Toxocara seropositivity, atopy and asthma: a study in Cuban schoolchildren

K. Kanobana[1], K. Vereecken[1], R. Junco Diaz[2], I. Sariego[3], L. Rojas[3], M. Bonet Gorbea[2] and K. Polman[1]

1 *Institute of Tropical Medicine, Antwerp, Belgium*
2 *National Institute of Hygiene, Epidemiology and Microbiology, Havana, Cuba*
3 *Institute of Tropical Medicine "Pedro Kourí", Havana, Cuba*

Abstract

INTRODUCTION Evidence suggests that human toxocariasis (HT) could stimulate the onset of allergic diseases such as asthma. More specifically, in subjects having a hypothetical 'atopic genotype', HT could boost preexistent allergy symptoms. We tested the latter hypothesis in Cuba, a country where both asthma and HT are prevalent.

MATERIAL AND METHODS In a group of Cuban school-aged children (n = 958), we investigated the association of *Toxocara* seropositivity and atopic status with asthma. *Toxocara* seropositivity was diagnosed with ELISA and atopy by allergen skin prick test. Both physician-diagnosed asthma and current wheeze, as determined by International Study of Asthma and Allergies in Childhood (ISAAC) questionnaire, were considered. Associations were assessed using multivariable logistic regression analyses, with either 'physician-diagnosed asthma' or 'current wheeze' as outcome variable.

RESULTS 40.1% of the children were *Toxocara* seropositive. Prevalences were 21.7% for current wheeze and 32.7% for physician-diagnosed asthma. The odds of having asthma were almost two times higher in atopic children, but only reached borderline significance (OR=1.90, CI 95%: 0.95–3.80 for physician-diagnosed asthma and OR=1.94, CI 95%: 0.98–3.85 for current wheeze). *Toxocara* seropositivity and physician-diagnosed asthma were associated (OR=1.51, CI 95%: 1.01–2.26). Moreover, in children without antibodies to *Toxocara*, being atopic was significantly associated with having physician-diagnosed asthma (OR=2.53, CI 95%: 1.63–3.90), while this association was not present in *Toxocara* positives (OR=1.38, CI 95%: 0.82–2.37).

CONCLUSION Our data confirm previous observations of higher *Toxocara* seropositivity rates in asthmatic children. *Toxocara* seropositivity appeared to abrogate the apparent association between atopy and asthma in Cuban children. Although this observation was limited to physician-diagnosed asthma, it challenges the hypothesis that HT stimulates the onset of allergic diseases such as asthma in atopic individuals.

keywords *Toxocara*, toxocariasis, asthma, atopy, Cuba

Introduction

The prevalence of asthma is characterised by geographical differences. It tends to be elevated in high-income countries and relatively rare in developing countries (Anonymous 1998). It has been postulated that helminth infections, which show an inverse geographical distribution, may in part be responsible for these differences – that is, they could suppress or inhibit the development of asthma (Cooper 2009).

Evidence from experimental and clinical studies suggests that human toxocariasis (HT), a cosmopolitan zoonotic helminth infection caused by the larval stage of the ascarid worms of dogs and cats (*Toxocara* spp.), could, in contrast to other helminths, promote the onset of allergic diseases such as asthma (Pinelli 2006; Cooper 2008). Humans, who are paratenic host, get infected by the accidental ingestion of embryonated eggs, which are shed in the environment by dog and cat faeces (Despommier 2003). HT-related syndromes include visceral, neural or ocular involvement, depending on the organs affected by the migration of the larvae.

Animal models suggest that the immunological inflammatory mediation caused by the migrating *Toxocara* larva reduces lung function in mice (Pinelli *et al.* 2008). Moreover, there are indications that HT is linked to chronic cough and asthma in humans. Examples are the notable association of *Toxocara* seropositivity with

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asthma and recurrent bronchitis in children in the Netherlands (Buijs *et al.* 1995, 1997) and with diminished lung function in a nationwide population survey in the USA (Walsh 2011). Still, some studies showed no association (e.g. Sharghi *et al.* 2001) and it remains to be determined whether HT is indeed an important risk factor for asthma in humans. A study in patients with clinical HT suggested that in subjects having a hypothetical 'atopic genotype', HT could boost pre-existent allergy symptoms and act as a 'developer', eliciting these signs in previously asymptomatic patients (Magnaval *et al.* 2006). The aim of this study was to further investigate this hypothesis in an epidemiological setting in Cuba, a country where both HT and asthma are prevalent.

In Cuba, nationwide studies have demonstrated that asthma occurs frequently. The 2004 National Survey on Asthma revealed a prevalence of 13% (Varona 2005). In a more recent study, designed to study the relationship between atopy, allergic diseases and helminth infections, more than 20% of schoolchildren were found to have asthma (Wordemann *et al.* 2008; Vereecken *et al.* 2012), which is among the highest prevalences in the world.

Here, we investigated the association between atopy, asthma and HT in the same study population of Cuban children. Details of the study design and methodologies have been described previously (Wordemann *et al.* 2006b). Data were complemented with *Toxocara* antibody data from a serological survey in the same study group (Sariego *et al.* 2012). In short, HT serology was conducted with a commercial ELISA kit to determine the presence of IgG to *Toxocara* larval excretory–secretory products (TES-IgG, Bordier Affinity products, Switzerland). Soil-transmitted helminth infections (STH; hookworm, *Trichuris trichiura* and *Ascaris lumbricoides*) were determined by stool examination. Atopy was defined as having a positive allergen skin prick testing to at least one of the seven following allergens: cockroach, house dust mite (2), cat, mixed tree, mixed grass and *Alternia alternata*. Two different methods were used to diagnose asthma. The first one was the International Study of Asthma and Allergies in Childhood (ISAAC) questionnaire, which is the most commonly used diagnostic method in epidemiological studies on atopic diseases in children. Current asthma was defined as an affirmative answer to the second core question on asthma ('wheezing during the last 12 months') and further referred to as 'current wheeze' (Asher & Weiland 1998). The second one was physician-diagnosed asthma, which is also often used as a standard method in asthma studies (Kemp *et al.* 1996). According to the Cuban National Asthma Guidelines, physician diagnosis of asthma is based on clinical examination and personal or family symptom history, supplemented with objective test results when available (Ministerio de Salud Pública 1999).

From a group of 1011 children aged 5 to 14 years from five schools from the municipality of San Juan y Martínez in western Cuba and 14 schools from the municipality of Fomento in central Cuba, 958 children with complete data on age, gender, asthma, atopy, STH infection and *Toxocara* seropositivity were included in this study.

All statistical analyses were conducted in STATA 10 IC software (Stata Corp., College Station, TX). A *P*-value of 0.05 or less was considered statistically significant. Statistical analyses were adjusted for the sampling design using school as primary sampling unit, that is, a survey proportion calculation was used to account for a clustering effect at school level (Wordemann *et al.* 2008). Data were analysed by logistic regression with either 'current wheeze' or 'physician-diagnosed asthma' as outcome variable. Independent variables included in the multivariable logistic model were *Toxocara* seropositivity, atopy, age (binary; below and above 10 years of age), gender and municipality. In addition, statistically significant associations with STH infections in the univariate analyses ($P < 0.05$), as well as significant interaction terms with *Toxocara* infection or age ($P < 0.1$), were added to the equation. The results of the analyses are summarized in Table 1.

The group consisted of 504 boys and 454 girls (52.6% and 47.7%, respectively). Most children were below 10 years of age ($n = 654/958$, 68.3%) and from the municipality of Fomento ($n = 739/958$, 77.1%). The overall prevalence of STH infections was 19.2% ($n = 184/958$); 5.0% ($n = 48/958$) were infected with *A. lumbricoides*, 9.7% ($n = 93/958$,) with *T. Trichura* and 8.9% ($n = 85/958$) with hookworm.

Antibodies to *Toxocara* spp. were detected in 40.1% of the children ($n = 384/958$), in accordance with previous findings (Sariego *et al.* 2012). Atopy was diagnosed in 21.5% ($n = 206/958$). Based on the ISAAC questionnaire, 22.4% ($n = 21/958$) had current wheeze, whereas the proportion of children with physician-diagnosed asthma was 32.7% ($n = 314/958$). Asthma outcomes can vary considerably depending on definition and methodology (Wordemann *et al.* 2006a).

Children infected with hookworm were less at risk of having asthma than those without (OR=0.53, CI 95%: 0.32–0.87 and OR=0.43, CI 95%: 0.27–0.69 for physician-diagnosed asthma and current wheeze, respectively). No other significant associations with STH infections were observed in the final model. These results are consistent with the outcome of a meta-analysis of observational studies on the association of helminth infections with asthma (Leonardi-Bee *et al.* 2006), except that our data did not reinforce the reported association of current

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Table 1 Associations with physician-diagnosed asthma and current wheeze†

	Physician-diagnosed ASTHMA (N=314)				Current Wheeze			
			OR (CI95%)				OR (CI95%)	
	Cases n	%	Unadjusted	Adjusted	Cases n	%	Unadjusted	Adjusted
Gender								
Male	180	57.3	1	–	116	53.9	1	–
Female	134	42.7	0.75 (0.59 0.95)*	0.77(0.62 0.96)*	99	46.0	0.93(0.76–1.15)	0.92(0.68–1.24)
Age								
<10 years	220	70.1	1	–	172	80.0	–	–
>10 years	94	29.9	0.88(0.60 1.30)	0.84(0.58 1.23)	43	20.0	0.46(0.32–0.66)*	0.38(0.25–0.57)*
Atopy								
No	229	72.9	1	–	140	69.3	–	–
Yes	85	27.1	1.60 (0.65 3.95)	1.90(0.95 3.80)	66	30.7	1.91(0.95–3.83)	1.94(0.98–3.85)
Toxocara								
No	174	55.4	1	–	124	57.7	–	–
Yes	140	44.6	1.31(0.95 1.83)	1.51(1.01–2.26)*	91	42.3	1.12(0.71–1.80)	1.13(0.69–1.84)
STH								
No	252	80.2	1	–	172	80.0	–	–
Yes	62	19.8	1.05(0.669 1.68)	–	43	20.0	1.07(0.67–1.70)	–
Ascaris lumbricoides								
No	289	92.0	1	1	198	92.1	–	1
Yes	25	8.0	2.33 (1.59 3.43)*	1.29(0.73–2.28)	17	7.9	1.97(1.39–2.79)*	1.11 (0.78–1.60)
Trichuris Trichuria								
No	284	90.4	1	–	189	87.9	1	–
Yes	30	9.5	0.97 (0.57 1.67)	–	26	12.1	1.39(0.86–2.24)	–
Hookworm								
No	295	93.9	1	1	205	95.3	1	1
Yes	19	6.1	0.56 (0.33 0.99)*	0.53(0.32–0.87)*	10	4.7	0.43(0.25–0.75)*	0.43(0.27–0.69)*
Municipality								
San Juan	109	34.7	1	–	84	39.1	–	–
Fomento	205	65.3	0.40 (0.28 0.53)*	0.36(0.23–0.56)*	131	60.9	0.35(0.23–0.52)*	0.33(0.20–0.53)*

OR, odds ratio; CI 95%, 95% confidence interval; –, not included in multivariable model; *Toxocara*, positive result in antibody ELISA on *Toxocara canis* excretory/secretory larval antigen; STH, soil-transmitted helminths; N, total number of asthma cases; n, number of asthma cases within subcategory; %=n/N*100, frequency of asthma cases within subcategory.
†Current wheeze, affirmative answer to the second core question on asthma in ISAAC questionnaire (Asher & Weiland 1998).
*$P < 0.05$; **$P < 0.1$.

A. lumbricoides infection with an increased risk of asthma.

The odds of having asthma were almost two times higher in atopic than in non-atopic children, independently of the asthma definition used, but the difference was only borderline significant (OR=1.90, CI 95%: 0.95–3.80 for physician-diagnosed asthma, OR=1.94, CI 95%: 0.98–3.85, $P = 0.056$ for current wheeze). A positive significant association with *Toxocara* antibody positivity was observed for physician-diagnosed asthma (OR=1.51, CI 95%: 1.01–2.26) but not for current wheeze (OR=1.12, CI 95%: 0.82–2.37).

Significant interactions with atopic status were observed for age ($P < 0.05$, for both asthma outcomes) as well as for *Toxocara* seropositivity ($P < 0.1$, for physician-diagnosed asthma only). Age-stratified analysis showed that the risk of having asthma was more pronounced in atopic children older than 10 years than in younger children (OR=3.7, CI 95%: 2.04–6.73 and OR: 6.41, CI 95%: 3.02–13.6 in children older than 10 years *vs.* OR=1.6, CI 95%: 1.05–2.42 and OR=1.9, CI 95%: 1.3–2.9 in younger children, for physician-diagnosed asthma and current wheeze, respectively). Moreover, stratification by *Toxocara* infection revealed that in children without *Toxocara* antibodies, being atopic was still significantly associated with having physician-diagnosed asthma (OR=2.53, CI 95%: 1.63–3.90), while this association disappeared in *Toxocara* seropositives (OR=1.38, CI 95%: 0.82–2.37).

A limitation of the study is that diagnosis of HT was restricted to the detection of antibodies, not allowing the distinction between current and past infection. The cross-sectional nature of the study hampers the identification of

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the temporal sequence of events regarding the onset of atopy, HT infection and the development of asthma. Lastly, we cannot exclude that unmeasured confounders, such as maternal smoking, parental allergic diseases and socio-economic level, may have influenced the study results. Nevertheless, we believe that our data give an indication of the association of *Toxocara* seropositivity with atopic status with asthma and have value in the design of future longitudinal studies.

In conclusion, the frequency of children with antibodies to *Toxocara* tended to be higher in asthmatic children, with the strength of the association depending on the definition of asthma used. Being atopic increased the risk of having asthma, and this was age dependent, with a higher risk in children older than 10 years. *Toxocara* seropositivity appeared to abrogate the apparent association between atopy and asthma in Cuban children. Although this observation was limited to physician-diagnosed asthma, it challenges the hypothesis that HT stimulates the onset of allergic diseases such as asthma in atopic individuals.

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Corresponding Author **Kirezi Kanobana**, Institute of Tropical Medicine, Dept of Biomedical Science, Unit of Medical Helminthology, Nationalestraat 155, 2000 Antwerp, Belgium. Tel.: +32-3-2470769; Fax: +32-3-2476359; E-mail: kkanobana@itg.be

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