

Association Between *Toxocara* spp. Infection and the Risk of Developing Rheumatoid Arthritis

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ABSTRACT

This study investigated the role of *Toxocara* spp. infection as a potential risk factor for developing rheumatoid arthritis (RA) in individuals from Doluya District, Saladin Governorate, Iraq, over the period of October 2022 to September 2023. Serum samples from 215 males and females, aged 5–58 years, were analyzed using *Toxocara* IgG ELISA. The prevalence of *Toxocara* spp. infection among the tested population was 19%, with notable differences observed based on age, gender, and pet ownership. The highest infection rate in females was recorded in the 5–15 age group (27.27%), while in males, the same age group showed a significantly higher prevalence of 42.85%. Pet ownership was identified as a major risk factor, with infection rates of 26.82% in those exposed to pets compared to 13.55% in non-owners. RA IgG levels were measured in infected and control groups to assess the potential link between *Toxocara* infection and rheumatoid arthritis. The mean RA IgG concentration in infected individuals was significantly elevated (0.6740 ± 0.3220) compared to the control group (0.2167 ± 0.0727), with a statistically significant difference ($p < 0.01$). These findings suggest that *Toxocara* spp. infection may play a contributory role in the development of rheumatoid arthritis, particularly in vulnerable populations with high exposure to infection risk factors such as pet ownership.

Keywords: Toxocariasis, *Toxocara*, Rheumatoid Arthritis, Spread of Toxocariasis

INTRODUCTION

Toxocariasis is a widespread zoonotic parasitic disease reported to have a worldwide distribution [1]. Caused by the larvae of *Toxocara*, *T. canis*, and *T. cati*, which are the main causes of infections in humans in tropical and subtropical areas worldwide [2]. The parasitic nematodes *T. canis* and *T. cati* are found in the intestines of dogs and cats as definitive hosts. Soil contamination with eggs is the major source of transmission to humans. Infection usually occurs in young children, particularly those aged between 2 and 7 years, who become susceptible due to the ingestion of eggs. The sources of such infection are infected soils, eating uncooked meat, and vegetables that may be contaminated with eggs, which in turn are ingested along with unsterilized fruits. Through the feces of infected dogs and cats, after ingestion, the eggs hatch in the front part of the small intestine and penetrate the intestinal mucous layer and then migrate through the intestine to be transmitted to various organs of the body such as the liver, lung, heart, brain, eye, and muscles through the bloodstream [3]. Rheumatoid Arthritis (RA) is a chronic autoimmune condition resulting from unexplained inflammatory processes [4].

The diagnosis of this disease is based on the presence of arthritis accompanied by fever for at least two weeks, and also typical features including skin rash, lymphadenopathy, hepatomegaly, and splenomegaly. Whenever children have fever, eosinophilia, and hypergammaglobulinemia, a pediatrician should be wary of toxocariasis because pets are typically found in houses and rural areas where cases are more likely to occur [5]. The influence of *Toxocara*, either as an inhibitory or as a risk factor for autoimmune diseases such as multiple sclerosis (MS) and rheumatoid arthritis (RA), has not yet been found, while the role of parasitic diseases in RA patients is still questionable [4]. Although they are considered possible reasons why helminth infections are connected to autoimmune diseases, there is a delicate balance of interaction among the immune system, the hygiene hypothesis, and socio-economic factors [6]. The present study

aimed to investigate whether *Toxocara* spp. play any role as a risk factor for rheumatoid arthritis development.

MATERIALS AND METHODS

This study took place in the Al-Dhuluiya District of Salah al-Din Governorate and was conducted from October 2022 to September 2023. Data were gathered through a questionnaire that asked for details such as name, age, gender, occupation, location of residence, and animal ownership status. For sample collection purposes, the Dhuluiya General Hospital laboratory and National Laboratory were utilized. 2. Blood sampling: For blood sampling, 5ml of venous blood was taken from subjects using a tourniquet with a 5ml medical syringe. The blood was transferred into lab tubes free of all impurities and left for about fifteen minutes at room temperature for the blood to coagulate.

It was then placed into a centrifuge at a speed of 3500 r/min. To separate the serum, pour it into Eppendorf tubes and keep it frozen at -20°C until analysis is done. Serological tests can be divided into three types: A - *Toxocara* IgG test, which is a diagnostic test for toxocariasis, used with a diagnostic kit from the Chinese company Sunlong Biotech; B - RA IgG examination, where the concentration of RA IgG in serum is measured using ELISA technology via Sunlong Biotech's own diagnostic kit. 4. Statistical analysis: The results were statistically analyzed using t-test and chi-square test. The comparison of means was performed using Duncan's multiple range test at $p < 0.05$ and $p < 0.01$ [7].

Results and Discussion

The objective of the present study is to determine the seroprevalence rate of *Toxocara* in Salah al-Din Governorate/Dhuluiya District during the period from December 2023 to September 2023. The research indicated that the detection rate of

Toxocara has reached a maximum of 19%. Among the sera of individuals. They were studied in different age groups ranging from five years to 58 years, regardless of sex or geographical location. By comparing the overall infection rate of 19% among different age groups in Al-Dhuluiya district in Saladin Governorate with other studies conducted in the same climatic conditions (hot and dry), this rate can be considered one of the highest. high.

Rates of spread of toxoplasmosis in Iraq and different countries. According to Aghamolaie et al. [8], the results of the current study are consistent with their findings. When age groups were compared, seropositivity rates for *T. canis* infection were found to be similar across all ages. The percentage among dog owners between 36 and 53 years old reached 74.6%, and among those between 54 and 71 years old it reached 75%, and among those between 72 and 89 years old it reached 76.9%, and among who are between 18 and 35 years old. years, reached 85%. However, this finding contradicts previous research suggesting that advanced age is an important risk factor for *T. canis* infection. In a study conducted in Iraq, Woodruff et al. [9] reported an adult toxocariasis rate of 7.3%. Infection rates ranging from 5.1% to 71.8% have been identified in different parts of the world, mainly due to variations in climatic, social, economic and cultural conditions [10]. These findings are consistent with earlier studies by Chieffi et al. [11] and Delai et al. [12]. The age group with the highest seropositivity for *T. canis* was 4-15 years, with a prevalence of 39.65%. One possible explanation for this could be the high occurrence of children and teenagers in public areas like parks, playgrounds, beaches, and sandboxes since these are stable places for the presence of *Toxocara* infection. Similarly, other reports state that exposure rates to *Toxocara* have been increasing along with age; this might be due to anti-*Toxocara* antibodies which act as a biological marker of prolonged immunological response and naturally boost up the gradual

population-wide immune buildup [13, 14]. Such studies have demonstrated that with each passing year, people have a higher seroprevalence towards *Toxocara* antibodies. According to an existing hypothesis, the emergence of this condition is caused by the accumulated number of years during which people live in highly polluted areas. These observations, however, may be due to repeated infections that facilitate the long-term development of the immune response through the establishment of immunological memory [15]. The infection rate in Iran for the age group of 47-57 years was recorded to be around 1.39-3.8% [16].

The reason for such high numbers compared to low numbers is due to the tough *Toxocara* eggshell, which enables it to survive outside the host for years, making it resistant to unfavorable environmental conditions. Similarly, a study that was done in a rural area of northern Brazil comprising 466 individuals aged from 5 to 90 years old found out that there is a higher seropositivity rate in children aged from 5 to 14 years, amounting to 36.6%, compared to older people, amounting to 22.5%. Also, in younger persons aged 10-19 years, there is a decline in early adulthood and a second rise during old age, showing a pattern of *Toxocara* spp. This pattern may be due to reduced exposure to infectious *Toxocara* spp. as one ages and subsequent decrease in antibody levels over time, while the increase in prevalence in the elderly is likely due to later life exposures related to agricultural or other outdoor wildlife activities [17]. This study determined the association between age and owning dogs and cats using ELISA to detect toxocariasis in Al-Dhuluiya District, Salah al-Din Governorate. Among the population, there was a peak infection rate of 26.82% among those who owned animals and a minimum of 13.55% recorded among those without any animals. The results from statistical analysis also indicated no statistically significant differences with a P-value > 0.05, as observed in Table 2.

Table 1: Percentage of People Infected with Toxocariasis Examined by Age Group

Age Groups	Number of Samples Tested	Number of Positive Samples	%
15-5	18	6	33.33
25-16	20	3	15
36-26	13	3	23.07
47-37	18	2	11.11
58-48	31	5	16.12
total	100	19	
Ns			
Pearson Chi-Square = 3.645 P-Value = 0.456			

Table 2: Study of Relationship Between Age Group of Toxocariasis Patients and Pet Ownership

Groups	Age Group	Number of Samples Tested	Positive Samples	%
Acquisition of Animals	5 -15	7	3	% 42.85
	16 – 25	12	2	% 16.66
	26 -36	5	2	% 40
	47-37	5	1	% 20
	58-48	12	3	% 25
	Total	41	11	% 26.82
Not Owning Animals	5 -15	11	3	% 27.27
	16 – 25	8	1	% 12.5
	26 -36	8	1	% 12.5
	47-37	13	1	% 7.69
	58-48	19	2	% 10.52
	Total	59	8	% 13.55
Statistical Analysis		Chi-Square = 0.403 P-Value = 0.879		
Ns				

It has been found that the results of the present study are in agreement with those of Shahat et al. [18], Phasuk et al. [19], and Na-

Ek et al. [10], where they concluded that *T. canis* seropositivity showed a significant association with contact with animals, and the highest

rates were among people who keep animals compared to those who do not keep animals, which is in accordance with many previous studies reporting significant associations between toxocariasis and contact with pets. On the other hand, contrary to research conducted by Zibaei et al. [20] and Delai et al. [12], they did not find any relationship between seroprevalence and animal ownership. This study's findings have demonstrated that there is a link between contracting toxocariasis and the presence of contact with dogs and cats. Furthermore, as stated earlier, people who raise dogs or cats become infected with *Toxocara* because sometimes these animals are kept in homes for various reasons without receiving full treatment and vaccination. On the other hand, they can also infect humans [16, 21]. The extent of environmental contamination with *Toxocara* eggs may increase due to a larger number of stray dogs and cats as pets at home. Soil is the major source of infection transmission in humans. People's behavior and health awareness, especially among children, can determine the levels of exposure to these infections because they correlate well with the number of dogs and cats in different age groups [22]. A high level of Rheumatoid arthritis IgG was found in the ELISA test, which is almost three times higher than in the control group. The percentage of levels among infected people reached 0.6740 ± 0.3220 compared to the control group's percentage level of 0.2167 ± 0.0727 . Results from statistical analysis revealed significant differences at a probability level of $p < 0.01$ as presented in Table 3.

Table 3: Rheumatoid Arthritis Concentrations in Toxocariasis Patients

Groups	RA-IgG
Infected Group	0.6740 ± 0.3220
Control Group	$0.0826 \pm 0.0637 \pm$
T Test	4.49
P-Value	0.01

There have been no prior investigations conducted locally on the prevalence of *Toxocara* infection among patients with rheumatoid arthritis, so there is no data to which these figures could be compared. The study revealed a highly significant increase in patients diagnosed with rheumatoid arthritis. A study conducted by Salem et al. [23] examined the incidence of rheumatoid arthritis and its relationship with gender and age groups. Results indicated that the infection rate among males with this disease was 30.2%, while females accounted for 69.8%. In terms of age, prevalence rates were found to be highest in

individuals between 10-13 years (41.2%), followed by those aged 5-9 years (35.3%) and 14-15 years (23.5%). Demographic variables didn't show any significant difference between patients diagnosed with arthritis and *Toxocara* infections. Exposure to animals such as dogs and cats, consumption of undercooked meat, or unwashed vegetables are considered potential risk factors for infection. Very limited evidence exists regarding the association between parasitic diseases and rheumatoid arthritis. In rheumatoid arthritis patients, *Toxocara* seroprevalence was notably higher than in the general population at 8.3%. Moreover, findings from multiple studies have revealed that joint inflammation is a prominent symptom among *Toxocara*-infected individuals [4]. The occurrence of parasitic infection and disease activity has been documented in some studies on the patient population suffering from rheumatoid arthritis. Additionally, this tendency toward negativity in the immune system and increased prevalence of all types of infections like *Toxocara* spp and *T. gondii* increases with prolonged duration of rheumatoid arthritis. There are some reports that show the presence of genetic background along with environmental factors to be responsible for rheumatoid arthritis. They have also made available information relating to *T. gondii* and *Toxocara* spp along with numerous other autoimmune disorders. The connection between arthritis severity and *Toxocara* infection lies in the parasite, which causes stimulation of pro-inflammatory cytokines like IL-33. There are several studies demonstrating that *T. gondii* might lead to symptoms as well as relapse of rheumatoid arthritis. Moreover, high levels of antibodies to *T. gondii* can be found in other autoimmune diseases such as primary biliary cirrhosis, thyroid disease, vasculitis, and so on. The findings of our research corroborate this association. Parasitic infection may be facilitated in the population with rheumatoid arthritis due to high levels of IL-10 and treatment-induced immunosuppression that are associated with susceptibility and persistence of parasites. Additionally, parasitic infection in rheumatoid arthritis patients might contribute to a higher degree of disease activity. Therefore, larger studies are needed to confirm whether parasitic infection plays a causal role in rheumatoid arthritis and disease activity, or whether RA treatment promotes parasite infestation. IL-10 is connected to parasite persistence during the chronic phase because it inhibits T cell and natural killer (NK) functions, which are needed for pathogen eradication [23].

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