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doi: <https://doi.org/10.15407/ubj94.05.077>TOXOCARIASIS IN CHILDREN
WITH DIGESTIVE SYSTEM DISEASESK. T. HLUSHKO[✉], H. A. PAVLYSHYN

I. Horbachevsky Ternopil National Medical University,
Department of Pediatrics No 2, Ternopil, Ukraine;
[✉]e-mail: glushko_kt@tdmu.edu.ua

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Toxocariasis is common among children and causes digestive diseases. The aim of the work was to study the development of toxocariasis in children with digestive diseases. The serum levels of specific IgG to Toxocara canis and Ascaris, levels of IL-4 and TNF- α were determined in 63 children. In addition, tests for parasites and a retrospective survey in order to assess sanitation and hygiene practices were conducted. All children were divided into two groups: group I includes 19 (30.2%) patients seropositive for toxocariasis, and group II – 44 (69.8%) children without any parasites. The level of IL-4 was higher in group I (18.0 ± 6.4 pg/ml) than in group II (7.2 ± 2.0 pg/ml) ($P < 0.001$). While the level of TNF- α did not differ significantly between groups I (4.5 ± 2.1 pg/ml) and II (3.6 ± 1.1 pg/ml) ($P > 0.05$). A higher incidence of Toxocara was observed among rural residents (78.9%). Children with toxocariasis more often had soil-pica (42.1%), played with dogs (100%), and did not wash their hands (84.2%) compared to the group of uninfected children. It was noted that hygienic habits and place of living contribute to Toxocara canis infection. IL-4 levels were considerably higher in group I than in group II that can be accounted for the host immune response activation, at the same time, the levels of TNF- α did not differ. Children without toxocariasis had also increased levels of IL-4, which may be related to past helminthic infection.

Key words: children, toxocariasis, digestive diseases, interleukin-4, tumor necrosis factor - α .

Toxocariasis is one of the common zoonotic helminth infections caused by the parasitic roundworms *Toxocara canis* (*T. canis*), the ascarids of dogs, and less frequently *Toxocara cati* (*T. cati*), the ascarids of cats. The spectrum of clinical symptoms in infected persons varies from asymptomatic infection to visceral organ infection caused by larval migration and is called “visceral larva migrans” [1, 2].

Humans may be infected with *Toxocara* by ingestion of embryonated eggs after contact with infected pets, soil contaminated with dog or cat feces or after consumption of infected raw or undercooked meat of other accidental hosts. In humans, the *T. canis* larvae do not develop to the adult stage but persist in tissues as the larval stage for many years [1-3].

Parasitic infections including *T. canis* and *T. cati* are often considered by scientists as neglected [1, 3, 4]. There are not enough studies on helminthiasis, in general, and toxocariasis, in particular, among children – and Ukraine is not an exception [5, 6]. For instance, the incidence and prevalence of toxocariasis in many countries including the USA, Europe and others is unknown [1, 3, 4, 6-8]. However, it is considered that there is a higher prevalence of *Toxocara* in tropical areas and rural settings.

The incidence of helminthiasis ranges from 64.8 to 303.2 per 100,000 in Ukraine. According to the Veterinary Service of Ukraine, the level of *Toxocara* infection in puppies is 87.4% and adult dogs 21.9% [6, 8]. There are currently no reliable statistics on the prevalence of toxocariasis among children or adults in Ukraine and in the Ternopil region, in particular.

However, children are considered to be especially vulnerable for acquiring infection because they are less likely to follow hygienic routing, more likely to eat soil and more frequently put contaminated fingers in their mouths either incidentally or intentionally and may play in contaminated environments such as public sandboxes or parks [1, 4]. Some children love dogs so much they may hug and kiss even stray animals.

Diagnosis of toxocariasis is typically confirmed by a combination of clinical signs, positive epidemiological history and laboratory results. Stool examination for *Toxocara* ova and larvae cannot be used because humans do not excrete them [1]. Serologic testing for antibodies against *Toxocara* is available. Only immunoglobulin G (IgG) is used because studies have shown that immunoglobulin M antibodies are not specific for human toxocariasis [9]. While specific anti-*Toxocara* IgG antibodies against larvae excretory/secretory (E/S) antigens using enzyme immunoassay are widely used [1]. On the other hand, the presence of antibodies does not always indicate present infection. At the same time, toxocariasis often presents in covert form and most infected people do not show specific signs of disease.

The systemic immune response to *Toxocara* infection is controlled by cytokines produced by Th1 and Th2 cells [1, 2, 10]. Interleukin-4 (IL-4), a well-known anti-inflammatory cytokine, promotes type II inflammatory response and provides antihelminthic protection by activating B cells and T cells proliferation, stimulating immunoglobulin E production, while tumor necrosis factor alpha (TNF- α) is a pro-inflammatory cytokine that is important for Th1-mediated protective immune responses [11]. TNF- α contributes to vasodilatation, edema formation, leukocyte adhesion to epithelium and oxidative stress in sites of inflammation. In addition, TNF- α takes part in different cellular events such as cell survival, proliferation, differentiation, apoptosis and necrosis. On the other hand, some studies referred TNF- α to Th2-mediated immune responses, but its role in it is unclear [11].

The Ternopil region is located in Western Ukraine, its economy is predominantly agricultural with a rural population.

The aim of this study was to investigate the seroepidemiology of toxocariasis, its clinical presentation and hygienic habits and risk factors of toxocariasis in children with digestive system diseases.

Materials and Methods

In the reported study, initially blood samples from 108 children with digestive system diseases, aged 2–17 years, were collected. All patients were recruited from the Ternopil Regional Children's Clinical Hospital. Serological tests were performed on blood samples to measure the level of specific anti-*Toxocara* immunoglobulin G (IgG) using enzyme-linked immunosorbent assay (ELISA) as a diagnostic test for toxocariasis. In order to exclude other parasitic infections, serum level of specific anti-*Ascaris* IgG using a commercial ELISA kit was used and stool tests for helminths and parasites were assessed.

Overall, a diagnostic level of anti-*T. canis* antibodies were detected in 19 (17.6%) children that did not get previous treatment and were included to the study to form Group I. As well, 44 (40.7%) patients were *Toxocara* - seronegative, did not have other parasitic infections and formed Group II. The clinical examination, immunological testing and retrospective survey were conducted for all subjects included in the study.

The serum levels of IL-4 and TNF- α were measured using ELISA kit in order to evaluate immune response; a retrospective survey of subjects and their caregivers in order to assess sanitation and hygiene practices were evaluated in 63 children included in the study. The questionnaire was developed by us according to epidemiology and risk factors of toxocariasis and other common parasites in children and evaluated by a group of experts. The Cronbach's alpha score was 0.71 for questions: "place of living," "eating unwashed vegetables," "drinking untreated water," "playing with dogs," "soil-pica," "biting nails," "washing hands" and "dropping toilets."

Parents or guardians of minors gave written informed consent and agreed to participate in the study, which was approved by the Bioethics Commission of the I. Horbachevsky Ternopil State Medical University (protocol No 24 from 27.08.2014).

Statistical data analysis was performed using the computer-based software package STATISTICA 10.0 (StatSoft Inc., Tulsa, OK, USA). The determination of data normality was done using the Shapiro–Wilk test or Kolmogorov–Smirnov test. Differences in continuous variables were analyzed using the Student's *t*-test (data expressed as mean \pm SD) or Mann–Whitney test (data expressed as median, range). The qualitative data were compared using the

Pearson's Chi-square (χ^2) test or Fisher's exact test. Spearman correlation was used to evaluate relationships involving ordinal variables. Differences were considered statistically significant if $P < 0.05$.

Results

Toxocara seropositive were 17.6% ($n = 19$) out of 108 children.

A total of 63 children with digestive diseases were included to the study: 19 Toxocara seropositive (Group I) and 44 children Toxocara seronegative and without other parasitic infections (Group II). Overall, the age of patients was 12.2 ± 3.8 years (range 3–17 years). The general characteristics of the study groups are presented in Table 1.

Increased anti-*Ascaris* IgG was in 10.5% of Group I and negative in Group II.

Past helminthic infections were common to both Group I (73.4%, $n = 14$) and Group II (72.7%, $n = 32$) ($P > 0.05$), their frequency is presented in Fig. There were no differences in types of past parasitic infections between Group 1 and Group 2.

Hygienic habits and risk factors of toxocariasis are presented in Table 2.

Main characteristics of clinical presentation of the subjects are presented in Table 3.

The serum levels of IL-4 and TNF- α in relation to study groups are presented in a Table 4.

Discussion

The incidence and prevalence of toxocariasis among children or adults in general are unknown in

many countries [1]. It is considered that frequency of toxocariasis depends on the frequency of dog and cat infections, environmental contamination with Toxocara eggs in public areas, climate, geographic location, income level, age of children, residence area and e.g.

In recent years, some research was conducted that assessed toxocariasis among children and adults. For instance, in the USA a national population-based study, NHANES III (1988–1994), revealed that among 20,395 tested persons, the Toxocara seroprevalence for individuals ≥ 6 years of age was 13.9% and generally did not decrease until 30–39 years [4]. A study performed in Mexico found that seroprevalence in children was 13.8%, while in adults it occurred rarely at 4.7% [12]. Chinese data showed that seroprevalence varied between 10.9–19.3% in different years [3]. At the same time, the seroprevalence in Polish children was relatively lower at 4.2% [7]. Simultaneously, the study conducted in the Carpathian region of Ukraine revealed that seroprevalence increased up to 42.5% [8]. Also, higher levels of seropositivity (from 45% to 98%) were found at rural African areas [13]. However, according to our results only 17.6% of children were seropositive. In addition, we consider that this assessment should be continued on more subjects in different regions of Ukraine because of different climate, geographical peculiarities and different ratio between rural and urban areas.

Other factors that can have an impact on Toxocara seropositivity are age of children, sex and

Table 1. The main characteristics of the study groups

Main characteristics	I group ($n = 19$), n (%)	II group ($n = 44$), n (%)	All patients ($n = 63$), n (%)	P
<i>Sex:</i>				
Boys	11 (57.9%)	19 (43.2%)	30 (47.6%)	0.28
Girls	8 (42.1%)	25 (56.8%)	33 (52.4%)	
<i>Place of residence:</i>				
Village	15 (79.0%)	23 (52.3%)	38 (60.3%)	0.04
Town	4 (21.0%)	21 (47.7%)	25 (39.7%)	
<i>Main pathology:</i>				
Gastroduodenal diseases	9 (47.4%)	28 (63.6%)	37 (58.7%)	0.23
Hepatobiliary diseases	10 (52.6%)	16 (36.4%)	26 (41.3%)	
Age, years, $M \pm SD$ (min; max)	10.9 ± 4.6 (3.0; 17.0)	12.8 ± 3.3 (6.0; 17.0)	12.2 ± 3.8 (3.0; 17.0)	0.15

P between II and I groups

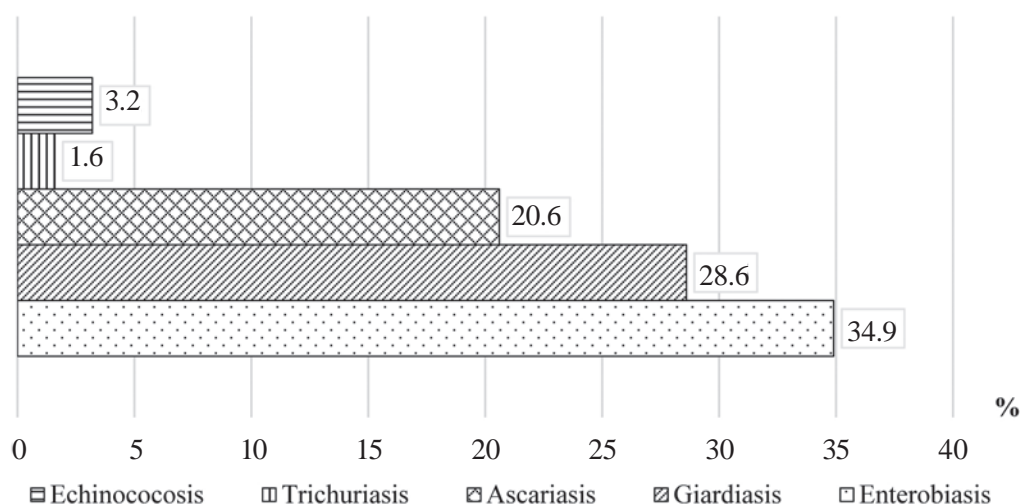


Fig. The frequency (%) of past helminthic infections among examined children ($n = 63$)

Table 2. Hygienic habits in the children of study groups

Hygienic habits	Groups of patients		<i>P</i>
	I group ($n = 19$)	II group ($n = 44$)	
Eating unwashed vegetables	17 (89.5%)	33 (75.0%)	0.19
Drinking untreated water	18 (94.7%)	37 (84.1%)	0.24
Eating uncooked meat	3 (15.8%)	8 (18.2%)	0.44
Playing with dogs	19 (100.0%)	24 (54.5%)	<0.001
Soil (sand) pica	9 (47.4%)	1 (2.3%)	<0.001
Biting nails	13 (68.4%)	23 (52.3%)	0.23
<i>Washing hands after toilet:</i>			
Do not wash	16 (84.2%)	0 (0.0%)	<0.001
Periodically do not wash	3 (15.8%)	17 (38.6%)	<0.001
Mainly wash	0 (0.0%)	27 (61.4%)	<0.001
Using of drop toilets	16 (84.2%)	23 (52.3%)	0.02
Participation in agricultural works (household farming) related to soil exposure	14 (73.7%)	32 (72.7%)	0.93

P between II and I groups

residence area. We found that mean age of infected subjects was 10.9 ± 4.6 years and did not differ between children aged 3–11 years (15.6%, $n = 8$ out 51) and those aged 12–17 years (19.3%, $n = 11$ out 57) ($P = 0.62$). While according to NHAMES III, seroprevalence was higher for persons 12–19 than 6–11 [4]. Seropositivity among Polish children was higher in school age (7.7%) than in preschoolers (3.0%) [7]. A Chinese study confirmed that children 10–11 years were seropositive (6.6%) more often in comparison with younger children [3]. In our opinion,

this may be due to higher caregiver's observation during younger ages.

Some studies have reported that persons with toxocariasis are more likely to be male [1, 14] or female [13]; however, we did not find any difference in patient's gender, as so in some other studies [1, 3, 7, 8].

Living in a rural area is considered a risk factor for toxocariasis and was shown in several studies [1, 4, 7, 8, 14]. Our results also confirm this as 79% of seropositive were rural residents, while 21% were

Table 3. The main clinical presentation of the study groups

Signs	I group (n = 19)	II group (n = 44)	P
<i>Complaints and physical examination</i>			
Abdominal pain (complaints)	13 (68.4%)	31 (70.5%)	0.87
Abdominal pain (physical exam)	14 (73.7%)	41 (93.2%)	0.03
Gallbladder signs (positive)	2 (10.5%)	22 (50.0%)	0.003
Hepatomegaly	16 (88.9%)	30 (71.4%)	0.14
Dyspeptic signs	9 (47.4%)	25 (56.8%)	0.49
Liquid feces	3 (15.4%)	1 (2.2%)	0.04
Intoxication clinical signs	10 (52.6%)	22 (50.0%)	0.84
Headache	3 (15.8%)	17 (38.6%)	0.07
<i>Laboratory and instrumental data</i>			
Eosinophils, %, M±SD, Me; 25%-75%	13.1±11.3 9.0; 7.0-14.0	5.4±4.4 4.0; 2.0-8.0	<0.001
Calcinates in liver	2 (10.5%)	2 (4.5%)	0.30
Calcinates in spleen	2 (10.5%)	0 (0.0%)	0.02

P between I and II groups, M – mean; SD – standard deviation; Me – median

Table 4. Serum interleukin-4 (IL-4) and tumor necrosis factor- α (TNF- α) levels in the examined children

Serum level of interleukins	Groups of patients		P
	I group (n = 19)	II group (n = 44)	
IL-4, pg/ml, M±SD, Me; 25%-75%	18.0 ± 6.4, 9.5; 8.4-15.7	7.2 ± 2.0, 7.8; 6.0-8.4	<0.001
TNF- α pg/ml, M±SD, Me; 25%-75%	4.5 ± 2.1, 1.3; 0.8-3.1	3.6 ± 1.1, 1.5; 0.7-3.6	>0.05

P between II and I groups, M – mean; SD – standard deviation; Me – median

urban. The explanation for this is that rural areas have a higher number of stray and domesticated dogs and cats, which may contribute to the higher degree of environmental contamination with *Toxocara* eggs. Income levels among rural residents generally are lower in comparison with urban residents. The incidence of using drop toilets and privet wells as a source of water is higher among village inhabitants. Poor hygiene habits in rural areas can also lead to a higher incidence of toxocariasis [1, 3, 4, 14].

Some of these findings were confirmed in our study. For instance, usage of drop toilets (84.2%) was related to *Toxocara* seropositivity, which also was observed in similar studies [14]. Furthermore, according to our results handwashing before eating or after visiting the toilet has been verified as a protective factor related to *Toxocara* seroprevalence. This also was found in other surveys [14].

While factors such as biting nails, consuming untreated water and unwashed vegetables were not confirmed to be risk factors for *Toxocara* infection

in this present study, some other researchers found these can lead to higher seropositivity [3, 14]. We suggest that further studies on more subjects should be done.

In several surveys exposure to soil was shown to be a risk factor of toxocariasis [1, 3, 4, 14], but the present study does not confirm this connection as household farming was almost equal among seropositive and seronegative children.

Other well-known investigated factors include playing with or owning dogs or cats (especially animal's less than 3 month of age), eating raw or undercooked meat and soil/sand pica [1, 3, 4, 8, 13, 14]. According to our results, geophagia and contact with dogs or cats were strongly connected with *Toxocara* seropositivity, while consumption of undercooked meat was not. It may be explained that meat was not contaminated with *Toxocara* larvae due to better control for products sold in the stores. Also, geophagia or sand pica and contact with dogs are higher risk factors due to soil/sand contamination with *Toxocara*

eggs as well as dogs being the main host for this parasitic infection [1].

Main clinical signs were liquid feces, eosinophilia and presence of multiple calcinates in liver and spleen. In two children who were seronegative for *Toxocara* and did not have other helminthiasis, calcinates in the liver were single and absent in the spleen. Eosinophilia is a typical finding and is a host's protective immune reaction for *Toxocara* larvae. The presence of multiple calcinates in liver and spleen is a sign of visceral larva migrans syndrome and occurs in patients with visceral toxocariasis. Calcinates also developed as a protective mechanism to localize larvae in the host's body.

Hepatomegaly and abdominal pain were common for both groups of subjects, which can be explained in that the parasitic negative group of patients had digestive system diseases – mainly chronic gastroduodenitis and functional biliary disorders. Although, with the presence of abdominal pain, positive gallbladder signs during objective examination were related to lower incidence of seroprevalence.

Other typical signs like wheezing, cough and fever were absent in this study [1].

In the present study, the assessment of cytokines levels showed that IL-4 level was significantly higher in *Toxocara* seropositive than in seronegative, while TNF- α did not differ. These results confirm that helminths modulate immune responses toward Th2-type predominance rather than induction of Th1-type response. Several studies demonstrate increased level of IL-4. For instance, Z. Araujo et al. also revealed rising IL-4 in *Toxocara* seropositive, but not among *Ascaris* seropositive [2]. The study performed by Mazur-Melewska K. et al., also found that IL-4 level increased in *Toxocara*-positive children, and in children with liver and spleen granulomas the level of IL-6 was increased [10]. According to Ierna MX, et al. [11], TNF was required for the induction of the Th2-type immune response against *Trichinella spiralis* and was important for the expulsion of helminths. According to our data, there was a highly increased level of TNF- α in children with multiple calcinates in liver and spleen and reached 27.5 pg/ml, while at the same time, IL-4 reached 42.8 pg/ml. In our opinion on the IL-4 and TNF- α levels, they impact different factors such as the amount of consumed infected eggs, immune response of host, age of children, presence of visceral larva migrans syndrome and previous helminthic or parasitic infection. For instance, in the parasite-

negative group, the level of IL-4 was significantly lower but twice as high than normal, while past helminthic infections among them were 72.7%.

Although, due to the limited number of examined subjects we suggest that further research should be conducted.

Conclusions. Toxocariasis is common among children and causes digestive diseases. It occurs more often among village inhabitant and has an impact on the immune response of children. In order to prevent spread of infections and control *Toxocara* infection, we must improve hygienic education of parents and children, require frequent disinfection of drop toilets, screen and treat dogs for *Toxocara canis* and protect public playgrounds and parks from animal feces contamination.

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Conflict of interest. Authors have completed the Unified Conflicts of Interest form at http://ukr-biochemjournal.org/wp-content/uploads/2018/12/coi_disclosure.pdf and declare no conflict of interest.

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ТОКСОКАРОЗ У ДІТЕЙ ІЗ ЗАХВОРЮВАННЯМИ ТРАВНОЇ СИСТЕМИ

К. Т. Глушко[✉], Г. А. Павлишин

Кафедра педіатрії №2, Тернопільський національний медичний університет імені І. Горбачевського МОЗ України, Тернопіль;
✉e-mail: glushko_kt@tdmu.edu.ua

Токсокароз є досить поширеним серед дітей і спричиняє захворювання органів травлення. Мета роботи полягала у дослідженні розвитку токсокарозу у дітей із захворюваннями органів травлення. У 63 дітей було визначено вміст специфічних IgG до *Toxocara canis* та *Ascaris lumbricoides*, сироваткові рівні IL-4 та TNF- α ; зроблено аналіз на гельмінти і паразити, а також проведено ретроспективне опитування для оцінки дотримання санітарно-гігієнічних пра-

вил. Дітей було поділено на дві групи: I група включала 19 (30,2 %) дітей з токсокарозом, II група – 44 (69,8 %) дитини без паразитів. Рівень IL-4 був вищим у I групі ($18,0 \pm 6,4$ пг/мл), ніж у II групі ($7,2 \pm 2,0$ пг/мл) ($P < 0,001$). Рівень TNF- α достовірно не відрізнявся між I ($4,5 \pm 2,1$ пг/мл) та II ($3,6 \pm 1,1$ пг/мл) групами ($P > 0,05$). Токсокароз частіше спостерігався серед жителів села (78,9%). У дітей з токсокарозом частішими були геофагія (42,1%), контакт з собаками (100 %), вони частіше не мили руки (84,2%) порівняно із групою неінфікованих. Відзначено, що гігієнічні звички та місце проживання сприяли зараженню *Toxocara canis*. Рівень IL-4 був значно вищим у групі I, ніж у групі II через активацію імунної відповіді хазяїна, тоді як рівень TNF- α не відрізнявся. Діти без паразитозів також мали підвищений рівень IL-4, що може бути пов'язано з перенесеними попередніми інфекціями.

Ключові слова: діти, токсокароз, захворювання травної системи, інтерлейкін-4, фактор некрозу пухлин- α .

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