

Short Communication

Toxocara canis* infection is associated with eosinophilia and total IgE in blood donors from a large Brazilian centre*V. C. C. Dattoli¹, S. M. Freire², L. R. Mendonça², P. C. Santos², R. Meyer² and N. M. Alcantara-Neves²**¹ Faculdade de Medicina, Universidade Federal do Acre, Acre, Brazil² Departamento de Biointeração, Instituto de Ciências da Saúde, Universidade Federal da Bahia, Bahia, Brazil**Summary**

OBJECTIVE To investigate the association between *Toxocara canis* infection and total IgE levels and eosinophilia in blood donors from a large Brazilian city.

METHODS Two hundred and sixty-eight blood donors from a government blood bank were tested. No helminth infection was diagnosed by parasitological stool examination. Total IgE levels and *T. canis* infection status were determined by ELISA. Eosinophil levels were determined using an automatic blood cell counter.

RESULTS *Toxocara canis* IgG antibodies were found in 124 (46.3%); 102 (38.0%) had eosinophilia $\geq 4\%$ and 29 (10.8%) had eosinophilia $\geq 10\%$, respectively; 140 (52.2%) individuals had total IgE antibodies above the cut-off levels. Both total IgE and eosinophil levels $\geq 10\%$ were positively associated with the infection.

CONCLUSION This study revealed a high prevalence of *T. canis* infection in blood donors, highlighting the need for screening for this infection. It also demonstrated that this population otherwise healthy has higher levels of blood eosinophils and total IgE and that both parameters are associated with *T. canis* infection.

keywords *Toxocara canis*, blood donors, eosinophilia, total IgE, prevalence

Human toxocariasis is caused by larvae of *Toxocara canis* or more rarely by *T. cati*, round worms of dogs and cats which colonise humans as paratenic hosts (Cypess *et al.* 1977). Most infected subjects do not show specific clinical manifestations and instead develop 'covert toxocariasis' (Taylor *et al.* 1987). In industrialised countries, high levels of total IgE and eosinophilia are frequently associated with allergy diseases; in developing countries, they are highly associated with parasitic infection (Cooper *et al.* 2008). Eosinophilia has been well-documented in humans infected by *T. canis*, even in asymptomatic subjects (Jacob *et al.* 1994) and is frequently accompanied by high levels of total IgE (Alonso *et al.* 2000). Antibody-dependent cell-mediated cytotoxicity (ADCC) is the main mechanism of killing parasites in which IgE plays an essential role (Capron & Capron 1994). The aim of the current study was to investigate the association between *Toxocara canis* infection and eosinophil levels and total IgE antibodies in healthy blood donors from Salvador, a major city of north-east Brazil.

The study population was composed of 306 blood donors (198 men and 108 women) from a government blood bank of Salvador-Bahia, Brazil. All subjects were approved to donate blood by Brazilian standard blood bank tests and had completed a standardised questionnaire. Eosinophil levels were determined using an automatic cell counter. The cut-off level of $\geq 4\%$ to express moderate eosinophilia was based on the conventional hemogram used in Brazilian laboratories. The 10% cut-off was arbitrarily chosen to express more severe eosinophilia. Total IgE concentration was obtained using a sandwich ELISA commercially available kit (RADIM, Ribeirão Preto, SP, Brazil), and the cut-off of 150 UI/ml was used following the manufacturer's recommendation. *Toxocara canis* IgG antibodies were detected by indirect ELISA using the excretory-secretory products of *T. canis* larvae as antigen (TcESLA), according to de Savigny (1975), and modified by Alcântara-Neves *et al.* (2008), in sera previously absorbed twice with *Ascaris lumbricoides* extract in the presence of 15 000 polyethylene glycol (Sigma, St Louis, MO, USA).

Briefly, puppies from parasite-infected bitches were treated with piperazine (100 mg/kg) and mineral oil. The uteri of adult *T. canis* females obtained were removed, and their eggs were incubated in 2% formalin until embryonation. The egg membranes were disrupted using glass beads, and the released larvae were purified by 15- μ m pore polystyrene membrane filtration. The larvae were cultured in RPMI medium (Sigma) at 37 °C in a CO₂ incubator, and the culture supernatants, which contained *TcESLA* and 1 mM phenylmethylsulfonyl fluoride (PMSF; Sigma), were cryopreserved at –70 °C until use. *TcESLA* was concentrated using Amicon filters (Millipore Corporate, Billerica, MO, USA) with pores permeable to molecules $\leq$ 3000 kDa and subsequently dialysed against phosphate buffered saline (PBS), pH 7.4. The protein content of the samples was determined using the Lowry technique (1951), and the antigen was aliquoted and cryopreserved at –70 °C until further use. The indirect ELISA was performed using 3.2 μ g/ml of *TcESLA* in carbonate/bicarbonate buffer, overnight at 4 °C. After blockage of unspecific reaction, the sera diluted at 1:1000 in a solution of PBS, containing 0.05 tween 20 and 2.5% faetal calf serum (PBS/T/FCS), were incubated with the antigen. Then, a biotinylated anti-human IgG/streptavidin-peroxidase (BD, Pharmingen, San Jose, CA, USA) was used as conjugate, and hydrogen peroxide and o-Phenylenediamine (Sigma) were used for developing the reaction.

The cut-off of the assay was set up using the mean plus three standard deviation of 10 sera from adults who had no contact with cats and dogs (negative controls). To verify the presence and influence of intestinal helminths in the blood cell counts, faecal samples were collected from all study participants and examined by the gravitational sedimentation (Hoffmann *et al.* 1934).

We performed a univariate analysis and a secondary stepwise, forward regression multivariate logistic analysis adjusted for the following confounding variables: gender, age, schooling level. Odds ratios (OR) with 95% confidence intervals (CI) were obtained. Data were analysed using the SPSS 16.0 software.

Of 306 subjects enrolled in the study, 268 with complete data set were included in the analysis. No differences were found between the studied and the excluded subjects in relation to the studied variables (data not shown). Relevant characteristics of this population are shown in Table 1. There were 198 (73.9%) men of whom 94 (35.1%) were aged 31–40 years; 200 participants (74.6%) had a schooling level of second grade or higher. Intestinal helminths were not found in this population. Eosinophil counts of $\geq$ 4% and $\geq$ 10% were observed in 73 (27.2%) and 29 (10.8%) of the subjects, respectively. Hundred and forty individuals (52.2%) had elevated total IgE, and 124 (46.3%) were positive for *T. canis* infection. No association was found between *T. canis* infection and age or

Table 1 Frequency and univariable and multivariable analyses of *Toxocara canis* infection with blood eosinophil levels and total IgE antibodies in blood donors from a large Brazilian urban centre

Variables N = 268 (frequencies %)	<i>T. canis</i> infection (46.3%)			
	n (%) / N	OR (95% CI)†	OR (95% CI)‡	P value§
Age (years)				
≤30 (38.1)	49 (48.0)/102	1	–	–
31–40 (35.1)	41 (43.6)/94	0.84 (0.48; 1.47)		
>40 (26.9)	34 (47.2)/72	0.97 (0.53; 1.77)		
Gender				
Female (26.1)	30 (42.9)/70	1	–	–
Male (73.9)	94 (47.5)/198	1.21 (0.70; 2.09)		
Schooling level				
1st grade or less (25.4)	36 (52.9)/68	1	–	–
Complete 2nd grade or more (74.6)	88 (44.0)/200	0.70 (0.40; 1.21)		
Total IgE (UI/ml)				
<150 (47.8)	49 (38.3)/128	1	1	
≥150 (52.2)	75 (53.6)/140	1.86 (1.14; 3.03)	1.77 (1.07; 2.92)	<0.05
Eosinophils				
<4% (61.9)	67 (40.4)/166	1	1	
4–9% (27.2)	37 (50.7)/73	1.52 (0.87; 2.64)	1.49 (0.84; 2.62)	>0.05
≥10% (10.8)	20 (69.0)/29	3.28 (1.41; 7.65)	3.14 (1.33; 7.43)	<0.05

†Univariable analysis.

‡Adjusted for: age, gender and schooling level.

§Qui square test.

gender in the studied individuals. Statistically significant associations were found between *T. canis* and $\geq 10\%$ eosinophilia (OR = 3.14; 95% CI = 1.33–7.43) and high total IgE production (OR = 1.77; 95% CI = 1.07–2.92).

The high proportion of men in our blood donor population was expected. Previous studies describing blood donor profiles have shown a low proportion of female blood donors (20–37%) (Gonçalez *et al.* 2003). High levels of eosinophilia and total IgE have been reported in non-affluent populations and have been attributed mostly to infection with intestinal helminths (Kayaba *et al.* 2001), although *T. canis* has also been associated with an increase in these Th2 immune system markers (Guardis *et al.* 2002), whereas we report high levels of eosinophilia (38.0%) and total IgE (52.2%) in a blood donor population free of intestinal helminths. We investigated whether *T. canis* infection was associated with abnormal amounts of blood eosinophils and total IgE in this otherwise healthy population living in Salvador, a city with a population of 2 800 000 in north-east Brazil. This study revealed a high prevalence of *T. canis* infection (46.3%). Similar investigations in Latin American countries such as Chile (Navarrete & Rojas 1998) and Argentina (Minvielle *et al.* 2003) reported lower infection prevalence in blood donor populations (5.3% and 12.3%, respectively). Clinical manifestations of *T. canis* infection are diverse and may include asthma-like symptoms and hepatomegaly (Jacob *et al.* 1994). In most *T. canis*-infected people, the infection is asymptomatic and undiagnosed, which is probably because of low larval burden (Schantz 1989). However, clinical cases of human toxocariasis in which the parasite survives for a long period in the host tissues have been reported as an important cause of hypereosinophilic syndromes (Overgaauw 1997). Blood eosinophils counts of $\geq 80\%$ of all white blood cells can occur in acute infections (Sapunar & Fardella 1999). Glickman *et al.* (1987) demonstrated that patients with eosinophilia and high levels of total IgE had a 82% chance of being infected with *T. canis*, which may be attributed to immunological mechanisms. Coffman *et al.* (1993) showed that parasite-derived antigens stimulate Th0 cells to become Th2 cells, leading to the production of IL-4 and IL-5, which stimulate IgE production and eosinophil proliferation and maturation, respectively.

Our study found a high prevalence of *Toxocara canis* infection among blood donors, highlighting the need for increased screening for this infection. The significant positive association between *T. canis* infection and eosinophil levels and IgE production could explain the high frequency of eosinophilia and total IgE production in healthy and non-helminth-infected subjects from non-affluent populations.

Acknowledgements

This study was conducted through the SCAALA (Social change, Asthma and Allergy in Latin America) initiative, funded by the Wellcome Trust and by the INCT/MCT/CNPq Programme. We thank CAPES, CNPQ and FAPESB for scholarships that supported some of the co-authors.

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