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Seroprevalence of *Toxoplasma gondii* and *Toxocara canis* in a human rural population of Southern Rio Grande do Sul

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ABSTRACT

Due to the growing population of pets, especially homeless dogs and cats, zoonoses still represent a significant public health problem. *Toxoplasma gondii* and *Toxocara* spp. are epidemiologically important zoonotic agents as they are etiological factors of human toxoplasmosis and toxocariasis, respectively. These parasites remain neglected even though they are substantially prevalent in rural areas. The aim of this study was to investigate *T. gondii* and *T. canis* seroprevalence and risk factors of seropositivity in a rural population in Pelotas municipality, Brazil. The study participants (n=344) were patients of a Basic Healthcare Unit (BHU) located in Cerrito Alegre. Blood samples were collected and tested for *T. gondii* antibodies by indirect immunofluorescence and *T. canis* antibodies by an indirect ELISA that targets an excreted-secreted antigen (TES). *T. gondii* seropositivity was 53.2%, with higher titers (1:256 - 1:1,024) in individuals who habitually eat pork, beef, or chicken, while *T. canis* seropositivity was 71.8% and concomitant *T. gondii* and *T. canis* seropositivity was 38.3%. Among the seropositivity risk factors assessed, only habitual undercooked meat consumption was significant (p = 0.046; OR = 3.7) for *T. gondii* and none of them were associated with *T. canis* seropositivity. Both parasites have a high prevalence in rural areas, which reinforces the need to invest in rural community education and health.

KEYWORDS: Rural. Toxocariasis. Toxoplasmosis.

INTRODUCTION

Due to a growing population of homeless pets, especially dogs and cats, zoonoses continue to represent a significant public health problem. The most common intestinal parasites in dogs and cats are protozoa from the genera *Giardia, Cystoisospora, Sarcocystis, Cryptosporidium*, and nematodes from the genera *Toxocara, Toxascaris, Ancylostoma, Uncinaria, Capillaria,* and *Trichuris*. In addition, cats are definitive hosts of the cosmopolitan protozoan *Toxoplasma gondii*. All of the above parasites are zoonotic agents, with *Toxoplasma gondii*, *Toxocara canis*, *Toxocara cati* having the highest epidemiological importance, as they are the etiological agents of toxoplasmosis and toxocariasis, respectively[1].

Human toxoplasmosis is a disease with worldwide distribution and high prevalence[2,3], as is human toxocariasis, also known as visceral larva migrans. Both infectionsmay present as ocular disease[4]. Even though these are important parasitic diseases, they remain neglected public health issues. Their clinical presentation is diverse as related to the immune response of the host to the parasite load and

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the affected organ[5]. Both parasites have similar routes of infection that include consumption of contaminated drinking water, raw fruits or vegetables, raw or undercooked meat, viscera contaminated with oocysts excreted by infected cats (*T. gondii*) or eggs eliminated by dogs (*T. canis*). The risk factors and behaviors in children include frequent contact with soil in playgrounds and schools, recreation rooms or sandboxes containing dog and cat feces, poor personal hygiene, geophagia, and the lack of parental supervision[5,6]. There are also other forms of transmission such as organ transplantation, blood transfusion, and vertical transmission in humans[7-10].

After invading the hosts, these parasites reach the liver or the eye producing ocular lesions, or neuronal manifestations. In Brazil, the seroprevalence of these pathogens varies according to geographic regions and biology of the local environment[11]. The rural population is more susceptible to infection by these parasites[12], since most of them live in poor conditions of sanitary infrastructure and hygiene, do not have adequate information on these diseases, as prevention methods. Moreover, inadequate child care an low rates of antiparasitic drugs in dogs and cats are important factores for the dissemination of these diseases.

Seroepidemiological studies in rural populations help in collecting information on parasitic diseases providing information to the affected population and helping in determining control measures and appropriate treatment regimens. This study, therefore, aimed to investigate the seroprevalence of *T. gondii* and *T. canis* in the rural population of Cerrito Alegre, a district of Pelotas, located in the Southern region of Rio Grande do Sul State, RS, Brazil.

MATERIALS AND METHODS

Samples and experimental design

Blood samples were collected from 344 patients at a Basic Healthcare Unit (BHU) in the town of Cerrito Alegre, RS (the 3rd district of the city of Pelotas) between March 2012 and February 2014, and the sera stored at -20 °C. The sample size was representative of all individuals in the study area. A survey of risk factors associated with the presence of parasitic infections was performed using a semi-structured questionnaire, that evaluated the following variables: gender, age, education, presence of cats and dogs at home, possessing or raising other animals (cattle, pigs, sheep, chickens, ducks), type of meat consumed, garden farming, habit of eating undercooked or raw meat, and consumption of processed meats or viscera.

The inclusion criteria for the study subjects were residence in the 3rd District of Pelotas, agreeing to participate in the study by signing the Informed Consent document (IC), allowing blood collection, and being at least 18 years of age. The project was approved by the Faculty of Medicine Ethics Committee UFPel (Nº OF.36/12).

Indirect immunofluorescence assay (IFA)

Seropositivity for *T. gondii* was tested using Imuno-Con Toxoplasmose kit (WAMA_Diagnóstica) an indirect immunofluorescence assay (IFA), according to the manufacturer's instructions. Briefly, wells of the IFA slides were filled with 20 µL of serum sample diluted in AD Buffer (1:30) and kept in a moist chamber for 30 min at 37 °C. Afterwards, the slides were washed three times for 10 min. with the kit buffer (100 mL of PBS diluted in 900 mL of distilled water) with stirring. The slides were then dried at 37 °C for 5 min, anti-human antibody conjugated to FITC added, and kept in a moist chamber for 30 min at 37 °C. The slides were washed thrice, each for 10 min. with stirring and subsequently dried at 37 °C for 5 min. Next, three drops of glycerin were added to each well, and a cover slip placed for fluorescence microscope reading. The samples that were positive in the lowest dilution (1:32) were subsequently tested at further dilutions of 1:64, 1:128, 1:256, 1:512, and 1:1024. Positive and negative control sera were used in all slides.

Enzyme-linked Immunosorbent assay (ELISA)

Serology testing for *T. canis* used TES antigens that were produced according to Santos *et al*.[13], but with modifications. Polystyrene 96-well plates were coated with 1 µg/mL of TES antigen dissolved in carbonate-bicarbonate buffer (pH 9.6) for 16 h. at 4 ºC. The free protein binding sites were blocked with skim milk powder (5% in PBS-T;0.05% Tween) for 1 h. at 37 °C, while sera were pre-adsorbed on total *Ascaris lumbricoides* antigen (AgSoAl; 23.7 mg/mL). All samples were evaluated in duplicate at a dilution of 1:100. Anti-mouse IgG human peroxidase conjugate (1: 5000 in PBS-T buffer; Sigma-Aldrich) was used as the secondary antibody. Both sera and conjugate were incubated for 1 h. at 37 °C. Plates were washed thrice with PBS-T between all test stages. Ortho-phenylenediamine (OPD) at a concentration of 0.4 mg/mL in citrate-phosphate buffer pH 4.0 with hydrogen peroxide (30v 0.01%) was used as the chromogen, the plate incubated at room temperature for 15 min in the dark and the reaction was quenched with 50 µL of 1N sulfuric acid. The reaction product was colorimetrically detected by taking absorbance

at 450 nm using a plate reader VICTOR X5 Multilabel (Perkin Elmer) with a 492 nm wavelength filter. Positive and negative control sera were added to plate in duplicate as controls.

Statistical analysis

The sample size was calculated based on the size of the adult population of the rural locality to be evaluated (2,000 individuals), considering a margin of error of 5% and a 95% confidence level. Thus, the sample size found was of 323 individuals.

All epidemiological data obtained from questionnaires were analyzed using Epi Info (ver. 6.04, CDC). To calculate associations, a logistic regression (unadjusted logistic regression) was used to estimate the odds ratio and their respective 95% confidence intervals for each outcome and their exposure variables. These analyzes were performed in the statistical package STATA 12.1 (StataCorp LP, College Station, USA). No adjusted analysis was performed for lack of associations in the crude analysis.

RESULTS

Serology for *T. gondii*

Of the 344 samples tested, 53.2% (n=183) were positive for *T. gondii.* Among the 225 individuals having cats, 54.7% (n=123) were seropositive for *T. gondii* while 50.4% (n=60) of seropositive individuals did not have any pets in the residence. Most of the patients habitually consumed meat (79.4%), and their seropositivity was determined to be 53.5%, compared to 52.1% in those who did not consume canned meats; this difference was not significant. Habitual consumption of undercooked meat was the only variable that was statistically associated with *T. gondii* seropositivity ($p = 0.04$) (Table 1).

Association analyses were performed using data from positive individuals alone to investigate the association between risk factor and an increase in titers of anti-*T. gondii* antibodies. These individuals were divided into two groups, namely: 1:32 -1:128 dilution (lower) and 1:512 - 1:1024 dilution (higher). This data classification enabled

Table 1 - Socio-demographic characteristics of the study population for the presence of anti-*Toxoplasma gondii* antibodies in a rural area of Southern Rio Grande do Sul region

Variables	Presence of anti-*T. gondii* antibodies		*P*	Unadjusted OR (CI95%)
	Negative (%)	Positive (%)		
Age			0.7925	
18-40 years	30 (45.5)	36 (54.5)		1
41 or more	113 (47.3)	126 (52.7)		0.93 (0.54;1.61)
Sex			0.2589	
Female	102 (49.3)	105 (50.7)		1
Male	59 (43.1)	78 (56.9)		1.28 (0.83;1.98)
Education			0.2850	
None to 8th grade	158 (46.5)	182 (53.5)		1
High School and University	3 (75)	1 (25)		0.29 (0.03;2.81)
Consumes canned meats	34 (47.9)	37 (52.1)	0.8371	
No	127 (46.5)	146 (53.5)		1
Yes				1.06 (0.63;1.78)
Cat owner			0.4529	
(no 119)	59 (49.6)	60 (50.4)		1
(yes 225)	102 (45.3)	123 (54.7)		1.19 (0.76;185)
Preparation and ingestion of meats			0.0459	
Well cooked (done)	158 (48.1)	171 (51.9)		1
Rare to medium/both	3 (20)	12 (80)		3.70 (1.02;13.3)
Consumes viscera*			0.4609	
No (191)	86 (45.1)	105 (54.9)		1
Yes (153)	75 (49.1)	78 (50.9)		0.85 (0.56;1.30)
Meat utilized in alimentation				
Chicken	119 (46.1)	139 (53.9)	0.8689	1.11 (0.68;1.82)
Pork	65 (42.5)	88 (57.5)	0.1512	1.37 (0.89;2.10)
Lamb	26 (47.3)	29 (52.7)	0.9482	0.98 (0.55;1.74)
Beef	128 (47.6)	141 (52.4)	0.8475	0.87 (0.52;1.45)

* Liver, kidney, heart

Table 2 - Association of anti-*Toxoplasma gondii* antibodies titers with the habit of ingesting bovine, porcine and chicken meat, in a rural population of Southern Rio Grande do Sul region

Titrations	Pork consumption			Beef consumption			Chicken consumption		
	Yes (%)	No (%)	p (OR)	Yes (%)	No (%)	p (OR)	Yes (%)	No (%)	p (OR)
1:32 to 1:128	63 (43.8%)	81 (56.2%)	0.0261 (2.29)	106 (73.6%)	38 (26.4)	0.0414 (3.14)	104 (72.2%)	40 (27.8%)	0.0301 (OR=3.37)
1:256 to 1:1024	25 (64.1%)	14 (35.9%)		35 (89.7%)	4 (10.3%)		35 (89.7%)	4 (10.3%)	

verification of the significance for the following risk factors, namely consumption of pork (OR = 2.29, $CI_{95\%}$ 1.10 - 4.78), beef (OR = 3.14, $CI_{95\%}$ 1.05 - 9.41) and chicken (OR = 3.37, $CI_{95\%}$ 1.12 - 10.08) and the results showed that individuals who habitually ingest these meats had higher titers (Table 2).

Serology for *T. canis*

Of the 344 samples tested, 71.8% (n=247) were positive for *T. canis*. No statistically significant differences were detected among the variables evaluated for seroprevalence of *T. canis* (71.8%) (Table 3).

Seropositivity for both *T. gondii* and *T. canis* was 38.3% (n=132), however no statistical difference was observed between the risk factors. In the population studied, a relationship between age and seropositivity of *T. canis* and *T. gondii* was found, most individuals older than 40 years presented a positive result for both parasites, and this index reached 73.8% and 51.4% in patients who were 60 years of age or older for toxocariasis and toxoplasmosis, respectively.

DISCUSSION

Deaths caused by infectious diseases continue to remain a public health problem worldwide and, particularly, in Brazil, despite a reduction in their numbers in recent decades. Only a few reports on the seroprevalence of infection by parasitic agents in rural areas are currently available. We found that 53.2% of the population were seropositive for *T. gondii* while 71.8% were seropositive for *T. canis* in the rural population of the city of Pelotas.

An association between individuals living in the countryside and seropositivity for toxoplasmosis has already been demonstrated[14]. Seropositivity rates for toxoplasmosis (56.7%), similar to those found in our study, have been reported in riverine populations in Brazil[15]. Among the risk factors evaluated for *T. gondii* and *T. canis*

Table 3 - Socio-demographic characteristics of the population studied for the presence of IgG antibodies anti-*Toxocara canis* in a rural area of Southern Rio Grande do Sul region

Variables	Presence of anti-*T. canis* antibodies		*P* value	Unajusted OR (CI95%)
	Negative(%)	Positive(%)		
Age*			0.6676	
18-40 years	20 (30.3)	46 (69.7)		1
41 or more	66 (27.6)	173 (72.4)		1.14 (0.63;2.07)
Sex			0.6898	
Female	60 (29.0)	147 (71.0)		1
Male	37(27.0)	100 (73.0)		1.10 (0.68;1.79)
Garden			0.8545	
No	34 (28.8)	84 (71.2)		1
Yes	63 (27.9)	163 (72.1)		1.05 (0.64;1.71)
Dog owner			0.4334	
No	10 (34.5)	19 (65.5)		1
Yes	87 (27.6)	228 (72.4)		1.38 (0.62;3.08)
Cat owner			0.7159	
No	35 (29.4)	84 (70.6)		1
Yes	62 (27.6)	163 (72.4)		1.10 (0.67;1.79)
Source of water**			0.7556	
Well or watering hole (322)	91 (28.3)	231 (71.7)		1
Tap water (19)	6 (31.6)	13 (68.4)		1.17 (0.43;3.18)

* Could not get all ages. ** Not all survey participants responded to their source of water

seropositivity, habitual eating of undercooked meat was the only significant risk factor (p = 0.045, OR = 3.7). Similarly, a study in pregnant women in Pelotas also described raw meat consumption as a significant risk factor for *T. gondii* seropositivity[16]. This association was also evident in a study conducted in the city of Arak (Iran) with 400 women, where handling or eating raw or undercooked meat was significantly associated with *T. gondii* seropositivity[17].

Among the seropositive cases aged between 18-40 years (n=36), 22 were women of childbearing age. This observation is important as parasitic infection during pregnancy is a major problem, especially during the first few months, when infection may result in miscarriage or birth defects. Some authors also cite issues that go beyond the complications of congenital infection. These include infertility mechanisms associated with *T. gondii*, such as the development of endometriosis, fetal rejection due to release of latent tachyzoites cysts in the endometrium, follicular aberrations in the ovaries, uterine atrophy, and hypothalamic dysfunction affecting reproduction that results from chronic toxoplasmosis[18].

The study shows that most individuals older than 40 years presented a positive result for both parasites, and this index was higher in patients aged 60 years or older. It should be noted that the increase in the elderly population in developing countries is drastically faster than in industrialized ones. As aging is associated with a higher prevalence of chronic and debilitating diseases (even in need of transplants), the use of drugs and immunobiological agents capable of altering immunity in the elderly becomes more frequent, and this immunosuppression may result in the reactivation of cysts of *T. gondii* and *T. canis*, among other tropical diseases typical of developing countries[19,20].

Analysis of *T. gondii* seropositive individuals showed statistical significance for the following risk factors, namely pork (OR = 2.29, $CI_{95\%}$ 1.10 - 4.78), beef (OR = 3.14, $CI_{95\%}$ 1.05 - 9.41), and chicken consumption (OR = 3.37, $CI_{95\%}$ 1.12 - 10.08) in individuals with high antibody titers, i.e., from 1:256 to 1:1024. Although antibody titration analysis was not carried out, other authors have reported that consumption of pork and beef is a significant risk factor for the presence of *T. gondii* antibodies[21,22].

The seroprevalence rate for *T. canis* was 71.8%. Similar rates have been observed in other regions in Brazil (52% and 65.4%)[23], while seropositivity in other countries has been reported to range from 12% to 86%[10,24,25]. These differences in seroprevalence are attributable to various reasons, such as location, sample size, and age of the study population, as well as the diagnostic tests used. A study carried out in the urban area, identified a seroprevalence rate between 52 and 65.4%, suggesting that country life with little health information increases the probability of *T. canis* infection[23]. The results presented here pertain to adults[24] and analyzed children[26] living in islands (86.75%) and urban zones (12.7% and 9.5%), and found completely divergent seroprevalence rates. It is interesting to note that the lowest seroprevalence rate was obtained in urban areas where health information is more available, while the higher seroprevalence rate was from a study in Majuro, one of the Marshall Republic Islands, where the population lives on agriculture, fishing, and the United States aid.

Contact with dogs did not affect *T. canis* seropositivity, as it was 72.4% in those with contact and 65.5% in those without. Similar figures of 60%, 62%, 66%, and 73.6% have been reported in studies from Brazil and Ghana on *T. canis* infection and its association with having pet dogs[23,25,27]. However, a study in rural Turkey found a significant association between having dogs at home and anti-*T. canis* antibodies[28]. The sources of water as an infection risk were also investigated as 231 seropositive patients (67%) consumed water from wells. Other studies have reported seropositivity rates of 13% and 88%[10,27] when unboiled and untreated water, respectively, was consumed; this may have contributed to infection with *T. canis*. The lack of significant association between risk factors and *T. canis* seropositivity may be related to the fact that more than 70% of the participants were seropositive, making it difficult to obtain statistical significance.

A study on children in Pelotas, Rio Grande do Sul, Brazil, found that more than 50% of the children were seropositive for *T. canis*[29]. It is noteworthy that *T. canis* eggs are frequently found in places where children assemble and play, such as parks, squares, and student-leisure areas in Pelotas and the Southern Rio Grande do Sul[11,30,31]. Since our study investigated adults, the chances of already having been in contact with infectious forms of the helminth tend to increase, due to the "exposure time" factor.

Concomitantly, the seropositivity of *T. gondii* and *T. canis* was 38.3%, and as already mentioned, both parasites have similar infection forms. Some studies indicate that the presence of antibodies to *T. canis* increases the risk of infection by *T. gondii*[32]. In the countryside of Sao Paulo, simultaneous infection rates by *T. canis* and *T. gondii* were found to be 14%[11].

We conclude that the rural population studied here displays high seropositivity for *T. gondii* and *T. canis* and that among the risk factors evaluated, only consumption of undercooked meat was a significant risk factor for *T. gondii* infection. Additionally, individuals who ingested pork, beef, or chicken meat had higher titers of anti-*T gondii* antibodies.

REFERENCES

1. Góralska K, Błaszkowska J. Parasites and fungi as risk factors for human and animal health. Ann Parasitol. 2015;61:207-20.
2. Dubey JP, Lago EG, Gennari SM, Su C, Joes JL. Toxoplasmosis in humans and animals in Brazil: high prevalence, high burden of disease, and epidemiology. Parasitology. 2012;139:1375-424.
3. Lopes AP, Dubey JP, Dardé ML, Cardoso L. Epidemiological review of Toxoplasma gondii infection in humans and animals in Portugal. Parasitology. 2014;141:1699-708.
4. Choi KD, Choi JH, Choi SY, Jung JH. Toxocara optic neuropathy: clinical features and ocular findings. Int J Ophthalmol. 2018;11:520-3.
5. Oliart-Guzmán H, Delfino BM, Martins AC, Mantovani SA, Braña AM, Pereira TM, et al. Epidemiology and control of child toxocariasis in the western Brazilian Amazon - a population-based study. Am J Trop Med Hyg. 2014;90:670-81.
6. Cassenote AJ, Lima AR, Pinto Neto JM, Rubinsky-Elefant G. Seroprevalence and modifiable risk factors for Toxocara spp. in Brazilian schoolchildren. PLoS Negl Trop Dis. 2014;8:e2830.
7. Dutra GF, Pinto NS, Avila LF, Telmo PL, Hora VP, Martins LH, et al. Evaluation of the initial and chronic phases of toxocariasis after consumption of liver treated by freezing or cooling. Parasitol Res. 2013;112:2171-5.
8. Maffrand R, Avila-Vázquez M, Princich D, Alasia P. Toxocariasis ocular congénita en un recién nacido prematuro. An Pediatr (Barc). 2006;64:599-600.
9. Moreira GM, Telmo PL, Mendonça M, Moreira AN, McBride AJ, Scaini CJ, et al. Human toxocariasis: current advances in diagnostics, treatment, and interventions. Trends Parasitol. 2014;30:456-64.
10. Rubinsky-Elefant G, Silva-Nunes M, Malafronte RS, Muniz PT, Ferreira MU. Human toxocariasis in rural Brazilian Amazonia: seroprevalence, risk factors, and spatial distribution. Am J Trop Med Hyg. 2008;79:93-8.
11. Prestes-Carneiro LE, Rubinsky-Elefant G, Ferreira AW, Araujo PR, Troiani C, Zago SC, et al. Seroprevalence of toxoplasmosis, toxocariasis and cysticercosis in a rural settlement, São Paulo State, Brazil. Pathog Glob Health. 2013;107:88-95.
12. Santos GM, Silva SA, Barbosa AP, Campos DM. Investigação soroepidemiológica sobre a larva migrans visceral por Toxocara canis em usuários de serviços de saúde de Goiânia – GO. Rev Patol Trop. 2009;38:197-206.
13. Santos PC, Lehmann LM, Lorenzi C, Hirsch C, Telmo PL, Mattos GT, et al. The seropositivity of Toxocara spp. antibodies in pregnant women attented at the university hospital in Southern Brazil and the factors associated with infection. PLoS One. 2015;10:e0131058..
14. Salamon D, Bulanda M. Toxoplasma gondii and women of reproductive age: an analysis of data from the Chair of Microbiology, Jagiellonian University Medical College in Cracow. Ann Parasitol. 2014;60:291-6.
15. Vitaliano SN, Mendonça GM, Sandres FA, Camargo JS, Tarso P, Basano SA, et al. Epidemiological aspects of Toxoplasma gondii infection in riverside communities in the Southern Brazilian Amazon. Rev Soc Bras Med Trop. 2015;48:301-6.
16. Cadermatori BG, Farias NA, Brod CS. Soroprevalência e fatores de risco à infecção por Toxoplasma gondii em gestantes de Pelotas, sul do Brasil. Rev Panam Infectol. 2008;10:30-5.
17. Mohammadi A, Shojaee S, Salimi M, Zareei M, Mohebali M, Keshavarz H. Seroepidemiological study of toxoplasmosis in women referred to Arak Marriage Consulting Center during 2012-2013. Iran J Public Health. 2015;44:654-8.
18. Aral Akarsu G, Elhan HA, Akarsu C. Retrospective evaluation of Toxoplasma gondii seropositivity in fertile and infertile women. Mikrobiyol Bul. 2011;45:174-80.
19. Gavazzi G, Herrmann F, Krause KH. Aging and infectious diseases in the developing world. Clin Infect Dis. 2004;39:83-91.
20. Lehner LJ, Staeck O, Halleck F, Liefeldt L, Bamoulid J, Budde K. Need for optimized immunosuppression in elderly kidney transplant recipients. Transplant Rev (Orlando). 2015;29:237-9.
21. Domingos A, Ito LS, Coelho E, Lúcio JM, Matida LH, Ramos Jr AN. Seroprevalence of Toxoplasma gondii IgG antibody in HIV/AIDS-infected individuals in Maputo, Mozambique. Rev Saude Publica. 2013;47:890-6.
22. Vitale M, Tumino G, Partanna S, La Chiusa S, Mancuso G, Giglia ML, et al. Impact of traditional practices on food safety: a case of acute toxoplasmosis related to the consumption of contaminated raw pork sausage in Italy. J Food Prot. 2014;77:643-6.
23. Souza RF, Dattoli VC, Mendonça LR, Jesus JR, Baqueiro T, Santana CC, 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.
24. Fu CJ, Chuang TW, Lin HS, Wu CH, Liu YC, Langinlur MK, et al. Seroepidemiology of Toxocara canis infection among primary schoolchildren in the capital area of the Republic of the Marshall Islands. BMC Infect Dis. 2014;14:261.
25. Kyei G, Ayi I, Boampong JN, Turkson PK. Sero-epidemiology of Toxocara canis infection in children attending four selected health facilities in the Central Region of Ghana. Ghana Med J. 2015;49:77-83.
26. Santarém VA, Leli FN, Rubinsky-Elefant G, Giuffrida R. Protective and risk factors for toxocariasis in children from two different social classes of Brazil. Rev Inst Med Trop Sao Paulo. 2011;53:66-72.
27. Ma G, Holland CV, Wang T, Hofmann A, Fan CK, Maizels RM, et al. Human toxocariasis. Lancet Infect Dis. 2018;18:e14–24.
28. Doğan N, Dinleyici EC, Bor O, Töz SO, Ozbel Y. Seroepidemiological survey for Toxocara canis infection in the northwestern part of Turkey. Turkiye Parazitol Derg. 2007;31:288-91.

29. Schoenardie ER, Scaini CJ, Brod CS, Pepe MS, Villela MM, McBride AJ, et al. Seroprevalence of Toxocara infection in children from southern Brazil. J Parasitol. 2013;99:537-9.
30. Amaral HL, Rassier GL, Pepe MS, Gallina T, Villela MM, Nobre MO, et al. Presence of Toxocara canis eggs on the hair of dogs: a risk factor for Visceral Larva Migrans. Vet Parasitol. 2010;174:115-8.
31. Moura MQ, Jeske S, Vieira JN, Corrêa TG, Berne ME, Villela MM, et al. Frequency of geohelminths in public squares in Pelotas, RS, Brazil. Rev Bras Parasitol Vet. 2013;22:175-8.
32. Marchioro AA, Colli CM, Ferreira EC, Viol BM, Araújo SM, Falavigna-Guilherme AL. Risk factors associated with toxoplasmosis and toxocariasis in populations of children from nine cities in southern Brazil. J Helminthol. 2015;89:428-32.