Journal of Tropical Pediatrics, 2017, 0, 1–6
doi: 10.1093/tropej/fmw094
Original paper

Seroprevalence of Toxocariasis In Children With Urticaria: A Population-Based Study

by Paula Mayara Matos Fialho,[1] Carlos Roberto Silveira Correa,[1] and Susana Zevallos Lescano[2]

[1]Faculdade de Ciências Médicas, Departamento de Saúde Coletiva, Campinas, São Paulo, Brasil
[2]Instituto de Medicina Tropical de São Paulo, Laboratório de Imunopatologia da Esquistossomose (LIM 06), Universidade de São Paulo, Cerqueira César, São Paulo 05403000, Brazil
Correspondence: Paula Mayara Matos Fialho, Departamento de Saúde Coletiva, Faculdade de Ciências Médicas, Universidade Estadual de Campinas, Brasil. Tel: +55 19 3521-9238. E-mail <paulamayara2@gmail.com>

ABSTRACT

Objective: This study described the prevalence of IgG class antibodies against *Toxocara* spp. and their association with urticaria in 2- to 12-year-old children.
Methods: This population-based cross-sectional study was conducted between May 2012 and September 2014. The study sample comprised 168 children. Blood samples were collected to verify the presence of toxocariasis by using ELISA to detect IgG antibodies. The guardians of the children were interviewed to characterize the presence or absence of other diseases, such as urticaria.
Results: The presence of urticaria was observed in 38% of participants. The seroprevalence of toxocariasis in this population was 16%. This study confirmed a positive association between urticaria and positive serology for *Toxocara* and a negative independent association with canine contact and the number of household residents.
Conclusions: There are no previous reports in the literature of a population-based study that correlates the presence of urticaria with serology for toxocariasis.

KEYWORDS: epidemiology, pediatrics, toxocariasis, tropical medicine, urticaria.

INTRODUCTION

Toxocariasis is a cosmopolitan zoonosis caused by the helminth species *Toxocara canis* and *Toxocara cati*, which infect dogs and cats, respectively. Humans are paratenic hosts, and contamination occurs by accidental ingestion of eggs in the embryonic stage [1]. Children aged between 2 and 12 years are most affected by this parasite [2, 3]. The principal ways in which children can be contaminated by *Toxocara* are the ingestion of vegetables contaminated with eggs of this parasite, contact with canine and feline cubs, consumption of meat and/or raw or undercooked viscera of chicken, ducks or cattle infected with *T. canis* larvae [4, 5].

Prevalence studies show that toxocariasis is present worldwide, and studies conducted in Brazil report that this prevalence has ranged from 4.2% to 65.4% [6–8]. Studies in several countries have shown that allergic reactions occur frequently in individuals with positive serology for *Toxocara*, suggesting that infection with this intestinal parasite contributes to the development of such reactions [9–12], including urticaria. An association between urticaria and parasitic infections has been already demonstrated by a variety of studies [13–15].

The present work aimed to describe the prevalence of IgG class antibodies against *Toxocara* spp.

© The Author [2017]. Published by Oxford University Press. All rights reserved. For Permissions, please email: journals.permissions@oup.com

and their association with urticaria in children aged between 2 and 12 years living in Campinas, São Paulo, Brazil.

MATERIALS AND METHODS

This population-based cross-sectional study was conducted between 2012 and 2014 in the district of São Marcos, Northern Campinas, São Paulo. The district has 17 474 inhabitants, 4148 of whom were children aged between 1 and 14 years.

The study population consisted of 200 children aged between 2 and 12 years identified from random household sampling, registered in the Sistema de Gerenciamento da Atenção Básica (SIGAB) of the Health Department of Campinas.

The sample size ($n = 200$) was calculated using the parameter of 50% presumed prevalence of factors to be described (asthma, toxocariasis), aiming to maximize the size of the sample. In this region, 100% of the households were registered in SIGAB. The population was estimated at 5000 inhabitants. Each household has, on average, one child in the age group of interest; thus, it was necessary to randomly choose 200 households. The selection was conducted using the Epi-info 6 program.

Ethical aspects and sociodemographic and clinical variables

After the necessary research clarifications and the signing of the informed consent form, one of the four trained interviewers asked the child's guardian the questions related to the questionnaire.

The questionnaire was divided into blocks and contained the variables described below:

a. Demographic and socioeconomic conditions: Date of birth; age (later stratified as children aged <5 years or >5 years); child's sex; number of people living in the residence; mother's schooling (in years); number of people living in the house who have an income; presence of a dog at home; presence of a cat at home; contact with dog; and contact with cat.
b. Behaviors and health-related conditions: Intradomiciliary smoking; respiratory symptoms in the past 15 days (to be considered positive if the child had gone to some health care service because of respiratory problems in the past month or if the child had any of the following respiratory symptoms: productive cough, noise or wheezing, or if the child woke up at night in the past 15 days owing to cough or shortness of breath); or urticaria (to be considered positive if the guardian made reference that the child presented, during the last month, any injury to the skin characterized by redness and swelling). To characterize the urticaria, the mother or the child's guardian was asked the following questions:

"Did your son or daughter go to see the doctor or any health service in the past year because of urticaria?"
"Does your son or daughter have any sort of sore, itchy, blistering or reddish stain on the skin?"

The study was approved by the Research Ethics Committee of the Faculty of Medical Sciences, State University of Campinas and the Tropical Medicine Institute of the University of São Paulo.

Collection of blood samples

Blood samples were collected from the children by digital puncture on filter paper (Whatman n° 3®). Data were collected in previously prepared booklets, using 1 cm wide strips of filter paper separated by cellophane strips, and then registered according to the sequence of collected samples. The child's ring finger was disinfected with 70% alcohol, and then the fingertip was pierced using a disposable lancet. Blood was collected by sliding the ribbon filter paper on the finger until it was completely absorbed. The strips were dried at room temperature and then were stored at −20°C.

Blood samples were sent to the laboratory, eluted and then subjected to ELISA with *T. canis* excretory-secretory (TES) antigen, to examine for IgG class antibodies.

TES antigen was prepared from the culture of second-stage larvae (L2) cultivated *in vitro* in accordance with the technique of de Savigny (1975) [16] with some modifications. Briefly, female worms collected from puppies were washed with tap water and dissected. Eggs were collected from the uterus and incubated in formalin 2% at 28°C for about 30 days, after which eggs were washed with saline solution and hatched with 0.5% hypochlorite acid solution in an Erlenmeyer flask with glass beads. Further steps required the use of a laminar flow bench. Larva were washed with saline solution and recovered with a Baermann apparatus, mixed with Eagle medium containing 80 μg/ml gentamicin in glass tubes, and incubated at 37°C. The supernatant containing TES Ag was collected once weekly when adding more sterile Eagle medium; protease inhibitor (phenylmethylsulfonyl fluoride) 5 mM was added to the antigen thus obtained and stored in aliquots at −20°C. About 900 ml of supernatant were concentrated 50–100 times in the Amicon apparatus, dialyzed against distilled water and ultracentrifuged. The protein concentration of antigen was 1.670 μg/ml, as determined by the method of Lowry et al. (1951) [17]. The resulting TES was aliquoted in vials and stored at −20°C until use.

All sera were absorbed with total *Ascaris suum* antigenic extract to avoid cross-reactions with common *Ascaris* antigens.

The cutoff density point in serological assays varied from 0.330 to 0.390 and was determined for every test using the mean of the optical density of 30 sera of the negative control group plus two standard deviations.

Statistical analysis

Analysis of variables compared proportions of children with and without urticaria. Associations between the independent variables and the urticaria were analyzed by using a chi-square test, and when the expected value of any case was <5, we applied Fisher's exact test. In addition, a logistic regression analysis was used to estimate the odds ratio of the variables studied with the variable presence of urticaria taken as the dependent variable. Initially, the independent variables that showed a significance level of 20% ($p < 0.20$) in association with the dependent variable were introduced, and those with $p < 0.10$ remained in the model. One-tailed testing was performed, and differences were considered statistically significant when $p \leq 0.05$. Statistical analyses were conducted with SAS 9.4 and Epi Info 7 software.

RESULTS

Among the 168 children studied, 64 (38%; 64 of 168) had urticaria. The seroprevalence of toxocariasis in this population was 16% (26 of 167) and was diagnosed using ELISA. The average age of children was 6.44 ± 3.55 years. Regarding sex, 63.7% were male and 36.3% were female. The prevalence of respiratory problems was 54.2% (91 of 168). Among the children studied, 4.8% (8 of 168) had been hospitalized because of respiratory problems.

Table 1 shows the variables related to socioeconomic, behavioral and health conditions regarding the presence or absence of urticaria. We found that 38.3% of boys and 37.7% of girls had urticaria, though a significant difference between the sexes was not identified ($p = 0.93$). The number of people in the household and the number of people testing positive for urticaria were significantly associated ($p = 0.03$). Among children with positive serology for *Toxocara*, 46.5% had urticaria ($p = 0.37$). Among the children that were hospitalized owing to respiratory problems, 62.5% were positive for urticaria ($p = 0.10$).

The results of logistic regression analysis are shown in Table 2. It was possible to verify the positive independent association between urticaria and toxocariasis. Contact with or the presence of a dog in the household, the number of people in the residence and residing with fewer than six people each showed a negative independent association with the presence of urticaria.

DISCUSSION

It was possible to observe a positive association between urticaria and positive serology for *Toxocara* in the children studied, and there was a negative independent association with canine contact and the number of household residents. The result of the present study has not, to the knowledge of the authors, been reported in any other population-based study. The categorization of children as having a positive history for urticaria was determined by

Table 1. Socioeconomic and behavioral profile and health conditions of 168 children evaluated for the presence of urticaria in Vila Esperança, São Marcos, Campinas-SP, 2012–14

Variables	Number	Frequency	Urticaria				*p**
			Positive		Negative		
	N	%	*N*	%	*N*	%	
Sex							
Male	107	63.7	41	38.3	66	61.7	0.93
Female	61	36.3	23	37.7	38	62.3	
Number of people in the residence							
<6	133	79.2	56	42.1	77	57.9	0.03
>6	35	20.8	8	22.9	27	77.1	
Number of people with work							
<3	152	90.5	56	36.8	96	63.2	0.30
>3	16	9.5	8	50.0	8	50.0	
Mother's level of education							
Did not attend school	124	73.8	52	41.9	72	58.1	0.08
Attended school	44	26.2	12	27.3	32	72.7	
Presence or contact with dog at home							
Yes	86	52.8	26	30.2	60	69.8	0.06
No	77	47.2	34	44.2	43	55.8	
Presence or contact with cat at home							
Yes	39	23.9	17	43.6	22	56.4	0.31
No	124	76.1	43	34.7	81	65.3	
Intradomiciliary smoking							
Yes	38	23.3	11	28.9	27	71.1	0.25
No	125	76.7	49	39.2	76	60.8	
Respiratory problems							
Yes	91	54.2	36	39.6	55	60.4	0.67
No	77	45.8	28	16.5	49	63.6	
Hospitalization owing to respiratory problems							
Yes	8	4.8	5	62.5	3	37.5	0.10**
No	160	95.2	59	36.9	101	63.1	
Toxocariasis							
Yes	26	15.6	12	46.2	14	53.8	0.37
No	141	84.4	52	36.9	89	63.1	

*p value of chi-square test.
**Fisher's exact test.

means of a questionnaire delivered by trained interviewers.

The positive serological association for *Toxocara* infection and urticaria has been described by Wolfrom et al. (1995) [14], who conducted a case-control study including 51 patients with chronic urticaria, where they found a strong association between this infection and dermatological manifestations. Although other studies have also already pointed out this association, this is not a consistent finding. For instance, in a study of 122 children between 6 and 15 years of age in the municipality of

Table 2. Logistic regression model for urticaria with 168 children living in Vila Esperança, São Marcos, Campinas-SP, 2012–14

Variables	OR	95% CI
Toxocariasis	2.51	1.12–5.58
Presence or contact with dog at home	0.56	0.332–0.97
Number of people in the household: <6	0.33	0.15–0.75

Uberlândia-Minas Gerais, Brazil, the authors found no association between toxocariasis and urticaria in atopic children [18].

The prevalence of urticaria in this study (38%) and positive serology for *T. canis* (16%) were similar to those found in other studies conducted in Brazil [18–21], but less than those found in other serological surveys in the same region [22].

The causes of dermatitis, including urticaria, are multifactorial, one of which is infection by intestinal parasites. The literature describes skin changes such as urticaria and pruritus as some of the clinical symptoms found in patients with toxocariasis [23].

Another important point detected in the analysis was the association between urticaria and the number of household residents. Living in a household with fewer than six people had a negative independent association compared with living in a household with more than six people, suggesting that living in a residence with fewer individuals protected the children from urticaria. This finding goes against the so-called 'Hygiene Hypothesis' formulated after a study conducted by Strachan (1989) [24] who studied children and young people up to 23 years of age, and found that children from larger families and older siblings were significantly more protected from allergic diseases. On the other hand, studies in the city of Salvador-Bahia [25] showed that allergic diseases occur in >30% of low-income populations, and in high-income segments of the population, these numbers reach 44%. It is worth noting that this study was conducted in a low socioeconomic region, where most homes consist of several members, and often not all members are from the same family.

Canine presence or contact also demonstrated a negative independent association with urticaria. That is to say, having a dog at home was a protective factor against urticaria. Some studies suggest that exposure to dogs or cats during childhood is associated with reduced risk of allergic diseases [26]. Thus, it can be suggested that contact with pets early in life would be a protective factor against allergy.

However, feline presence or contact did not show any association with urticaria. Reports in the literature of the association between allergies and the contact/presence of dogs and/or cats are common. The existence of stray dogs and cats is common in the study area, and most children in this locality play with the animals.

Sex and age were not associated with urticaria. The average age was about 6 years. These results are similar to those presented by other authors, as this age group is the one with the highest exposure to the environment and allergen exposure [3].

One limitation of the present data is that there are many dogs wandering in the surroundings. These dogs are least cared for and possibly most parasitized. However, soil contamination by *Toxocara* eggs was not studied in this work. Thus, another factor that may be associated is that having a dog at home or in possession of the family can ward off wandering or surrounding dogs.

It should be noted that this was one of the few population-based studies that used a randomly sampled population. This methodology minimizes bias and makes it possible for the results to be reproduced in the reference population.

There are no previous reports in the literature of a population-based study that correlates the presence of urticaria with serology for toxocariasis.

In conclusion, our findings confirmed the association of urticaria with toxocariasis. Infection by *Toxocara* should always be considered in diagnosis of chronic urticaria.

ACKNOWLEDGMENTS

We thank the Fundação de Amparo à Pesquisa do Estado de São Paulo (Grant no. 2012/14134-6) for the PhD scholarship for PMMF; we also thank Dr Guita Rubinsky Elefant for her valuable assistance during the ELISA procedure.

REFERENCES

1. Rubinsky-Elefant G, Hirata CE, Yamamoto JH, *et al*. Human toxocariasis: diagnosis, worldwide seroprevalences and clinical expression of the systemic and ocular forms. Ann Trop Med Parasitol 2010;104:3–23.
2. Alderete JM, Jacob CM, Pastorino AC, *et al*. Prevalence of toxocara infection in schoolchildren from the Butantã region, São Paulo, Brazil. Mem Inst Oswaldo Cruz 2003;98:593–7.
3. Figueiredo SDP, Taddei JAAC, Menezes JJC, *et al*. Estudo clínico-epidemiológico da toxocaríase em população infantil. J Pediatr (Rio J) 2005;81:126–32.
4. Carvalho EAA, Rocha R. Toxocariasis: visceral larva migrans in children. J Pediatr (Rio J) 2011;87:100–10.
5. Morimatsu Y, Akao N, Akiyoshi H, *et al*. familial case of visceral larva migrans after ingestion of raw chicken livers: appearance of specific antibody in bronchoalveolar lavage fluid of the patients. Am J Trop Med Hyg 2006;75:303–6.
6. Souza RF, Dattoli VCC, Mendonça LR, *et al*. Prevalência e fatores de risco da infecção humana por *Toxocara canis* em Salvador, Estado da Bahia. Rev Soc Bras Med Trop 2011;44:516–9.
7. Guilherme EV, Marchioro AA, Araujo SM, *et al*. Toxocariasis in children attending a Public Health Service Pneumology Unit in Paraná State, Brazil. Rev Inst Med Trop São Paulo 2013;55:189–92.
8. Correa CRS, Bismarck CM. Toxocariasis: incidence, prevalence and the time serum remains positive in school children from Campinas, SP, Brazil. J Trop Pedriatrics 2010;56:215–6.
9. Buijs J, Borsboom G, Renting M, *et al*. Relationship between allergic manifestations and Toxocara seropositivity: a cross-sectional study among elementary school children. Eur Respir J 1997;10:1467–75.
10. Sharghi N, Schantz PM, Caramico L, *et al*. Environmental exposure to *Toxocara* as a possible risk factor for asthma: a clinic-based case-control study. Clin Infect Dis 2001;32:e111–6.
11. Chan PW, Anuar AK, Fong MY, *et al*. *Toxocara* seroprevalence and childhood asthma among Malaysian children. Pediatr Int 2001;43:350–3.
12. Pinelli E, Brandes S, Dormans J, *et al*. Infection with the roundworm Toxocara canis leads to exacerbation of experimental allergic airway inflammation. Clin Exp Allergy 2008;38:649–58.
13. Heratizadeh A, Werfel T, Kapp A. Atopische dermatitis: hygiene-hypothese. Hautarzt 2006;57:576–85.
14. Wolfrom E, Chêne G, Boisseau H, *et al*. Chronic urticaria and Toxocara canis. The Lancet N.d 345:196.
15. Dal T, Çiçek M, Uçmak D. Seroprevalence of IgG anti-Toxocara canis antibodies and anti-Fasciola sp. antibodies in patients with urticaria. Clin Ter 2013;315–7.
16. Savigny DH. In vitro maintenance of Toxocara canis larvae and a simple method for the production of Toxocara ES antigen for use in serodiagnostic tests for visceral larva migrans. J Parasitol 1975;61:781–2.
17. Lowry OH, Rosebrough NJ, Farr AL, *et al*. Protein measurement with the Folin phenol reagent. J Biol Chem 1951;193:265–75.
18. Grama DF, Lescano SZ, Pereira Mota KC, *et al*. Seroprevalence of *Toxocara* spp. in children with atopy. Trans R Soc Trop Med Hyg 2014;108:797–803.
19. Manini MP, Marchioro AA, Colli CM, *et al*. Association between contamination of public squares and seropositivity for Toxocara spp. in children. Vet Parasitol 2012;188:48–52.
20. Prestes-Carneiro LE, Rubinsky-Elefant G, Ferreira AW, *et al*. Seroprevalence of toxoplasmosis, toxocariasis and cysticercosis in a rural settlement, São Paulo State, Brazil. Pathog Glob Health 2013;107:88–95.
21. Cassenote AJF, de Abreu Lima AR, Pinto Neto JM, *et al*. Seroprevalence and modifiable risk factors for toxocara spp. in Brazilian Schoolchildren. PLoS Negl Trop Dis 2014;8:e2830.
22. Anaruma Filho F, Chieffi PP, Corrêa CRS, *et al*. Human toxocariasis: a seroepidemiological survey in the municipality of Campinas (SP), Brazil. Rev Inst Med Trop São Paulo 2002;44:303–7.
23. Gavignet B, Piarroux R, Aubin F, *et al*. Cutaneous manifestations of human toxocariasis. J Am Acad Dermatol 2008;59:1031–42.
24. Strachan DP. Hay fever, hygiene, and household size. BMJ 1989;299:1259–60.
25. Baqueiro T, Pontes-de-carvalho L, Carvalho FM, *et al*. Asthma and rhinitis symptoms in individuals from different socioeconomic levels in a Brazilian city. Allergy Asthma Proc 2007;28:362–7.
26. Ownby DR, Johnson C, Peterson EL. Exposure to dogs and cats in the first year of life and risk of allergic sensitization at 6 to 7 years of age. JAMA 2002;288:963–72.