SEROPREVALENCE OF IgG ANTIBODIES OF HUMAN TOXOCARIASIS AND ASOCIATED RISK FACTORS IN KHANH HOA PROVINCE

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Background: Human toxocariasis is a zoonotic parasitic disease caused by Toxocara canis and T. cati roundworm larvae. While many studies have shown that dog and cat owners are at a higher risk of acquiring Toxocara spp. infection, there is no available evidence regarding the seroprevalence of Toxocara spp. infection among dog and cat owners in Khanh Hoa. Therefore, this study aims to investigate the prevalence of anti-IgG of Toxocara spp. infection and associated risk factors for visceral larval migrans among dog owners in three different areas of Khanh Hoa province..

Subjects and methods: A descriptive cross-sectional study was conducted from 2022 to 2024 in three localities representing different socio-geographic areas (urban, rural, and mountainous) of Khanh Hoa province. A total of 1502 blood samples were collected from local individuals aged 5 to 75 for this study. Serum anti-T. canis IgG antibodies were detected using a commercial enzyme-linked immunosorbent assay (ELISA) kit: NovaLisaTM, Toxocara canis IgG ELISA. Simultaneously, a survey using structured questionnaire about information on risk factors associated with Toxocariasis was conducted among 720 people (aged 18 and over) from 1,502 individuals whose bloods were taken for testing.

Results and conclusions: A total of 1,502 blood samples were examined for the presence of antibodies to dog and cat roundworm larval infection. Results revealed an overall positive rate of 57.66% across the three localities, with variations depending on geography. In Khanh Vinh, a mountainous district, the infection rate was 75.44%, and in its commune Khanh Trung, it was 81.20%. For the two urban wards, the rates were significantly lower (Phuoc Long Ward: 44.40% and Van Thanh Ward: 54.18%). A structured questionnaire was used to investigate risk factors among 720 individuals (aged 18 years old and over) out of the 1,502 individuals whose bloods were tested. Results indicated that 389 of them tested positive for the ELISA test. Infection rates varied among age groups: under 25 years old (6.94%), 25-50 years old (66.78%), and over 50 years old (37.28%). Notably, 49.87% of infected individuals owned dogs or cats, 93.32% regularly consumed raw vegetables, and 25.71% frequently handled dogs or cats. The high infection rate of dog and cat roundworm larvae in Khanh Hoa underscores the presence of several identified high-risk factors.

Keywords: *Human Toxocariasis infection, risk factors, Khanh Hoa province.*

INTRODUCTION

Toxocara infection in humans is a neglected parasitic disease with public health implications, transmitted accidentally from animals to human through the ingestion of Toxocara eggs or larvae. Once in the body, larvae travel through the bloodstream to various organs, including the eyes, liver, intestine, and other organs, leading to significant diseases or syndromes. At Khanh Hoa Hospital of Tropical Diseases, during the period from 2016 to 2019, the number of individuals seeking examination due to symptoms associated with Toxocarasis infection accounted for 74.50% in the Elisa Toxocara IgG test samples. Despite being considered a common disease, comprehensive investigations into the current infection status and related factors are lacking. Moreover, there have been

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no implemented measures to prevent this disease in local areas. Therefore, it is essential to conduct a study to determine the prevalence of Toxocara infection in the community and identify the risk factors for this parasitic infection in Khanh Hoa province. This research aims to contribute to minimizing pathogen pollution in the environment and safeguarding the health of the local population.

SUBJECTS AND METHODS

Population of the study and sample size

For seroprevalence: The study population consisted 1502 individuals aged from 5 to 75 years old from different households in 6 communes (or wards) in 3 different socio-geographical areas of Khanh Hoa Province. The sample size was determined using a single-proportion population as follows:

$Z^2p(1 - p) / d^2$

where p is the seroprevalence from a previous study, d is the margin of error, and Z is the standard score, which corresponds to 1.96. This formula was calculated based on a prevalence rate of 17% from a previous study (27), with a margin of error of 2% and a confidence level of 95%. The calculated sample size was 1500.

Interpretation of Results for Toxocara antibodies Elisa test:

Cut-off point was set at 10 NTU (NovaTec Units).

Positive > 11NTU

Equivoqual: 9 - 11 NTU

Negative: < 9NTU

For questionnaire and survey: The questionnaire consisted of 32 questions to collect information on the general demographic data of the dog owner (gender, age, district, , occupation, and education), information on the dog (number of dogs, species, health check/ vaccination, deworming,), and information on possible risk factors (playing with the dog; kissing or touching the dog; location of feces discharge; feces management; and habits of hand washing before a meal, after soil contact, and after contact with the dog).

Data entry and processing: using PASW Statistics 18.

RESULTS

Toxocara Canis infection rate

Communes, Wards

Select three localities representing urban, rural, and mountainous areas of Khanh Hoa province. For each locality, choose two wards or communes. Randomly select 250 people from each ward (commune) to have their blood tested for Elisa IgG.

Table 1. Toxocariasis infection in communes/wards

Commune/City	Commune/Ward	Number of people tested	Number of positive cases
Nha Trang	Phuoc Long Ward	250	111 (44.40%)
	Van Thanh Ward	251	136 (54.18%)
Dien Khanh	Dien Son commune	250	123 (49.20%)
	Dien An commune	250	118 (47.20%)
Khanh Vinh	Khanh Vinh Town	251	175 (69.72%)
	Khanh Trung Commune	250	203 (81.20%)
Total		**1502**	**866 (57.65)**

In 1502 blood sample tests, the Toxocariasis infection rate accounted for 57.65%. This rate was nearly equal to that reported in Do Trung Dung's study (2016)[1]: 58.70%, higher than the rates found in Le Dinh Vinh Phuc's study (2021)[3]: Ben Tre 10.80%, Dong Nai 10.00%, Long An 9.20%, Ba Ria Vung Tau 7.50%, Binh Thuan 5.80%, and Binh Duong 5.00%. Comparatively, the positivity rate in Kondo's study (1998)[9] conducted in Japan was 1.6%, which is lower than the rate observed by Magnaval (1994)[12] on La Reunion island (92.8%).

Districts, towns

Table 2. Toxocariasis infection in districts/cities

Districts/Cities	Number of people testing	Number of positive cases
Nha Trang city	501	247 (49.30%)
Dien Khanh district	500	241 (48.20%)
Khanh Vinh district	501	378 (75.44%)
Total	**1502**	**866 (57.65%)**

Among 866 positive Toxocara canis IgG Elisa samples, the positivity rates in different communes and districts were as follows:

- Nha Trang: 49.30%.

- Dien Khanh: 48.20%.

- Khanh Vinh: 75.44%.

Out of the three randomly chosen local areas in Khanh Hoa province tested to determine the prevalence of Toxocara infection, Khanh Vinh district exhibited the highest infection rate at 75.44%. This rate was nearly equal to that found in Pham Thi Thu Hoai's study (2014)[2], which reported a rate of 74.90% in children in Yen Lac commune, Yen Dinh district, Thanh Hoa. Additionally, it was higher than the rate reported in Liao's study (2010)11 in Swaziland (44.6%) and lower than the rate in Rai's study (1996)13 in Nepal (81%).

Risk factors of Toxocara infection

Association of age groups and Toxocara infection

Age-groups divided by quartile:

Group 1: < 25 years old (6.94%).

Group 2: 25 - 50 years old (55.78%).

Group 3: > 50 years old (≥ 37.28%).

Table 3. Age group infected with Toxocariasis

Results	Age group			Total
	Group 1: < 25 years old	Group 2: 25 - 50 years old	Group 3: > 50 years old	
Positive	27 (55.10%)	217 (61.13%)	145 (45.89%)	389 (54.03%)
Negative	22 (44.90%)	138 (38.87%)	171 (54.11%)	331 (45.97%)
Total	49	355	316	720
Chi-Square = 15.659 ; p = 0.000: statistically significant difference.				

The age group 25 - 50 had the highest Toxocara infection rate at 55.78%, while the age group under 25 years old had the lowest infection rate at 6.94%. This result aligns with the findings of Le Dinh Vinh Phuc's study (2021)[3]. However, it differs from the results of Coelho LMPS and Silva MV (2004)[6], where the infection rate in children under 18 years old in Sorocala city, Sao Paulo state, Brazil, was reported as 38.30%.

Association between sex and Toxocariasis infection

Table 4. Sex and Toxocariasis infection

Test result	Sex		Total
	Male	Female	
Positive	112 (50.68%)	277 (55.51%)	389 (54.02%)
Negative	109 (49.32%)	222 (44.49%)	331 (45.98%)
	221	499	720
Chi-Square = 1.440 ; p = 0.230			

The results showed that the Toxocariasis infection rate was higher in females (55.51%) and a little lower in males(50.68%). This indicates that the results were nearly equal to those in Le Dinh Vinh Phuc's study (2021)[3], where the rates were reported as 62.50% for females and 37.50% for males. Likewise, the results were also similar to those of Cao Van Huyen's study (2018)[4], where the infection rates were 52.70% for males and 47.30% for females.

Association between consumption of raw vegetables and Toxocariasis infection

Table 5. Frequent consumption of raw vegetables and Toxocariasis infection

Test result	Consumption of raw vegetables		Total
	Yes	No	
Positive	363	26	389
Negative	306	25	331
	669	51	720
Chi-Square: 0.2052 ; p = 0.651: no statistical difference			

Results demonstrated that there is no statistical difference between people who frequently consumed raw vegetables and those did not. Comparing this result to Phan Anh Tuan's study, an association was found between raw vegetable consumption and Toxocara infection[5].

Association of owning dogs, house cats and Toxocariasis infection

Table 6. dogs, cats and Toxocarisais infection

Test result	Dogs and house cats		Total
	No	Yes	
Positive	195 (49.49%)	194 (59.51%)	389 (54.03%)
Negative	199 (50.51%)	132 (40.49%)	331 (45.97%)
	394	326	720
Chi-Square = 7.207a; p = 0.007: statistically significant different			

Results showed that people who keep dogs and cats have a higher risk of Toxocariasis infection than those who do not, and the difference was statistically significant. The investigation result in An Phu hamlet (Cu Chi district, Ho Chi Minh city) showed that areas with households owning free-running dogs have 38% of people infected with Toxocarasis. The Toxocara spp. infection rate was 2 times higher in the group of people owning dogs or cats[10].

Table 7. Frequent hugging dogs, cats and Toxocariasis infection

Test result	Hugging dogs, cats		Total
	No	Yes	
Positive	289 (52.45%)	100 (59.17%)	389 (54.03%)
Negative	262 (47.55%)	69 (40.83%)	331 (45.97%)
	551	169	720
Chi-Square: 2.352; p = 0.125: Not statistically different			

T. canis eggs were identified in 10.70% of dog's hair samples, including embryonated eggs with larvae, in a study conducted in Egypt[7]. This poses a potential risk of direct transmission of Toxocariasis from domesticated dogs to humans during close-contact activities. Children who usually play with dogs have a greater risk of Toxocara spp. infection than those who do not, with an odds ratio (OR) of 1.83[8].

DISCUSSION

Toxocariasis in humans is a neglected zoonotic parasite that affects millions of pediatric and adolescent populations globally, especially in tropical and subtropical regions. This is the first serological investigation of Toxocara spp. infection among dog and cat owners in Khanh Hoa. The present study showed that the overall seroprevalence of T. canis infection was 57.65%. Compared with the infection rate of Toxocara spp. at 55%, the overall infection rate of Toxocara canis larvae in humans in two communes (Duc Phong, Duc Chanh) in Mo Duc district (Quang Ngai) is 17.3% (Bui Van Tuan, Doctoral thesis, 2018). This infection situation is also much higher than research projects in Iran (20.43%) and Egypt (29.85%)[14]. In addition, the results of our study were also higher than the globally reported seroprevalence (19.0%) and the pooled seroprevalence in Southeast Asia (34.1%)[15], Thailand (58.2%)[17], Vietnam (45.2%)[16], and the Philippines (49%)[17]. However, the seroprevalence rate in this study was lower than that in other regions, including Makoko, Nigeria (86.1%)[18], and the Republic of the Marshall Islands (86.75%)[19].

Several elements contribute to Toxocara spp. infection, including geographic, socioeconomic, climatic, environmental, and cultural factors. The seropositivity rates of Toxocara spp. infection were similar across all age groups: < 25 years old (55.10%), 25 - 50 years old (61.13%), and over 50 years old (45.89%). However, this result contrasts with several studies that showed older age as a significant risk factor for Toxocara spp. infection[13,21,22]. Serum Toxocara IgG can persist with age, increasing the detection of Toxocariasis via serology over time. A possible explanation for these similar seropositivity rates among different age groups may be that dog owners came in contact with T. canis eggs at a young age, as IgG persists over a long period of life. Presumably, the tropical climate of the province is suitable for the development of the infective larval stage of Toxocara spp. eggs. Dogs are the most popular pets in the region, with a large number of free-running dogs in the area. We hypothesized that the soil, the number of dogs in the areas, and the human-animal bond influenced the seropositivity rate in this study.

Humans acquire Toxocara spp. by accidentally ingesting infected eggs, which can be found in several public places[23]. This situation is evident in the analysis of the consumption of raw vegetables. There is no significant difference in the infection rates between people who consume raw vegetables and those who do not. This study suggests that people might be infected with Toxocara spp. in ways other than by the consumption of raw vegetables and emphasizes the necessity of hand hygiene to prevent parasite eggs from entering the human body via the fecal-oral route.

The study had several limitations. First, ELISA is a method for detecting anti-T. canis IgG antibodies, which is not the reference laboratory test for T. canis detection, Toxocara spp. larval excretory-secretory antigens Western blot, and secondly, it could yields false-positive results due to cross-reactivity with other helminths, especially *A. lumbricoides*[24]. A descriptive cross-sectional survey in 383 pupils from grade 3 to grade 5 in two villages in another district of Khanh Hoa province (Ninh Hoa district) showed a prevalence of *Ascaris* in lumbricoides infection (15.1%). Therefore, the probability of false positivity should be considered. However, this is a cross-sectional research, and the seroprevalence and the risk factors were evaluated concurrently and not over a period of time; hence, the true causes and effects might not be strongly determined.

CONCLUSIONS

- The Toxocara infection rate in Khanh Hoa province was 57.65%, with Khanh Vinh district having the highest infection rate at 75.44%.

- The age group with the highest infection rate was 25 - 50 years old.

- The rate of women infected with Toxocariasis is higher than that of men.

- Results demonstrated that there is no statistical difference between people who frequently consumed raw vegetables and those did not.

- Families owning dogs and cats have a statistically significantly higher risk of Toxocara infection than households that do not.

- People who hug dogs and cats have a higher risk of Toxocara infection than those who do not.

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