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Asian Pacific Journal of Tropical Biomedicine

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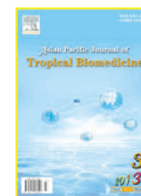

Document heading doi:

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Anti-*Toxocara* spp. antibodies in an adult healthy population: serosurvey and risk factors in Southeast Brazil

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PEER REVIEW

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Comments

The article was well-written, interesting, and well- conducted in respect to determination of the sample, the laboratory analysis methodology and analysis of the results. The study brought interesting data. Even though it is primarily applicable to a local situation and on a specific population group, it makes important methodological contributions in its research area, especially the data analysis.

(Details on Page 215)

ABSTRACT

Objective: To evaluate the frequency of anti-*Toxocara* spp. antibodies in an adult healthy population. **Methods:** The study was performed by interviewing 253 blood donors, from 19 to 65 years of age, in a hematological centre in Presidente Prudente, São Paulo, southeast Brazil. A survey was applied to blood donors in order to evaluate the possible factors associated to the presence of antibodies, including individual (gender and age), socioeconomic (scholarship, familial income and sanitary facilities) and habit information (contact with soil, geophagy, onycophagy and intake of raw/undercooked meat) as well as the presence of dogs or cats in the household. ELISA test was run for detection of the anti-*Toxocara* spp. IgG antibodies. Bivariate analysis followed by logistic regression was performed to evaluate the potential risk factors associated to seropositivity. **Results:** The overall prevalence observed in this study was 8.7% (22/253). Contact with soil was the unique risk factor associated with the presence of antibodies (P=0.0178; OR=3.52; 95% CI=1.244-9.995). **Conclusions:** The results of this study reinforce the necessity in promoting preventive public health measures, even for healthy adult individual, particularly those related to the deworming of pets to avoid the soil contamination, and hygiene education of the population.

KEYWORDS

Toxocariasis, Larva migrans, Seroprevalence, Diagnostic, Epidemiology

1. Introduction

Toxocariasis is a widespread zoonosis caused by the ascarid nematodes *Toxocara canis* (*T. canis*) and *Toxocara cati*, which primarily infect dogs and cats, respectively[1]. Human toxocariasis is a soil-transmitted helminthic infection. *Toxocara* eggs are released into the environment with the faeces of parasitised pets, and these eggs may be embryonate and accidentally be ingested by humans, particularly children who often play with contaminated soil. Many authors have reported different rates of *Toxocara* infections in both children and adults in different countries. Although human toxocariasis is highly prevalent in disadvantaged countries, some authors have focused

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Foundation Project: Supported by Universidade do Oeste Paulista (Unoeste) (Grant No. 184/2009).

Article history:
Received 11 Dec 2012
Received in revised form 16 Dec, 2nd revised form 19 Dec, 3rd revised form 27 Dec, 2012
Accepted 10 Feb 2013
Available online 28 Mar 2013

on the global importance of this zoonosis, which remains underestimated and neglected, even in developed countries[2].

The clinical spectrum of human toxocariasis is broad and ranges from asymptomatic infection to severe organ injury, including hepatic, pulmonary, ophthalmic and neurological disturbances. Some risk factors have been associated with toxocariasis, including gender, age, socioeconomic status, contact with pets, and ingestion of raw meat. Nevertheless, the results of different studies on the toxocariasis risk factors have been largely inconsistent until now[3].

Blood donors have been considered as a model to study the seroprevalence of infectious diseases in the healthy adult population[4-6]. The prevalence of anti-*Toxocara* spp. antibodies in this population has been studied on some continents. In Europe, the seroprevalence ranged from 1% in Spain to 13.65% in the Slovak Republic[7,8]. While in Oceania, the seroprevalence varied from (0.70±1.65)% in New Zealand to 7.0% in Australia[5,9]. In South America, the rates varied from 10.6% to 38.9% in Argentina, respectively[10,11]. In Brazil, there is a reported rate of 46.3% in northeast Brazil[12]. However, little is known about the risk factors for toxocariasis in voluntary blood donors.

Based on these statements, this study was conducted to assess both the seropositivity and risk factors for *Toxocara* spp. infection in an adult healthy population from southeast Brazil.

2. Materials and methods

2.1. Study area

The study was conducted from January to May of 2010 at a haematological centre in the municipality of Presidente Prudente, within the state of São Paulo, southeast Brazil (22°10′30″S, 51°25′28″W). The estimated population of this municipality in 2010 was approximately 207 610 inhabitants that were living in both urban and rural areas[13].

2.2. Subjects

A total of 253 voluntary blood donors ranging from 19 to 65 years old were included in this survey. The number of individuals to be enrolled was established using the statistical software Epi Info, version 6.0, with an estimated seroprevalence of 15%, an absolute error of 4.5 and a 95% confidence interval (95% *CI*).

To avoid the possibility of participation of multiple people from the same family, the individuals were included in the study by using a systematic sampling selection from the record numbers at the haematological centre.

The criteria for the inclusion of subjects followed the recommendations of the Brazilian National Health Vigilance Agency (ANVISA-Resolution 153/2004) that coordinates the Program of Blood and Blood Products in Brazil. All individuals included were considered as healthy individuals. During the clinical trial, all blood donors were asked to provide informed consent for their participation in the study, and a short questionnaire interview was conducted to gather information to determine the epidemiology of toxocariasis, including factors such as gender, age, educational or academic level, family income, sanitary facilities, pet ownership (dogs and/or cats), behavioural habits (onychophagy or geophagy), and intake of either raw or undercooked meat.

2.3. Sample collection

After standard blood collection in a polyethylene donation bag, the residual blood in the tubing was collected into 5.0 mL serum collection vacuum tubes. The tubing was manually clamped at the bag to prevent backflow of blood and/or anticoagulant from the bag into the tubing. The samples of blood were centrifuged at 3 300 r/min for 7 min, and the obtained serum was mixed immediately with a buffered glycerin solution of the same volume (Merck, USA) and stored at −32 °C.

A universal flask container (120 mL) was provided to each individual for stool collection. The stool samples were collected at the household by a researcher on the day after the interview.

2.4. Antigen preparation

T. canis excretory-secretory larval antigens (TES) were obtained according to the method described elsewhere[14], with some modifications[15]. Briefly, *T. canis* eggs were collected from the uterus of female adult worms and were embryonated by incubating them in 2% (v/v) formalin at 28 °C for approximately 1 month. Infective eggs were artificially hatched, and the larvae were recovered and maintained *in vitro* at 37 °C in serum-free Eagle′s medium. At weekly intervals, the culture supernatant containing the TES was collected in sterile flasks and replaced with fresh culture medium. All of the supernatants were treated with 200 mmol/L of the protease inhibitor phenyl-methyl-sulfonyl fluoride (Sigma, St. Louis, USA), concentrated with Amicon Ultrafiltration units (Millipore, Danvers, USA), dialysed against distilled water, centrifuged at 12 000 r/min for 60 min at 4 °C, and filtered with 0.22 μm Millipore membranes.

2.5. Preincubation of sera with *Ascaris suum* adult worm extract (AWE)

To remove antibodies elicited by exposure to *Ascaris* that could cross-react with *Toxocara* antigens, the test samples were preincubated with an AWE of *Ascaris suum*[15]. Briefly, adult worms recovered from a porcine intestine were macerated in distilled water, and NaOH was added to a final concentration of 0.15 mol/L. After a 2 h incubation at room temperature, the mixture was neutralised with 6 mol/L HCl, the lipids were removed from the extract with ether, and the extract was centrifuged at 12 000 r/min for 20 min at 4 °C. The aqueous phase was removed and filtered through a 0.22 μm Millipore membrane. All sera were pre-incubated with a final concentration of 25 μg/mL AWE in 0.01 mol/L PBS (pH 7.2) containing 0.05% (v/v) Tween 20 (PBS-T) (Sigma, St. Louis, USA) for 30 min at 37 °C before use in the ELISA.

2.6. ELISA

Serum samples were tested for IgG antibodies to TES by ELISA at a dilution of 1:320, as previously described

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