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Seroprevalence of *Toxocara* at Tra Vinh University Hospital in Vietnam

T.B. NGUYEN[1], T.T.H. NGUYEN[2], S.Q. HUYNH[3], T.V. PHU[1], A.M. TAHA[4], D. NGUYEN[5], H.M. LE[2], H.N. NGUYEN[2], L.T.K. NGUYEN[2], N.T. TRAN[2]

[1]Faculty of Medicine and Pharmacy, Tra Vinh University, Tra Vinh, Vietnam
[2]Public Health Department, Can Tho University of Medicine and Pharmacy, Can Tho, Vietnam
[3]Department of Emergency, Can Tho S.I.S General Hospital, Can Tho, Vietnam
[4]Faculty of Medicine, Fayoum University, Fayoum, Egypt
[5]Massachusetts General Hospital, Corrigan Minehan Heart Center, Harvard Medical School, Boston, MA, USA

Abstract. – OBJECTIVE: The study aims to assess the seroprevalence of Toxocariasis and its associated risk factors among individuals attending the outpatient department at Tra Vinh University Hospital, Vietnam, in 2022.

SUBJECTS AND METHODS: A cross-sectional survey was conducted among outpatients of Tra Vinh University Hospital. Toxocariasis diagnosis was based on the Enzyme-Linked Immunosorbent Assay (ELISA) performed at the hospital's laboratory department. We assessed the seroprevalence of Toxocariasis and evaluated associated risk factors, including demographics and certain behaviors.

RESULTS: Of the 249 participants surveyed, 165 tested positive for Toxocariasis, yielding a seroprevalence of 66.3% (95% CI: 60.4-72.1). Multivariate analysis revealed that age groups up to 30 and 30-60 years had higher odds of Toxocariasis infection, with adjusted odds ratios (aOR) of 2.52 (95% CI: 1.04-6.11) and 3.21 (95% CI: 1.44-7.15) respectively. Additionally, individuals residing in rural areas and those in contact with dogs or cats had increased risks, with aORs of 2.21 (95% CI: 1.21-4.01) and 2.04 (95% CI: 1.10-3.79), respectively. Notably, hand washing before eating emerged as a protective factor against Toxocariasis, presenting an aOR of 0.38 (95% CI: 0.19-0.76).

CONCLUSIONS: Our findings underscore a significant seroprevalence (66.3%) of Toxocara spp. among outpatients at Tra Vinh University Hospital. Proactive measures, including hand hygiene before meals and after pet interactions, are advocated. There is a pronounced need for community-level epidemiological surveillance for human Toxocariasis.

Key Words:
Seroprevalence, Toxocariasis, Tra Vinh, Vietnam.

Abbreviations
ELISA: Enzyme-Linked Immunosorbent Assay; IgG: Immunoglobulin G; T-seroprevalence: prevalence of anti-Toxocara serum antibodies.

Introduction

Toxocariasis is caused by a series of related nematode species (ascarids) that infect dogs and cats worldwide. Eggs from these roundworms are commonly found in human habitations as dogs and cats live with humans as pets, making them a common environmental contaminant. In contrast, countless others run wild throughout the streets of most urban centers. The eggs in dog and cat feces become infectious within weeks after they are deposited in the local environment (e.g., sandboxes, city parks, public beaches, etc.). Humans, particularly children, frequently ingest these eggs accidentally and become infected. In contrast to their definitive hosts, human infection remains occult, often resulting in disease caused by the migrating larval stages[1]. Recent epidemiological research[2,3] has estimated that ~1.4 billion people worldwide, particularly in subtropical and tropical regions, are infected with or exposed to *Toxocara* species, indicating that human Toxocariasis is a neglected tropical disease. The estimated global anti-*Toxocara* serum antibodies (referred to as T-seroprevalence) was 19.0% (95% CI: 16.6-21.4%; 62,927/265,327); seroprevalence was the highest in the African region (37.7%; 25.7-50.6%) and the lowest in the Eastern Mediterranean region (8.2%; 5.1-12.0%). The pooled seroprevalence was 34.1% (20.2-49.4%) in Southeast Asia,

Corresponding Author: Tuyen Thi Hong Nguyen, MD; e-mail: nthtuyen@ctump.edu.vn

24.2% (16.0-33.5%) in the Western Pacific, 22.8% (19.7-26.0%) in the American; and 10.5% (8.5-12.8%) in the European regions (4). A significantly higher T-seroprevalence was associated with a lower income level, lower human development index, lower latitude, higher humidity, higher temperature, and higher precipitation (p-value < 0.001). Potential risk factors associated with seropositivity to *Toxocara* included male gender, living in a rural area, young age, close contact with dogs, cats, or soil, consumption of raw meat, and drinking untreated water[4].

In Vietnam, some studies[5-8] determining the seropositivity prevalence for Toxocariasis were conducted in hospitals and communities but only in a few locations. A study[5] at Central Highlands University Hospital indicated that the seropositivity prevalence for Toxocariasis in humans was 57.8%; in Medic Medical Center Laboratory, Ho Chi Minh City, Vietnam, in 2012, this prevalence was 45.2%[6]. A cross-sectional survey[7] was conducted on 1,327 people, followed by KAP surveys with 400 household representatives in 2 communes of Mo Duc district, Quang Ngai province, in 2016. Data were collected from the sero-immunological test (ELISA-IgG) and face-to-face interviews using the pre-designed questionnaires. The results indicated that the overall infection proportion was 17.3%, of which Duc Phong and Duc Chanh communes' prevalence was 17.9% and 16.7%, respectively. A significant difference was shown in the seroprevalence between patients aged 15 and under 15 years old (19.9% *vs.* 12.8%, $p < 0.05$). In these studied communes, 54.5% of households reported raising dogs (almost free-ranged, 83.5%); only 11.9% of those households dewormed their dogs. Food sources contaminated with *Toxocara canis* larvae have the potential to cause ascariasis in humans. The findings of a study[8] conducted in Can Tho, Vietnam revealed that less than 50% of street food vendors adhere to food safety regulations.

Tra Vinh province is located in the Mekong Delta of southern Vietnam. In Tra Vinh, 29 ethnic groups reside. The Kinh represent 69% of the population, while the Khmer account for 29%. The majority of the people work in agriculture, forestry, and fishery, which accounts for 46.89%[9]. Based on a preliminary investigation[10] at Tra Vinh University Hospital, eight out of every ten individuals tested positive for Toxocariasis when they underwent serology checks. We have not identified any recent studies that indicate the seroprevalence of Toxocariasis in humans in Tra Vinh, Vietnam. Although it is estimated that tens of millions of people are exposed to or infected with *Toxocara spp.*, the epidemiological data on Toxocariasis remains limited[11]. Consequently, we undertook this study to determine the seroprevalence and assess some related factors of Toxocariasis among individuals visiting the outpatient department at Tra Vinh University Hospital.

Subjects and Methods

Study Design

This cross-sectional study was carried out from August - September 2022 at Tra Vinh University Hospital.

Sample Size

The sample size was determined using the formula for estimating a proportion. where n is the smallest sample size to be achieved in the study; p is the expected prevalence of toxocariasis; d is the absolute error: 5%; $Z_{(1-\alpha/2)}$ is Z statistic for a level of confidence of 95%. This formula was calculated based on a prevalence of Toxocariasis of 80% from a previous initial investigation[10]. The smallest sample size to be achieved in the study was 246. The expected response rate was 10%. The total number of people to be investigated was 274. We surveyed 249 people.

Exclusion criteria: patients who returned for parasite re-examining suffering from acute and chronic infections, cancer, and autoimmune diseases. Patients who could not give interviews (people with mental illness, deaf and mute people). The participants were selected using a systematic random sampling technique in which every 10th person visiting the outpatient department at Tra Vinh University Hospital was included.

Data Collection

Interviewing techniques

Study participants were given a clear explanation of the purpose of the study and agreed to participate. Questions in Vietnamese included socio-demographic profile, personal history, behavior and other life habits.

The questionnaire mainly consisted of three parts:

1) The first part of the questionnaire comprised questions related to age, sex, ethnicity, religion, accommodation, education, jobs, income, and personal history.

2) The second part of the questionnaire comprised questions related to personal behavior and other life habits.

In this study, we used the RIDASCREEN® *Toxocara* IgG test, a kit ELISA that detects specific IgG antibodies in human serum infected with *Toxocara* spp. All tests were performed at the hospital's laboratory using IgG ELISA kits according to the manufacturer's instructions. The RIDASCREEN® *Toxocara* IgG test, R-Biofarm test kit, has been approved by the European Union for the quality and safety of *in vitro* medical devices. The test has a sensitivity of 100% and a specificity of 90.7%[12].

Ethical Approval

Ethical approval was obtained by the Medical Ethics Committee of Tra Vinh University, Vietnam (Approval No.16/HĐĐĐ, 23/08/2022) before data collection could begin. Study participants were informed about the study objectives and gave informed consent. All participants had the right to withdraw from the study at any time. The anonymity and privacy of the participants were maintained throughout the data collection and reporting process. Care was taken to ensure that no harm or discomfort was caused to any study participant.

Statistical Analysis

After detecting and correcting (or removing) corrupt or inaccurate records from a record set, data were entered into Epi Data 3.1 software and transferred to STATA 17 software for data analysis (StataCorp LLC, College Station, TX, USA). Descriptive statistics (percentage and frequencies) were calculated to assess the percentage and the positive ELISA test. The Chi-square test for the difference in prevalence between the two groups. Then, the multivariable logistic regression model was used to assess the relationship between different factors and the prevalence of toxocariasis. The independent variables from the Chi-square test in univariate analysis with a p-value < 0.5 were included in the model. The reported results are adjusted OR (aOR) and 95% confidence intervals (95% CI). A p-value < 0.05 was considered significant.

Table I. Participant's characteristics (n = 249).

Socio-demographic characteristics	Frequency	Percentage
Age group (years)		
≤ 30	69	27.7
31-60	144	57.8
> 60	36	14.5
Gender		
Male	70	28.1
Female	179	71.9
Ethnicity		
Kinh	212	85.1
Others	37	14.9
Religion		
Yes	61	24.5
No	188	75.5
Residence		
Countryside	158	63.4
City	91	36.6
Occupation		
Farmer	47	18.9
Others	202	81.1
Education		
≤ 12 years	179	71.9
> 12 years	70	28.1
Household economy		
Poor and near-poor	19	7.6
Other	230	92.4

Results

249/278 people voluntarily participated in the study; the response rate was 91%. Table I presents some characteristics of the study participants. The mean age was 42.5, the median age was 43, the lowest was 12, and the highest was 80. People aged 30 and under, 31-60, and 61 and older were 27.7, 57.8, and 14.5%, respectively. Females, people living in rural areas, and Kinh were 71.9%, 85.1%, and 63.4%, respectively.

Table II shows that the percentage of study subjects positive for Toxocariasis is 66.3% (95% CI: 60.4-72.1). Table III presents the relationship

Table II. Seroprevalence of *Toxocara* antibodies among outpatients (n = 249).

Samples	N	%	95% CI
Positive	165	66.3	60.4-72.1
Negative	84	33.7	27.9-39.6

Table III. The Seroprevalence of Toxocariasis by selected demographic factors and behavior characteristics among outpatients at Tra Vinh University Hospital.

Values	Total	T-seroprevalence		*p*
		Yes (%)	No (%)	
Age group (years)				
≤ 30	69	42 (60.9)	27 (39.1)	
31-60	144	106 (73.6)	38 (26.4)	0.006
> 60	36	17 (47.2)	19 (52.8)	
Gender				
Male	70	49 (70.0)	21 (30.0)	0.436
Female	179	116 (64.8)	63 (35.2)	
Ethnicity				
Kinh	212	139 (65.6)	73 (34.4)	0.577
Others	37	26 (70.3)	11 (29.7)	
Religion				
Yes	61	40 (65.6)	21 (34.4)	0.895
No	188	125 (66.5)	63 (33.5)	
Residence				
Countryside	158	117 (74.0)	41 (26.0)	0.001
City	91	48 (52.7)	43 (47.3)	
Occupation				
Farmer	47	34 (72.3)	13 (27.7)	0.328
Others	202	131 (64.8)	71 (35.2)	
Education				
≤ 12 years	179	122 (68.2)	57 (31.8)	0.313
> 12 years	70	43 (61.4)	27 (38.6)	
Household economy				
Poor and near-poor	19	13 (68.4)	6 (31.6)	0.836
Other	230	152 (66.1)	78 (33.9)	
Drinking water				
Treated	71	48 (67.6)	23 (32.4)	0.778
Untreated	178	117 (65.7)	61 (34.3)	
Eating fresh vegetables				
Yes	215	146 (67.9)	69 (30.1)	0.168
No	34	19 (55.9)	15 (44.1)	
Eating raw meat				
Yes	191	122(63.9)	69 (36.1)	0.148
No	58	43 (74.1)	15 (25.9)	
Washing hands before eating				
Yes	178	108 (60.7)	70 (39.3)	0.003
No	71	57 (80.3)	14 (19.7)	
Owning dogs or cats				
Yes	141	96 (68.1)	45 (31.9)	0.488
No	108	69 (63.9)	39 (36.1)	
Contact with dogs or cats				
Yes	185	131 (70.8)	54 (29.2)	0.010
No	64	34 (53.1)	30 (46.9)	
Other worm infections				
Yes	12	7 (58.3)	5 (41.7)	0.551
No	237	158 (66.7)	79 (33.3)	

between some population characteristics of the study subjects and the prevalence of Toxocariasis in univariate analysis. We recorded two characteristics related to the seroprevalence: the participants' age group and the place of residence. The prevalence of Toxocariasis of those aged 30 and under, 30-60 years old, and over 60 was 60.9%, 73.6%, and 47.2%, respectively (p = 0.006). People living in rural and urban areas had a prevalence of Toxocariasis of 74% and 52.7%,

respectively (p = 0.001). Other characteristics such as gender, ethnicity, religion, occupation, education, and income have not been related to the prevalence of Toxocariasis. We recorded two behaviors related to the prevalence: handwashing before eating and after the contact with dogs or cats. Those who have wash their hands before eating and do not wash their hands before eating have a prevalence of Toxocariasis at 60.7% and 80.3%, respectively (p = 0.003). Those who interacted with dogs or cats and did not have contact with dogs or cats had a prevalence of Toxocariasis of 70.8% and 53.1%, respectively (p = 0.01). The seroprevalence of those drinking untreated water, eating raw vegetables, eating raw meat, and owning dogs or cats was 67.6%, 67.9%, 63.9%, and 68.1%, respectively. However, behavioral characteristics were not associated with the prevalence of Toxocariasis.

We selected factors associated with Toxocariasis at p-values below 5% in univariate analysis to further analyze in a multivariate logistic regression model. Four variables were associated with the T-seroprevalence in the multivariable logistic regression model and are shown in Table IV. Three related factors that increase the infection likelihood for *Toxocara* spp. were the age group, living place, and contact with cats or dogs. Those aged 30 and younger, 30-60 years old, had an aOR of 2.52 (95% CI: 1.01-6.11) and 3.51 (95% CI: 1.44-7.15), respectively, compared with those over 60 years old ($p < 0.05$). Living in rural areas and having contact with cats or dogs had aORs of 2.21 (95% CI: 1.21-4.01) and 2.04 (95% CI: 1.1-3.79), respectively ($p < 0.05$). Those who washed their hands before eating had an aOR of 0.38 (95% CI: 01.9-0.76) with a p-value of 0.006.

Discussion

The results of our study showed that the T-seroprevalence among people visiting the outpatient department at Tra Vinh University Hospital was 66.3% (95% CI: 60.4-72.1). This prevalence was relatively high; approximately 2/3 of people who came to the clinic were positive for this parasite. The notable finding of a high seropositivity prevalence (66.3%) against *Toxocara* spp. among individuals who visited our University Hospital in 2022 underscores the significance of maintaining a heightened index of suspicion for pathologies potentially linked to larvae. This emphasizes the need for continued vigilance and diligent consideration of larva-related conditions within Vietnam healthcare practices. However, this was not representative of the community. The result prompts the need for community-level research. The T-seroprevalence in the present study was significantly higher than in some other studies[5,6,13-15] in Vietnam. The prevalence of Toxocariasis in Tay Nguyen University Hospital was 57.8%[5], in Medic Medical Center Laboratory, Ho Chi Minh City, was 45.2%[6], in Hanoi Medical University Hospital was 59%[13] and was also higher than the reported infection prevalence of 30.2-33.3% in other parts of Vietnam[14,15]. The results

Table IV. Multiple logistic regression (final model) analysis of risk factors associated with the seroprevalence of Toxocariasis among outpatients at Tra Vinh University Hospital

Values	aOR	95% CI for aOR		p
		Lower	Upper	
Age group (years)				
≤ 30	2.52	1.04	6.11	0.040
31-60	3.21	1.44	7.15	0.004
> 60	1			
Residence				
Countryside	2.21	1.21	4.01	0.009
City	1			
Washing hands before eating				
Yes	0.38	0.19	0.76	0.006
No	1			
Contact with dogs or cats				
Yes	2.04	1.10	3.79	0.024
No	1			

obtained in Vietnam demonstrate a significantly high and variable seropositivity rate for *Toxocara* spp, across various locations in the country. One possible hypothesis for this phenomenon is that geographical and climatic factors create favorable conditions for the larval development of these worms in the soil. Furthermore, lifestyle factors, such as dog ownership and the practice of allowing dogs to roam freely, may also contribute to the situation. Petting dogs and cats also facilitate the accidental transmission of diseases from animals to humans. Our research showed that the percentage of households keeping dogs or cats is up to 68.1%. Multiple studies[7] conducted in Vietnam have uncovered a prevalent trend of households keeping dogs and allowing them to roam freely. The prevalence of *Toxocara* spp. in our study approximated that in Brazil[16] at 64.6% ($p > 0.05$). However, the T-seroprevalence in Tra Vinh appears to be higher compared to other countries such as Nigeria[17], Iran[18], North America[19], Mexico[20], Central African Gabon[21], and China[22]. We also found that the T-seroprevalence in our study was lower than 79.3% and 71.8%, which were reported in Columbia[23] and Southern Rio Grande do Sul[24] ($p < 0.05$), respectively.

In the present study, the T-seroprevalence in males was 70% and in females was 64.8%, but the difference was not statistically significant; this was also found in some other studies in Vietnam[13-15] and Columbia[23]. However, some studies[6] showed that females were more affected than males). Other population characteristics, such as ethnicity, religion, employment, education, and household economy, have not been associated with the prevalence of Toxocariasis in our study. The age group was related to the seropositivity prevalence in univariate and multivariate analysis. Multivariate analysis showed that the age group under 31, and 31-60 had a higher risk of Toxocariasis than those over 60 with aOR = 2.52 (95% CI: 1.04-6.11), aOR = 3.21 (95% CI: 1.44-7.15), respectively. This finding is consistent with other studies[7-22] in which age was found to be related to the prevalence of Toxocariasis in humans. People aged up to 60 could be a group of workers who are more likely to be exposed to environmental factors than people over 60 years old could be an explanation for this difference.

This study also revealed that people in rural areas (including cities and towns) were likelier to have Toxocariasis than those in urban areas with aOR = 2.21 (95% CI: 1.21-4.01). This finding is similar to other studies in China[25] and Turkey[26]. Gastrointestinal infections are more commonly affected in rural areas than in urban areas. Therefore, interventions on Toxocariasis should focus on this population. Eating raw vegetables and consuming raw/undercooked meat are typical eating behaviors of Vietnamese people, but in this study, we have not found an association between Toxocariasis and these behaviors. A study[27] in Thailand also found no association between the habit of eating raw vegetables and T-seroprevalence. However, a study[25] in China reported that consuming raw/undercooked meat was a risk factor for *Toxocara* infection.

In univariate analysis, we noted several factors significantly related to the T-seroprevalence. Regarding modifiable risk behaviors, handwashing before eating appeared to be the only significant protective factor associated with *Toxocara spp.* infection in both the univariate and multivariate analyses. People practicing handwashing before eating were less likely to be infected with *Toxocara spp.* than those who did not, with aOR = 0.38 (95% CI: 0.19-0.76) and ($p = 0.006$). This effect was also observed in previous studies in Vietnam[5], and Thailand[27]. This study emphasizes the importance of hand hygiene to prevent parasite eggs from entering the body. Contact with pets, especially dogs and cats, is a typical behavior of Vietnamese people[7]. Contacting dogs and cats increased the T-seroprevalence with aOR = 2.04 (95% CI: 1.11-3.79) and a *p*-value of 0.024. This risk behavior was also recognized in many studies in Vietnam[5-7] and worldwide[19]. Therefore, proper personal hygiene, such as washing hands after contact with pets, is a critical behavior to control the infection of *Toxocara spp.*

Despite the potential contributions of this study to the T-seroprevalence among people visiting the outpatient department at Tra Vinh University Hospital, the following limitations still need to be addressed. First, an ELISA test, which is not the gold standard, was used to detect anti-*Toxocara spp.* IgG antibodies; *Toxocara spp.* larval excretory-secretory Western blot could show false positive results due to cross-reactivity with other helminths, especially *A. lumbricoides*[28]. Second, the study design was cross-sectional, and T-seroprevalence and risk factors were evaluated simultaneously; thus, true causes and effects might not be powerfully demonstrated. Third, the study was conducted only in outpatients presenting to the hospital; therefore, the T-seroprevalence in the hospital may not reflect the status of Toxocariasis in the community.

Conclusions

The seropositivity prevalence against *Toxocara spp.* was found to be relatively high (66.3%) among individuals visiting Tra Vinh University Hospital, Vietnam, in 2022. Some demographic characteristics, such as age, living in the countryside, and individual behavior (washing hands before eating and after contact with dogs and cats), influence the prevalence of toxocariasis. Therefore, proper hygiene is a fundamental measure to control human Toxocariasis. Epidemiological surveillance for human Toxocariasis should be carried out at the community level.

Conflict of Interest

The authors declare that they have no conflict of interests.

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Ethics Approval

Ethical approval was obtained by the Medical Ethics Committee of Tra Vinh University, Vietnam (Approval No. 16/HĐĐĐ, 23/08/2022) before data collection could begin.

Informed Consent

Study participants were informed about the study objectives and gave informed consent. All participants had the right to withdraw from the study at any time. The anonymity and privacy of the participants were maintained throughout the data collection and reporting process. Care was taken to ensure that no harm or discomfort was caused to any study participant.

Availability of Data and Materials

The data sets during and/or analyzed during the current study are available from the corresponding author on reasonable request.

Authors' Contribution

All authors made critical contributions to the multidisciplinary follow-up and management of patients in the cohort and the drafting of the study. TBN and TTHN designed the details of the study. DN, TVP, TTHN, and AMT provided the data. TBN, TTHN and SQH investigated the data and ensured accurate and strict exclusions according to the study criteria. The analysis was carried out by TTHN interpreted the analysis and wrote the paper. DN, TVP, TTHN, HML, HNN, LTKN, NTT and AMT contributed to the critical evaluation and revision of the manuscript. TBN and TTHN shaped the final version, and all authors approved the final version of the article.

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