Neglected toxocariasis among eosinophilic children: A cross-sectional study in Shibin El Kom, Menoufia Governorate, Egypt

Original Article

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ABSTRACT

Background: Determination of the prevalence of human toxocariasis is a priority considering the sustained developmental goals of WHO by 2030 and the recent climatic changes.
Objective: To detect the seroprevalence of toxocariasis among eosinophilic children in Shibin El Kom, Menoufia Governorate, Egypt.
Subjects and Methods: The study included 300 randomly selected eosinophilic children for whom stool analysis was performed. Ninety-six of the children positive for other parasitic infections were excluded from the study to avoid cross-reactivity. The remaining 204 children with negative stool analysis were subjected to the detection of *Toxocara* antibodies using ELISA.
Results: Seropositivity of *Toxocara* antibodies among examined children was 15.7% (32/204); and 84.3% (172/204) were negative. Their ages ranged from 5 to 17 years, with a mean age of 9.16±3.11 years. Suspicious clinical history was recorded in 94.1% (192/204) of children; 15.7% (32/204) had history of recurrent fever of unknown origin; 21.6% (44/204) children were asthmatic; and cutaneous manifestations were observed in 2% (4/204) of cases. None of the patients had ocular or hepatic disorders. The history of direct contact with pets was present in 15.7% (32/204) of patients with non-significant differences to negative children.
Conclusion: Our study reported a relatively higher prevalence of seropositivity to toxocariasis among children with eosinophilia in Shibin El Kom City, Menoufia Governorate, Egypt. Implementing studies on a larger scale would provide more knowledge on the spread of this parasite in Egypt, and enhance advanced approaches to improve its control.
Keywords: children; Egypt; ELISA; eosinophilia; neglected diseases; toxocariasis.

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INTRODUCTION

In 2022, the WHO adopted a strategy to eliminate neglected tropical diseases from the years 2021 to 2030. Therefore, addressing these infections that lack attention is a priority to achieve improvement in control and management[1]. Toxocariasis is now recognized as the fourth parasitic illness of comparable significance after ascariasis, trichuriasis, and hookworm infections. The primary focus of global initiatives aimed at controlling human soil-transmitted helminth infections[2].

Ascarid worms that infect dogs (*T. canis*) and cats (*T. cati*), are the causative parasites of human toxocariasis, a neglected zoonotic illness that affects populations worldwide[3]. *Toxocara* eggs are highly prevalent in the environment and are detected in the soils of numerous subtropical and tropical locations in Asia, Africa, and the Americas[2]. Human toxocariasis seroprevalence ranged from 0.6% in Canada to 86% in Nigeria[4]. Previous reports suggested that stray and domestic dogs and cats played a significant role in the development of toxocariasis among Egyptians, with toxocariasis occurring particularly in low-income populations with inadequate hygiene standards[5]. Toxocariasis can also arise by other routes than contact with pets. The swallowing of embryonated eggs in raw vegetables, contaminated soil, or the accidental consumption of *Toxocara* larvae in raw or undercooked meat or paratenic hosts' livers[6]. Due to common behaviors like geophagia, playing in the dirt, close contact with animals that have eggs in their fur, poor personal hygiene, and lack of parental supervision, render children the most vulnerable group to contract toxocariasis[7].

In human toxocariasis, there is no maturation beyond the larval stage, and the migratory larvae induce severe local immune responses and allergic reactions in several organs, including the liver, lungs, eyes, and brain, leading to visceral, covert, ophthalmic, and cerebral toxocariasis[8]. In most instances, children may exhibit no symptoms, or they may experience signs of asthma in the first two conditions. Other symptoms may also include fever, cough, hepatomegaly, stomach discomfort, or

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skin manifestations[9]. Eosinophilia is the prominent characteristic of the host's immune reaction towards the migrating larvae of both species[4]. Diagnosing toxocariasis in humans is difficult due to the mentioned diverse and nonspecific clinical signs. In addition, most eosinophilia cases are incorrectly classified as high eosinophilia syndrome, which results in inappropriate treatment if serological tests for *Toxocara* spp. are not performed[10].

On the other hand, if not diagnosed and properly treated, human toxocariasis usually leads to visceral larva migrans (VLM) with several symptoms such as arrhythmia, hypoxia, and ocular manifestations. Ocular larva migrans is typically characterized by impaired vision, floaters, photophobia, leukocoria, and ocular inflammation. Cardiac-associated toxocariasis is an uncommon but serious and life-threatening VLM condition that recently received increased attention[11,12].

The present study aimed to assess the existing rate of specific immunity to toxocariasis among children with eosinophilia and to investigate the association between eosinophilia and human toxocariasis.

SUBJECTS AND METHODS

This cross-sectional study was conducted at the Outpatient Clinic of the Pediatrics Department, Menoufia University hospitals during the period from October 2022 to October 2023.

Study design: Stool and blood samples were collected from eosinophilic children after fulfilling a questionnaire. Stool samples were examined, and blood samples were assessed for hematological parameters, quantification of total IgE level and ELISA for *Toxocara* IgG antibodies presence.

Patients: We enrolled 300 eosinophilic children referred to the Outpatient Clinic of the Pediatrics Department, Menoufia University hospitals. The recruited children met the inclusion criteria of having an absolute eosinophilic count above 500 cm^3 and free stool samples from intestinal parasites. Stool analysis was performed for all the recruited children. Parasitic infection was detected in 32% (96/300) children, so they were excluded from the study, and the remaining 204 children with negative stool analysis were subjected to measurement of *Toxocara* antibodies by ELISA (Fig. 1). Relevant information regarding patient demographics, such as age, gender, and contact with pets was recorded.

Study questionnaire: The questionnaire included demographic data (age, gender, and residential status), disease details (eosinophilia rate, and clinical symptoms and signs such as unexplained fever, eye, lung, skin, and liver issues), and possible risk factors, e.g., interaction with canines.

Stool examination: Each child provided three consecutive fecal samples, collected one day apart. Stool samples were microscopically examined first by wet mount and stained with iodine to exclude intestinal protozoa that may be associated with cutaneous manifestations. Stool analysis was also examined after processing by formalin-ethyl acetate concentration procedure[13]. To avoid potential cross-reactions, positive samples with other helminth and protozoal infections were excluded. Additionally, stool culture, floatation methods, and acid-fast staining[13] were performed to exclude strongyloidiasis, hookworm infections, and cryptosporidiosis.

Hematological studies: Two separate venous blood samples were taken from the children under study. The first blood sample (~2 ml) was collected with EDTA anticoagulant for the complete blood count (CBC) and evaluation of the eosinophil count. Eosinophilia, defined as values over 400/mm^3, indicated elevated eosinophil absolute count[14]. The second blood sample (~2 ml) was subjected to centrifugation, to separate serum that was maintained at -20°C for the detection of anti-*Toxocara* IgG antibodies and measurement of the total IgE level.

Identification of anti-*Toxocara* IgG antibodies: Serum samples were tested for specific IgG antibodies using the commercial human *T. canis* antibody ELISA kit (Sun Long Biotech Co., LTD, Cat # SL2825Hu) as per the manufacturer's instructions[15]. Test efficacy was determined by comparing the average value of the positive control, which should be equal to or greater than 1.00, with the average value of the negative control, which should be equal to or less than 0.10. The computation of the crucial value (cut-Off) was calculated by addition of 0.15 to the average value of the negative control. If the optical density (OD) value proved to be less than the cut-off value, the sample was classified as negative for human *Toxocara*-IgG. When the OD value proved greater than or equal to the specified threshold (cut-off), the sample classified as positive for human *Toxocara*-IgG.

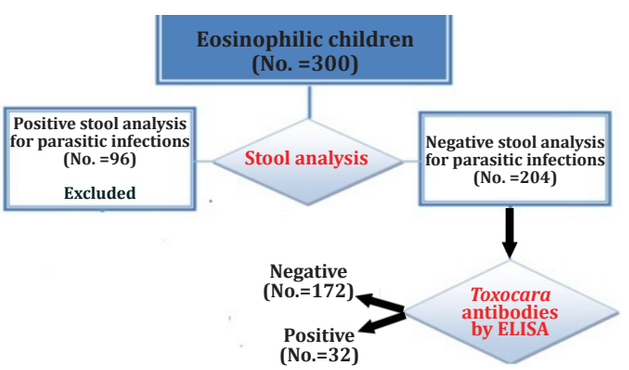

Fig. 1. Flow chart for the recruited eosinophilic children.

Quantification of total IgE concentration: The quantification of total IgE was conducted using the Cobas e 411 immunoassay analyzer (Roche Diagnostics, Germany). The assay utilized electrochemiluminescence (ELC) technology with compatible kits (Roche Elecsys IgE II, Germany) according to the manufacturer instructions[16]. The OD was measured at a wavelength of 450 nm. The standard curve was constructed, and the concentration of the samples was calculated based on the average absorbance obtained from the standard curve.

Statistical analysis: Data was collected and analyzed using the Statistical Package for Social Science (SPSS), (Statistical Package for Social Science) program for statistical analysis version 21; IBM Corporation, Armonk, NY, USA. Quantitative data were presented as mean ± standard deviation (SD) (minimum-maximum). Qualitative data were expressed as frequency and percent (%). The chi-square test was used to measure the association between qualitative variables. Student's t-test was used to compare continuous parametric variables between two groups. The Mann–Whitney U test was used for continuous non-parametric variables between two groups. Pearson's and Spearman's correlation were used to assess the correlation between quantitative variables, as appropriate. Statistical significance was considered at $P \leq 0.05$.

Ethical considerations: This research inquiry was approved by the Research Ethics Committee of the Institute of National Liver Disease (NLI IRB procedure N. 00366/2022). The research followed the relevant principles and restrictions specified in the Declaration of Helsinki. All participants' guardians were informed about the objectives of the study, and their informed consent was acquired and included. Children with positive toxocariasis and/or positive stool analysis were informed and treated.

RESULTS

Out of the 204 children, 124 (60.8%) were males, and 80 (39.2%) were females. Their ages ranged from 5 to 17 years, with a mean age of 9.16±3.11 years. Full history taken from the children's guardians revealed that out of the 204 examined children, 192 (94.1%) had a suggestive clinical history. Among the examined children, 32 (15.7%) had a past record of repeated episodes of fever of unexplained cause; 44 children (21.6%) were diagnosed with asthma, while four children (2%) exhibited cutaneous symptoms. All the individuals were free from ocular or hepatic problems. Direct interaction with pets was documented in 32 (15.7%) cases (Table 1).

The mean hemoglobin (Hb) level for the 204 children ranged from 9-16 gm/dl with a mean Hb level of 11.67±1.41 gm/dl. Total leucocytic count ranged from 4-17*10^3/cm^3 (8.53±2.82*10^3). The platelet count was within normal ranges for all children (343±105*10^3). The absolute eosinophilic count ranged from 500-1000 (752±151). The total quantification of IgE ranged from 2-1365 (145±242) (Table 1).

Screening for *Toxocara* IgG antibody titer in all the recruited children revealed positivity in 32(15.7%); 172 (84.3%) were negative. On comparing the

Table 1. Demographic, clinical, and laboratory data of *Toxocara* IgG positive and negative children.

Variable	Study children (No.= 204)		Statistical analysis *P* value
	Toxocara IgG positive No. = 32 (15.6%)	*Toxocara* IgG negative No. = 172 (84.4%)	
	Mean±SD, No. (%)	Mean±SD, No. (%)	
Male **Female**	20 (62.5%) 12 (37.5%)	104 (60.5%) 68 (39.5%)	0.829
Age (years)	8.75 ± 1.81	9.23 ± 3.3	0.240
Asymptomatic **Symptomatic**	4 (12.5%) 28 (87.5%)	8 (4.7%) 164 (95.3%)	0.055
Fever of unknown origin	16 (50.0%)	16 (9.3%)	< 0.001*
Asthmatic children	4 (12.5%)	40 (23.3%)	0.174
Cutaneous manifestations	4 (12.5%)	0 (0.0%)	< 0.001*
Direct contact with pets	8 (25.0%)	24 (14.0%)	0.115
Hb	11 ± 1.24	11.7 ± 1.41	0.002*
WBCs	10.88 ± 3.34	8.09 ± 2.49	< 0.001*
Platelet count	379 ± 88.3	337 ± 106	0.020*
Absolute eosinophilic count	737 ± 168	755 ± 148	0.568
Total IgE	118 ± 142	150 ± 256	0.329

*: Significant (P<0.05).

demographic, clinical, and laboratory data between children with *Toxocara* IgG positive and *Toxocara* negative antibodies, the presence of a history of fever of unknown origin and cutaneous manifestations were significantly higher in the *Toxocara* IgG positive group ($P<0.05$). Also, the WBCs and platelet counts were significantly elevated with toxocariasis ($P<0.05$), while the absolute eosinophilic count and total IgG showed comparable levels between both groups ($P>0.05$). In contrast, asthmatic manifestations did not differ significantly between both groups ($P>0.05$) and history of direct contact with pets did not show significant variation ($P>0.05$). On the other hand, the mean Hb level was significantly lower in children with positive *Toxocara* IgG antibodies ($P<0.05$) (Table 1).

In those patients with positive serum *Toxocara* IgG antibodies, the antibody titer negatively correlated with the Hb level and with both the absolute eosinophilic count and total IgE ($P<0.05$). The absolute eosinophilic count negatively correlated with age and Hb level, and IgG positively correlated with the total IgE ($P<0.05$) (Table 2).

In the *Toxocara* IgG positive patients, 12 (37.5%) had eosinophilia count of <700, and in 20 (62.5%) the count was >700. Eosinophilia was significantly higher in males and in patients with a history of direct contact with pets ($P<0.05$). The Hb level was significantly lower, and the total IgE was significantly higher in patients with high eosinophilia ($P<0.05$) (Table 3).

Table 2. Correlation between *Toxocara* IgG antibody titer, absolute eosinophilic count, total IgE, and the patients' ages and laboratory parameters.

Variable	*Toxocara* **IgG positive children (No.=32)**					
	***Toxocara* antibody titer (IgG)**		**Absolute eosinophilic count**		**Total IgE**	
	R	***P* value**	**R**	***P* value**	**R**	***P* value**
***Toxocara* IgG antibody titer**			0.927	<0.001*	0.452	0.009*
Age	0.395	0.025	-0.385	0.030*	-0.247	0.173
Hb	-0.617	<0.001*	-0.547	0.001*	-0.129	0.483
WBCs	-0.169	0.356	0.150	0.412	0.001	1
Platelet count	-0.095	0.604	0.049	0.788	0.001	1
Absolute eosinophilic count	0.927	<0.001*			0.741	<0.001*
Total IgE	0.452	0.009*	0.741	<0.001*		

***:** Significant ($P<0.05$); **R:** For correlation.

Table 3. Demographic, clinical, and laboratory data of *Toxocara*-positive children with eosinophilia > or ≤ 700.

Variables	*Toxocara* **IgG positive children (No.=32)**		**Statistical analysis *P* value**
	Eosinophilia ≤700 No. = 12 (37.5%)	**Eosinophilia >700 No. = 20 (62.5%)**	
Male **Female**	4 (33.3%) 8 (66.7%)	16 (80.0%) 4 (20.0%)	0.021*
Age (years)	8.67 ± 0.49	8.80±2.28	0.804
Symptomatic	12 (100.0%)	16 (80.0%)	0.271
Fever of unknown origin	8 (66.7%)	8 (40.0%)	0.144
Asthmatic children	0 (0.0%)	4 (20.0%)	0.271
Cutaneous manifestations	0 (0.0%)	4 (20.0%)	0.271
Direct contact with pets	0 (0.0%)	8 (40.0%)	0.014*
Hb	11.67 ± 0.492	10.60 ±1.39	0.004*
WBCs	11.33 ± 5.14	10.60 ± 1.66	0.640
Platelet count	383 ± 121	377 ± 64	0.871
Total IgE	14.67 ± 13.86	181 ± 148	<0.001*

***:** Significant ($P<0.05$).

DISCUSSION

Recent global warming and urbanization may apparently influence prevalence of toxocariasis, presenting it as a dominating infection[2]. In terms of the results attained in the current study, the overall seropositivity of anti-*Toxocara* IgG in all the children examined was 15.7%. Generally, toxocariasis is lower in developed temperate countries such as the USA (3.6%) [17] and China (5.1%)[18], but higher in tropical countries with lower-middle-income, e.g., Nigeria (92.4%)[19], Honduras (88.6%)[20], and Vietnam (59.0%)[21]. Recent reports from Alexandria Governorate, representing Lower Egypt, recorded a higher seropositivity of 29.87% compared to 9.02% from Qena Governorate, representing Upper Egypt[22].

To the best of our knowledge, the present study is the first to report seropositivity to toxocariasis among eosinophilic children in Shibin El Kom city, Menoufia Governorate, representing one of the major governorates of the Delta region. The variation in *Toxocara* seropositivity observed in previous studies may be attributed to the diverse ecological characteristics of the ecosystems in the investigated locations, as well as the different diagnostic procedures employed in each study[23].

In the current research, out of 32 cases that tested positive for anti-*Toxocara* IgG, four were asthmatic (12.5%) and four had cutaneous manifestations (12.5%). The findings of this study aligned with the results reported in Damietta, Egypt by Temsah *et al*.[3] who reported that anti-*Toxocara* IgG seropositivity was non-significant in negative children regarding asthma as a presenting symptom, and was significant regarding cutaneous manifestations. On the other hand, two studies[24,25] reported a relatively higher prevalence of toxocariasis in asthmatic children at a rate of 42% and 17% respectively.

The current study examined the controversial nature of behavioral and sociodemographic data. The obtained result showed no statistically significant connection between anti-*Toxocara* IgG seropositivity and the variables of sex and socioeconomic level in all the examined children which is consistent with the findings of several studies[3,9,25]. Despite other reported significant correlation between contact with pets and seropositivity to infection with *Toxocara*, the present work did not record this positive relation, which contrasted the above-reported studies and agreed with another two studies[26,27]. These findings may be attributed to other sources of infection transmission as eggs deposited in sand or soil with a high tolerance level since they can endure without hatching for several months, even in cold temperatures. Furthermore, once the eggs have hatched, larvae can remain viable for at least four weeks[28].

Elevation of blood eosinophil absolute counts over 500 cells/µl is the acknowledged criterion for toxocariasis. In severe instances, the blood leukocyte count may elevate to 100.000/mm^3, with 80-90% of these being eosinophils[29]. Furthermore, other research findings indicated that 68% of individuals with unidentified eosinophilia were accurately diagnosed with specific anti-*Toxocara* antibodies[30]. In the current research, eosinophilia above 700/µl was significantly correlated with anti-*Toxocara* IgG antibody titer and total IgE. However, we recorded no significant correlation between positive and negative eosinophilic children with toxocariasis.

One additional and significant finding in the present work was the positive correlation between fever, elevated WBCs, and toxocariasis. Fever, an atypical symptom of toxocariasis, was also the most commonly observed indication in younger children in a study in which the investigators attributed the cause of fever to viral infections and emphasized the difficulty and necessity of doubt when diagnosing toxocariasis. Additionally, they indicated that relying just on physical examination and abnormal laboratory test results may not be sufficiently diagnostic, and that it is necessary to analyze the patient's history and epidemiological data to make an accurate diagnosis[31].

In conclusion, this study demonstrated a relatively high prevalence rate of *Toxocara* seropositivity among eosinophilic children in in Shibin El Kom city, Menoufia Governorate, Egypt, confirming that toxocariasis is a crucial contributing factor for eosinophilia in children. It is imperative firstly to assess the repercussions of this overlooked parasitic illness on public health; secondly, Pediatricians in Egypt should be mindful of considering toxocariasis as a frequent differential diagnosis for bronchial asthma and eosinophilic pneumonia. Additional research is required to investigate the relationship between toxocariasis and other patient groups. It is recommended to conduct extensive studies in various locations in Egypt, as well as increase the sample size. Furthermore, studies are recommended to control human toxocariasis through recognizing other different routes of transmission than dealing with pets, such as soil examination. Another important health approach is to investigate animals and humans together in the same area.

Author contributions: All authors contributed to the initiation and conception of the study. The materials were generated, and the data was collected and assessed by Gouda MA, AboShabaan HS, Rizk MS, Ibrahim AF. Gouda MA authored the initial draft of the work, while the other authors provided input on earlier versions. The writers conducted a comprehensive review and unanimously approved the final version of the work.

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