13.33%, and Kotha at 6.67% came next. The elevated seroprevalence in Al-Kifl directly correlates with the high canine infection rates in the same locality, highlighting the influence of rural environments and close contact with domestic or stray dogs on human exposure, even though the Chi-square analysis revealed no statistically significant difference among these regions (P = 0.7613) (Chen *et al*., 2018). Male dog owners had a greater seroprevalence of 20% compared to females at 14.2%, according to research on the impact of sex on human exposure; however, this difference was not statistically significant (P = 0.5066). Males are more likely to inadvertently consume embryonated eggs due to gender-related behavioral patterns including farming, outdoor jobs, and more frequent direct contact with canines or contaminated soils (Sowemimo, 2012). Lastly, the age-wise immunological analysis showed that the youngest age group (5–15 years) had the highest seropositivity rate (38.46%), while the older age groups had much lower exposure rates (10.53% in the 16–26 group, 18.75% in the 27–37 group, 7.69% in the 38–48 group, and 14.29% in the 49–59 group). Despite the fact that there was no statistically significant difference between the age groups (P = 0.2293), A significant public health problem is the notable surge in children between the ages of 5 and 15. Children are the most susceptible to larvae migration syndromes because of their unique behavioral characteristics, which include poor personal hygiene, insufficient handwashing, geophagia, and playful, unrestrained contact with puppies, which are the main shedders of *T. canis* eggs (Chen *et al*., 2018; Saravanan *et al*., 2016).

CONCLUSION

According to the study's findings, *Toxocara canis* is a highly common zoonotic parasite in Babylon Province that poses a serious threat to public health and animal welfare. The molecular results verify that over half of dogs are sick, with puppies serving as the main reservoir and a significant source of environmental pollution because of vertical transmission pathways. The study shows a significant immunological seroprevalence of 17.33% among dog owners, with youngsters between the ages of 5 and 15 being the most exposed due to behavioral risk factors. Additionally, the circulating strains' evolutionary stability is highlighted by the high genetic identity (99.4% to 100%) between local and foreign isolates. These results underscore the urgent need for integrated control strategies, including regular deworming of dogs, management of stray populations, and increasing public health awareness to mitigate the transmission of toxocariasis in the region.

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