CHAPTER 7

Respiratory atopic disease, *Ascaris*-IgE and tuberculin test in urban South African children

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Abstract

Background

Epidemiological relation of intestinal helminth infection and atopic disease, both associated with a Th2 immune response, is controversial, since it has been reported that helminth infection may either suppress or predispose to atopic disease. This relation has not been tested in an area with high burden of *M. tuberculosis* (*M. tuberculosis*) infection, a known Th1 stimulating infection.

Objective

To study the association of intestinal helminth infection and atopic disease in a community where helminth infection is endemic and *M. tuberculosis* infection high.

Methods

Three-hundred and fifty-nine randomly selected children aged 6-14 years from a poor urban suburb were tested with allergy questionnaire, skin prick test (SPT) to common aeroallergens, *Ascaris* specific IgE (*Ascaris*-sIgE), fecal examination for pathogenic intestinal helminths and tuberculin skin testing (TST). Histamine bronchoprovocation was tested in the group of children aged 10 years and older. Results were corrected for demographic variables, socioeconomic status, parental allergy, environmental tobacco smoke (ETS) exposure in the household, recent anthelminthic treatment and for clustering in the sampling unit.

Results

Ascaris-sIgE was elevated in 48% of children, *Ascaris* eggs were found in 15% and TST was positive in 53%. Children with elevated *Ascaris*-sIgE had significantly increased risk of positive SPT to aeroallergens, particularly house dust mite, atopic asthma (*ever* and *recent*), atopic rhinitis (*ever* and *recent*), and increased atopy-related bronchial hyperresponsiveness. In children with negative TST (< 10 mm), elevated *Ascaris*-sIgE was associated with significantly increased risk of atopic symptoms ($OR_{adjusted}$ 6.5; 1.9-22.4), while in those with positive TST (≥ 10 mm) this association disappeared ($OR_{adjusted}$ 0.96; 0.4-2.8).

Conclusions

These results suggest that immune response to *Ascaris* (*Ascaris*-sIgE) may be a risk factor of atopic disease in populations exposed to mild *Ascaris* infection and that *M. tuberculosis* infection may be protective against this risk, probably by stimulation of anti-inflammatory networks.

Introduction

Although infection with helminths can stimulate polyclonal IgE synthesis, their role in the development of atopic disease remains uncertain.[1,2] The epidemiological relation of helminth infection and atopic disease, both associated with a T-helper (Th) 2 immune response, is controversial, since it has been reported that helminth infection may either suppress atopic disease[3-6] or predispose to it.[7,8] Anthelminthic treatment may influence atopic conditions, or modulate the severity of symptoms or sensitization.[9-11] It is generally assumed that the prevalence of allergies is low in populations heavily infested with helminth.[12-15]

The effect of intestinal helminth infection on atopic symptoms seems to depend on the duration and intensity of infection.[12,13] With chronic and intense infection, atopic symptoms may be suppressed, while mild and intermittent infection may result in enhanced reaction to environmental allergens and atopic response.[12,13]

Previous epidemiological surveys often used different definitions of atopy, which made it difficult to compare results. Recently the World Allergy Organization (WAO) published a new definition of atopic disease as clinical allergic symptoms in combination with a positive allergy skin prick test (SPT) and/or an elevated serum IgE antibody level,[16] which makes it possible to define atopic diseases more uniformly.

Although *Mycobacterium tuberculosis* (*M. tuberculosis*) infection is high in most helminth-infested populations in the developing world, the possible influence of *M. tuberculosis*, a strong stimulator of Th1 immunity, on the effect of helminth infection on the development of atopic disease has not been investigated.

In a resource-poor urban suburb of South Africa, where helminth infection is endemic and tuberculosis (TB) incidence high,[17,18] we investigated the associations of *Ascaris lumbricoides* infection (specific-IgE or eggs), and atopic sensitization to common aeroallergens or atopic diseases in the pediatric population, using the new WAO definitions. In addition, we tested the influence of positive tuberculin skin test (TST) on the associations investigated.

Materials and methods

This cross-sectional study was conducted between June and October 2003, in an established epidemiological research-site in Cape Town, South Africa, with a population of 36.334 in 2001 (Statistics South Africa: Western Cape, Census 2001) and predominantly of mixed ancestry. Although most of the families live in brick houses and have access to clean water and electricity, the general socioeconomic conditions are poor. The prevalence of childhood asthma is 10.8 -13.3% and of allergic rhinitis 16 %.[19,20] The vaccination policy in South Africa requires all children to receive a single dose of *M. bovis* BCG-vaccine during the first week of life. Based on the WHO criteria, neonatal BCG vaccination in the study area is universal ($\geq$ 90%).

All children aged 6-14 years living in a 15% randomly selected sample of household-addresses were enumerated and invited to participate in the study. Exclusion criteria in-

cluded known immune-compromising disease (such as HIV), active tuberculosis disease, pregnancy or parental refusal. Parents or legal guardians gave written, signed, informed consent.

Under the supervision of a trained field-worker, each parent completed a written validated International Study of Asthma and Allergies in Children (ISAAC) Phase I questionnaire on previous (ever) and recent (<12 months) symptoms of asthma or allergic rhinitis. In addition, the questionnaire included questions on socioeconomic variables, parental allergic history, BCG immunization, anthelminthic treatment, environmental tobacco smoke (ETS) exposure in the household and HIV-status.

The proportion of HIV-1-infected persons in the area is among the lowest in South Africa.[21] Children with questionnaire-reported positive HIV-status were excluded, because of the possible effect on T cell immunity. The presence of BCG scar was noted in each child.

SPT to eight common aeroallergens (ALK-Abelló, Denmark) based on local allergen exposure (house dust mite (HDM), Bermuda grass, Rye grass, cat dander, dog dander, *Alternaria alternata*, *Cladosporium herbarium* and *Aspergillus*) together with a positive (histamine chloride 10mg/ml) and negative (glycerol) control were performed after completion of the questionnaire. A positive SPT reaction was defined as a mean wheal diameter of ≥ 3mm in excess of the negative control.

Total serum IgE and *Ascaris*-sIgE levels were measured with the CAP RAST (Pharmacia®, Uppsala, Sweden). Due to the lack of reference values for non-Caucasian children for this analysis total serum IgE level higher than the median value of the children in the study and *Ascaris*-sIgE level ≥ 0.35 IU/ml was considered elevated. Two stool samples, taken at least 24 hours apart, were collected from each child. A portion (0.5-1 gram) was weighed, added to formalin, concentrated by formalin-ether sedimentation and examined by light microscopy. The presence of helminth eggs was defined as helminth infection. Helminth infection intensity (eggs per gram (epg) stool) was classified according to the proposed WHO classification (WHO/CTD/SIP/98.1).

The histamine bronchoprovocation test was used to assess bronchial hyper-responsiveness (BHR). The spirometry (with a calibrated Jaeger Masterscope, software version: 4.52i) was repeated until the best of three reproducible baseline measurements of forced expiratory volume at 1 second (FEV_1) was obtained, this was taken as reference. During the pilot spirometry testing, a majority of the children less than 10 years of age failed to achieve reproducible readings within the allocated time-frame of the test. This was mainly due to lack of motivation or difficulty with understanding the instructions. Because of this difficulty the study coordinators decided to limit the histamine bronchoprovocation test to all children 10 years of age and older. The test was conducted according to the modified Cockcroft protocol, as described by Steinbrugger.[22] It consisted of a 2-minute inhalation of nebulized histamine (Pari LC PLUS nebulizer) through a mouthpiece of a Pari-Boy (37:00; 50 Hz; 1.3 bar −2700 l/min; Pari, Starnberg, Germany). During inhalation, the nose was closed by a clip. The histamine concentration (0.03 to 7.8 mg/ml) was doubled in a standardized way and the FEV_1 measured after inhalation of each concentration. The test was continued until a decrease in FEV_1 from baseline of 20% or the maximum dose was reached. The response to the histamine challenge was expressed as $PC_{20;}$ the concentration

that caused a fall in FEV_1 of 20% from baseline calculated by linear interpolation of log-linear dose-response curve. A subject was regarded as having an increased BHR if PC_{20} was ≤ 8.0 mg/ml. Children with a FEV_1 fall of ≥ 10 % after histamine challenge were treated with two inhalations of 200µg salbutamol and FEV_1 measurement was repeated 5 minutes later.

The research-site has a very high TB incidence.[18] The TB notification rate was 341 per 100.000 for new smear-positive TB and 612 per 100.000 for bacteriological confirmed TB in 2002. Tuberculin reactivity becomes apparent in 3-6 weeks after initial *M. tuberculosis* infection and may remain positive for the lifetime of the individual.[23] A positive TST reaction is an accepted hallmark of primary infection with *M. tuberculosis*.[23] TST response was documented in the children. TST was performed by injecting 2 TU (tuberculin units) of PPD RT 23 (Statens Serum Institut, Copenhagen, Denmark) and measuring the transverse induration diameter after 2 to 3 days. In accordance with the American Thoracic Society guidelines, a positive TST was defined as an induration of 10 mm or more.[24]

Definition of atopic disease outcome variables

Allergic symptoms represent ISAAC-questionnaire-reported symptoms. A positive SPT was used to differentiate children with atopic symptoms from those with non-atopic symptoms. Atopic asthma and rhinitis were defined according to the new WAO definition, as questionnaire reported asthma or allergic rhinitis together with a positive SPT.[16] Atopy-related BHR was defined as increased BHR with positive SPT (BHR with SPT).

Statistical analysis

Bivariate data analyses were performed using the chi-squared test (SPSS 11.0). Regression analyses were performed with the generalized estimated equation (GEE) logistic regression (LR) (STATA 8.0), using reported allergic symptoms, SPT reactivity, atopic diseases or BHR with SPT as the dependent variable and *Ascaris*-sIgE as the independent variable. The association was adjusted for possible confounding variables: demographic (age and gender), genetic- (parental allergic history), socioeconomic- (household income), environmental (ETS exposure in the household) factors and anthelminthic treatment. In addition, the association was stratified for the presence or absence of a positive TST, in a LR model. Results were corrected for clustering (> 1 child per household) in the sampling unit. The sampling unit in the study is the household. There was a total of 201 households in the study, with an average of 1·5 children per household. For the analysis, each child was coded and linked to the specific household. The study protocol was approved by the Ethics Review Board of the University of Stellenbosch.

Results

Of 418 enumerated children aged 6-14 years, 359 (86%) were enrolled in the study, 39 had moved away from the area, 14 refused consent, 3 were pregnant and 3 exceeded the age limit. The median age was 11.0 (range, 6-14) years. The children excluded from the study did not differ in age or gender from those included.

Table 1 describes the most important characteristics of the 359 children analyzed, together with prevalences of reported allergic symptoms, atopic sensitization, atopic diseases, increased BHR, helminth infection and anthelminthic treatment, serum IgE antibody levels, parental allergic history, positive TST reaction, environmental tobacco smoke (ETS) exposure in the household and BCG immunization. All children in the study reported to have received neonatal BCG immunisation. The prevalence of BCG scar did not differ between children with positive TST and those with negative TST.

Of children with helminth infection, 14.8% (53) were infected with *Ascaris* and 40% (144) with *Trichuris trichiura (Trichuris)*. Other helminth eggs found were *Enterobius vermicularis* in 4 children and *Hymenolepsis nana* in 1. The median infection intensity and IgE values were log-transformed because the values were not normally distributed. The median (range) infection intensity among the children infected with *Ascaris* was 182 (0-5888) epg and *Trichuris* 58 (0-10.000) epg. The median (range) total IgE level was 143.00 IU/ml (2.33-18080.00) and *Ascaris*-sIgE 0.35 IU/ml (<0.35-63.10). Fifty percent (178) of children had an elevated total IgE level and 48% (171) an elevated *Ascaris*-sIgE level. Of children who underwent histamine bronchoprovocation 55 % (133) had increased BHR and 13.3% (32) had both increased BHR and positive SPT. TST was positive in 53% (179) of children. The TST distribution of the children was unimodal and the median TST size in those with any TST induration was 18 mm (range 1-30.5). The prevalence of parental allergic history or recent (<12 months) anthelminthic treatment did not differ between children with elevated *Ascaris*-sIgE or *Ascaris* eggs in the stool and those without.

Children with *Ascaris* eggs in the stool were more likely to have an elevated *Ascaris*-sIgE level (adjusted odds ratio [OR_{adj}] 2.4; 95% CI 1.3-4.5) and had a higher mean log *Ascaris*-sIgE ($p< 0.005$) than those without. In contrast, children with *Trichuris* infection were not more likely to have an elevated *Ascaris*-sIgE level (OR_{adj} 1.50; 95% CI 0.92-2.80) or a higher mean log *Ascaris*-sIgE ($p = 0.10$).

Ascaris-sIgE and atopic disease

Children with elevated *Ascaris*-sIgE had a higher frequency of allergic symptoms, atopic diseases, positive SPT to HDM, BHR and BHR with SPT than those without elevated *Ascaris*-sIgE. For reported asthma ever ($p < 0.05$), atopic asthma *ever* ($p < 0.05$) and *recent* ($p < 0.005$)), atopic rhinitis *ever* ($p < 0.05$) and *recen*t ($p < 0.05$), positive SPT to HDM ($p < 0.05$) and BHR ($p < 0.05$) the differences were significant.

Figure 1 shows a significant linear association of log *Ascaris*-sIgE and the frequency of atopic diseases, positive SPT to HDM or BHR with SPT in the children. Tabl**e 2** shows that children with elevated *Ascaris*-sIgE had significantly increased risk of atopic sensitization, particularly HDM and atopic diseases than those without. They also showed a trend to have higher risk of reported allergic symptoms, BHR and BHR with SPT. The increase in risk of atopic disease or positive SPT for HDM observed in children with elevated *Ascaris*-sIgE remained significant after adjusting for possible confounding variables (**Table 3**). In a linear regression analysis, log *Ascaris*-sIgE was significantly inversely related to log PC_{20} (Regression coefficient [ß] = –0.206, SE 0.06, $p = 0.02$).

Table 1. Characteristics of the study population (total = 359)

		Number/ Total	(%)
Demographics:			
Sex	Male	188/359	(52.4)
	Female	171/359	(47.6)
Age (years)	6-10	167/359	(46.5)
	11-14	192/359	(53.5)
Respiratory allergic symptoms:			
Asthma			
ever	yes	49/359	(13.6)
recent (≤ 12 months)	yes	27/359	(7.5)
Allergic rhinitis			
ever	yes	41/359	(11.4)
recent	yes	34/359	(9.5)
Atopic sensitization:			
Skin prick test (SPT) reaction ≥ 3 mm	house dust mite	48/359	(13,4)
	rye grass	17/359	(4.7)
	Alternaria	15/359	(4.2)
	Bermuda grass	12/359	(3.3)
	cat	9/359	(2.5)
	Aspergillus	4/359	(1.1)
	dog	4/359	(1.1)
	Cladosporium	3/359	(0.8)
	any allergen	66/359	(18.4)
Respiratory atopic diseases (*):			
Atopic asthma			
ever	yes	16/359	(4.5)
recent	yes	11/359	(3.1)
Atopic rhinitis			
ever	yes	15/359	(4.2)
recent	yes	14/359	(3.9)
Histamine bronchoprovocation (†):			
Increased BHR	yes	133/242	(54.9)
Increased BHR with positive SPT	yes	32/242	(13.3)
Helminth infection:			
Helminth egg in stool sample	*Ascaris lumbricoides*	53/359	(14.8)
	Trichuris trichiura	144/359	(40.1)
Anthelminthic treatment ≤ 12 months	yes	75/359	(20.9)
Serum IgE antibody levels (IU/ml):	*Ascaris* specific-IgE (≥ 0.35)	171/ 357	(47.9)
	total IgE (≥ median:143.0)	178/357	(49.9)
Parental allergic history	yes	83/359	(23.1)
Environmental exposures:			
Positive tuberculin skin test (TST)	≥10 mm	179/337	(53.1)
Environmental tobacco smoke (ETS) exposure in the household	yes	217/359	(60.4)
BCG immunization	yes	359/359	(100)

(*) Defined as combination of reported allergic symptom and a positive SPT, according to the new World Allergy Organization nomenclature.

(†) Histamine challenge was performed only in children ≥ 10 years of age (explanation in the methods section).

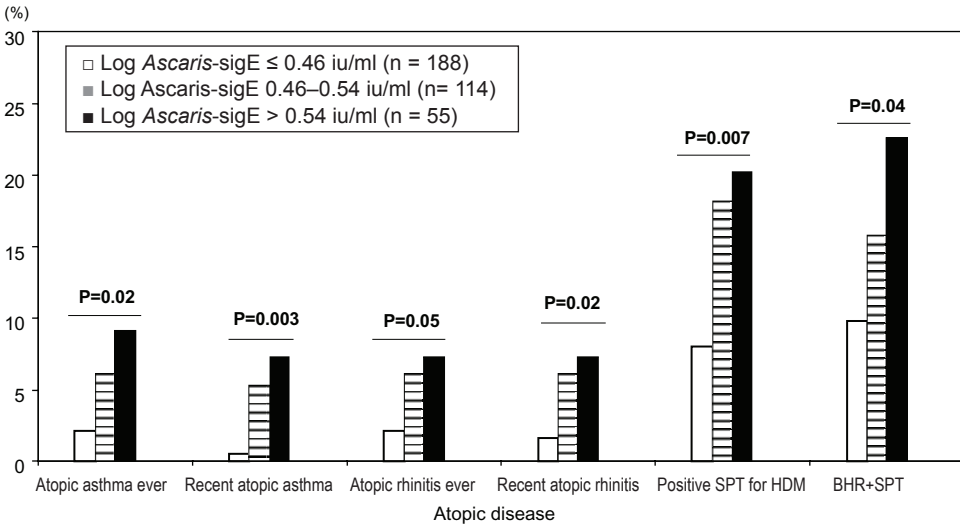

Fig. 1. Frequency of atopic diseases, positive skin prick test (SPT) to HDM and BHR with positive SPT showing direct linear increase with log *Ascaris* speciific IgE (*Ascaris*-sIgE) level (p value for linear relation)

Children with *Ascaris* eggs in the stool did not have an increased risk of positive SPT (OR 0.57; 95% CI 0.23-1.40), atopic asthma (OR 0.85; 95% CI 0.81-0.86), atopic rhinitis (OR1.04; 95% CI 0.22-4.82), or atopy-related BHR (OR 0.74; 95% CI 0.21-2.60) than those without. This did not change after adjusting for the possible confounding variables.

TST, *Ascaris*-sIgE and atopic disease

Adjusted stratified analysis in a LR model, according to TST reactivity showed that in children with negative TST elevated *Ascaris*-sIgE was associated with increased risk of atopic symptoms (OR_{adj} 6.5; 95 % CI 1.9-22.4), while in those with a positive TST elevated *Ascaris*-sIgE was not associated with increased risk (OR_{adj} 0.96; 95% CI 0.4-2.8). There was a significant interaction-factor between a positive TST and an elevated *Ascaris*-sIgE ($p = 0.02$) in the LR model.

Discussion

This study in children from a resource-poor urban suburb of South Africa shows a significant association of elevated *Ascaris*-sIgE level and increased risk of atopic asthma, atopic rhinitis or hypersensitivity to common aeroallergens. It also shows that a positive TST, a hallmark of *M. tuberculosis* infection, may influence the association of *Ascaris*-sIgE and atopic disease. In children with negative TST elevated *Ascaris*-sIgE was associated with increased risk of atopy, while in those with positive TST no increased risk was observed.

Table 2. Crude and adjusted risk of allergic symptoms, atopic sensitization and atopic diseases in all children and in those with elevated *Ascaris* specific IgE (*Ascaris*-sIgE ≥ 0.35 IU/ml).

		All children (n = 359)	Children with elevated *Ascaris*-sIgE (n = 171)	Crude OR	95% CI	Adjusted OR*	95% CI
Allergic symptoms and BHR:							
Asthma							
ever	no	308	141 (45.8)	1		1	
	yes	49	30 (61.2)	1.87	1.01-3.46	1.79	0.90-3.53
recent	no	330	155 (47.0)	1		1	
	yes	27	16 (59.3)	1.64	0.74-3.65	1.55	0.64-3.77
Allergic rhinitis							
ever	no	316	147 (46.5)	1		1	
	yes	41	24 (58.5)	1.62	0.84-3.13	1.51	0.71-3.18
recent	no	323	150 (46.4)	1		1	
	yes	34	21 (61.8)	1.86	0.90-3.85	1.9	0.85-4.26
Increased BHR	no	109	41 (37.6)	1		1	
	yes	131	67 (51.1)	1.74	1.04-2.91	1.67	0.93-3.00
Atopic sensitization:							
HDM	no	309	138 (44.7)	1		1	
	yes	48	33 (68.8)	2.73	1.42-5.22	3.81	1.76-8.26
Any positive SPT	no	292	133 (45.5)	1		1	
	yes	65	38 (58.5)	1.68	0.98-2.90	2.09	1.15-3.79
Atopic diseases (†):							
Atopic asthma							
ever	no	341	159 (46.6)	1		1	
	yes	16	12 (75.0)	3.4	1.1-10.9	3.97	1.19-13.23
recent	no	346	161 (46.5)	1		1	
	yes	11	10 (90.9)	11.5	1.5-90.7	14.26	1.78-114.02
Atopic rhinitis							
ever	no	343	160 (46.8)	1		1	
	yes	15	11 (73.3)	3.1	1.0-10.0	4.56	1.19-17.48
recent	no	342	160 (46.6)	1		1	
	yes	14	11 (78.6)	4.2	1.2-15.3	8.64	1.91-39.11
Increased BHR with SPT(‡)	no	208	89 (42.8)	1		1	
	yes	32	19 (59.4)	2.0	0.9-4.2	2.08	0.90-4.84

(*) Adjusted odds ratios (OR) were calculated with the logistic regression model (Generalized Estimated Equation, GEE) using allergic symptoms, atopic sensitization, atopic diseases or BHR + SPT as the dependent variable and elevated *Ascaris*-sIgE together with confounding variables: age, gender, parental allergic history, average household income, anthelminthic treatment in the past 12 months, environmental tobacco smoke (ETS) exposure in the household and tuberculin skin test reactivity as the independent variable. In addition, correction was made for clustering (> 1 child per household) in the sampling unit.

(†) Atopic disease was defined using the new World Allergy Organization nomenclature as reported allergic symptom and positive skin prick test (SPT).

(‡) Only 240 children aged ≥ 10 years of age with complete data on histamine bronchoprovocation and *Ascaris*-sIgE were included in the analysis.

Table 3. Crude and adjusted risk of atopic disease outcomes, positive skin test to house dust mite (HDM) and *Ascaris* specific IgE ***(****Ascaris*-sIgE), together with confounding variables used in the logistic regression model.

	Adjusted OR (95% CI)				
Variable (categories)	**Atopic asthma ever**	**Recent atopic asthma**	**Atopic rhinitis ever**	**Recent atopic rhinitis**	**Positive SPT t o HDM**
Ascaris-sIgE (IU/ml)					
< 0.35	1.0	1.0	1.0	1.0	1.0
≥ 0.35	3.97 (1.19-13.23)	14.26 (1.78-114.02)	4.56 (1.19-17.48)	8.64 (1.91-39.12)	3.81 (1.76-8.26)
Age (yr)					
6-10	1.0	1.0	1.0	1.0	1.0
11-14	1.83 (0.55-6.13)	3.0 (0.60-14.89)	3.04 (0.71-12.94)	4.94 (0.98-25.04)	2.26 (1.04-4.89)
Gender					
Male	1.0	1.0	1.0	1.0	1.0
Female	1.75 (0.59-5.21)	1.17 (0.86-15.06)	3.61 (0.07-1.16)	2.99 (0.70-12.73)	1.85 (0.89-3.85)
Household income (SAR)					
< 800	1.0	1.0	1.0	1.0	1.0
≥ 800	2.04 (0.48-8.62)	2.72 (0.52-14.16)	3.99 (0.94-16.88)	6.16 (1.17-32.43)	1.84 (0.65-5.20)
Parental allergy					
No	1.0	1.0	1.0	1.0	1.0
Yes	1.75 (0.55-5.56)	1.29 (0.30-5.57)	1.13 (0.26-4.91)	1.56 (0.33-7.40)	1.46 (0.65-3.30)
TST (mm)					
<10	1.0	1.0	1.0	1.0	1.0
≥ 10	0.62 (0.21-1.83)	0.43 (0.12-1.58)	0.12 (0.02-0.64)	0.05 (0.01-0.41)	0.89 (0.43-1.82)
Recent helminthic treatment					
No	1.0	1.0	1.0	1.0	1.0
Yes	0.29 (0.03-2.50)	0.48 (0.05-4.67)	0.71 (0.13-3.96)	0.98 (0.16-5.98)	1.18 (0.48-2.93)
Current smoke exposure					
No	1.0	1.0	1.0	1.0	1.0
Yes	0.92 (0.30-2.88)	0.81 (0.21-3.10)	0.24 (0.07-0.90)	0.17 (0.04-0.79)	0.26 (0.26-1.20)

Adjusted odds ratios (OR_{adj}) were calculated with the Generalized Estimated Equation (GEE) logistic regression (LR) model (STATA 8.0), using atopic asthma, atopic rhinitis and positive SPT to house dust mite (HDM) as the dependent variable and *Ascaris*-sIgE and the other confounding variables in the table as independent variable. In addition, correction was made for clustering (> 1 child per household) in the sampling unit. Atopic disease was defined using the new World Allergy Organization nomenclature as reported allergic symptom and a positive skin prick test (SPT).

These findings suggest that elevated *Ascaris*-sIgE may be a risk factor of respiratory atopic disease and is in support of previous studies in South Africa,[8,14,25] South America[10] and China.[7]

Although it has been hypothesized that polyclonal IgE produced during helminth infection may block allergic reaction by either suppressing antigen-specific IgE production or

by saturation of IgE receptors on mast cells,[10,26-28] other studies which demonstrated lack of saturation of mast cell capacity in vitro or *in vivo* argue against this mechanism.[29,30] Recent evidence suggests that chronic infections, such as helminth and *M. tuberculosis* may lead to $CD4^+$ regulatory T cell (Treg) stimulation with subsequent production of high levels of anti-inflammatory cytokines, which may inhibit allergic inflammation.[13] This effect may depend on the intensity and persistence of infection.[13,31] With intense and persistent infection, atopic responses may be suppressed by strong stimulation of Tregs and anti-inflammatory cytokines.[13] In contrast, mild helminth infection (as in our study population) may lead to moderate or no stimulation of anti-inflammatory networks and this may result in an enhanced reaction to environmental allergens and atopic response.[13,31]

The mild intensity of *Ascaris* infection in our study population may be explained by the frequent single-dose anthelminthic therapy with benzimidazoles being used by children in the area.[32] This reduces the intensity of parasitic infestation and interrupts the persistence (or chronicity) of infection. The higher prevalence of *Trichuris* infection, which is less sensitive to a single dosage of anthelminthic therapy, supports this assumption.[32]

A rather surprising finding is that the presence of *Ascaris* eggs in the stool was not associated with increased prevalence of atopic disease. This observation may reflect important differences in the immunological profile between *Ascaris* eggs in the stool and elevated *Ascaris*-sIgE, especially in populations where intestinal helminth infection is of mild intensity. This is supported by reports that an elevated *Ascaris*-sIgE level and the presence of *Ascaris* eggs in the stool may measure different entities.[33] An elevated *Ascaris*-sIgE level reflects the competency of the host Th-2 immune response to *Ascaris* antigen. The presence of *Ascaris* eggs in the stool reflects active infection, although the impact of this infection on the host immune response may depend on different factors, including the severity and chronicity of infection.[13,31] The lack of association between *Ascaris* eggs and atopic disease may partly be related to the low prevalence and mild intensity of *Ascaris* eggs in the children.

Our findings differ from those of another research group who reported a lower prevalence of hypersensitivity to HDM in African children with urinary schistosomiasis.[30] The authors considered the anti-inflammatory cytokine, IL-10 produced during *Schistosoma* infection to be responsible for the effect. The enhanced production of IL-10 seen with chronic tissue helminth infections such as filariasis and schistosomiasis has not been observed in ascariasis.[34] This, and the mild intensity of helminth infection, may account for the different observations.

Consistent with previous findings, we found that a positive TST was inversely associated with the risk of atopic disease.[35] This is in support of recent evidence that chronic infections, such as *M. tuberculosis* may down-regulate the atopic response through the stimulation of anti-inflammatory networks.[13,31] The strong median TST reaction (18 mm) in the children indicates hypersensitivity to natural *M. tuberculosis* infection which is highly prevalent in the community and not to BCG or environmental mycobacteria which generally stimulate a weaker TST reaction.[23,24,35,36] Our present findings contrast with the results of a study in Gambian children, in which no inverse association was found between positive TST and atopy in children.[37] The difference in findings could be explained by the fact that a larger proportion of children in our study had a strongly positive TST size than in the

Gambian study. This is supported by observations that the size of TST is directly related to serum IFN-γ level, suggesting that increased serum IFN-γ concentration may partly explain the inverse association between *M. tuberculosis* infection and atopy.[38] Furthermore, the new globally accepted WAO definition of atopic rhinitis was used in our study.[16] This contrasts with the Gambian study in which atopy was defined as a positive SPT only.

Particular strength of this study is the use of objective measures (SPT) to differentiate children with atopic and non-atopic symptoms, based on the new WAO definitions.[16] Most previous studies defined atopic outcome either as a positive SPT or an elevated serum IgE only.[6,11] The influence of *M. tuberculosis* infection was taken into account, and this, to our knowledge, has not been reported previously in this context. The main limitations are the cross-sectional design and questionnaire-based diagnosis of allergic symptoms. Limitations inherent to the cross-sectional design are recall-bias and the inability to analyze temporal relationship of *Ascaris* eggs, *Ascaris*-sIgE, the development of atopic disease and TST reactivity. Questionnaire-based limitations were reduced by using the validated ISAAC questionnaire and collecting questionnaire data before performing objective tests. The reliability of questionnaire-generated data is supported by the fact that the prevalences of allergic symptoms were comparable to those of the ISAAC study in Cape Town.[20] That reliable spirometric tests could not be achieved in younger children does not bias the results, but rather reflects an important difficulty which may be encountered in performing such a standardized test in otherwise healthy voluntary children. Although the factors responsible for the high frequency of increased BHR were not investigated, it may be related to the high prevalence of ETS exposure and other non-atopic environmental airway pollutants in the area. ETS is a known risk factor for airway hyper-responsiveness in children.[39]

In conclusion, this study shows a significant association of elevated *Ascaris*-sIgE and atopic sensitization and symptoms, suggesting that elevated *Ascaris*-sIgE may be an environmental risk factor of atopic manifestation, particularly in helminth-infected children with mild infection. It also shows the influence of natural *M. tuberculosis* infection on the association, which may be attributed to stimulation of anti-inflammatory immune networks during *M. tuberculosis* infection. There is a need for prospective community-based studies to confirm these findings.

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