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Risk factors for *Toxocara* spp. seroprevalence and its association with atopy and asthma phenotypes in school-age children in a small town and semi-rural areas of Northeast Brazil

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ABSTRACT

Toxocara canis, *Toxocara cati*, are roundworms that live in the intestines of dogs and cats, respectively, and are predominantly agents of human toxocariasis. Studies have suggested that *Toxocara* spp. seroprevalence increases levels of total and aeroallergen-specific IgE (sIgE), asthma prevalence and asthma morbidity. Nevertheless, other work reported a negative association between *Toxocara* spp. seropositivity with skin hypersensititity and a positive association with sIgE. The objective of the present study was to evaluate risk factors for acquiring *Toxocara* spp. infection and to investigate possible significant association between its seroprevalence with atopy and asthma. Students from elementary schools, residents in a small town and its surroundings of Northeast Brazil, underwent blood sampling to measure levels of anti-*Toxocara* spp. IgG, peripheral blood eosinophils, and specific IgE to aeroallergens. We used univariable and multivariable logistic regression analyses to assess possible risk factors for *Toxocara* spp. seropositivity and its association with atopy, wheeze/asthma with asthma phenotypes, in a sample of 791 elementary school children aged 6–13 years. *Toxocara* spp. seroprevalence reached 63.6%; 49.9% had sIgE; 7.2% and 3.3% had atopic wheeze/asthma and non-atopic wheeze/asthma respectively. Risk factors associated with *Toxocara* spp. seropositivity were: contact with dogs (adj. OR 2.33; 95% CI = 1.70–3.19) and cats (adj. OR 3.09; 95% CI = 2.10–4.55), and male sex (adj. OR 2.21; 95% CI = 1.62–3.02). The presence of anti-*Toxocara* IgG was statistically associated with blood eosinophils >4% and >10% (adj. OR 1.84; 95% CI = 1.33-2.55 and adj. OR 2.07; 95% CI = 1.45–2.97, respectively), and atopy (adj. OR 2.00; 95% CI = 1.49–2.68), but it was not associated with wheeze/asthma. Concluding, the results obtained in this study showing the association of *Toxocara* spp. seroprevalence with sIgE may suggest a possible immunological cross-reactivity between IgE epitopes from *Toxocara* spp. and aeroallergens.

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1. Introduction

Over the last few decades, there has been an increase in the prevalence of allergic respiratory diseases such as asthma and rhinitis worldwide. Data from the ISAAC (International Study of Asthma and Allergy in Childhood) Phase III studies showed increase in the prevalence of these diseases in developing and recently in industrialized countries in Africa, Latin America and parts of Asia (Beasley, 1998).

Various explanations have been put forward to explain temporal trends of increasing prevalence of atopy and allergic diseases in these regions including: 1) changes in living environment associated with urbanization resulting in increased exposure to environmental allergens such as dust mites and cockroaches (Rodriguez et al., 2011); and 2) the hygiene hypothesis that explains such epidemiological trends in terms of decreasing exposure to infections in early childhood leading to impaired regulation of the inflammatory response (Strachan, 1989). Human helminth infections, that are highly prevalent in tropical populations living in conditions of poverty and poor hygiene, have been proposed to play a key role in the regulation of allergy in populations where these infections are endemic (Maizels, 2013). The interaction between host and parasite during chronic helminth infections results in an immune regulatory environment that suppresses host allergic effector responses responsible for parasite killing, and it has been suggested that such modulation of allergic inflammation may affect atopy and allergic diseases (Pfefferle and Renz, 2014; Pontes-de-Carvalho et al., 2013). In contrast, the frequent or seasonal exposures to helminths that do not cause chronic infections, have been associated with increased allergic inflammation (Santos et al., 2013; Alcântara-Neves et al., 2010), and the same appears to be true for zoonotic helminth infections such as toxocariasis that are unable to complete their life cycle in the human host (Buijs et al., 1997).

Toxocariasis is a human infection caused mostly by the intestinal roundworms *Toxocara canis* and *Toxocara cati* parasites of dogs and cats, respectively, and is transmitted to humans through the ingestion of embryonated eggs. Other *Toxocara* species exist but they rarely or never were found infecting humans (Bowman and Hendrix, 2008). Humans serve as paratenic hosts for *T. canis* and *T. cati,* in whom the parasites are unable to develop to adulthood. Toxocariasis is a cosmopolitan infection, present mainly in developing countries in populations living in conditions of poverty and poor hygiene (Overgaauw and van Knapen, 2013).

Most of human *Toxocara* spp. infections are asymptomatic (covert toxocariasis-CT) and morbidity caused by the infection is believed to depend on parasite burden and host immune response (Macpherson, 2013). When symptomatic, it can occur in three clinical forms: visceral larva migrans (VLM), ocular larva migrans (OLM), and neurological toxocariasis (NT). These clinical forms are not restricted to *Toxocara species* infection since some other helminth larvae may migrate in the human organs and systems, causing visceral larva migrans (Beaver et al., 1952; Fragoso et al., 2011; Gavignet et al., 2008). The asymptomatic form of human toxocariasis may be associated with low cognition (Walsh and Haseeb, 2012) and immunomodulation (Mendonça et al., 2012; Maizels, 2013).

Some studies have observed significant association between *Toxocara* spp. seroprevalence and increased levels of total and aeroallergen-specific IgE (sIgE), allergen skin test prick reactivity (SPT), asthma prevalence and asthma morbidity (Kanobana et al., 2013; Buijs et al., 1997). However, other studies observed no significant associations between the presence of anti-*Toxocara* spp. IgG antibodies and allergic markers (Sharghi et al., 2001; Fernando et al., 2009). Our group, studying children living in the Brazilian city of Salvador, has reported previously a negative association between *Toxocara* spp. seropositivity and SPT, and a positive association with sIgE (Mendonça et al., 2012). *Toxocara* spp. infection appeared to mediate at least partly the disassociation found between sIgE and SPT, but was not associated with atopic or non-atopic wheeze/asthma (Mendonça et al., 2012). In the present study, we estimated the prevalence of *Toxocara* spp. infection, identified risk factors associated with this infection, and evaluated possible associations between *Toxocara* spp. infection and atopy and asthma in a population of students attending elementary schools in rural and urban areas of a small town in Northeast Brazil.

2. Material and methods

2.1. Study area and population

We performed a cross sectional study in São Francisco do Conde (SFC), a small city within the metropolitan region of Salvador in Northeast Brazil. The estimated population of this municipality in 2010 was approximately 36,677 inhabitants.

The study was conducted between August and December 2010. A total of 1187 children and adolescents between 6 and 13 years old, from public schools with 150 or more students (8/22 schools), located in semi-rural and urban areas, were enrolled, of whom 791 students with data available for all study variables were included in the present analysis.

The parents or legal guardians signed an informed consent and the study protocol was approved by the Research Ethics Comitee of Maternity Climério de Oliveira, Federal University of Bahia (UFBa), Salvador-BA under registration CEP.004/2010.

2.2. Clinical and epidemiological data collection

History of allergy was collected by interviewing parents or guardians, using an ISAAC Phase III Portuguese-adapted questionnaire. Data on sanitation, social class and risk factors for toxocariasis were collected using a previously validated questionnaire (Strina et al., 2003).

2.3. Clinical samples

Stool samples were collected and examined by spontaneous sedimentation (Golvan et al., 1974) and Kato-Katz (Katz et al., 1972). Blood samples were collected for eosinophil counts and sera were stored for measurement of allergen-specific IgE and anti-*Toxocara* spp. IgG.

2.4. Obtaining excretory-secretory antigens of T. canis larvae (TES)

The excretory/secreted factors of the *T. canis* larvae (TES antigen) were prepared as described previously (de Savigny and Tizard, 1977; Alcântara-Neves et al., 2014). In summary, *T. canis* eggs were obtained from adult female worms and incubated in 3% formalin for approximately 28 days until full embryonation. The eggs were induced to hatch after treatment with 5% sodium hypochlorite and agitation in presence of RPMI 1640 (Sigma-Aldrich, St. Louis, MO, USA). The larvae were cultivated in RPMI 1640 supplemented with penicillin (1000 U/mL), streptomycin (1 mg/mL), amphotericin (2.5 μg/mL) and gentamicin (0.2 mg/mL), free from fetal bovine serum and kept in an incubator with 5% CO_2 at 37° C. Culture supernatants were harvested weekly, treated with 0,2 M phenylmethylsulfonyl fluoride (PMSF, Sigma, St. Louis, MO, USA), and stored at −70 °C. The TES was concentrated by ultrafiltration through a 3000 kD filter (Millipore Corporate, MA, USA) at 4 °C; dialyzed against phosphate buffered saline, pH 7.4 (PBS), and centrifuged at 6000*g* for 10 min. Protein concentration of TES was

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determined by Lowry's method (1951), before aliquoting and storage at −70 °C until use.

2.5. Absorption of sera with Ascaris lumbricoides antigens

To minimize reactions caused by immunologic IgG cross-reactivity between anti-*A. lumbricoides* and anti-*Toxocara* spp. antibodies, sera were incubated (1 in 5 dilution) with crude extracts of *A. lumbricoides* adult worms in the presence of PBS containing 15% polyethylene glycol (PEG 15,000, Sigma Chemical Co., San Louis, MO, USA) and 0.1% azide. The serum was homogenized for 30 min and then centrifuged at 724 *g* for 10 min at 4 °C. The supernatant was removed and kept at −70 °C until assayed. Because we have observed previously that sera depleted of *A. lumbricoides* antibodies do not contain cross-reactive IgG antibodies to *Trichuris trichiura* extract (Mendonça et al., 2013), sera were not absorbed also against *T. trichiura* antigens.

2.6. Immunoassay for the detection of anti-Toxocara spp. IgG

To detect anti-*Toxocara* spp. IgG antibodies in sera, we used an indirect ELISA as described previously (Mendonça et al., 2013). High-binding polystyrene 96-well microplates, (Costar, Corning, N.Y.) were incubated with TES at a concentration of 3 μg/mL in carbonate bicarbonate buffer (100 mM, pH 9.6) at 4 °C overnight. Blocking of non-specific binding was done with PBS containing 0.05% tween 20 (PBS/T), 10% fetal calf serum (PBS/T/10%FCS) in a humidified chamber for 1 h at room temperature. Sera diluted at 1:1000 were added to the wells. After washing, anti-human IgG biotinylated-conjugate (BD Pharmingen, San Diego, CA, USA) was added at a dilution of 1:4000 in PBS/T/2.5%FCS followed by streptavidin-peroxidase (BD Pharmingen, San Diego, CA, USA) diluted at 1:500. All reagents were diluted in PBS/T/2.5%FCS, incubated for 60 min at room temperatures (except for the last step, for 30 min), and washed thrice with PBS/T. Finally the chromogen OPD (*ortho*-phenylenediamine; Merck & Co., Inc., White House Station, NJ, USA) was added at a concentration of 0.04 mg/mL. The reaction was stopped with 25 μl of 2 N H_2SO_4 and the optical density was read at 450 nm in a microplate reader. The assay cut-off value of OD = 0.22 was calculated as the mean optical density (0.129) plus three standard deviations (0.0318) using results obtained from ten sera from students with blood eosinophil levels below 2%, negative parasitological stool examinations, and negative specific IgE for aeroallergen assays. Because this assay, does not discriminate between different species of *Toxocara* spp. infections (Kennedy et al., 1987), we used the results of this assay as a marker for past or present infection with both *Toxocara* species which infect usually humans.

2.7. Determination of specific IgE to aeroallergens

Serum levels of specific IgE antibodies to aeroallergens were determined by Immunocap (Phadia Diagnostics AB, Uppsala, Sweden) immunofluorescence assay using the ImmunoCAP-100 instrument (lmmunoCap system, Phadia AB, Uppsala, Sweden). Specific IgE to *Blomia tropicalis* (D201) and Phadiatop aeroallergens (Pollen extracts, fungi extracts, dog and cat epithelia and *Dermatophagoides* spp.) was measured and expressed in kU/L (1 kU/L = 2.4 ng/mL of IgE). A positive test was defined as sIgE ≥ 0.70 kU/L.

2.8. Definitions of atopy and asthma phenotypes

Students with IgE to *B. tropicalis* or to the Phadiatop set of allergens >0.70 kU/L were defined as atopic. They were classified as having current wheeze using the phase III ISAAC questionnaire data (wheezing in the last 12 months) and were considered to have wheezing/asthma if parents reported wheezing in the previous 12 months plus at least one of the following: (I) previous diagnosis of asthma; (II) wheezing with exercise; (III) ≥4 episodes of wheezing; (IV) waking up at night because of wheezing. Students were allocated to one of four groups: 1) atopic wheezers/asthmatics; 2) non-atopic wheezers/asthmatics; 3) atopic non-wheezers/asthmatics; and 4) non-atopic/non-wheezers/asthmatics.

2.9. Statistical analysis

Statistical Package for the Social Sciences (SPSS) version 19.0, was used to construct the database and do the analysis. Data were entered in duplicate after all the questionnaires had been reviewed and any coding errors had been corrected. Only the students with complete data were included in the present analysis. Potential risk factors for *Toxocara* spp. seroprevalence investigated were: gender, age, maternal schooling, income, school location, contact with dog and cat, and active infections with *A. lumbricoides* and *T. trichiura*. For studying the risk factor of *Toxocara* spp. seroprevalence on allergy markers, we performed univariable analysis for each outcome (eosinophilia, total IgE aeroallergen-specific and wheezing/asthma), followed by multivariable models for each outcome in which variables with $P < 0.2$ in univariable analysis were considered and those with $P < 0.05$ were included in the final model using a backward step-wise procedure. The variables considered in multivariable analyses were: maternal education, family income, parental asthma history, school locality, intestinal helminth infection and presence of dog and cat at home, all of them analysed as categorical variables. *A priori* variables included in the multivariable models were age and gender. The association between anti- *Toxocara* spp. IgG and wheezing/asthma phenotypes was analyzed by multivariate logistic analysis using non-atopic non-wheezers/non-asthmatics and atopic non-wheezers/non-asthmatics as reference groups for non-atopic versus atopic wheezing/asthma, respectively (Mendonça et al., 2012).

3. Results

We analyzed data from 791 children with complete data of the 1187 subjects initially enrolled. Preliminary analysis using Chi^2 did not show any difference in prevalence of atopy and wheeze/asthma between those students included and excluded from the analysis (data not shown).

Table 1 shows the frequencies of possible risk factors for *Toxocara* seropositivity and wheezing/asthma. Overall, 63.6% of subjects were seropositive for anti-*Toxocara* spp. The seropositivity was statistically and positively associated with male gender, contact with dogs and cats. On the other hand, significant associations were not observed for the other variables studied as potential risk factors for the anti-*Toxocara* spp. IgG antibodies seroprevalence, nor for wheezing/asthma prevalence.

Table 2 shows the association between the positivity and levels of *Toxocara* ssp. IgG with blood eosinophils. Anti-*Toxocara* IgG was found in 63.6% of the total students, and 74.8% and 37.9% had eosinophilia above 4% and 10% respectively. There was a positive and statistically significant association in both crude and adjusted analyses between the seropositivity and eosinophila in both levels. Stratification by titres of anti-*Toxocara* spp. IgG showed that increasing titres were associated with a greater risk of eosinophilia.

Table 3 shows that a positive association was also observed between *Toxocara* spp. seropositivity and the presence of atopy to *B. tropicalis* IgE, Phadiatop allergens, and any aeroallergens in both

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Table 1
Associations between potential risk factors and IgG seropositivity to *Toxocara* spp. and wheezing/asthma.in 791 students, aged 6–13 years enrolled in public schools in Northeast Brazil.

Studied variables	n (%)	Anti-*Toxocara* spp. IgG seropositivity n (%)	Crude OR (95% CI)	[a]Adjusted OR (95% CI)
Gender				
Female	379 (47.9)	210 (55.4)	**1**	**1**
Male	412 (52.1)	293 (71.1)	**1.98 (1.48–2.65)**	**2.21 (1.62–3.02)**
Age in years				
10–<13	383 (48.4)	247 (64.5)	1	
6–<10	408 (51.6)	256 (62.7)	0.90 (0.68–1.21)	–
Maternal schooling				
1st grade or less	439 (55.5)	283 (64.5)	1	–
Incomplete 2nd grade	317 (40.1)	199 (62.8)	0.93 (0.69–1.25)	–
Complete 2nd grade or more	35 (4.4)	21 (60.0)	0.82 (0.40–1.67)	–
Family income				
≥1	454 (57.4)	302 (66.5)	1	
<1	337 (42.6)	201 (59.7)	0.78 (0.58–1.04)	–
School location				
Rural	254 (32.1)	158 (62.2)	1	
Urban	537 (67.9)	355 (66.1)	1.17 (0.86–1.61)	–
Dog contact				
No	295 (37.3)	147 (49.8)	**1**	
Yes	496 (62.7)	356 (71.8)	**2.56 (1.90–3.45)**	**2.33 (1.70–3.19)**
Cat contact				
No	562 (71.0)	317 (56.4)	**1**	**1**
Yes	229 (29.0)	186 (81.2)	**3.34 (2.30–4.86)**	**3.09 (2.10–4.55)**
Ascaris and/or *Trichuris infection"*				
No	445 (69.4)	263 (59.1)	**1**	**1**
Yes	196 (30.6)	136 (69.4)	**1.58 (1.10–2.26)**	**1.22 (1.03–1.87)**

The student number was 721 for all the variables, except for *Ascaris* and/or *Trichuris* infections which was 641. Numbers in bold are those statistically significants.
[a] Adjusted for gender, age, dog, school location, maternal schooling, family income, cat and helminth infections.

Table 2
Associations of *Toxocara* spp. seropositivity with eosinophilia in 791 students aged 6–13 years enrolled in public schools in Northeast Brazil.

Anti-*Toxocara* IgG	n (%)	Eosinophilia (4%)		n (%)	Eosinophilia (10%)	
		Anti-*Toxocara* spp. IgG seropositivity n (%)	[a]Adjusted OR (95% CI)		Anti-*Toxocara* spp. IgG seropositivity n (%)	[a]Adjusted OR (95% CI)
Negative	288 (36.4%)	166 (57.6)	**1**	288 (36.4%)	58 (20.1)	**1**
Positive	503 (63.6%)	376 (74.8)	**1.84(1.33–2.55)**	503 (63.6%)	191 (37.9)	**2.07 (1.45–2.97)**
Level of anti-*Toxocara* IgG						
Neg < 0.22	288 (36.4%)	166 (57.6)	1	288 (36.4%)	58 (20.1)	**1**
≥0.22 ≤ 1	381 (48.2%)	288 (75.6)	1.23 (0.86–1.77)	381 (48.2%)	135 (35.4)	**1.32 (1.12–2.23)**
≥1	122 (15.4%)	88 (72.1)	**1.60 (1.03–2.44)**	122 (15.4%)	56 (45.9)	**1.55 (1.03–2.33)**

[a] Adjusted for age, sex, maternal education, monthly household income, living in urban vs. rural areas, and helminth infections. Numbers in bold are those statistically significant.

Table 3
Association between *Toxocara* spp. and atopy measured by presence of aeroallergen-specific IgE in 791 students aged 6–13 years enrolled in elementary public schools in Northeast Brazil.

Anti-Toxocara spp. IgG	Phadiatop[a] IgE ≥ 0.70 KU/L)		*B. tropicalis* specific IgE ≥ 0.70 KU/L)		Any allergen IgE ≥ 0.70 KU/L	
	n (%)/N	OR (95% CI)[**]	n (%)/N	OR(95% CI)[**]	n (%)/N	OR(95% CI) (95% CI)
Negative (n = 288; 36.4%)	82 (28.5)/288	1	65 (22.5)/288	**1**	123 (42.7)/288	**1**
Positive (n = 503; 63.6%)	251(49.9)/503	**1.95 (1.40–2.72)**	286 (56.8)/503	**1.85 (1.31–2.62)**	342 (67.9)/503	**2.00 (1.49–2.68)**
Level of anti- *Toxocara* IgG						
< 0.22 (n = 288; 36.4%)	82 (28.5)/288	**1**	65 (22.6)/288	**1**	123 (42.7)/288	**1**
≥0.22 ≤ 1(n = 381; 48.2%)	197 (51.7)/381	**1.62 (1.19–2.22)**	249 (65.3)/381	**1.44 (0.90–2.12)**	247 (64.8)/381	**1.78 (1.34–2.36)**
≥1 (n = 122; 15.4%)	54 (44.3)/122	1.33 (0.88–2.02)	37 (30.3)/122	**1.39 (1.28–2.43)**	95 (77.9)/122	**1.55 (1.06–2.7)**

[a] IgE specific to *Blomia tropicalis* (D201) and to Phadiatop aerollergens (pollen extracts, fungi extracts, dog and cat epithelia and *Dermatophagoides* spp.) measured by immunoCAP.
[**] ORs Adjusted for age, sex, maternal education, family income, living in urban and rural areas and intestinal helminth infections. Numbers in bold are those statistically significants.

crude and adjusted analyses. Stratification by titres of anti-*Toxocara* spp. IgG also showed positive and significant statistically associations with the allergens IgE except for the crude analyses of the association with the Phadiatrope allergen which showed a positive but not significant statistically association.

There was no association between *Toxocara* spp. seropositivity and anti-*Toxocara* IgG stratification levels with wheezing/asthma and wheezing/asthma phenotypes (Table 4), nor with/wheezing/asthma morbidity (data not shown).

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Table 4
Associations between anti-*Toxocara* spp. serum IgG with wheezing/asthma and with wheezing/asthma phenotypes in 791 students aged 6–13 years enrolled in public schools in schools in Northeast Brazil.

Studied variables	Wheezing/asthman (%)/N		Crude OR(95% CI)	[a]Adjusted OR(95% CI)
Anti-*Toxocara* spp. IgG seropositivity				
No	28 (9.7)/288		1	1
Yes	55 (10.9)/503		1.16 (0.72–1.88)	1.14 (0.69–1.90)
Anti-*Toxocara* spp.	Non-atopic wheeezers (N = 26)		Atopic wheeezers (N = 57)	
	[b]Reference group: non-atopic, non-wheeezers N = 346		[b]Reference group: atopic, non-wheeezers N = 362	
	n (%)/N	[a]OR (IC 95%)	n (%)/N	[a]OR (IC 95%)
Negative	10 (6.3)/158	1	13 (13.2)/98	1
Positive	16 (8.5)/188	1.18 (0.49–2.87)	44 (16.6)/264	1.23 (0.62–2.48)
Serum levels				
Negative < 0.22	10 (6.3)/158	1	13 (13.2)/98	1
≥ 0.22 ≤ 1	12 (8.6)/139	1.36 (0.60–3.62)	29 (15)/194	0.86 (0.48–1.53)
≥ 1	4 (8.1)/49	0.60 (0.17–2.16)	15 (21.4)/70	1.27 (0.63–2.55)

[a] ORs adjusted for sex, age, income, maternal education and nutritional status, and intestinal helminth infections.
[b] Reference groups for analysis.

4. Discussion

Despite the importance of *Toxocara* spp. infections for human health, toxocariasis is considered a neglected disease by the World Health Organization (Nelson et al., 1996), and is little recognised as a significant problem by public health institutions in developing countries (Noordin et al., 2005). In the present study, we observed a seroprevalence of *Toxocara* spp. infection of 63.6%, in a population of schoolchildren attending elementary schools in urban and semi-rural areas in Northeast Brazil and did not observe a statistically significant difference in prevalence between areas. Previous studies have provided similar or slightly lower prevalence estimates from the same region of Brazil: Mendonça and collaborators (2013) in a cross-sectional analysis of 1309 children aged 4–11 years living in urban areas of Salvador observed a prevalence of 48.4%, while Souza et al. (2011) estimated a prevalence of 59.9% in 338 children and adults also in urban Salvador with a higher prevalence observed among the lower social classes.

Several studies have suggested that contact with dog is the main risk factor acquisition of *T. canis* infection, because this animal is a direct source for the transmission of embryonated *T. canis eggs* (Loukas et al., 2000; Schnieder et al., 2011; Strube et al., 2013). Associations with cat exposure are less frequently described although we did observe cat exposure to be a significant risk factor independent of that of dogs. Although, cats tend to bury their faeces, they are also a recognised risk factor and the control of stray cats and treatment of pet cats should be included as public health measures for the control of human toxocariasis.

We observed a two-fold increase in risk of *Toxocara* spp. seroprevalence in boys compared to girls. Previous studies conducted in children and adolescents have shown that boys are at higher risk of infection with *Toxocara* spp. than girls (Roldán et al., 2010), probably because they tend to be more active out of doors and exposed to environments contaminated with *Toxocara* spp. eggs (Alonso et al., 2000; Romero Núñez et al., 2013; Wiśniewska-Ligier et al., 2012).

Previous studies have shown that socioeconomic status is an important determinant of *Toxocara* spp. seroprevalence (Aguiar-Santos et al., 2004; Alvarado-Esquivel, 2013). However, in the present study we did not observe such an effect using monthly household income and maternal schooling to represent socioeconomic status. The population of São Francisco do Conde is characterized by having poor socioeconomic conditions, where a high proportion of the population had a monthly household income below the minimum wage of U$ 200 per month in 2010 and where only a minority of mothers had completed the 2nd grade. The lack of effect observed in this study might be explained by the relative homogeneity of the study populations with respect to socioeconomic factors unlike previous studies where clearer social stratification was present (Souza et al., 2011; Mendonça et al., 2012; Negri et al., 2013; Mendonça et al., 2013).

Helminthic infections induce Th2-type immune response, causing the production of IL4, IL-5, and IL-13, which lead to the production of IgE, eosinophilopoiesis, and mucus production. Human *Toxocara* spp. infection is characterized by presenting, among laboratory findings, high levels of blood eosinophils (Mazur-Melewska et al., 2012; Pinelli and Aranzamendi, 2012). In our study we observed the occurrence of eosinophilia above 4% and 10% to be greater in the students' positive for anti-*Toxocara* spp. IgG, even after adjustment for co-infections with *A. lumbricoides* and *T. trichiura*. These data are similar to the findings of Dattoli et al. (2011), who found elevated levels of eosinophils in individuals infected with *Toxocara* spp. and without evidence of other helminth infections.

Several previous studies have found positive significant associations of *Toxocara* spp. seropositivity with asthma (Buijs et al., 1997; Kanobana et al., 2013). We also explored potential associations between *Toxocara* spp. seroprevalence and markers of allergy. Multivariable analysis revealed that individuals seropositive for *Toxocara* spp. were more likely to have specific IgE to aeroallergens (adj. OR 1.95, 95% CI = 1.40–2.72) including to *B. tropicalis* (adj. OR 1.85; 95% CI: 1.31–2.62), supporting the findings of a previous study of children in urban Salvador (Mendonça et al., 2012), were it was found a positive association with sIgE and negative association wuth skin sensibility, but no association with wheezing/asthma. They attributed this last finding to the predominance of non-atopic wheezing/asthma in their population which was determined by the population attributed factor (PAF) being 22.0% (Cunha et al., 2010). In our population the PAF was 30.4% (data not shown) which was also low and could explain the absence of association between atopic wheezing/asthma and *Toxocara* spp. seroprevalence. In fact these two populations although the former lives in urban periphery and the later lives in small town and its semi-rural areas, they are both from non-affluent communities and live nearby.

The positive association found between IgG anti-*Toxocara* spp. seroprevalence and sIgE may be due to cross-reactivity between IgE epitopes of *Toxocara* spp. antigens and those of environmental allergens. Several previous studies have demonstrated cross-reactivity between mites and helminths. Ponte et al. (2011) have shown that *A. lumbricoides* antigens give rise to the production of IgE cross-reactive with antigens of *B. tropicalis*. Acevedo et al. (2009) attributed this cross-reaction to epitopes of tropomyosin and glutanione-S transferase shared by *A. lumbricoides* and *B. tropicalis*. Another possibility would be the presence of cross-reactive carbohydrate epitopes. Helminth carbohydrates stimulate the synthesis of IgE that may cross-react with IgE specific to aeroallergens, and

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which may be less effective in inducing degranulation of mast cells and basophils (Ponte et al., 2011).

We also did not observe an association with non-atopic asthma suggesting that in this population the occurrence of pulmonary toxocariasis causing asthma-like symptoms is uncommon.

In the present study we did perform skin prick test (SPT) to aeroallergens and did not measure the concentration of IL-10 of peripheral blood culture of the studied population. A previous study in urban Salvador observed a dose-dependent inverse association between titres of anti-*Toxocara* spp. antibodies and SPT, and also a positive association between *Toxocara* spp. seroprevalence and the presence of aeroallergen-specific IgE (Mendonça et al., 2012). The protection against SPT was attributed to the immunomodulatory effects of *Toxocara* spp. Reinforcing these findings, Alcântara-Neves et al. (2014) observed a dose-response increase in IL-10 production by peripheral blood leukocytes from children infected with increasing numbers of helminth species including *Toxocara* spp.

A hypothesis to explain this immunomodulatory role of toxocariasis would be competition for high affinity receptors IgE receptors (FcεRI) on effector cells between parasite-induced IgE (both polyclonal and parasite-specific IgE) and aeroallergen-specific IgE in which FcεRI become saturated with parasite-induced IgE. Such competition could decrease effector cell sensitivity to aeroallergen-induce activation leading to control of allergic inflammatory reaction. However a criticism of this hypothesis was put forward by who showed that only extremely high levels of polyclonal IgE, rare in most populations, can cause such an effect-only levels of total IgE in excess up 10,000 ng/mL impaired basophil degranulation. Another hypothesis put forward to explain the protection conferred by helminth infections to atopy and asthma is the immunomodulation occurred in their chronic phase known as Th2-modified response where high production of IL-10 and TGF-β cytokines are produced, to allow the parasite survival in their hosts (Maizels, 2013); Smits et al. (2010) and as shown by Alcântara-Neves et al. (2014) *Toxocara* spp. seropositivity was found positively associated with increased IL-10 production by blood cells.

In conclusion, our data show that human *Toxocara* spp. seroprevalence is associated with contact with dogs and cats, sources of infection with this zoonosis, and male sex. We observed also an association of *Toxocara* spp. seroprevalence with peripheral blood eosinophilia and the presence of specific IgE to aeroallergens, but not with wheezing/asthma. The association with aeroallergen-specific IgE may be a consequence of cross-reactivity between parasite-specific and aeroallergen IgE.

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