Tropical Medicine and International Health doi:10.1111/j.1365-3156.2007.01988.x

VOLUME 13 NO 2 PP 180–186 FEBRUARY 2008

Association of atopy, asthma, allergic rhinoconjunctivitis, atopic dermatitis and intestinal helminth infections in Cuban children

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Summary

OBJECTIVE To examine the relationship of past and current intestinal helminth infections with asthma, allergic rhinoconjunctivitis, atopic dermatitis and atopy.

METHODS Cross-sectional study of 1320 children aged 4–14 years from two Cuban municipalities. Helminth infections were determined by stool examination and parental questionnaire. Asthma, rhinoconjunctivitis and atopic dermatitis were diagnosed by International Study of Asthma and Allergies in Childhood questionnaire, asthma additionally by spirometry, atopy by skin prick testing.

RESULTS Questionnaire-based frequencies were 21% for asthma, 14% for allergic rhinoconjunctivitis and 8% for atopic dermatitis. According to spirometry, 4% had asthma; 20% had a positive skin prick test. A history of infection for *Enterobius vermicularis* was associated with increased risk of atopic dermatitis (OR 1.88, $P = 0.001$) and allergic rhinoconjunctivitis (OR 1.34, $P = 0.046$), and hookworm with increased risk of allergic rhinoconjunctivitis (OR 2.77, $P = 0.021$). A positive stool examination for *Ascaris lumbricoides* infection was negatively associated with atopic dermatitis (OR 0.22, $P = 0.007$). Asthma and atopy were unrelated to helminth infections.

CONCLUSION Current *A. lumbricoides* infection protects against atopic dermatitis in Cuban children, while past infection with *E. vermicularis* and hookworm are risk factors for allergic rhinoconjunctivitis and/or atopic dermatitis. Apparently, interactions differ depending on the type of helminth and atopic disease and on the time of helminth infestation.

keywords allergic rhinoconjunctivitis, asthma, atopic dermatitis, atopy, helminth infections

Introduction

The observation that atopic diseases are very common and helminthic infections relatively uncommon in developed countries and that the inverse is true in many developing countries has led to the speculation that the two phenomena may be inversely associated. The idea is part of a broader hypothesis suggesting that exposure to infections in early childhood reduces the risk of developing allergies, the so-called 'hygiene hypothesis'. However, the relationship between atopic diseases and helminthic infection remains uncertain and controversial (Nyan *et al.* 2001; Sharghi *et al.* 2001; Palmer *et al.* 2002; Cooper *et al.* 2004; Van den Biggelaar *et al.* 2004).

Most studies have been on intestinal helminth infection in relation to asthma and atopy, and little is known on the association with other atopic outcomes such as atopic dermatitis and allergic rhinoconjunctivitis (Huang *et al.* 2002; Schäfer *et al.* 2005). Moreover, they focussed on the current exposure to helminth infections, which makes it difficult to identify the temporal sequence between the two phenomena. In this study, we examined the association of past and current intestinal helminth infections with asthma, atopic dermatitis, allergic rhinoconjunctivitis and atopy among schoolchildren in two Cuban municipalities where helminth infections are prevalent and atopic disease levels are reported to be very high (Masoli *et al.* 2004; Wördemann *et al.* 2006a). We also investigated the contribution of other common risk factors which have been found to be associated with atopic diseases and/or atopy in literature (Bufford & Gern 2005).

Methods

Study group

A cross sectional study was performed between 2003 and 2004 in San Juan y Martínez, a municipality in the West of Cuba and in Fomento, a municipality in the center of the island. In San Juan y Martínez all children (n = 398) from five, and in Fomento all children (n = 922) from 14 primary schools were included in the study. Using Survey Select, SAS version 8.0 (SAS Institute Inc., Cary, NC, USA), schools were randomly selected after stratification for municipality (San Juan y Martinez or Fomento) and area (urban or rural); all children from each school were included. Both municipalities are rural mountainous areas which have been reported to be endemic for helminthiasis (Escobedo *et al.* 2007; Wördemann *et al.* 2006b).

Informed written consent was obtained from the parents of each child. The study was approved by the ethical committees of the Institute of Tropical Medicine in Antwerp, Belgium, the Pedro Kouri Institute of Tropical Medicine (IPK) and the National Institute for Hygiene, Epidemiology and Microbiology (INHEM) in Havana, Cuba.

Study design

All participating children were submitted to spirometry before and after exercise, skin prick testing and stool examination. Furthermore, a parent or guardian of each child was interviewed using an extended version of the standard Spanish version of the ISAAC questionnaire (Asher *et al.* 1995). ISAAC definitions of atopic diseases were used: current asthma was defined as an affirmative answer to the second ISAAC core asthma question on current wheeze (The ISAAC Steering Committee 1998); allergic rhinoconjunctivitis was diagnosed as defined by Strachan *et al.* (1997) and atopic dermatitis as defined by Williams *et al.* (1999) (an affirmative answer to the second and third core question of the ISAAC rhinitis or dermatitis questionnaire, respectively). Additional questions concerned factors that have been described to be associated with allergic sensitisation or atopic diseases in literature (Bufford & Gern 2005): intake of antibiotics during first year of life, pet ownership or contact during first year of life, pet ownership or contact at present, gastroesophageal reflux (either frequent vomiting, or pyrosis, or sourly smelling breath, or regular refused feeding during infancy), smoking in the household, family atopy (atopy of father, mother or sibling), presence of siblings, pre-school daycare attendance and being breast-fed during infancy.

Atopy was defined as a positive skin prick test reaction to at least one of the applied allergens. Skin prick testing was performed using extracts of seven allergens that have been used worldwide by ISAAC (*Dermatophagoides pteronyssinus*, *D. farinae*, cat dander, mixed tree, mixed grass, *Alternaria alternata,* and cockroach) produced by ALK, Nieuwegein, The Netherlands. Histamine (10 mg/ml) was used as a positive and allergen diluent as a negative control. The extracts and controls were placed on the volar side of the left forearm using separate ALK lancets. Skin response was measured after 15 min, considering the largest weal dimension (3 mm or larger) in the absence of significant reactivity of the diluent control as a positive reaction.

To demonstrate bronchial hyperresponsiveness (BHR), spirometry was performed before and 5 and 10 min after exercise (6 min free running) according to ATS (American Thoracic Society) guidelines (The American Thoracic Society, Medical Section of the American Lung Association 1987; The American Thoracic Society 2000) in all children (4–14 years old) using a handheld computerized spirometer (Spirobank, MIR, Rome, Italy). If at baseline the FEV1 was lower than 70% of the predicted value as defined by the European Respiratory Society and Knudson *et al.* (1976), or if the FEV1 had fallen more than 15% either 5 or 10 min after exercise, spirometry was considered abnormal. In such a case the child received a bronchodilator to aid recovery, and spirometry was repeated after 20 min to make sure that for all children the baseline values were obtained again.

One fresh stool sample was collected from each child for one direct smear and two 25 mg Kato Katz examinations (Katz *et al.* 1972). Current helminth infections were defined by the presence of helminth eggs (*Ascaris lumbricoides*, *Trichuris trichiura*, hookworm or *Enterobius vermicularis*) detected by either of the two methods. Furthermore, a history of helminth infections was taken for each subject. The parents were asked if their child ever had a parasite infection, and if yes, which parasite. If the parents did not know the name of the parasite, its appearance was described to them (e.g. *lombrices pequenos* or *lombricillas* for 'small white worms', i.e. *E. vermicularis*, or *lombrices blancos grandes* for 'large white worms', i.e. *A. lumbricoides*). No pictures were used.

For all statistical computations STATA for Windows, version Intercooled STATA 9 was used. A P-value of ≤0.05 was considered statistically significant. Statistical analyses were adjusted for the sampling design using the school as primary sampling unit. Municipality (San Juan y Martinez or Fomento) and area (rural/urban) were included as stratification variables. Factors identified as statistically significant in the univariate analyses were entered into a stepwise logistic regression model adjusted for age (linear), sex, municipality, area and household income (≤250 pesos, or >250 pesos).

Results

A total of 1320 children were sampled from 19 schools. The age range of the participating children was 4–14 years (median 8 years); 679 boys (51%) and 641 girls (49%). Response rate to the questionnaires was 100% (1320/1320) and 99–100% for the other tests (1319/1320 for skin prick test, 1308/1320 for spirometry). Of the 1313 children who provided stool samples, 83 (6%) were positive for *A. lumbricoides*, 126 (10%) for *T. trichiura*, 121 (9%) for hookworm, and 36 (3%) for *E. vermicularis*; 295 (22%) children were positive for at least one of these helminths. Geometric mean egg counts for helminth positive children were 947.1 eggs per gram of feces (epg) for *A. lumbricoides*, 133.6 epg for *T. trichiura* and 210.5 epg for hookworm; eggs were not counted for *E. vermicularis*. Of the 1320 children whose parents were interviewed, 349 (26%) had a history for *A. lumbricoides*, 5 (<1%) for *T. trichiura*, 39 (3%) for hookworm and 763 (58%) for *E. vermicularis*. 279 (21%) of the children were reported to have current wheeze, 54 (4%) had an abnormal spirometry, 267 (20%) showed a positive reaction to the skin prick test, 179 (14%) had allergic rhinoconjunctivitis as defined by Strachan and 110 (8%) had atopic dermatitis as defined by Williams.

After adjusting for age, sex, municipality, urban/rural background and income, history of hookworm infection was associated with increased risk of allergic rhinoconjunctivitis, and a history of infection with *E. vermicularis* with increased risk of atopic dermatitis and allergic rhinoconjunctivitis. A positive stool examination for *A. lumbricoides* was inversely related to atopic dermatitis. *Trichuris trichiura* did not have a significant effect on any of the atopic diseases, neither by questionnaire nor by stool examination. Skin prick test reactivity, current wheeze and spirometry were not significantly associated with any of the examined helminths on multiple regression (Tables 1 and 2).

Discussion

Despite evidence that helminth infections and atopic diseases are inversely associated (Scrivener *et al.* 2001; Cooper *et al.* 2004; Van den Biggelaar *et al.* 2004) the relationship between the two is still not clear (Palmer *et al.* 2002). Previous studies have provided conflicting evidence showing that helminthiases either cause (Palmer *et al.* 2002), inhibit (Scrivener *et al.* 2001; Cooper *et al.* 2004; Van den Biggelaar *et al.* 2004) or are unrelated to atopic diseases (Sharghi *et al.* 2001; Davey *et al.* 2005; Cooper *et al.* 2006); or that atopic diseases protect from helminth infections (Nyan *et al.* 2001). Our study confirms the existence of different associations between helminth infections and atopic diseases, the nature of which appears to depend on the type of helminth infection and atopic disease studied. Also, the time of worm infestation seems to play a role as suggested by the questionnaire based results in comparison with those based on stool examination. Unfortunately, prior data on stool examination were not available, so we had to rely on questionnaires to obtain information on the children's history of helminth infections, as has been done by others (Palmer *et al.* 2002; Schäfer *et al.* 2005). We are, however, aware of the limitations of this approach. The method is rather unspecific and insensitive, with the risk of measuring the effect of conspicuous and heavy worm infections only. The cross-sectional study design and potential information and recall bias in the questionnaire data do not allow making any strong temporal associations. Longitudinal studies are certainly a more valid approach to examine the causal association between helminth infections and atopic diseases. Nevertheless, we think that our data do give an indication of the importance of time of worm infestation in this relationship. In general, the results of cross sectional studies have an important value in the design of new longitudinal studies.

We found a negative association between current *A. lumbricoides* infection and atopic dermatitis, suggesting that *A. lumbricoides* might protect against atopic dermatitis. However, the inverse cannot be excluded either, especially since a history of infection with *A. lumbricoides* did not have any effect on atopic dermatitis. A bi-directional inverse relationship between helminthic infections and atopic dermatitis was reported by Schäfer *et al.* (2005), who used questionnaire data on the history of atopic dermatitis and worm infestations including extra questions on their time of onset. Taking into account the temporal sequence of the onset of atopic dermatitis and time of infestation, they found prior worm infestations to be negatively associated with atopic dermatitis and prior atopic dermatitis to be negatively associated with worm infestations.

Data on the relationship between intestinal parasites and atopic dermatitis and allergic rhinoconjunctivitis are scarce (Huang *et al.* 2002; Schäfer *et al.* 2005). We found a history of infection with *E. vermicularis* to be associated with an increased risk of allergic rhinoconjunctivitis and atopic dermatitis, the latter in contrast to Schäfer *et al.*, who reported a negative association between prior infestation with *E. vermicularis* and atopic dermatitis in East German schoolchildren.

We did not find any association between current *E. vermicularis* infection and atopic dermatitis or allergic rhinitis. Huang *et al.* (2002) had similar results for atopic dermatitis, but reported a negative association with allergic rhinitis and asthma. But in Huang's study current infection with *E. vermicularis* was based on tape test

Table 1 Results of the univariate analysis for associations between atopic diseases and helminth infections as well as other common risk factors. Univariate logistic regression was adjusted for age, sex, municipality, urban/rural background and income; *n* = number of children within subgroup; % = percentage of children within the subgroup who show the outcome

		Allergic rhinoconjunctivitis				Atopic dermatitis			Current wheeze			Spirometry			Skin prick test		
		n	%	OR	*P*-value	%	OR	*P*-value	%	OR	*P*-value	%	OR	*P*-value	%	OR	*P*-value
Risk factor																	
History of parasite infection																	
Ascaris lumbricoides	Yes	349	23.3	1.153	0.342	9.7	1.217	0.383	29.5	1.574	0.019	6.3	1.557	0.023	19.5	1.279	0.100
	No	969	14.5			7.8			18.1			3.2			20.6		
Hookworm	Yes	39	37.5	3.008	0.019	5.1	0.593	0.209	30.8	1.541	0.287	5.1	1.129	0.893	12.8	0.725	0.248
	No	1279	16.0			8.4			20.8			4.0			20.6		
Enterobius vermicularis	Yes	763	18.0	1.503	0.002	10.2	1.890	0.001	22.7	1.352	0.092	3.8	0.975	0.938	20.1	0.849	0.363
	No	554	14.3			5.8			19.0			4.3			20.8		
Current parasite infection																	
Any helminth	Yes No	295	18.6	1.084	0.660	6.4	0.700	0.256	22.7	1.012	0.932	3.0	0.586	0.178	18.3	0.882	0.426
		1017	16.1			9.0			20.8			4.3			20.9		
A. lumbricoides	Yes	83	23.5	0.916	0.686	2.4	0.222	0.008	26.5	0.948	0.846	2.4	0.378	0.165	13.3	0.888	0.729
	No	1230	15.9			8.8			20.8			4.1			20.9		
Hookworm	Yes	121	14.1	0.967	0.894	9.1	1.207	0.710	17.4	0.763	0.174	3.3	0.748	0.613	19.0	0.753	0.130
	No	1192	16.5			8.3			21.6			4.0			20.6		
E. vermicularis	Yes	36	12.5	0.880	0.760	5.6	0.632	0.460	16.7	0.902	0.795	0	Too few samples		11.1	0.456	0.149
	No	1277	16.4			8.5			21.3			4.0			20.7		
Trichuris trichiura	Yes	126	22.8	1.324	0.275	5.6	0.588	0.094	29.4	1.455	0.056	4.0	0.881	0.761	20.6	1.086	0.629
	No	1175	15.8			8.8			20.4			4.0			20.6		
Other common risk factors																	
Antibiotics first year	Yes	643	22.8	2.152	0.001	10.9	1.897	0.002	29.1	2.520	0.000	4.7	1.299	0.255	20.0	1.076	0.468
	No	676	10.3			5.9			13.5			3.3			20.3		
Current pet ownership	Yes	1045	16.0	0.960	0.888	7.8	0.705	0.031	20.9	0.956	0.850	4.0	0.982	0.969	20.6	0.876	0.508
	No	276	18.1			10.5			22.1			4.0			19.2		
Current pet contact	Yes	1140	15.8	1.095	0.706	8.3	1.040	0.888	20.4	0.947	0.818	4.5	6.390	0.022	20.8	0.799	0.514
	No	181	21.9			8.3			26.0			1.1			17.1		
Previous pet ownership	Yes	811	14.2	0.740	0.112	8.1	0.938	0.843	20.7	0.948	0.647	4.8	1.915	0.051	21.3	1.020	0.858
	No	510	20.2			8.6			21.8			2.8			18.6		
Previous pet contact	Yes	514	16.1	1.104	0.700	9.0	1.197	0.479	22.4	1.229	0.155	5.3	1.884	0.129	22.0	1.054	0.733
	No	807	16.7			7.9			20.3			3.2			19.2		
Symptoms of reflux	Yes	716	21.0	2.073	0.001	10.1	1.496	0.056	26.0	1.929	0.000	4.9	1.777	0.009	20.0	0.937	0.604
	No	604	10.3			6.3			15.4			3.0			20.1		
Smoking in household	Yes	645	16.4	0.987	0.928	7.4	0.736	0.104	19.9	0.820	0.223	3.3	0.665	0.223	20.9	1.116	0.302
	No	676	16.5			9.2			22.3			4.7			19.7		

Table 1 (*Continued*)

		Allergic rhinoconjunctivitis				Atopic dermatitis			Current wheeze			Spirometry			Skin prick test		
		n	%	OR	*P*-value	%	OR	*P*-value	%	OR	*P*-value	%	OR	*P*-value	%	OR	*P*-value
Family atopy	Yes	603	21.1	1.910	0.002	10.5	1.602	0.023	29.9	2.934	0.000	5.8	2.712	0.000	22.4	1.326	0.100
	No	713	12.4			6.5			13.7			2.5			18.5		
Presence of siblings	Yes	1086	17.3	1.275	0.228	8.7	1.400	0.235	21.6	1.172	0.355	4.1	1.176	0.696	19.7	0.931	0.742
	No	216	13.7			7.0			19.5			3.2			21.3		
Daycare attendance	Yes	231	16.8	1.318	0.109	8.2	1.092	0.692	24.8	1.441	0.048	3.0	0.701	0.395	19.1	0.857	0.190
	No	1090	16.4			8.4			20.4			4.2			20.1		
Breastfeeding	Yes	1134	16.7	1.528	0.127	8.4	1.530	0.419	20.7	0.740	0.426	4.1	0.611	0.462	20.2	1.489	0.433
	No	53	18.4			5.7			28.3			7.6			11.3		

Table 2 Results of the final multivariate logistic regression models for associations between atopic diseases and helminth infections as well as other common risk factors. Factors identified as statistically significant at the 5% level in univariate analysis were entered into a stepwise forward logistic regression model. Multiple logistic regression was adjusted for age, sex, municipality, urban/rural background and income irrespective of their significance level. Only significant results are shown

Variable/risk factor	OR	95% CI	*P*-value
Allergic rhinoconjunctivitis			
Hookworm (history)	2.81	1.23–6.42	0.017
Enterobius vermicularis (history)	1.34	1.00–1.79	0.048
Symptoms of reflux	1.74	1.24–2.44	0.003
Family atopy	1.67	1.07–2.59	0.025
Antibiotics first year	1.80	1.28–2.53	0.002
Atopic dermatitis			
E. vermicularis (history)	1.86	1.34–2.58	0.001
Ascaris lumbricoides (feces)	0.23	0.08–0.68	0.011
Family atopy	1.56	1.09–2.24	0.017
Antibiotics first year	1.61	1.10–2.35	0.017
Current pet contact	0.68	0.48–0.98	0.039
Current wheeze			
Family atopy	2.60	2.00–3.38	<0.001
Antibiotics first year	2.11	1.55–2.86	<0.001
Symptoms of reflux	1.59	1.20–2.11	0.003
Abnormal spirometry before or after exercise			
Current pet contact	6.56	1.30–33.13	0.025
Family atopy	2.77	1.94–3.97	<0.001

examination. We only used stool examination, which is much less sensitive (Tsibouris *et al.* 2005) and may thus explain the lack of an effect of current *E. vermicularis* infection on atopic diseases in our study population.

In our Cuban study population, a history of infection with hookworm was associated with an increased risk for allergic rhinoconjunctivitis. To our knowledge we are the first to find an association between hookworm infections and allergic rhinoconjunctivitis.

Trichuris trichiura was not related to any atopic disease. These results are in line with the results by Dagoye *et al.* (2003), who found *T. trichiura* to be unrelated to wheeze in contrast to other helminths. Especially helminths with a systemic phase in their life cycle could influence atopic disease (Scrivener *et al.* 2001; Dagoye *et al.* 2003).

Asthma in Cuban children was not related to helminthiasis, whether defined as current wheeze or as abnormal spirometry. The percentage of children with bronchial hyperresponsiveness found with spirometry (4%) and of those with current wheeze reported by parents (21%) differed considerably, as has been described before (Wördemann *et al.* 2006a). Both methods have limitations and their use for the diagnosis of asthma, separately or

together, has been debated (Demissie *et al.* 1998; Remes *et al.* 2002; Gruchalla *et al.* 2003). Also skin prick test reactivity was not affected by helminths, confirming the results of Palmer *et al.* (2002) and Cooper *et al.* (2006). Skin prick test reactivity in relation with helminth infections may depend on intensity and chronicity of helminth infections, such that heavy and chronic helminth infections protect from atopy (Lau & Matricardi 2006). Thus the lack of an effect of worm infection on skin prick test reactivity in our study and those of Palmer *et al.* (2002) and Cooper *et al.* (2006) might be due to the rather lower intensities and prevalences than those in which skin prick test reactivity was inversely related with helminthiasis (Cooper *et al.* 2003; Van den Biggelaar *et al.* 2004).

We also examined the effect of other common risk factors for atopic diseases and/or atopy (Bufford & Gern 2005). Antibiotic intake during the first year of life was significantly associated with an increased risk for current wheeze, atopic dermatitis and allergic rhinoconjunctivitis in our study population. An interference of antibiotic intake with intestinal flora and a subsequent impact on the development of atopy and atopic diseases has been suggested before (Hart *et al.* 2002). Yet the association between antibiotic intake and atopic diseases could also be inverse, with an increased antibiotic intake due to allergic symptoms wrongly diagnosed as bacterial infections or due to respiratory infections requiring antibiotic treatment in asthmatic children, as suggested by Bufford and Gern (2005). Symptoms of reflux were associated with allergic rhinoconjunctivitis and current wheeze. The latter has been found by Nordenstedt *et al.* (2006) in Norway as well and seems to be due to heightened bronchial reactivity and microaspirations in patients with reflux.

Current animal contact was negatively associated with atopic dermatitis. In literature animal contact is generally described to have a protective effect against atopic diseases (Bufford & Gern 2005). Other risk factors, such as breastfeeding, sibling size, daycare attendance, smoking in household, pet ownership or contact during first year of life did not have a significant impact on atopic diseases. A recent overview shows that associations between the above mentioned risk factors and atopic diseases often are inconsistent (Bufford & Gern 2005).

Conclusion

Current *A. lumbricoides* infection protects against atopic dermatitis in Cuban children, while past infection with *E. vermicularis* and hookworm are risk factors for allergic rhinoconjunctivitis and/or atopic dermatitis. Apparently, interactions differ depending on the type of helminth and atopic disease and on the time of helminth infestation.

Acknowledgements

We thank all children, parents, teachers, school staff as well as the staff in the policlinics, the health authorities and all field workers who participated in this study.

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