Mediterranean Journal of Hematology and Infectious Diseases

Original Article

Seroprevalence of *Toxocara Canis* in the city of Catania, Italy

Alessandra Nicoletti[1], Calogero Edoardo Cicero[1], Antonia Mantella[2], Loretta Giuliano[1], Cristina Rascunà[1], Vincenza Paradisi[3], Alessandro Bartoloni[2], Mario Zappia[1] and Vito Sofia[1].

[1] Department of Medical and Surgical Sciences and Advanced Technologies "G.F. Ingrassia", Section of Neurosciences, University of Catania, Catania, Italy.
[2] Department of Experimental and Clinical Medicine, Infectious and Tropical Diseases Unit, University of Florence, Florence, Italy.
[3] Italian Society of General Medicine (SIMG), Catania, Italy.

Competing interests: The authors have declared that no competing interests exist.

Abstract. Toxocariasis is one of the most common helminthiases worldwide. However, there is a lack of data regarding Southern Italy. We have evaluated the seroprevalence and associated environmental factors of toxocariasis in a sample of adults living in the city of Catania.
Presence of anti-*Toxocara canis* IgG antibodies was searched using an ELISA test using excretory/secretory antigens. Environmental risk factors have been evaluated with a face-to-face questionnaire.
Two hundred eighty-seven subjects (193 [67.3%] women, mean age 48.1±15.6 years) were enrolled, and presence of anti *T. canis* antibodies was found in 23 participants, of whom 18 (78.3%) were women with a mean age of 51.1±14.0 years, giving a seroprevalence of 8.0% (95%CI 5.4-11.7). At multivariate analysis, a positive association for subjects with more than three siblings (adjOR 3.17; 95%CI 1.09-9.25) was recorded.
Our study confirms that exposition to *T. canis* is frequent also in urban areas of western countries.

Keywords: Toxocara canis; Helminths; Epidemiology.

Citation: Nicoletti A., Cicero C.E., Mantella A., Giuliano L., Rascunà C, Paradisi V., Bartoloni A, Zappia M., Sofia V. Seroprevalence of *Toxocara Canis* in the city of Catania, Italy. Mediterr J Hematol Infect Dis 2019, 11(1): e2019031, DOI: http://dx.doi.org/10.4084/MJHID.2019.031

Published: May 1, 2019 **Received: February 6, 2019** **Accepted: April 13, 2019**

Correspondence to: Alessandra Nicoletti, Department G.F. Ingrassia, Section of Neurosciences University of Catania, Via Santa Sofia 78, 95123 Catania. Tel. +390953782783. E-mail: anicolet@unict.it.

Introduction. Human toxocariasis is due to the larval stages of the ascarids *Toxocara canis* and *Toxocara cati*, common roundworms of dogs and cats respectively. It is one of the most prevalent helminthiases worldwide, especially in settings where the man-soil-dog relationship is particularly close.[1] *T. canis* parasites the small intestine of the dog, its main host that can become infected via the placenta or by contact with contaminated feces. The female *T. canis* produces up to 200 000 eggs per day, releasing them to the environment through feces.[2] Humans can be infected by direct contact with dogs, by the ingestion of contaminated food or soil or by eating infected meat of paratenic hosts.[1] While the large majority of infections are thought to be asymptomatic, visceral larva migrans (VLM) and ocular larva migrans (OLM) are the most common clinical manifestations,[3] even if two less severe syndromes have been described: the covert toxocariasis, which is more common in children, and common toxocariasis that was reported in adults.[4] However, *T. canis* has also been identified in the Central Nervous System, leading to a wide variety of

neurological manifestations collectively termed neurotoxocariasis. [5] In western countries toxocariasis is part of the so-called "neglected infections of poverty" because of its distribution in low income areas of the United States of America (USA)[6] and, for Europe, among eastern countries and the southern regions of European countries, both areas with lower socioeconomic levels, compared with the rest of Europe.[7] Toxocariasis has a seroprevalence of up to 90% in tropical settings and, for western countries, ranging from 35% to 42% in rural areas and between 2% and 5% in urban areas.[8] In Italy, the only two studies have been carried out reporting a seroprevalence of 4.0% in a northern Italian region[9] and a seroprevalence of 1.6% in the Marche region.[10]

The aim of the present study was to describe the seroprevalence of *T. canis* and the association between demographic and environmental factors in a sample of the adult population in the city of Catania.

Materials and Methods. The study has been performed in Catania, Italy, a city of the Sicily, which is located at a mean altitude of about 30 m above sea level and has an area of 181 km^2. Its official population is 293,104 inhabitants.[11] Participants over 14 years old were selected using a multi-stage sampling method. The study is part of a larger case-control study aimed to evaluate the role of both environmental and genetic factors and the risk of Multiple Sclerosis (MS) in the population of Catania. Background and methods have been extensively reported elsewhere.[12]

After enrolment in the study, a blood sample was collected. Samples have been coded and processed to obtain serum aliquots and then stored at -20°C in the laboratories of the "Azienda Ospedaliera Policlinico Vittorio Emanuele." Serum samples have been shipped in dry ice to the laboratories of the Infectious Diseases Institute (Malattie Infettive e Tropicali, AOU Careggi) of the University of Florence and have been analysed by a biologist blinded to the status of the participants. Specific *T. canis* IgG has been detected with a commercial ELISA kit (Ridascreen *Toxocara* IgG; R-Bio farm, Milan, Italy) using excretory/secretory antigens (*Toxocara* excretory-secretory antigen [TES-Ag]) from second-stage *T. canis* larvae.[13] A face-to-face semi-structured standardized questionnaire about demographic and environmental factors has been administered to all the participants.

All the analyses have been conducted with the software STATA 12.0. For the prevalence of anti-*T. canis* antibodies the 95% CI have been calculated. Quantitative variables were described using mean and standard deviation. The difference between means and the difference between proportions were evaluated by the *t-test* and the chi-square test, respectively.

Unconditional logistic regression analysis was performed, and for each study variable, we calculated OR, 95% CI, and *p-value* (two-tailed test, α=0.05). Multivariate analysis was conducted to investigate the independent effect of risk or protective factor after adjustment for one or several other factors or to adjust for confounding variables. Parameters associated with the outcome at the univariate analysis with a threshold of P = 0.25 were included in the model. The model was manually constructed using the likelihood ratio test (LRT) to compare the log-likelihood of the model with and without a specific variable. Sex, age, and education have been considered *a priori* confounder variables. Whenever variables were dichotomized, the cutoffs were derived from the median value of the pooled distribution. Each participant was asked to sign an informed consent. The study has been approved by the Local Ethical Committee (code 64/2018/PO).

Results. At the end of the recruitment process, 300 subjects have been enrolled. After the research of anti-*T. canis* antibodies in serum, results of 13 participants were deemed unreliable due to incorrect storage of the samples, and thus these subjects have been dropped from the final analysis, with a final sample of 287 participants.

Subjects had a mean age of 48.1±15.6 years, and 193 (67.3%) were women. The majority of them were professionals (n=103, 36.4%) or housewives (n=79, 28.0%). The demographic characteristics of the population are reported in **Table 1**.

Presence of anti-*T. canis* antibodies were found in 23 subjects (18 women, 78.2%; mean age 51.1±14.0 years) resulting in seroprevalence of 8.0% (95%CI 5.4-11.7). At the univariate analysis, no association has been found with sex, age, profession or owning pets; a number of siblings were significantly associated with *T. canis* seropositivity with an OR of 3.46 (95%CI 1.41-8.47) for subjects with more than 3 siblings. A close association was found at multivariate analysis adjusting by age, sex and education (adj OR 3.17; 95%CI 1.09-9.25) as shown in **Table 2**.

Discussion. Toxocariasis is one of the most prevalent helminthiases worldwide and even if the parasite tends to be more prevalent in tropical settings, in urban areas of Western countries seroprevalence ranges from 2 to 5%.[8]

This is the first study carried out to assess the seroprevalence of toxocariasis in a sample of the adult population (over 14 years) in the city of Catania, Sicily. We found a seroprevalence of 8.0%, a value slightly higher with respect to data reported in other urban areas of European countries,[8] as well as to those previously reported in two earlier studies carried out Italy, 1.6 in Central Italy in 2003[10] and 4.0 in Northern Italy in 1990.[9]

Table 1. Demographic characteristics of the population.

	Subjects *T. canis* negative (n=264)	**Subjects *T. canis* positive (n=23)**
Age	47.8±15.7	51.1±14.0
Sex (women), n (%)	175 (66.3)	18 (78.3)
Profession, n (%)		
Professional/Office job	95 (36.5)	8 (34.8)
Industry/Agriculture	39 (15.0)	3 (13.0)
Tradesman	8 (3.1)	1 (4.4)
Artisan	7 (2.7)	0
Housewife	70 (26.9)	9 (39.1)
Unemployed	9 (3.5)	0
Student	12 (4.6)	0
Other	20 (7.7)	2 (8.7)
Marital status, n (%)		
Married	196 (74.5)	17 (73.9)
Not married	44 (16.7)	2 (8.7)
Divorced/Widower	23 (8.8)	4 (17.4)
Education, n (%)		
Primary school	58 (22.0)	8 (34.8)
Secondary school	86 (32.6)	8 (34.8)
High school	80 (30.3)	5 (21.7)
University	40 (15.2)	2 (8.7)
Cat ownership, n (%)	88 (33.3)	4 (17.4)
Dog ownership, n(%)	127 (48.1)	8 (34.8)
Number of siblings	3.3±2.3	4.5±3.1
< 3 siblings	170 (64.9)	8 (34.8)
>3 siblings	92 (35.1)	15 (65.2)

n, number.

The higher seroprevalence found in the city of Catania can be explained by the environmental factors such as the degree of humidity and temperature that increase the overall survival of *T. canis* eggs in the soil.[14] However, another critical aspect of being considered is environmental contamination due to the presence of infected dog feces that contribute to the dissemination of *T. canis* eggs. While we have no data about the prevalence of *T. canis* infected dog feces in the urban areas of Catania, a study conducted in the neighbouring city of Messina, that shares with Catania the same environmental factors, found a prevalence of *T. canis* eggs of 3.6% in a random sample of dog feces.[15] Furthermore, another contribution to the increased seroprevalence is the increased presence of dogs living in the households, considering the high level of infestation they bear, up to a prevalence of 76% in puppies in a recent study conducted in Italy.[16]

Presence of *T. canis* IgG was assessed using a TES-ELISA, considered the standard serological test commonly used in epidemiological surveys to determine the seroprevalence of *T. canis* in a defined population. While we are aware of the limitations of ELISA test, such as the low specificity, we believe that the lack of a confirmatory Western Blot, considered the gold standard procedure,[14] has not influenced our results because the low specificity depends on the frequent cross-reactions with other nematode infections

Table 2. Univariate and multivariate analysis of variables associated with the presence of anti-*Toxocara canis* antibodies.

	Univariate analysis			Multivariate analysis		
	OR	**95% CI**	**P-value**	**adjOR**	**95% CI**	**P-value**
Age	1.01	0.98-1.04	0.331	0.99	0.96-1.03	0.891
Sex (women), n (%)	1.83	0.65-5.09	0.247	1.58	0.55-4.51	0.392
Profession, n (%)						
Professional/Office job	-			-		
Industry/Agriculture	0.91	0.23-3.62	0.898	-		
Tradesman	1.48	0.16-13.40	0.725	-		
Artisan	-			-		
Housewife	1.52	0.56-4.15	0.407	-		
Unemployed	-			-		
Student	-			-		
Other	1.18	0.23-6.01	0.836	-		
Marital status, n (%)						
Married	-			-		
Not married	0.52	0.11-2.35	0.399	-		
Divorced/Widower	2.00	0.62-6.47	0.245	-		
Education, n (%)						
Primary school	-			-		
Secondary school	0.67	0.23-1.89	0.456	0.98	0.32-3.01	0.980
High school	0.45	0.14-1.45	0.184	0.80	0.21-3.01	0.751
University	0.36	0.07-1.79	0.214	0.82	0.13-4.93	0.834
Cat ownership, n (%)	0.42	0.13-1.27	0.126	-		
Dog ownership, n(%)	0.57	0.23-1.40	0.224	-		
Number of siblings	-					
< 3 siblings	1	-	-	-	-	-
>3 siblings	3.46	1.41-8.47	**0.006**	3.17	1.09-9.25	**0.034**

n, number; adjOR, adjusted Odds Ratio.

such as *Ascaris lumbricoides*, filariasis or strongyloidiasis, which are rare in western countries.[14] In our sample T. *canis* seropositivity was not associated with age , as expected according to recent literature[17]
Furthermore in our sample pet ownership was not associated with the risk of *T. canis* seropositivity, probably because the risk of being infected by the direct contact with domestic animals has been showed to be low.[18]

On the other hand, the number of siblings (more than 3) was significantly associated with *T. canis* seropositivity. Even if we have not a clear explanation for this association, it is possible to hypothesize that a larger number of siblings could act as a proxy indicator of a lower socio-economic level, a risk factor for being infected with *T. canis* eggs.[17] The size of the study and the selection of a sample from the general population using an equal probability selection method (a multi-stage sampling) represent the main strengths of our study.[12] However, we are aware that the sex and age distribution of the sampled population may not be entirely representative of the general population. It should be underlined in fact that this study is part of a larger population-based case-control study on MS and to this reason, healthy subjects were recruited matched by age and sex with the enrolled MS cases.[12]

Conclusions. Toxocariasis is a neglected disease, but our study confirms that exposition to *T. canis* is

frequent also in the urban area of western countries. It should be stressed that even if the majority of infections are asymptomatic, *T. canis* can also lead to a wide range of neurological manifestations (neurotoxocariasis) and that due to the high seroprevalence recorded in our population, its diagnosis should be taken into account in the clinical setting.

Acknowledgements. We are grateful to the Italian Society of General Medicine (SIMG) for its support, and in particular to Anna Salvo MD, Guglielmo Travaglianti MD, Gaetano Profeta MD, Carmelo Di Gregorio MD, Antonino Rizzo MD, Nuccia Spada MD, Giovanni Cappello MD, Salvatore Amato MD, Marco Ciancio MD, Melchiorre Fidelbo MD, Cettina Persano MD, Valeria Polizzi MD, Giovanni Marotta MD, Maurizio D'Urso MD for their participation in the study.

Funding. This research was funded by the Department of Medical and Surgical Sciences and Advanced Technologies "G.F. Ingrassia", University of Catania, Italy (Piano Triennale di Sviluppo delle Attività di Ricerca Scientifica del Dipartimento 2016-18).

References:

1. Ma G, Holland CV, Wang T, Hofmann A, Fan CK, Maizels RM, Hotez PJ, Gasser RB. Human toxocariasis. Lancet Infect Dis. 2018 Jan;18(1):e14-e24. https://doi.org/10.1016/S1473-3099(17)30331-6
2. Glickman LT, Schantz PM. Epidemiology and pathogenesis of zoonotic toxocariasis. Epidemiol Rev. 1981; 3:230-250. https://doi.org/10.1093/oxfordjournals.epirev.a036235
3. Schantz PM, Glickman LT. Toxocaral visceral larva migrans. N Engl J Med. 1978; 298(8):436-439. https://doi.org/10.1056/NEJM197802232980806 PMid:622118
4. Taylor MR, Keane CT, O'Connor P, Girdwood RW, Smith H. Clinical features of covert toxocariasis. Scand J Infect Dis. 1987; 19(6):693-696. https://doi.org/10.3109/00365548709117206
5. Deshayes S, Bonhomme J, de La Blanchardière A. Neurotoxocariasis: a systematic literature review. Infection. 2016; 44(5):565-574. https://doi.org/10.1007/s15010-016-0889-8
6. Hotez PJ. Neglected infections of poverty in the United States of America. PLoS Negl Trop Dis. 2008; 2(6):e256. https://doi.org/10.1371/journal.pntd.0000256
7. Hotez PJ, Gurwith M. Europe's neglected infections of poverty. Int J Infect Dis. 2011 15(9):e611-619. https://doi.org/10.1016/j.ijid.2011.05.006 PMid:21763173
8. Fan C-K, Holland CV, Loxton K, Barghouth U. Cerebral Toxocariasis: Silent Progression to Neurodegenerative Disorders? Clin Microbiol Rev. 2015; 28(3):663-686. https://doi.org/10.1128/CMR.00106-14
9. Genchi C, Di Sacco B, Gatti S, Sangalli G, Scaglia M. Epidemiology of human toxocariasis in northern Italy. Parassitologia. 1990; 32(3):313-319. PMid:2132443
10. Habluetzel A, Traldi G, Ruggieri S, Attili AR, Scuppa P, Marchetti R, Menghini G, Esposito F. . An estimation of Toxocara canis prevalence in dogs, environmental egg contamination and risk of human infection in the Marche region of Italy. Vet Parasitol. 2003, 113(3-4):243-252. https://doi.org/10.1016/S0304-4017(03)00082-7
11. Istituto Nazionale di Statistica, 2011. 15° Censimento generale della popolazione.
12. Nicoletti A, Messina S, Bruno E, Mostile G, Quattrocchi G, Raciti L, Dibilio V, Cappellani R, D'Amico E, Sciacca G, Lo Fermo S, Paradisi V, Patti F, Zappia M. Risk factors in multiple sclerosis: a population-based case-control study in Sicily. Background and methods. Neurol Sci. 2016 37(12):1931-1937. https://doi.org/10.1007/s10072-016-2685-8
13. de Savigny DH, Voller A, Woodruff AW. Toxocariasis: serological diagnosis by enzyme immunoassay. J Clin Pathol. 1979; 32(3):284-288. https://doi.org/10.1136/jcp.32.3.284 PMid:372253 PMCid:PMC1145636
14. Fillaux J, Magnaval J-F. Laboratory diagnosis of human toxocariasis. Vet Parasitol. 2013; 193(4):327-336. https://doi.org/10.1016/j.vetpar.2012.12.028 PMid:23318165
15. Risitano AL., Brianti E., Gaglio G., Ferlazzo M., Giannetto S. Environmental contamination by canine feces in the city of Messina: parasitological aspects and zoonotic hazards. In Proceedings of LXI Congress of the Italian Society for Veterinary Science (S.I.S.Vet.). Salsomaggiore Terme, Italy: 135-136.
16. Corda A, Tamponi C, Meloni R, Varcasia A, Parpaglia MLP, Gomez-Ochoa P, Scala A. Ultrasonography for early diagnosis of Toxocara canis infection in puppies. Parasitol Res. 2019 Mar;118(3):873-880. https://doi.org/10.1007/s00436-019-06239-4 PMid:30706166
17. Berrett AN, Erickson LD, Gale SD, Stone A, Brown BL, Hedges DW. Toxocara Seroprevalence and Associated Risk Factors in the United States. Am J Trop Med Hyg. 2017; 97(6):1846-1850. https://doi.org/10.4269/ajtmh.17-0542 PMid:29016316 PMCid:PMC5805073
18. Poeppl W, Herkner H, Tobudic S, Faas A, Mooseder G, Burgmann H, Auer H. Exposure to Echinococcus multilocularis, Toxocara canis, and Toxocara cati in Austria: a nationwide cross-sectional seroprevalence study. Vector Borne Zoonotic Dis. 2013; 13(11):798-803. https://doi.org/10.1089/vbz.2012.1283 PMid:24107202