

Ascaris lumbricoides Infection Is Associated with Increased Risk of Childhood Asthma and Atopy in Rural China

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There is growing international interest in the possible relationships between helminthic infection and allergic disease, although the nature of the relationships remains uncertain and controversial. The interrelationships of current and past infection with *Ascaris lumbricoides* and asthma and atopy were investigated in a cross-sectional sample of 2,164 children between the ages of 8 and 18 years from Anqing Province, China. The children were sampled from a larger family-based study of the genetics of asthma. The prevalence of either a history of or a positive stool examination for *Ascaris* was 24.5%. Asthma was defined for analytic purposes using previously validated, stringent criteria including airways responsiveness to methacholine. Independently of the other factors assessed, infection with *A. lumbricoides* was associated with increased risk of asthma ($p < 0.001$), an increased number of skin tests positive to aeroallergens ($p < 0.001$), and an increased dose-response slope to methacholine ($p = 0.003$). The association of sensitization to common aeroallergens with increased asthma risk was enhanced in those children infected with *Ascaris*, and such infection was associated with an increased risk of asthma independent of sensitization to aeroallergens in this selected population. These data suggest a complex relationship between ascariasis and susceptibility to childhood asthma among predisposed children that may involve an interaction with the immune response to inhaled aeroallergens.

Keywords: asthma; atopy; helminthic infection; child; China

Asthma, the most common chronic disease among children in developed nations (1), carries a substantial direct and indirect economic cost worldwide (2). Whereas most asthma originates in childhood, the natural history of asthma is poorly understood. It has been widely observed that the prevalence of asthma and other allergic diseases has risen over the past two decades in industrialized nations (3). However, the baseline prevalence of childhood asthma is substantially lower in developing countries (1). Little is known about the etiology of asthma in mainland China.

In developing tropical countries, endemic helminthic infection is a major public health problem (4). Ascariasis is among the most common helminthic infections worldwide; the global infection burden was recently estimated to be approximately 1.5 billion people, representing an overall prevalence of 36% (4). A recent nationwide survey in China suggested that *Ascaris lumbricoides* infection was the most common helminthiasis, with an

overall prevalence of 47% and an estimated 531 million infections, and was most prevalent in children between 5 and 19 years old (5).

The epidemiologic observation that asthma is very common and helminthic infection relatively uncommon in developed countries and that the converse is true in many developing countries has led to speculation that the two phenomena may be inversely associated (6–8). However, the relationship between asthma and helminthic infection remains uncertain. Previous studies in endemically parasitized non-Chinese populations have variously argued that helminthiasis causes asthma (9–14), inhibits asthma (15–17), or is unrelated to asthma (18, 19).

The aim of this study was to investigate the association of infection with *A. lumbricoides* (round worm) and asthma and atopy in a rural Chinese population of children from families ascertained on the basis of asthma. A related aim was to explore possible associations between the pathogenic mechanisms underlying atopic disease and immune responses to helminthic infection in the Chinese population.

METHODS

The study was conducted in families of asthmatic subjects from the eight rural counties of Anqing Province, China, and has been previously described (20–22). The current study population comprised all children from these families who were aged between 8 and 18 years and had parasite data available.

Helminthic Infection

A history of helminthic infection with *A. lumbricoides* was taken for each subject. Examination of stool specimens for ova and parasites was performed at the Parasitology Laboratory of Anhui Medical University using the Kato–Katz thick-smear technique (23).

Spirometry and Airways Responsiveness

Spirometry, anthropometry (height, weight, and body mass index [BMI]), and methacholine challenge were measured using standard techniques (24, 25) as previously described (21). Airways responsiveness was expressed as the two-point dose-response slope (DRS) of methacholine response against percentage fall in FEV₁ (26).

Total Serum IgE Levels and Allergy Skin Testing

Serum total IgE levels were determined by means of the UniCAP 100 system (Pharmacia and Upjohn, Kalamazoo, MI). Forearm skin testing was performed (27) with the following allergens: cockroach, house dust, mixed grasses, cured tobacco leaf, mixed trees, polyvalent molds, *Dermatophagoides pteronyssinus*, *Dermatophagoides farinae*, *Artemisia vulgaris*, and mulberry silk waste (henceforth referred to as silk). A test was considered positive if the diameter of the skin wheal was 3 mm or more after subtraction of the negative control. Skin reactivity was expressed as the sum of the positive skin reactivity tests in each individual.

Definition of Asthma

All subjects were administered a standardized questionnaire (modified American Thoracic Society—Division of Lung Disease) (28). For analytical purposes, our primary definition of asthma used a stringent

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algorithm (28) based on the presence of all of the following: affirmative answers to the questions "Do you have asthma?" and "Has your asthma been confirmed by a doctor?"; at least two respiratory symptoms (cough, wheezing, dyspnea, and nocturnal cough/wheezing/dyspnea) or a history of recurrent asthma attacks; and airway hyperresponsiveness to methacholine, defined as a fall of 20% or more in FEV₁ from baseline following administration of up to 25 mg methacholine/ml.

Statistical Methods

Generalized estimating equations (GEE) (29) were used to appropriately adjust for familial correlations and to investigate the bivariate and multivariate relationships between measures of ascariasis with the following outcomes: asthma; number of skin tests positive to aeroallergens; and the DRS to methacholine. Skin test reactivity was also used as an explanatory covariate in certain models. Total serum IgE levels were only available on a subsample of the population ($n = 835$, 39%), and were therefore used in bivariate analyses but not in the multivariate modelling.

The total serum IgE levels and the DRS were skewed with a long right-hand tail and were log_e transformed before analysis. A constant was added to each DRS measurement to allow log_e transformation when the measure was less than or equal to zero. Cigarette smoking history in parents and children was categorized as: 0 = never smoked; 1 = past smoker; 2 = current smoker. Asthma, sex, and positive skin test reactivity to each aeroallergen were analyzed as binary variables. All other variables were analyzed as continuous.

Analysis and data management were performed using STATA v5 (Stata Corporation, College Station, TX) and SPlus v4.5 (Mathsoft Inc., Cambridge, MA).

RESULTS

Subject Characteristics

The study originally included 3,372 subjects 18 years of age or younger in 1,617 families of asthmatic subjects in Anqing, China. The present analyses were limited to the 2,164 children (in 1,152 families) aged between 8 and 18 years for whom there was information on helminthic infection. The characteristics of participating children are summarized in Table 1. The average number of children per family was 1.9; this was less than

TABLE 1. CHARACTERISTICS OF THE STUDY POPULATION ($n = 2,164$)

	% (n)*
Male sex	49.3 (1,066)
Current cigarette smoking	0.9 (19)
Past cigarette smoking	7.6 (165)
Paternal smoking	
Current cigarette smoking	69.3 (1,500)
Past cigarette smoking	8.6 (187)
Maternal smoking	
Current cigarette smoking	3.6 (78)
Past cigarette smoking	0
Asthma†	10.1 (219)
Past or current infection with <i>A. lumbricoides</i>	24.6 (533)
At least one positive skin reactivity test	40.3 (872)
PD ₂₀ ≤ 25 mg/ml methacholine	41.8 (905)
	Mean (SD)
Age, yr	12.08 (2.64)
Total serum IgE level, IU/ml‡	157.19 (141.02 – 173.36)
Number of positive skin reactivity tests	1.03 (1.59)
Height, m	1.39 (0.14)
Weight, kg	32.91 (10.40)
BMI, weight/height ²	16.70 (2.40)

* Percentage of all subjects ($n = 2,164$).

† Physician diagnosed asthma, history of either two or more respiratory symptoms or asthma attacks in the preceding 12 months, and airway responsiveness to methacholine (PD₂₀ ≤ 25 mg/ml).

‡ $n = 835$ samples; geometric mean and 95% confidence intervals (± 1.96 SEM).

the minimum sibship size of 2 enrolled in the parent study due to the exclusion of children not matching the entry criteria for the current analysis. A positive history or stool examination of *A. lumbricoides* infection was present in 15.9% ($n = 345$) and 12.2% ($n = 264$) of subjects, respectively. The concordance between a positive history of infection and a positive examination result for *Ascaris* was poor (Cohen's Kappa was 0.02). Therefore, infection with *Ascaris* within each individual was also analyzed by use of a variable that was coded as positive if either the history or the examination was positive. Serum total IgE levels ranged from 2.0 to 10,137.5 IU/ml. The number of positive skin tests ranged from 0 to 9; 40% of subjects were sensitized to at least one aeroallergen (Table 1). Cigarette smoking had a low prevalence among the children, was very common among fathers, and was almost absent in the mothers (Table 1).

Bivariate GEE analysis suggested that higher BMI (odds ratio [OR] = 0.82/kg/m², 95% confidence interval [CI] = 0.77–0.88, $p < 0.001$), increasing height (OR = 0.05/m, 95% CI = 0.02–0.14, $p < 0.001$), and increasing age (OR = 0.87/yr, 95% CI = 0.82–0.92, $p < 0.001$) were closely associated with decreased risk of asthma. Neither male sex (OR = 1.05, 95% CI = 0.80–1.40, $p = 0.71$) nor cigarette smoking history (OR = 1.40, 95% CI = 0.94–2.08, $p = 0.10$) was significantly associated with asthma risk. Paternal (OR = 1.08, 95% CI = 0.89–1.31, $p = 0.44$) or maternal (OR = 0.73, 95% CI = 0.43–1.23, $p = 0.24$) smoking history was not significantly associated with asthma risk in offspring. As has been previously shown in this population (22), bivariate analysis indicated that log_e total serum IgE levels (OR = 1.23/log_e IU/ml, 95% CI = 1.07–1.40, $p = 0.003$) and the number of skin tests positive to aeroallergens (OR = 1.10/positive skin test, 95% CI = 1.01–1.19, $p = 0.03$) were positively associated with asthma risk.

Association of Helminthic Infection with Asthma

Bivariate GEE analysis suggested that subjects having either a positive history or a positive stool examination for *Ascaris* had an increased risk of asthma (OR = 1.85, 95% CI = 1.37–2.49, $p < 0.001$). Analyzed separately, both a positive history (OR = 1.82, 95% CI = 1.28–2.59, $p = 0.001$) and a positive stool examination (OR = 2.26, 95% CI = 1.58–3.23, $p < 0.001$) for *A. lumbricoides* were associated with an increased risk of asthma.

Multivariate GEE analyses confirmed these associations. Independently of other potential predictors, a positive history or stool examination for *A. lumbricoides* was the best overall predictor of increased asthma risk (Table 2, Model 1). This relationship was also independent of the association of asthma with sensitization to aeroallergens (Table 2, Model 2). Further multivariate analysis suggested an important interaction between the number of skin tests positive to aeroallergens and a positive history or stool examination for *A. lumbricoides* (OR = 1.25, 95% CI = 1.13–1.37, $p < 0.001$). The results suggested that the association of sensitization to aeroallergens with asthma risk was enhanced in those subjects who had a pos-

TABLE 2. ASSOCIATION OF ASTHMA WITH PAST OR CURRENT ASCARIS LUMBRICOIDES INFECTION AND SKIN REACTIVITY TO AEROALLERGENS

Model	Explanatory Variables	OR (95% CI)	p Value
1*	Positive history or examination for <i>A. lumbricoides</i>	1.71 (1.26 – 2.31)	0.001
2*	Positive history or examination for <i>A. lumbricoides</i>	1.63 (1.19 – 2.22)	0.002
	Number of positive skin tests	1.11 (1.02 – 1.20)	0.01

* Model also adjusted for height and BMI.

Results from multivariate generalized estimating equation analyses.

TABLE 3. PERCENTAGES OF ASTHMA* IN NONALLERGIC/ALLERGIC SUBJECTS AND THOSE INFECTED/NONINFECTED WITH *ASCARIS LUMBRICOIDES*

	Nonallergic (0 positive skin tests)	Allergic (≥ 1 positive skin tests)
Negative history or examination for <i>A. lumbricoides</i>	9.8 (n = 82/835) [†]	9.0 (n = 53/587) [‡]
Positive history or examination for <i>A. lumbricoides</i>	12.4 (n = 32/258) [§]	21.3 (n = 46/216)

* Physician diagnosed asthma, history of either respiratory symptoms or asthma attacks in the preceding 12 months and airway responsiveness to methacholine ($PD_{20} \leq 25$ mg/ml).

[†] 86 missing values.

[‡] 52 missing values.

[§] 25 missing values.

^{||} 46 missing values.

itive history or examination for *A. lumbricoides* infection (Table 3).

Association of Helminthic Infection with Atopy and Airways Responsiveness

Bivariate GEE analysis indicated that a positive stool examination (OR = 1.43, 95% CI = 1.11–1.86, $p = 0.006$) but not a positive history (OR = 1.04, 95% CI = 0.81–1.32, $p = 0.77$) for *A. lumbricoides* infection was associated with increased total serum IgE levels in the subsample of children with IgE levels available. The geometric mean total IgE in those with a positive stool examination was 206.0 IU/ml and in those without a positive stool examination was 147.0 IU/ml. Multivariate GEE analysis indicated that a positive stool examination or positive history for *Ascaris* infection was associated with increased number of positive skin reactivity tests (Regression coefficient [β] = 0.32, SD = 0.08, $p < 0.001$; model adjusted for sex and age) and increased log_e dose–response slope to methacholine challenge ($\beta = 0.10$, SD = 0.03, $p = 0.003$; model adjusted for sex and height). Multivariate analysis of individual allergens indicated that a positive history or stool examination for *A. lumbricoides* was associated ($p < 0.001$) with sensitization to all of the aeroallergens tested, with the exception of *D. pteronyssinus*, *D. farinae*, mold, and cockroach (data not shown).

DISCUSSION

Our study was designed to investigate the interrelationships between asthma, atopy, and helminthic infection in rural China in a large sample of children from asthmatic families and has shown that infection with *A. lumbricoides* was closely associated with increased risk of childhood asthma, increased airways responsiveness to methacholine, and sensitization to common aeroallergens.

The major limitations of our study are its cross-sectional design and potential information biases relating to the collection of questionnaire data. The cross-sectional nature of the data meant that a temporal relation between ascariasis and the development of asthma could not be demonstrated. However, our sample size was large, standardized protocols were employed, and *Ascaris* stool examination data, physiological indices of airways responsiveness and atopy, and anthropometric data were measured objectively. Further, we used a stringent algorithm for defining ‘asthma’ for our analysis that had been previously validated in the Anqing population (28). There was only a limited and sporadic history of anti-*Ascaris* treatment, and medication use for asthma was virtually absent (no use of corticosteroids) (20) in the rural Anqing population at the time of this study, making it unlikely that our results were strongly confounded by treatment. Stools were also examined for schistosomiasis, ancylosomiasis, and enterobiasis; however, the prevalence of these parasites was too low ($< 3\%$) to be analyzed further in this study. Finally, the familial aggregation of asthma, atopy, and helminthic infection and the potential interrela-

tionships between the genetic and environmental components of variance of these outcomes could not be investigated in the current study population due to the strong ascertainment scheme and the lack of quantitative worm burden data.

Although our findings may not be generalizable to the general population of Anqing due to the selected nature of the population, they do apply to children at high risk of developing asthma (relatives of asthmatic subjects). The prevalence of asthma among children in the Anqing general population is less than 5% (21); a prohibitively large sample size would therefore be needed to study the relation between ascariasis and asthma in the Chinese general population.

This cross-sectional, family-based cohort study is, to our knowledge, the first to investigate the interrelationships of childhood asthma, asthma-associated physiological traits, and helminthic infection in a Chinese population. The inconsistent results of previous studies investigating the association between asthma and helminthiasis (7, 8) may relate to their relatively small sample sizes, to the lack of objective physiological measures related to asthma, and to the lack of a stringent definition of asthma.

Our results are consistent with previous studies in non-Chinese populations that have suggested an association between helminthiasis and an increased risk of asthma (9–14, 30, 31) and suggest that ascariasis may act to potentiate the T_H2 immune response characteristic of childhood asthma (8). Lifestyle changes and improved public health in industrialized nations such as the United States have resulted in a relatively low prevalence of parasitic infection but have also been associated with a marked increase in the incidence and severity of asthma and other atopic disorders (8). Our study raises the possibility that immune responses to parasitization and the predisposition to atopic disease may share common mechanisms. The close association of ascariasis with increased total serum IgE levels, sensitization to common aeroallergens, and increased airways responsiveness to methacholine were consistent with this finding and provide evidence of two potential mechanisms for the observed association of *Ascaris* infection with childhood asthma.

Both experimental studies (32) and studies in humans (33) have demonstrated that infection with *A. lumbricoides* can potentiate sensitization to allergens. Our study has shown the association of increased risk of childhood asthma with an interaction between enhanced sensitivity to common inhaled allergens and *Ascaris* infection. This suggests that the association of *Ascaris* infection with asthma, increased skin test reactivity, and increased airways responsiveness could reflect a pathway to airway inflammation related to potentiation of immune responses to aeroallergens (32). This effect could be due to an immediate influence of *Ascaris* infection or to a predisposition to the clinical expression of allergies in those subjects predisposed to more persistent *Ascaris* infection. Our study suggests that immune responses to parasitization and the predisposition to asthma may share common causal pathways. There is consistent evidence from family studies

and animal models that both asthma and resistance to helminthic infection are under substantial genetic control (7, 8, 34, 35). As other studies have suggested, such pathways may well involve shared genetic determinants (8, 36).

Previous epidemiological studies have hypothesized that the discordant relationship between the high prevalence of atopy and the low prevalence of asthma and other allergic disease in the Chinese population is due to the action of other adjuvant factors in determining the clinical expression of asthma (37). Our finding that *Ascaris* infection was associated with asthma independently of aeroallergen sensitization (Table 2, Model 2) and with airways responsiveness suggests that helminthic parasitization may represent one such adjuvant factor. This second potential pathway involving *Ascaris* infection may influence airways responsiveness independently of atopy and, in addition to potentially shared genetic determinants, could in part be due to acute pulmonary hypersensitivity responses caused by the tissue-migratory juvenile lung stage of the parasite (31).

A model of the possible influence of helminthic infection on the allergic reactivity of tropical populations has been proposed by Lynch (38). With mild helminthiasis, reactivity to specific allergens is elevated by the nonspecific potentiation of IgE synthesis against environmental antigens and possibly also by direct reactivity against the parasite. This increase in allergic reactivity reaches a peak, after which further stimulation results in suppressive effects on reactivity to specific allergens that may be due to saturation of mast cell Fc receptors and/or an inhibition of specific IgE synthesis by the polyclonal response (15, 39–43). The overall prevalence of *Ascaris* infection and the mean total serum IgE levels were suggestive of light-to-moderate infection intensities in the majority of the children studied. Further, several previous studies and nationwide surveys of China found that the intensity of *Ascaris* infection was generally light (5, 44–46). Together with the positive relationship between *Ascaris* infection and increased skin test reactivity in our study, these observations are consistent with the hypothesis that potentiation of the specific immune response and concomitant increased susceptibility to allergic disease may be associated with moderate levels of helminthic infection, and heavy infection may produce the opposite effect (38).

Alternatively, our finding may mean that the ecologic relationship between lower asthma prevalence and increased parasitism observed in many developing countries (6) reflects confounding factors such as diet, exposure to bacterial infections, and antibiotic use (8). Asthma and other allergic diseases are the result of complex interactions between largely unknown genetic and environmental mechanisms. *Ascaris* infection, although associated with increased asthma risk, likely interacts with many other exposures and individual genetic predispositions and therefore may have only a relatively small etiologic fraction at the population level.

Asthma is increasing in prevalence, and hence in clinical and economic importance, in China (47). Whereas asthma is relatively uncommon compared with industrialized countries, the absolute numbers of asthmatic children are very significant given the enormous population base of China. The prevalence of infection with *A. lumbricoides* in China is highest in children, suggesting that public health measures should be targeted at this age group (5). Our study suggests that there may be some value in an evaluation of the effects of targeted antihelminthic treatment on the expression of asthma in children living in endemically parasitized areas of China.

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Online Supplement for:

***ASCARIS LUMBRICOIDES* INFECTION IS ASSOCIATED WITH INCREASED RISK OF CHILDHOOD ASTHMA AND ATOPY IN RURAL CHINA**

METHODS

This study was conducted in Anqing Province, China between July 1, 1994 and January 26, 1998 in collaboration with Anhui Medical University and the Anqing Health Bureau and has been previously described (E1, E2). Anqing has three urban areas and eight rural counties, with a total area of 15,000 km² and a total population in 1990 of 5.8 million (urban 9%; rural 91%). Anqing was selected for this study because of the homogeneity of its large population with regard to ethnicity, occupation, diet, and environment and the stable resident population of its villages over several centuries. The prevalence of asthma among children in the Anqing general population is less than 5% (E3).

The study was approved by the Institutional Review Boards of the Brigham and Women's Hospital, the Anhui Medical University Center for Ecogenetics and Disease Control, and the Harvard School of Public Health. Informed consent was obtained from the parents of all participating children younger than 12 years and from all participating children 12 years or older.

Study Population

Families of asthmatic subjects from the eight rural counties of Anqing were originally enrolled in a study of the genetics and epidemiology of asthma through a multistage process described in detail elsewhere (E3). In brief, cases of physician-diagnosed asthma had to meet the following criteria for a family to be eligible: (1) the patient had a history of repeated onset of wheeze with dyspnea but was asymptomatic between two events, and (2) the patient's respiratory symptoms

were relieved by bronchodilator use. Using this definition of asthma, families were ascertained for inclusion in the study using the following criteria: (1) presence of at least two siblings with physician-diagnosed asthma who were 6 years or age or older; (2) availability of both parents; and (3) not more than one parent with physician-diagnosed asthma. The current study population was subsampled from the original study population and comprised all children from these families who were aged between 8 and 18 years and had parasite data available.

Questionnaire

Individual and family histories of respiratory symptoms, respiratory health status, medication use, occupational history, home environment, demographic information, and cigarette smoking were assessed at the interview using a standardized questionnaire (modified American Thoracic Society—Division of Lung Disease).

Helminthic Infection

A history of helminthic infection with *Ascaris lumbricoides* was taken for each subject. Examination of stool specimens for ova and parasites was performed at the Parasitology Laboratory of Anhui Medical University using the Kato-Katz thick-smear technique (E4). Stools were also examined for schistosomiasis, ancylostomiasis, and enterobiasis, however the prevalence of these parasites was too low (< 3%) to be analysed further in this study.

The concordance between a positive history of infection and a positive examination result for *Ascaris* was poor (Cohen's Kappa was 0.02). Therefore, infection with *Ascaris* within each individual was also analysed by use of a variable that was coded as positive if either the history or the examination was positive.

Spirometry and Airways Responsiveness

Spirometry was performed in the sitting position using a noseclip and ATS “Snowbird Guideline” approved equipment (Schiller, Switzerland), calibrated daily. The best forced expiratory volume in one second (FEV₁) was measured according to the guidelines of the American Thoracic Society (E5).

Response to methacholine challenge was assessed using the modified Chatham protocol (E6). Airways responsiveness testing was performed in all subjects whose FEV₁ was > 60% of predicted. Subjects were asked to avoid bronchodilator use for at least 4 hours before testing, unless symptoms required bronchodilator treatment. Airways responsiveness was expressed as the two-point dose-response slope (DRS) of methacholine response against percentage fall in FEV₁ (E7).

Height and weight were measured by standard methods at the time of spirometric assessment after subjects removed their shoes and outerwear. Height was measured to the nearest 0.1 cm on a portable stadiometer. Weight was measured to the nearest 0.1 kg with the subject standing motionless on the scale. Body mass index (BMI) was calculated for each subject as weight (in kg) divided by the squared height in meters.

Total Serum IgE Levels and Allergy Skin Testing

Serum total IgE levels were determined by means of the UniCAP 100 system (Pharmacia & Upjohn, Kalamazoo, MI). Skin testing was performed with a slightly modified version of the semiquantitative puncture method developed by Santilli and coworkers (E8). In addition to histamine and saline controls, the following allergens were applied to the skin of the forearm:

cockroach, house dust, mixed grasses, cured tobacco leaf, mixed trees, polyvalent molds, *Dermatophagoides pteronyssinus*, *Dermatophagoides farinae*, *Artemisia vulgaris*, and mulberry silk waste (unprocessed silk threads that are broken during reeling, henceforth referred to as silk). The silk, *A. vulgaris*, polyvalent molds, and house dust antigens were provided by the Department of Allergy of Peking Union Medical College (Beijing, China); the remaining antigens were purchased from Bayer Corp (Spokane, WA). The preparation of the silk antigen has been described elsewhere (E9). A test was considered positive if the diameter of the skin wheal was ≥ 3 mm after subtraction of the negative control. Skin reactivity was expressed as the sum of the positive skin reactivity tests in each individual.

Statistical Methods

Statistical analysis investigated associations of infection with *Ascaris lumbricoides* with the following outcomes: asthma; number of positive skin tests to aeroallergens; and the DRS to methacholine. Skin test reactivity was also used as an explanatory covariate in certain models. Total serum IgE levels were only available on a subsample of the population ($n = 835$, 39%) and were therefore used in bivariate analyses but not in the multivariate modelling.

For analytical purposes, our primary definition of asthma used a stringent algorithm (E2) based on the presence of all of the following: affirmative answers to the questions “Do you have asthma?” and “Has your asthma been confirmed by a doctor?”; at least two respiratory symptoms (cough, wheezing, dyspnea, or nocturnal cough/wheezing/dyspnea) or a history of recurrent asthma attacks; and airway hyperresponsiveness to methacholine, defined as a $\geq 20\%$ fall in FEV_1 from baseline following administration of ≤ 25 mg of methacholine/ml.

The total serum IgE levels and the DRS were skewed with a long right-hand tail and were $\log_e$ transformed before analysis. A constant was added to each DRS measurement in order to allow $\log_e$ transformation when the measure was ≤ 0 . Asthma, sex, and positive skin test reactivity to each aeroallergen were analysed as binary variables. Cigarette smoking history in parents and children was categorized as: 0 = never smoked; 1 = past smoker; 2 = current smoker. All other variables were analysed as continuous.

Generalised estimating equations (GEE) (E10) were used to investigate the bivariate and multivariate relationships and predictive value of measures of helminthic infection with the outcomes of interest and to appropriately adjust for the familial correlations present in these data. Both forwards and backwards stepwise procedures were used to select a set of independent predictors. The modelling included an investigation of the need for interaction or polynomial terms and examination of the effect of observations with high regression leverage.

Analysis and data management was carried out using STATA v5 (Stata Corporation) and SPlus v4.5 (Mathsoft Inc.). Formal statistical significance was defined at the conventional 5% level.

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