

Research article

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## Sero-epidemiology of toxocariasis among school aged Children in Ho Chi Minh City: Preliminary results from a two phases community study

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### Abstract

**Background:** Children are especially vulnerable to *Toxocara* infestation and its severe complications, however, there has not been any published data on the disease prevalence and treatment effectiveness in pediatric population. This paper was to present preliminary results of the study to determine the prevalence of toxocariasis in children aged 3 to 15 in Ho Chi Minh City (HCMC) and its associated factors.

**Methods:** A cross-sectional study was conducted between March and April 2023. Study was carried out at public schools, where participants were taken blood samples and given clinical examination to look for toxocariasis. Participants diagnosed with the infestation were invited to have treatment with Albendazole. Data was collected and analyzed to answer questions about toxocariasis prevalence and its associated factors.

**Results:** Anti - *Toxocara* antibodies were detected in 14.2% (140/986) of the study population. There were significant differences in percentage of serologically positive children among different grades, districts and carer's educational level. Risk factors for sero-positivity based on multivariable analysis were educational level of carers, contact with dogs and/or cats, and improper collecting and disposing of dogs and/or cats stool.

**Conclusion:** This was the community-based prevalence study of toxocariasis in a pediatric population in Vietnam. Implementation of preventive measures such as public education, routine faecal examinations and chemotherapeutic treatment of animals is highly recommended.

**Keywords:** Toxocariasis, Children, *Toxocara* infestation, Vietnam.

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### 1. INTRODUCTION

*Toxocara canis* and *Toxocara cati* are among the most widely distributed parasitic worms and have a wide zoonotic impact [1, 2]. Toxocariasis is endemic Southeast Asia, where the climate is generally warm and ideal for the proliferation of soil-transmitted helminthes [3]. Despite its widespread presence, Toxocariasis is among the neglected tropical diseases (NTDs)

[4], hence the scarcity in data. The trend is reflected in Vietnam, where only sporadic data on the disease is available, and it suggests that toxocariasis is prevalent among the population. The sero-prevalence of toxocariasis is 42.9% to 59% in patients who visited hospitals for helminthiasis examinations in Vietnam, which makes this the dominant soil-transmitted helminth infestation diagnosed [5, 6]. In Hanoi,

the second biggest city in Vietnam, a study on urban households with pet dogs and cats found that 37.7% cats and 47.8% dogs were infected with *Toxocara spp.*, and 58.7% people living in those households were seropositive of *Toxocara* antibodies [7].

Vietnam has made some significant progress in limiting NTDs, among those is achieving the World Health Organization target in deworming children [8]. Ho Chi Minh City, the most populous city with the largest children population in Vietnam, has maintained this achievement through regular deworming doses administered in schools for all school-aged children. However, more needs to be done because there is still an increasing wave of migration from other provinces, which contributes to its growing population and introduces more challenges to maintain people's health. Due to its developing infrastructure and distinct culture, its citizens, especially those living in poverty, are still vulnerable to soil-transmitted helminthiasis from exposures of street food contamination and unhygienic living conditions [6, 9].

Besides socioeconomic and environmental risk factors, significant behavioral risk factors for toxocariasis include contact with soil, history of geophagia, and poor hygiene [10-12]. Children are more susceptible to infestation, which can be due to their immune system not fully developed and lack of attention to hygienic practices [2, 10]. The lack of data on toxocariasis in Vietnam in general has also persisted [6, 15]. Therefore, we proposed a study to (i) determine the seroprevalence of *Toxocara spp.* infestation among children aged 3 to 15 in HCMC and (ii) determine the effectiveness and safety of albendazole as treatment for symptomatic toxocariasis in children aged 3 to 15 in HCMC.

## 2. METHODS

### 2.1. Study design

The study was carried out in two phases with different designs: The first phase was a cross-sectional study followed by a quasi-experimental study. The data collection for the first phase of the study was conducted at schools including public kindergarten, primary

and secondary schools during school days through collaboration with HCMC department of education and training to ensure participation from parents and students. The laboratory testing and the second phase were conducted at Children's Hospital 2.

All children aged 3 to 15 from each participating school were eligible to join, except those who had history of allergic reactions to substances of phlebotomy equipment (alcohol, latex, etc.) or who were unable or refused to fast for at least 8 hours prior to blood collection. The students and whose parents or legal guardians provided consent were included in phase 1. For the intervention phase, the children who were diagnosed with common toxocariasis and the parents or immediate caregivers of the children were invited to continue with the study.

The sample size for phase 1 was calculated using the formula for prevalence. With the desired level of confidence of 95%, precision of 0.04, the expected prevalence of 19.3% [16], and the design effect for cluster sampling is 2.0, the minimum sample size was 768 participants. From our experience in conducting research in school children, we estimate that the acceptance rate was approximately 80%, yielding approximately 960 students to achieve the required sample size. For phase 2, we planned to include all participants diagnosed with toxocariasis from the cross-sectional phase. Based on a study of anti-*Toxocara* antibodies in children aged 1 to 18 in urban cities in China, the average sero-prevalence of *Toxocara* infestation was 19.3% [16]. Therefore, we expected to have at least 186 participants with positive results to continue with phase 2. All participants in phased 2 were diagnosed of toxocariasis, thus they all received treatment and there was no randomization due to ethical restrictions.

The multistage cluster sampling method was applied to recruit participants. In the first stage, we divided the population based on two criteria: geographic areas - urban and rural, and school grades (from preschool year 1 to 3, to grade 9). There are 16 urban districts, 1 city and 5 rural districts in HCMC. According to the 2019 Population and Housing Census, the ratio of

children aged 3 to 15 years living in urban areas to those living in rural areas in HCMC was 3:1. In terms of grades, we assumed that the population in each grade did not vary significantly, so we recruited the same number of participants from each grade. Based on the second assumption that the distribution of schools is in accordance with the ratio of population in urban and rural areas, we chose three schools in urban districts and one in rural districts in each level (preschool, primary school, or secondary school) to be included in the study. In each school, we recruited 20 participants from each grade total number of cases.

## 2.2. Data collection

Blood samples were collected by nurses from Children's Hospital 2. Each participant was required to give 4ml of whole blood, which was divided into three tubes as follows: 0.5ml for total blood counts, 1.5ml for liver and kidney function tests, 2ml for enzyme-linked immunosorbent assay (ELISA) test for anti-*Toxocara* antibodies. To test for anti-*Toxocara* antibodies, we used the AccuDiag™ *Toxocara* IgG ELISA Kit designed for qualitative detection of IgG antibodies to *Toxocara* in human serum. The kit has been approved for such usage by the Vietnam Ministry of Health. The sensitivity and the specificity of the test kit are 87.5% and 93.3%, respectively, according to the instructions for using of Diagnostic Automation/ Cortez Diagnostics Inc manufacturer.

Six months after the completion of treatment, all phase 2 participants were tested with the same set of examinations to determine the efficacy of the treatment and detect other possible long-term effects on bio-physiological functions. Parents or immediate caregivers of the children were asked to complete a questionnaire using provided tablets on the day of blood sample collection. The questionnaire was designed using Kobo toolbox, and collects information on participant's sex, age, address, parents' income and education, parent's knowledge on toxocariasis prevention, clinical symptoms and consequences, and child's history of exposure to toxocariasis risk factors.

The diagnosis criteria were based on Vietnam Ministry of Health (MOH) guidelines

on treatment for toxocariasis [17]. A suspected case was defined as having a history of close contact with dogs and/or cats or having other risk factors of toxocariasis and the following symptoms: itching, Urticaria; headache, abdominal pain, or indigestion; pain or numbness; fever; wheezing. These symptoms may be accompanied by one or more of the following symptoms: hepatomegaly, pneumonia, chronic abdominal pain, focal neurological disorders, vision loss, retinal damage. A confirmed case of toxocariasis in this study was defined as a suspected case with anti-*Toxocara* antibodies detected in blood serum using ELISA, with or without eosinophilia.

In Vietnam, according to the guidelines of the Ministry of Health in 2020, treatment of common toxocariasis for adults and children is albendazole 10-15 mg/kg/day and not more than 800 mg/day for 5-14 days, divided into 2 times/day, drink after eating.

The study was registered at ClinicalTrials.gov with Trial registration Identifier NCT05208333. This was also approved by the Institutional Review Board of Pham Ngoc Thach University of Medicine and HCMC Department of Health. Informed consent was obtained from all study participants and their parents for each phase of the study.

## 2.3. Data management and analysis

Data were processed and analysed using the STATA software (version 15). To determine the relationship between variables, t-test was used to compare means of independent variables; Chi-square test was used to compare proportions. Odds ratios (ORs) and 95% confidence intervals (CIs) were calculated for risk factors. Uni-variate analysis was started to assess the association between positive seroprevalence and independent variables. Only variables that were significantly associated with positive seroprevalence in children (t-test, with p-value < 0.2) in this model were retained for sub-sequent steps. The multiple regression model was used to determine possible risk factors and the outcome. Crude odds ratios (ORs) were compared with adjusted ORs (aORs) to decide which variables should be kept in the model.

### 3. RESULTS

From March 2023 to April 2023, a total of 986 pupils were studied in the cross-sectional phase including 474 (48.07%) boys and 512 (51.93%) girls with an average age of  $9.79 \pm 3.26$  years old. The infected pupils were continued to be examined and had re-tested after six months. As the treatment process is on going, this paper only shows preliminary results from the cross-sectional study.

**Table 1:** Characteristics of studied subjects

	Urban		Rural		Total	
	Number	Percentage (%)	Number	Percentage (%)	Number	Percentage (%)
Gender						
Boys	294	46.97	180	50.00	474	48.07
Girls	332	53.03	180	50.00	512	51.93
Total	626	100	360	100	986	100
Grade						
Kindergarten	93	14.86	116	32.22	209	21.2
Primary school	319	50.96	120	33.33	439	44.52
Junior high school	214	34.18	124	34.44	338	34.28
Total	626	100	360	100	986	100

Number of pupils participated in the study were not much different between two sexes. However, number of pupils participated in the study were significantly different among grades: kindergarten, primary schools and junior high schools.

**Table 2:** Prevalence of anti - *Toxocara* antibodies in the studied sample

Characteristics	Total n = 986	Positive results (95% CI) n = 140	p-value
Gender			0.5*
Girls	512	15% (12%, 18%)	
Boys	474	14% (11%, 17%)	
Grade			< 0.001\$
Kindergarten	209	9.1% (5.7%, 14.0%)	
Primary school	439	11.0% (8.4%, 15.0%)	
Junior high school	338	21.0% (17.0%, 26.0%)	
Studied district			< 0.001*
Thu Duc	293	12.0% (8.6%, 16.0%)	
Nha Be	360	23.0% (19.0%, 28.0%)	
Binh Thanh	220	6.8% (4.0%, 11.0%)	
District 1	113	7.1% (3.3%, 14.0%)	
Carer's educational level			< 0.001\$

Characteristics	Total n = 986	Positive results (95% CI) n = 140	p-value
No schooling/Primary school	9	44.0% (15.0%, 77.0%)	
Junior/Senior high school	490	18.0% (15.0%, 22.00%)	
University or higher	487	9.9% (7.4%, 13.0%)	
Carer's occupation			0.6*
Officer	299	13.0% (9.3%, 17.0%)	
Agriculture related jobs	17	18.0% (4.7%, 44.0%)	
Others	670	15.0% (12.0%, 18.0%)	

\* Results of chi-square test; \$Results of chi-square for trend

Anti - *Toxocara* antibodies were detected in 14.20% (140/986) of the study population. There were significant differences in percentage of serologically positive children among different grades, districts and carer's educational level whilst prevalence of anti - *Toxocara* antibodies was not different between gender or among carer's occupation.

**Table 3:** Risk factors and their relationships with *Toxocara* sero-positivity children

Characteristics	n	OR1	95% CI3	P	aOR2	95% CI3	p-value
Carer's educational level	986			< 0.001			< 0.001
No schooling/Primary school		1	-		1	-	
Junior/Senior high school		0.27	0.07-1.12		0.26	0.07-1.11	
University or higher		0.14	0.04-0.57		0.11	0.03-0.48	
Carer's occupation	986			0.64			0.076
Officer		1	-		1	-	
Agriculture related jobs		1.47	0.33-4.77		0.67	0.14-2.42	
Others		1.19	0.80-1.80		0.54	0.32-0.92	
Consumption of fresh vegetables	986			0.12			
Frequently		1	-		-	-	
Infrequently		0.73	0.48-1.15		-	-	
No		0.58	0.35-0.97		-	-	
Wash vegetables	986			0.46			
Properly		1	-		-	-	

Characteristics	n	OR1	95% CI3	P	aOR2	95% CI3	p-value
No		1.17	0.77-1.74		-	-	
Contact with dogs or cats	986			< 0.001			< 0.001
Frequently		1	-		-	-	
Infrequently		0.76	0.48-1.22		0.78	0.48-1.26	
No		0.35	0.23-0.54		0.32	0.21-0.51	
Consumption of cooked foods	986			0.47			
No		1	-		-	-	
Yes		0.66	0.24-2.31		-	-	
Flushing worms for dogs or cats	986			0.80			
No		1	-		-	-	
Yes		0.94	0.56-1.52		-	-	
Collect and dispose of dog or cat stool properly	986			0.056			0.009
No		1	-		1	-	
Yes		0.62	0.36-1.01		0.50	0.28-0.85	
Washing hands after contacting with soil	986			0.26			
No		1	-		-	-	
Frequent		0.80	0.55-1.18		-	-	
Washing hands before eating	986			0.005			0.059
No		1	-		1	-	
Frequent		0.55	0.37-0.83		0.65	0.43-1.02	

1OR = Odds Ratios; 2aOR = adjusted OR; 395% CI = 95% Confidence Interval

Results from uni-variate analyses showed that the following variables were significantly related to *Toxocara* sero-positivity: Educational level of carers, contact with dogs or cats,

washing hands before eating ( $p < 0.05$ ). After controlling for other factors in multi-variate analyses, educational level of carers, contact with dogs or cats, and collect and dispose of

dog or cat stool properly remained significantly related to *Toxocara* sero-positivity.

#### 4. DISCUSSION

Toxocariasis is a neglected parasitic disease that can cause serious health problems. The prevalence in Ho Chi Minh City (HCMC) children was similar in Iran [18] and Mexico [19], or even lower than that in Indonesia [20], Cuba [21] and Brazil [22]. This may be due to the similar climate in these countries. Furthermore, the high percentage of children exposed to infestation in the population (might be due to the high humidity, moist soil and suitable temperature for development of *Toxocara* eggs) could explain the higher prevalence of the last three countries listed above. Economic status may also affect the epidemiology compared with country with economic difficulties such as Cuba. Nevertheless, the sensitivity and specificity of the ELISA method varies and strongly depends on the target antigens and antibodies chosen by the manufacturer. Due to the lack of an alternative confirmation method, such as western blot, and the possible cross-reactivity with other infective agents, the few false-positive results cannot be ignored.

In general, the prevalence rate in healthy individuals (not eosinophilic subjects) is diverse in different countries. The seroprevalence has been reported with relatively higher exposure levels in some countries including 29.8% in Nigeria [23], 76.6% in Taiwan [24], 63.2% in Indonesia [20], and 50.6% in Brazil [25]. These countries also have relatively similar economic status that may play an important role in *Toxocara* epidemiology via its impact on public health and sanitation.

Among socio-demographic factors, carer's educational level at university or higher (aOR = 0.11, 95% CI = 0.03, 0.48) had a lower odds of sero-prevalence. These results are consistent with the study by Mohammad Reza et al. in Iran [12, 26]. The knowledge accompanied with the high level of education might be the explanation for this finding. In the present study, neither age nor gender seemed to be important factors related to a positive serology. Similar findings have been reported in Iran and Nigeria

[23, 26] while some reports indicated that boys had a greater opportunity to acquire *Toxocara* infestation, as they had more contact with dogs [27]. Sero-prevalence of *Toxocara* infestation in school-aged children did not increase with age, perhaps due to the sample size in each age being too small to find a discrepancy in age vs. sero-prevalence.

In the present study, frequent contact with dogs and/or cats, and collecting or disposing of dogs and/or cats stool improperly were risk factors for toxocariasis in children. They probably acquired the infestation through inadvertently ingesting eggs contaminating a dog's body. A recent study indicated that dogs infected with *T. canis* might infect people by direct contact because of the high density of embryonating and embryonated eggs in their fur [28]. The present results further suggested that *Toxocaral* infestation in HCMC school children was also acquired by the ingestion of soil containing infective eggs, confirming the earlier report that contamination of soils with *T. canis* eggs was relatively high. As proven in another study [19], hygiene habits, housing area and customs were factors that primarily influenced the variation of sero-prevalence of *T. canis*, and the risk of transmission increases with the degree of environmental pollution. Humans as paratenic hosts get infected through the fecal-oral route by the accidental ingestion of embryonated eggs through contact with dogs, cats, and soil and also consumption of contaminated raw vegetables or undercooked meat from other paratenic hosts [29]. Contamination with *Toxocara spp.* from the land in parks, playgrounds, gardens and beaches at home or at school is considered a critical factor for toxocariasis, as well as contact with dogs and/or cats.

This is community-based prevalence study of toxocariasis in a pediatric population in Vietnam. The study was conducted because of the lack of epidemiological data on this infestation in children in Vietnam, especially in HCMC. A limitation of the study is that diagnosis of human toxocariasis was restricted to the detection of antibodies, not allowing the distinction between current and past

infestation. The cross-sectional nature of the study hampers the identification of the temporal sequence of events.

## 5. CONCLUSION

The results of this investigation revealed a sero-prevalence of 14.20 % in children of HCMC, Vietnam. We observed a higher percentage of sero-positivity in junior high school students, in those studying in Nha Be District and those whose parents had no schooling or just finished primary school. Implementation of preventive measures such as public education, routine faecal examinations and chemotherapeutic treatment of animals is highly recommended.

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