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

**Short title:** Risk factors for *Toxocara* spp. seroprevalance and its association with allergy

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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 *Toxocara* infection and of human visceral larva migrans (VLM), diagnosed by detection of serum IgG against these parasites. Studies have suggested that *Toxocara* spp. seroprevalance increased levels of total and aeroallergen-specific IgE (sIgE), as well as skin test prick reactivity (SPT) to aeroallergens, asthma prevalence and asthma morbidity. Nevertheless, others reported previously a negative association between *Toxocara* spp. seropositivity and SPT, and, a positive association with sIgE. The objective of the present study was to evaluate risk factors for acquiring *Toxocara* sppinfection and to investigate possible significant associations between its seroprevalancewith atopy and asthma. Students from elementary schools residents in São Francisco do Conde, a small town of northeast Brazil, underwent blood sampling to measure levels of anti-*Toxocara* spp. IgG, peripheral blood eosinophilia, and atopy by measurement of specific IgE to aeroallergens. We used univariable and multivariable regression analyses to assess possible risk factors for *Toxocara* spp. infection and the association of its seropositivity with atopy or asthma, and polytomous logistic regression to assess its possible association with asthma phenotypes, in a sample of 791 elementary school-age children aged 6-13 years. *Toxocara* spp seroprevalence reached 63.6%, eosinophilia >4% and >10% was present in 74.8% and 38.0% respectively; 49.9% anti-allergen IgE (sIgE); atopic wheeze/asthma of 7.2% and non-atopic wheeze/asthma of 3.3%. Risk factors associated with *Toxocara* sppinfection 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. *Toxocara* spp. Anti-*Toxocara* seroprevalancewas positively associated with eosinophilia >4% and >10%, and atopy, but itwas not associated with atopic or non-atopic wheeze/asthma. We observed a high seroprevalence of anti-*Toxocara* spp IgG in school children associated with male sex and exposure to dogs and cats. *Toxocara* spp*.* seroprevalancewas not associated with wheeze/asthma but was associated with eosinophilia and the presence specific IgE to *Blomia tropicalis*, the latter suggestive of possible immunological cross-reactivity between IgE epitopes from *Toxocara* spp. and aeroallergens.

**Key-words:** Toxocariasis, Risk factors, Specific IgE, Atopy, Asthma.

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 and microbes 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 et al., 2004). 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, in 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 (Lopez et al., 2009, 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 istransmitted to humans through the ingestion of embryonated eggs. Other *Toxocara* species exist but they rarely or never were found infecting humans (Bowman., 2008). Humans serve as paratenic hosts for *Toxocara* *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 et al., 2012) and immunomodulation (Mendonça et al., 2012, Maizels., 2013).

Some studies have observed significant association between *Toxocara* spp. seroprevalanceand 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 of 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 with atopy and asthma in a population of students attending elementary schools in rural and urban areas in Northeastern Brazil.

1. **MATERIAL AND METHODS**
   1. **Study area and population**

We did 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 1,187 children and adolescents between 6-13 years old, from public 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. Only elementary schools in this area with 150 or more students were included (8/22 schools).

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.

* 1. **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).

* 1. **Clinical samples**

A stool sample was 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.

* 1. **Obtaining excretory-secretory antigens of *Toxocara canis* larvae (TES)**

The excretory/secreted factors of the T. canis larve (TES antigen) was prepared as described previously (de Savigny et. al., 1975; Alcantara-Neves et al., 2008). 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 hipochlorite and agitation in presence of RPMI 1640 (Sigma-Aldrich, St. Louis, MO, USA. The larvae were cultivated in RPMI 1640 supplemented with penicillin (1,000 U/ml), streptomycin (1mg/ml), amphotericin (2.5µg/mL) and gentamicin (0.2 mg/ml), free from foetal bovine serum and kept in an incubator with 5% CO2 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 ºC. 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 6,000 g for 10 minutes. Protein concentration of TES was determined by Lowry`s method (1951), before aliquoting and storage at -70 °C until use.

* 1. **Absorption of sera with *Ascaris lumbricoides* antigens**

To minimize reactions caused by immunologic IgG cross-reactivity between anti-*A. lumbricoides* andanti-*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 minutes and then centrifuged at 5,724 g for 10 minutes at 4 °C. The supernatant was removed and kept at -70 °C until assay. 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 3μg/ 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% foetal calf serum (PBS/T/10%FCS) in a humidified chamber for 1 hour 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 minutes at room temperatures (except for the last step, for 30 minutes), 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 25μl of 2N H2SO4 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 plus three standard deviations 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.

* 1. **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 aerollergens (Pollen extracts, fungi extracts, dog and cat epithelia and *Dermatophagoides* spp.) was measured and expressed in kU/L (1kU/L=2.4 ng/mL of IgE). A positive test was defined as sIgE ≥ 0.70 kU/L.

* 1. **Definitions of atopy and asthma phenotypes**

Students with IgE to *Blomia 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 wheeze/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 seroprevalanceinvestigated 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.  *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).

1. **RESULTS**

We analysed data from 791 children with complete data of the 1187 subjects initially enrolled. Preliminary analysis using Chi2 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 seroprevalance IgG antibodies, nor with wheezing/asthma.

Table 2 shows the association between the positivity and levels of Toxocara ssp IgG with blood eosinophils. Eosinophilia above 4% was observed in 74.8% and above 10% was in 38.0% of students. There was a positive and statistically significant association in both crude and adjusted analyses between the seropositivity and and eosinophila in both levels. Stratification by titles of anti-*Toxocara* spp*.* IgG showed that increasing titles were associated with a greater risk of eosinophilia.

Table 3 shows that a e positive association was also observed between *Toxocara* spp. seropositivity and the presence of atopy to *Blomia tropicalis* IgE, Phadiatop allergens, and any aeroallergens in both crude and adjusted analyses., Stratification by titles of anti-*Toxocara* spp*.* IgG showed also positive and statistically associations with the allergens IgE except for the crude analyses of the association with the Phadiatrope allergen which showed a positive but not statistically significant 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).

1. **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 toxocariasis in a population of schoolchildren attending elementary schools in urban and semi-rural areas in Northeaster Brazil of 63.6%, 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 1,309 children aged 4-11 years living in urban areas of Salvador observed a prevalence of 48.4%, while Souza collaborators (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 associated with toxocariasis, 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. seroprevalancein 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 (Liao and colleagues, 2011; Roldan 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. seroprevalance(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 *Ascaris* and *Trichuris*. This data corroborates the findings found by Dattoli (2011), who found elevated levels of eosinophils in individuals infected with *Toxocara* spp. and without evidence of other helminth infections.

We also explored potential associations between *Toxocara* spp. seroprevalance 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) and *B. tropicalis* (adj. OR 1.85; 95% CI: 1.31 - 2.62), and support the findings of a previous study of children in urban Salvador (Mendonça et al., 2012). An explanation for this association may be 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 and collaborators (2011) have shown that *A. lumbricoides* antigens give rise to the production of IgE cross-reactive with antigens of *B. tropicalis*. Acevedo and colleagues (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 which may be less effective in inducing degranulation of mast cells and basophils.

Several previous studies have found positive significant associations of *Toxocara* spp*.* seropositivity with asthma (Buijs et al., 1997, Kanobana et al., 2013). Our data do not support these findings, we did not observe an association with atopic asthma consistent with findings of a previous study of children in urban Salvador (Mendonça et al., 2012). 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 not do skin prick testing (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 titer of anti-*Toxocara* spp antibodies and SPT, and also a positive association between *Toxocara* spp. seroprevalanceand 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, Alcantara-Neves and collaborators (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 (Fc£RI) on effector cells between parasite-induced IgE (both polyclonal IgE and parasite-specific) and aeroallergen-specific IgE in which Fc£RI 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 Mitre and collaborators (2005) 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. The production of immunosuppressive cytokines like IL-10 and TGF-β by regulatory cells populations, induce B cells to class switch from IgE to IgG4. IgG4 can block the binding of IgE to Fc£RI, imparing effector cell degranulation (Jutel et al., 2013). IL-10 may also act by suppressing the activity and function of eosinophils by inhibiting the production of IL-5 by Th2 cells (Jutel et al., 2003). Another hypothesis which needs to be evaluated and which we have proposed, is the presence of cross-reactivity between self and parasite antigens that could induce a long-lasting modulation of anti-parasite immune responses even in the absence of the parasite. Helminth infections have a vast repertoire of proteins, many of which are shared with epitopes from other organisms, such as environmental allergens, leading to cross-reactivity, since the processing and presentation of polypeptide per CD pathway MHC class II CD4+ T cells is degenerated (Pontes-de-Carvalho et al., 2013).

In conclusion, our data show that human *Toxocara* spp. seroprevalance 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. seroprevalancewith peripheral blood eosinophilia and the presence of specific IgE to aeroallergens, but not with atopic and non-atopic 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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**REFERENCES**

Acevedo, N., Sánchez, J., Erler, A., Mercado, D., Briza, P., Kennedy, M., Fernandez, A., Gutierrez, M., Chua, K.Y., Cheong, N., Jiménez, S., Puerta, L., Caraballo, L., 2009. IgE cross-reactivity between Ascaris and domestic mite allergens: the role of tropomyosin and the nematode polyprotein ABA-1. Allergy 64, 1635-1643.

Aguiar-Santos, A. M., Andrade, L. D., Medeiros, Z., Chieffi, P. P., Lescano, S. Z. & Perez, E. P. 2004. Human toxocariasis: frequency of anti-Toxocara antibodies in children and adolescents from an outpatient clinic for lymphatic filariasis in Recife, Northeast Brazil. *Rev Inst Med Trop Sao Paulo,* 46**,** 81-5.

Alcântara-Neves, N. M., Badaró, S. J., dos Santos, M. C., Pontes-de-Carvalho, L. & Barreto, M. L. 2010. The presence of serum anti-Ascaris lumbricoides IgE antibodies and of Trichuris trichiura infection are risk factors for wheezing and/or atopy in preschool-aged Brazilian children. *Respir Res,* 11**,** 114.

Alcântara-Neves, N. M., de S G Britto, G., Veiga, R. V., Figueiredo, C. A., Fiaccone, R. L., da Conceição, J. S., Cruz, Á., Rodrigues, L. C., Cooper, P. J., Pontes-de-Carvalho, L. C. & Barreto, M. L. 2014. Effects of helminth co-infections on atopy, asthma and cytokine production in children living in a poor urban area in Latin America. *BMC Res Notes,* 7**,** 817.

Alonso, J. M., Bojanich, M. V., Chamorro, M. & Gorodner, J. O. 2000. Toxocara seroprevalence in children from a subtropical city in Argentina. *Rev Inst Med Trop Sao Paulo,* 42**,** 235-7.

Alvarado-Esquivel, C. 2013. Toxocariasis in waste pickers: a case control seroprevalence study. *PLoS One,* 8**,** e54897.

Beasley, R. Worldwide variation in prevalence of symptoms of asthma, allergic rhinoconjunctivitis, and atopic eczema: ISAAC. The Lancet, v. 351, n. 9111, p. 1225-1232, 1998. ISSN 0140-6736.

Beaver, P. C., Snyder, C. H., Carrera, G. M., Dent, J. H. & Lafferty, J. W. 1952. Chronic eosinophilia due to visceral larva migrans; report of three cases. *Pediatrics,* 9**,** 7-19.

Bowman D. G. 2008. **Feline Clinical Parasitology. pp 286,**

Buijs, J., Borsboom, G., Renting, M., Hilgersom, W. J., van Wieringen, J. C., Jansen, G. & Neijens, J. 1997. Relationship between allergic manifestations and Toxocara seropositivity: a cross-sectional study among elementary school children. *Eur Respir J,* 10**,** 1467-75.

Fernando, D., Wickramasinghe, P., Kapilananda, G., Dewasurendra, R. L., Amarasooriya, M. & Dayaratne, A. 2009. Toxocara seropositivity in Sri Lankan children with asthma. *Pediatr Int,* 51**,** 241-5.

Fragoso, R. P., Monteiro, M. B., Lemos, E. M. & Pereira, F. E. 2011. Anti-Toxocara antibodies detected in children attending elementary school in Vitoria, State of Espírito Santo, Brazil: prevalence and associated factors. *Rev Soc Bras Med Trop,* 44**,** 461-6.

Gavignet, B., Piarroux, R., Aubin, F., Millon, L. & Humbert, P. 2008. Cutaneous manifestations of human toxocariasis. *J Am Acad Dermatol,* 59**,** 1031-42.

Golvan, Y., Combes, C., Bayssade-Dufour, C. & Nassi, H. 1974. [Cercaria of Ribeiroia marini (Faust and Hoffman, 1934), antagonistic trematode of Schistosoma mansoni and inducing castration of the host-mollusca, vector of human schistosomiasis]. *C R Acad Sci Hebd Seances Acad Sci D,* 279**,** 405-8.

Jutel, M., Akdis, M., Budak, F., Aebischer-Casaulta, C., Wrzyszcz, M., Blaser, K. & Akdis, C. A. 2003. IL-10 and TGF-beta cooperate in the regulatory T cell response to mucosal allergens in normal immunity and specific immunotherapy. *Eur J Immunol,* 33**,** 1205-14.

Jutel, M., Van de Veen, W., Agache, I., Azkur, K. A., Akdis, M. & Akdis, C. A. 2013. Mechanisms of allergen-specific immunotherapy and novel ways for vaccine development. *Allergol Int,* 62**,** 425-33.

Kanobana, K., Vereecken, K., Junco Diaz, R., Sariego, I., Rojas, L., Bonet Gorbea, M. & Polman, K. 2013. Toxocara seropositivity, atopy and asthma: a study in Cuban schoolchildren. *Trop Med Int Health,* 18**,** 403-6.

Katz, N., Chaves, A. & Pellegrino, J. 1972. A simple device for quantitative stool thick-smear technique in Schistosomiasis mansoni. *Rev Inst Med Trop Sao Paulo,* 14**,** 397-400.

Kennedy, M. W., Maizels, R. M., Meghji, M., Young, L., Qureshi, F. & Smith, H. V. 1987. Species-specific and common epitopes on the secreted and surface antigens of Toxocara cati and Toxocara canis infective larvae. *Parasite Immunol,* 9**,** 407-20.

Loukas, A., Hintz, M., Linder, D., Mullin, N. P., Parkinson, J., Tetteh, K. K. & Maizels, R. M. 2000. A family of secreted mucins from the parasitic nematode Toxocara canis bears diverse mucin domains but shares similar flanking six-cysteine repeat motifs. *J Biol Chem,* 275**,** 39600-7.

Macpherson, C. N. 2013. The epidemiology and public health importance of toxocariasis: a zoonosis of global importance. *Int J Parasitol,* 43**,** 999-1008.

Maizels, R. M. 2013. Toxocara canis: molecular basis of immune recognition and evasion. *Vet Parasitol,* 193**,** 365-74.

Mazur-Melewska, K., Mania, A., Figlerowicz, M., Kemnitz, P., Służewski, W. & Michalak, M. 2012. The influence of age on a clinical presentation of Toxocara spp. infection in children. *Ann Agric Environ Med,* 19**,** 233-6.

Mendonça, L. R., Figueiredo, C. A., Esquivel, R., Fiaccone, R. L., Pontes-de-Carvalho, L., Cooper, P., Barreto, M. L. & Alcantara-Neves, N. M. 2013. Seroprevalence and risk factors for Toxocara infection in children from an urban large setting in Northeast Brazil. *Acta Trop,* 128**,** 90-5.

Mendonça, L. R., Veiga, R. V., Dattoli, V. C., Figueiredo, C. A., Fiaccone, R., Santos, J., Cruz, Á., Rodrigues, L. C., Cooper, P. J., Pontes-de-Carvalho, L. C., Barreto, M. L. & Alcantara-Neves, N. M. 2012. Toxocara seropositivity, atopy and wheezing in children living in poor neighbourhoods in urban Latin American. *PLoS Negl Trop Dis,* 6**,** e1886.

Negri, E. C., Santarém, V. A., Rubinsky-Elefant, G., Giuffrida, R. & Vieira da Silva, A. 2013. Anti-Toxocara spp. antibodies in an adult healthy population: serosurvey and risk factors in Southeast Brazil. *Asian Pac J Trop Biomed,* 3**,** 211-6.

Nelson, S., Greene, T. & Ernhart, C. B. 1996. Toxocara canis infection in preschool age children: risk factors and the cognitive development of preschool children. *Neurotoxicol Teratol,* 18**,** 167-74.

Noordin, R., Smith, H. V., Mohamad, S., Maizels, R. M. & Fong, M. Y. 2005. Comparison of IgG-ELISA and IgG4-ELISA for Toxocara serodiagnosis. *Acta Trop,* 93**,** 57-62.

Overgaauw, P. A. & van Knapen, F. 2013. Veterinary and public health aspects of Toxocara spp. *Vet Parasitol,* 193**,** 398-403.

Pfefferle, P. I. & Renz, H. 2014. Microbial exposure and onset of allergic diseases - potential prevention strategies? *Allergol Int,* 63**,** 3-10.

Pinelli, E. & Aranzamendi, C. 2012. Toxocara infection and its association with allergic manifestations. *Endocr Metab Immune Disord Drug Targets,* 12**,** 33-44.

Pontes-de-Carvalho, L., Mengel, J., Figueiredo, C. A., Alcântara-Neves, N. M. & Social Changes, A. t. a. A. i. L. A. S. s. S. G. 2013. Antigen Mimicry between Infectious Agents and Self or Environmental Antigens May Lead to Long-Term Regulation of Inflammation. *Front Immunol,* 4**,** 314.

Rodriguez A., Vaca M, Oviedo G, Erazo S, Chico ME, Teles C, Barreto ML, Rodrigues LC, Cooper PJ. Thorax. 2011. Urbanisation is associated with prevalence of childhood asthma in diverse, small rural communities in Ecuador. 2011 Dec;66(12):1043-50.

Romero Núñez, C., Mendoza Martínez, G. D., Yañez Arteaga, S., Ponce Macotela, M., Bustamante Montes, P. & Ramírez Durán, N. 2013. Prevalence and Risk Factors Associated with Toxocara canis Infection in Children. *ScientificWorldJournal,* 2013**,** 572089.

Santos, L. N., Gallo, M. B., Silva, E. S., Figueiredo, C. A., Cooper, P. J., Barreto, M. L., Loureiro, S., Pontes-de-Carvalho, L. C. & Alcantara-Neves, N. M. 2013. A proteomic approach to identify proteins from Trichuris trichiura extract with immunomodulatory effects. *Parasite Immunol,* 35**,** 188-93.

Schnieder, T., Laabs, E. M. & Welz, C. 2011. Larval development of Toxocara canis in dogs. *Vet Parasitol,* 175**,** 193-206.

Sharghi, N., Schantz, P. M., Caramico, L., Ballas, K., Teague, B. A. & Hotez, P. J. 2001. Environmental exposure to Toxocara as a possible risk factor for asthma: a clinic-based case-control study. *Clin Infect Dis,* 32**,** E111-6.

Souza, R. F., Dattoli, V. C., Mendonça, L. R., Jesus, J. R., Baqueiro, T., Santana, C. e. C., Santos, N. M., Barrouin-Melo, S. M. & Alcantara-Neves, N. M. 2011. [Prevalence and risk factors of human infection by Toxocara canis in Salvador, State of Bahia, Brazil]. *Rev Soc Bras Med Trop,* 44**,** 516-9.

Strina, A., Cairncross, S., Barreto, M. L., Larrea, C. & Prado, M. S. 2003. Childhood diarrhea and observed hygiene behavior in Salvador, Brazil. *Am J Epidemiol,* 157**,** 1032-8.

Strube, C., Heuer, L. & Janecek, E. 2013. Toxocara spp. infections in paratenic hosts. *Vet Parasitol,* 193**,** 375-89.

van Ree, R. & Yazdanbakhsh, M. 2007. Allergic disorders in African countries: linking immunology to accurate phenotype. *Allergy,* 62**,** 237-46.

Wiśniewska-Ligier, M., Woźniakowska-Gęsicka, T., Sobolewska-Dryjańska, J., Markiewicz-Jóźwiak, A. & Wieczorek, M. 2012. Analysis of the course and treatment of toxocariasis in children-a long-term observation. *Parasitol Res,* 110**,** 2363-71.

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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** | | | | |
| **Studiedvariables** | **n (%)** | **Anti-*Toxocara spp.* IgG seropositivity n(%)** | **Crude OR**  **(95% CI)** | **\*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 (62.5) | 0.90 (0.68-1.21) | -- |
| **Maternal schooling** |  |  |  |  |
| 1st grade or less | 439 (58.1) | 283 (64.5) | 1 | -- |
| Incomplete 2nd grade | 317 (41.9) | 199 (62.8) | 0.93 (0.69-1.25) | -- |
| Complete 2nd grade or more | 35 (7.2) | 21 (60.0) | 0.82 (0.40-1.67) | -- |
| **Family income** |  |  |  |  |
| ≥ 1 | 454 (57.1) | 302 (66.6) | 1 |  |
| < 1 | 337 (42.6) | 201 (59.7) | 0.78 (0.58-1.04) | -- |
| **School location** |  |  |  |  |
| Rural | 254 (32.1) | 158 (62.4) | 1 |  |
| Urban | 537 (67.9) | 345 (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) | 262 (59.8) | **1** | **1** |
| Yes | 196 (30.6) | 136 (69.4) | **1**.**58 (1.10-2.26)** | **1.22 (1.03-1.87)** |
| \*Adjusted for gender, age, dog, school location, maternal schooling, family income, cat and helminth infections. -  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. | | | | |

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| **Table 2. Assoctions of *Toxocara* spp. seropositivity with eosinophilia in 791 students aged 6 to 13 years enrolled in public schools in Northeast Brazil** | | | | | | |
| **Anti-*Toxocara* IgG** | **Eosinophilia (4%)** | | | | **Eosinophilia (10%)** | |
| **n (%)** | **Anti-*Toxocara* spp. IgG seropositivity**  **n(%)** | **\* AdjustedOR**  **(95% CI)** | **n (%)** | **Anti-*Toxocara* spp. IgG seropositivity n(%)** | **\*Adjusted OR (95% CI)** |
| Negative | | 288 (36.4%) | 163 (56.9) | **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 (38.0) | **2.07**  **(1.45-2.97)** |
| **Level of anti-*Toxocara* IgG** | | | | | | | |
| Neg < 0.22 | | 288 (36.4%) | 163 (56.9) | 1 | 288 (36.4%) | 58 (20.1) | **1** |
| ≥ 0.22 ≤ 1 | | 381 (48.2%) | 288 (75.6) | 1.23  (0.86- 1.77) | 369 (46.7%) | 135 (36.6) | **1.32**  **(1.12-2.23)** |
| ≥ 1 | | 122 (15.4%) | 88 (72.1) | **1.60**  **(1.03-2.44)** | 134 (16.9) | 56 (41.8) | **1.55**  **(1.03-2.33)** |
| \* 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. | | | | | | | |

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| **Table 3 - A1ssociation between *Toxocara* spp and atopy measured by presence of aeroallergen-specific IgE in 791 students aged 6 to 13 years enrolled in elementary public schools in Northeast Brazil** | | | | | | |
| **Anti-*Toxocara***  **spp IgG** | **Phadiatop\* 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)** |
| 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 (22.5)/ 288 | **1** | 123 (42.7)/ 288 | **1** |
| ≥ 0.22 ≤ 1  (n= 381; 48.2%) | 200 (50.5)/396 | **1.62**  **(1.19- 2.22)** | 225 (61.0) /369 | **1.44**  **(0.90- 2.12)** | 234 (63.4) /369 | **1.78**  **(1.34-2.36)** |
| ≥ 1  (n=122; 15.4%) | 51 (60.6)/107 | 1.33  (0.88-2.02) | 61 (45.5) /134 | **1.39**  **(1.28- 2.43)** | 108 (62.0)/134 | **1.55**  **(1.06-2.7)** |
| \* 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. | | | | | | |

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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 to 13 years enrolled in public schools in schools in Northeast Brazil** | | | | |
| **Studied variables** | **Wheezing/asthma**  **n (%)/N** | | **Crude OR**  **(95% CI)** | **\*Adjusted OR**  **(95% CI)** |
| **Anti-*Toxocara spp.* IgG seropositivity** | | | | |
| No | 28 ( 9.72 ) /288 | | 1 | 1 |
| Yes | 55 ( 10.93 ) /503 | | 1.16  (0.72-1.88) | 1.14  (0.69-1.90) |
| **Anti-*Toxocara* spp IgG** | **Non-atopic wheeezers (N=26)** | | **Atopic wheeezers (N=57)** | |
| **Seropositivity** | **\*\*Reference group: non-atopic, non-wheeezers** N **=346** | | **\*\*Reference group: atopic, non-wheeezers**  N **= 362** | |
|  | **n (%)/N** | **\*OR**  **(IC 95%)** | **n (%)/N** | **\*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) |
| \*ORs adjusted for sex, age, income, maternal education and nutritional status, and intestinal helminth infections. \*\*Reference groups for analysis. | | | | |