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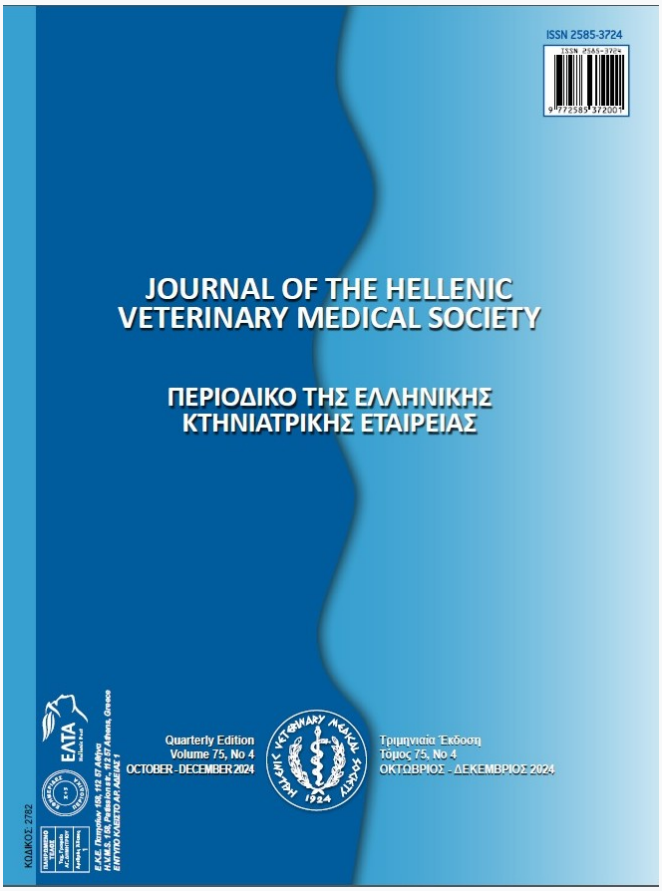


# Serodiagnosis of toxocariasis by ELISA Test in handlers of dogs and cats in Karbala city, Iraq

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Research article
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# Serodiagnosis of Toxocariasis by ELISA Test in handlers of dogs and cats in Karbala city, Iraq

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**ABSTRACT :** The gold standard for diagnosing Toxocariasis in humans is the visual identification of larvae in tissues and organs; the reference test for immunodiagnosis is an enzyme-linked immunosorbent assay that detects immunoglobulin-G antibodies against the excretory-secretory antigen of Toxocara. The current investigation was carried out from July to October 2023 in order to first determine the seroprevalence of Toxocariasis in the population of Karbala City and to document its association with a few epidemiological parameters. An enzyme-linked immunosorbent test (ELISA) approach was used in the human investigation. The study analyzed 50 blood samples from dog and cat handlers using an enzyme-linked immunosorbent assay (ELISA). The total seroprevalence of Toxocariasis was 9 (18%). The results showed that 5 (20%) more cat owners than dog owners had the infection, compared to 4 (16%) cat owners. The infection is more common in male handlers (23%), compared to female handlers (12.5%), with the maximal seroprevalence (44.4%) found in the age range of 5 to 15 years. In comparison to urban areas (8.6%), Toxocariasis is more common in rural areas (25.9%). The association between sex and lifestyle and the incidence of Toxocariasis in humans was not statistically different ($p > 0.05$) while significant with age. The importance of this study was about investigating and proving the presence of the infection in Iraq, especially in Karbala Governorate, since most of the infections are without signs at the beginning of the infection, in addition to showing the importance of dealing carefully with domestic animals, which may be a source of transmission of the infection to breeders. The study also indicated that the infection risk rate is equal for genders and lifestyle, and the possibility of infection is the same for those who live in the countryside or the city.

***Key words***: ELISA; *Toxocara canis*;*Toxocara cati*; Serodiagnosis;Toxocariasis

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## INTRODUCTION

Human Toxocariasis is one of the most widespread parasitic infections in the world and more common in poor and tropical nations. Ingesting the eggs shed on the faces of the specific dog or cat host infection results in infection of humans. Although Toxocariasis in humans can cause a variety of clinical symptoms, the two most commonly reported clinical symptoms are visceral and ocular larval migration. The quantity and migration routes of Toxocara larvae determine the majority of the clinical symptoms and consequences associated with infection with this parasite. The roundworms *Toxocara canis* and *Toxocara cati* are the cause of the significant but little-known tropical disease Toxocariasis. The adult Toxocara worms ascarid nematodes that belong to the Toxocaridae family and genus Toxocara inhabits the small intestines of various permanent hosts. For instance, infects the canine hosts, both domestic (as dogs) and wild (as foxes and wolves), whereas the animals of the Felid family act as final hosts for *T. cati*. Humans unintentionally consume eggs that contain infectious third-stage larvae of Toxocara species, which results in Toxocariasis (Ma *et al.*, 2018).The infection spreads to humans when the eggs found in canine or feline feces are consumed by people (Magnaval *et al.*, 2022). Humans behave abnormally as hosts in terms of the full life cycle; they can become infected through unwashed hands, raw vegetables, or direct contact with infectious (embryonated) eggs in contaminated soil (gardens, sandpits, and playgrounds) (Rostami *et al*., 2019). In humans, infectious larvae hatch after being consumed as eggs and then pass through the intestine, travel to different organs throughout the body via the circulatory system, and infect more tissues. However, larval stages do not mature into adult worms but instead wander the body for months or even years, causing damage to any infected tissues (Fecková *et al*., 2020). Toxocara species do not proliferate within their human hosts; instead, they live in host tissues in an immature state. The symptoms of Toxocariasis are brought on by the larvae dying in human tissue and the inflammatory response that follows (Joy *et al*., 2017). The amount and location of Toxocara larvae, which are divided into four groups: ocular larvae, visceral larvae, migration, covert form, and neural larvae, determine the infection's non-specific clinical indications and symptoms (Fecková *et al*., 2020). The most significant symptoms in adults are fever, weakness, and gut pain in the majority of cases with visceral larvae migration. Asthma, blindness, eosinophilia, encephalitis, abdominal discomfort, and hepatomegaly are among the symptoms of a severe infection in young humans. According to (Magnaval *et al.*, 2022) some individuals experience neurological disturbances such as nausea, vomiting, limb and muscle discomfort, cough, pneumonia, wheezing, fatigue, and lymphadenitis. Larvae of Toxocara migrate through blood to different organs, where they inflict harm and inflammation. The majority of larvae go through the liver and lungs, which can cause symptoms including fever, hepatomegaly, coughing, decreased appetite, and asthma (Fecková *et al*., 2020), where endophthalmitis, retinal granulomas, and blindness are brought on by larval migration to the eye. Hypergamma-globinaemia, leukocytosis, and eosinophilia are present. The illness can range in severity from an infection that causes no symptoms to death (Joy *et al*., 2017). The diagnosis and control of Toxocariasis are not easy, as the larvae migrate into the tissues, remaining dormant or hypobiotic parasites. Since it is very challenging to directly examine for visceral larvae in a biopsy, directed serum tests are used. In advance of these tests, therefore, to produce highly specific and sensitive results from the second-stage larvae secreted (Noordin *et al*., 2020). The immunoglobulin, mostly IgG but also, to a lesser extent, IgM and IgE, is produced in response to the infection, which has shown the ELISA test to be a sensitive and specific test in the diagnosis of VLM (Mazhab-Jafari *et al*., 2019).

There has been relatively little research done on Toxocariasis in humans in Iraq. Research on Toxocariasis is scarce; for example, Al-Dabbagh et al.'s work (Al-Saeed *et al*., 2014) in Mosul, (Al-Asady & Al-Nasiri, 2020) in the Biji-Salah Al-Din province, and (Hadi, 2017) in Baghdad, and no study in Karbala, Iraq, examined the prevalence of the infection. This is due to the difficulty of diagnosing cases, as most cases do not show signs, and the signs are different depending on your safety and the presence of the larval stage of the parasite. According to the above data, the aim of the study is to measure the prevalence of Toxocariasis infection in Karbala city's dog and cat handlers using ELISA-IgG and research the connection between the infection and a few epidemiological factors (age, gender, and living region).

## MATERIALS AND METHODS

### Collection of Blood Samples

50 blood samples in total were taken at random from 25 dog and 25 cat owners. Each blood sample (3 ml of

blood) was placed in a gel tube together with the date, location, age, and sex information before being transported to the lab to prepare the sera in a cool box. 50 blood samples for this experiment, utilize human serum or plasma (citrate or heparin) samples. The samples should be retained at 2-8 °C if the assay is run within five days of sample collection; if not, if samples were frozen during storage, properly mix the thawed samples before testing; otherwise, they should be aliquoted and stored at deep freezing temperatures (-70--20 °C). Do not repeatedly freeze and thaw. The inactivation of samples with heat is not advised (Gheorghe, 2018).

**Serum preparation and ELISA test**

For ten minutes, the serum was separated by centrifugation at 3000 rounds per minute, and the serum was gently aspirated by pipette contents into sterile, dry test tubes with labels; store at -20°C until needed.

**Serological study**

Serological study was conducted for detection of Toxocara IgG antibodies in human.

**Enzyme linked immunosorbent assay (ELISA) Kit**

ELISA kits were purchased from Demeditec Diagnostics GmbH Company in Germany country.

**Sample preparation**

IgG Sample Dilution Buffer was used to dilute all samples 1+100; to do this, mix 10µL of sample with 1 mL of IgG Sample Dilution Buffer in tubes. The mixture was then well stirred using a vortex.

**Reagent preparation**

Washing Buffer (20x conc.) was diluted with 190 mL of distilled water and 10 ml of Washing Buffer. TMB Substrate Solution must be kept out of direct sunlight and at 4 °C even if it is ready for use.

**Assay procedures**

Well A1 was designated for the substrate blank, and 100 µL of standards, controls, and diluted samples were distributed into their appropriate wells. The kit's foil was used to cover the wells, and they were then incubated for an hour at 37 °C. After the incubation period was up, the foil was taken off, the wells' contents were aspirated, and 300 µL of washing buffer was used to wash each well three times. All wells, with the exception of Substrate Blank Well A1, received 100 µL of the conjugate, which was then incubated for 30 minutes at room temperature (25°C). The steps for washing were repeated in step 3. Each well received 100 µL of TMB substrate solution, which was then incubated in the dark for precisely 15 minutes at room temperature (25 °C). All of the wells' colors changed from blue to yellow when 100 µL of Stop Solution was applied. The absorbance at 450 nm was measured using an ELISA reader (Biobase, China) 30 minutes after the stop solution was added. The following formula was used to determine the results, with the cut-off being the mean absorbance value of the cut-off. Control determinations: Sample (mean) absorbance value x 10/cut-off = [units = U].

**Calculation**

The average absorbance value of the Cut-off Control calculations is known as the Cut-off.

**Statistical Analysis**

The SAS (2018) software was utilized to ascertain the impact of variance variables on the study parameters. In this study, the chi-square test was utilized to compare percentages (0.05 and 0.01 likelihood) statistically significant (Marasinghe & Koehler, 2018).

## RESULTS

According to the study's results, the total seroprevalence of Toxocariasis is 18% based on the measurement of IgG antibodies by ELISA. Nine positive cases were identified from 50 blood samples (serum) obtained from the handlers of 25 dogs and 25 cats.

**Table 1.** The precision of the standards

| Cut-off | absorbance (OD/ 450nm) |
|---|---|
| Substrate blank | 0.005 |
| Negative control | 0.01 |
| Cut off | 0.48 |
| Positive control | 1.1 |
| Interpreted positive value | > 11 U |
| Interpreted Suspected value | 9 - 11 U |
| Interpreted Negative value | < 9 U |

**Table 2.** Descriptive statistics results of ELISA

| | absorbance (OD/ 450nm) | |
|---|---|---|
| state | Negative | Positive |
| Number of samples | 41 | 9 |
| Minimum titer (U) | 0.19 | 10.83 |
| Maximum titer (U) | 8.33 | 14.17 |
| Range | 8.14 | 3.34 |
| Mean titer (U) | 3.292 | 12.81 |
| Std. Deviation | 2.224 | 1.5 |
| Std. Error of Mean | 0.3707 | 0.75 |

**Total Rate of Toxocariasis in Human by ELISA Test**

The study showed that the total rate of infection with Toxocariasis in 50 blood (3 ml) samples was 18%, collected from 25 handlers of cats and 25 handlers of dogs. In this study, we found that the infection rates between cat handlers and dog handlers were (20%) and (16%), respectively.

**Prevalence of Toxocariasis infection according to sex**

The result of the study recorded that the differences between males and females infection rates are statistically not significant ($p > 0.05$). And the infection rate was higher in males (23%), than in females (12.5%), as indicated in table 4.

**Prevalence of Toxocariasis infection according to age group (year)**

It can be seen from the current study's results that the IgG antibody titer differs among the participants' age groups. Interestingly, the infection with the highest seroprevalence was (44.4%) within the age group 5-15 years, followed by seroprevalence of 33.3% within the age group 16-26 years, and Toxocariasis with the lowest seroprevalence of 11.1% within the

**Table 3.** Total Prevalence of infection with Toxocariasis in Human in Karbala city

| | No. samples | Positive | Percentage % |
|---|---|---|---|
| handlers of cats | 25 | 5 | 20 |
| handlers of dogs | 25 | 4 | 16 |
| Total | 50 | 9 | 18 |
| **Tested samples** | **Number (%)** | **mean IgG titer (U)** | |
| Positive | 9 | 12.81 | |
| Suspected | none | none | |
| Negative | 41 | 3.292 | |

**Table 4.** Prevalence of Toxocariasis infection according to sex

| gender | No. | IgG -ELISA test | | X2 | P-value |
|---|---|---|---|---|---|
| | | No. of positive (Patient) | Percentage % | | |
| male | 26 | 6 | 23 | 0.720 | 0.396(NS) |
| female | 24 | 3 | 12.5 | | |
| total | 50 | 9 | 18 | | |

NS: No significant difference at ($p > 0.05$)

**Table 5.** The value of IgG titer (using IgG- ELISA test) According to age group (year)

| age | No. | Percentage % | X2 | P-value |
|---|---|---|---|---|
| 5-15 | 4 | 44.4 | 4 | 0.261(NS) |
| 16-26 | 3 | 33.3 | | |
| 27-37 | 1 | 11.1 | | |
| 38-48 | 1 | 11.1 | | |
| Total | 9 | | | |

NS: No significant difference at ($p > 0.05$)

**Table 6.** Prevalence of Toxocariasis infection according to its type of living area

| area | No. | Positive | Percentage % | X2 | P-value |
|---|---|---|---|---|---|
| Urban | 23 | 2 | 8.6 | 2.49 | 0.114(NS) |
| Rural | 27 | 7 | 25.9 | | |
| total | 50 | 9 | 18 | | |

NS: No significant difference at ($p > 0.05$)

age groups 27-37 and 38-48. (Table 5). Based on statistical analysis, there was significant difference in the overall prevalence of toxocariasis between age groups ($p > 0.05$).

**Prevalence of Toxocariasis infection according to its type of living area in Karbala city**

According to Table 8, the prevalence of toxocariasis is higher in rural areas (25.9%) than in urban areas (8.6%) in Karbala city, Iraq. As table (6) indicates, no significant difference ($p > 0.05$) was found.

## DISCUSSION

This study is considered the first research to evaluate the prevalence of human Toxocariasis in the Iraqi city of Karbala. In this investigation, Toxocariasis seroprevalence was 18%, as determined by Toxocara IgG-ELISA antibodies. Seroprevalence (18%) was discovered in a Dutch community survey (Mughini-Gras *et al*., 2016), which is comparable to our findings in terms of other research. Al-Saeed et al. (2014) observed a lower seroprevalence in Iraq, reporting 30.8% occurrences in their study in Mosul. A different study conducted in Baghdad City found that 40.5% of cases were documented (Hadi, 2017). In contrast to Al-Asady and Al-Nasiri's, 6.81% (Al-Asady & Al-Nasiri, 2020) findings, which estimated the seroprevalence in the Kirkuk province to be 6.81%, our data show that seroprevalence is significantly higher. A few differences were found when the most recent Toxocariasis seroprevalence results were compared to those of other studies conducted around the world. However, the seroprevalence of Toxocariasis identified in the current study is greater than that found in two prior Iranian studies: 4.4% in health centers in the Lorestan province by (Mahmoudvand *et al*., 2015) and 1.70% in Sistani and Baluchistan by (Shahraki & SARDAR, 2014). The current investigation found that men (23%) had a higher seroprevalence of Toxocariasis than did women (12.5%). Similar findings from earlier studies have been reported (Hadi, 2017) found Male serum samples, 12 were positive (23.07%) and of 40 female samples 7 were positive (17.50) for Toxocariasis .However, other research (Iddawela *et al*., 2017) indicates that females are more prevalent than males. According to (Pinelli *et al*., 2011), Men have a greater seroprevalence of Toxocariasis than women due to traditional male behavior and activities, particularly their vocations like farming. Regarding the correlation between age and the prevalence of toxocariasis, authors have drawn a variety of conclusions. While some people claimed there was no obvious connection between age and the disease, others claimed that toxocariasis affects youngsters more commonly than adults. The highest seroprevalence of Toxocariasis is found in the age categories of 5 to 15 years, followed by 16 to 26 years (33.3%), while the lowest seroprevalence is found in the age groups of 27 to 37 years and 38 to 48 years (11.1%). Our study was supported by the findings of several preceding studies (Al-Asady & Al-Nasiri, 2020; Hadi, 2017). It is well known that Toxocara species can spread orally from animal to human through infected eggs found in soil contaminated by dog and cat feces. Soil contaminated with dog and cat feces poses a serious risk to humans (Khalil *et al*., 2021). Youngsters are more likely to engage in dirty play, have poor personal hygiene, purposefully or inadvertently put their hands and fingers in their mouths, have immature immune systems, and repeatedly contract infections, which cause antibodies to persist (Airs *et al*., 2023).The increase in pediatric Toxocariasis infections, according to (Kaul *et al*., 2022), may be due to contaminated dog and cat feces on playfields.(Gürel *et al*., 2005), who discovered 18.9% of Toxocara *spp*. eggs in playfields, provided evidence to support this theory. Adults with the infection have a higher chance of coming into contact with contaminated soil because they are more likely to have previously resided in a rural area. As a result, it is thought that the results in elderly patients are a reflection of earlier infections (Airs *et al*., 2023). But determining whether seroprevalence in older age groups is the result of recent, mild infections or older infections with lingering antibodies is difficult. According to the current study, toxocariasis seroprevalence was higher in rural areas (25.9%) than in urban areas (8.6%). The results of our study agreed with those of other studies by (Doğan *et al*., 2007)

Study found that cases were 0.71 percent in urban regions and 16.97% in rural areas. Additionally, another study (Mahmoudvand *et al*., 2015) , which recorded cases in rural regions at 11.9% and in urban areas at 2.1%, showed comparable outcomes. It is a reasonable assumption that rural places are more dangerous than metropolitan ones (Eslahi *et al*., 2017). The high rate of infection in rural regions from bare hands in soil and/or sand (particularly playground sand pits) and from owning cats or cattle, along with generally low standards of education and cleanliness, as well as frequent geophagy behavior among young people, are likely to contribute to this high incidence (Hadi, 2017).

## CONCLUSIONS

The prevalence of Toxocariasis in humans in Karbala is 18% by using ELISA on the 50 blood samples for IgG antibody detection. The infection rate with Toxocariasis in males is higher than in females, and the small age group is more likely to be infected, with no significant difference regarding sex, while significant difference with age . The infection rate with Toxocariasis in rural more than in urban with non-significant difference statistical.

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## CONFLICT OF INTEREST

The authors declare no competing interest.

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