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Original Paper

Ascaris lumbricoids Infection as a Risk Factor for Asthma and Atopy in Rural Bangladeshi Children

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Abstract: Controversy persists as to whether helminth infections cause or protect against asthma and atopy. The aim of this study was to investigate the effects of helminth infection on asthma and atopy among Bangladeshi children. A total of 912 children aged 4.5 years (mean = 54.4, range = 53.5–60.8 months) participated in a cross-sectional study nested into a randomized controlled trial in Bangladesh. Ever-asthma, ever-wheezing and current wheezing were identified using the International Study of Asthma and Allergies in Childhood questionnaire. Current helminth infection was defined by the presence of helminth eggs in stools, measured by routine microscopic examination. Repeated *Ascaris* infection was defined by the presence of anti-*Ascaris* IgE ≥ 0.70 UA/ml in serum measured by the CAP-FEIA method. Atopy was defined by specific IgE to house dust mite (anti-DP IgE) ≥ 0.70 UA/ml measured by the CAP-FEIA method and/or positive skin prick test (≥ 5 mm). Anti-*Ascaris* IgE was significantly associated with ever asthma (odds ratio (OR) = 1.86, 95% CI: 1.14–3.04, highest vs. lowest quartile; *P* for trend 0.016). Anti-*Ascaris* IgE was also significantly associated with positive anti-DP IgE (OR = 9.89, 95% CI: 6.52–15.00, highest vs. lowest; *P* for trend < 0.001) and positive skin prick test (OR = 1.69, 95% CI: 1.01–2.81, highest vs. lowest, *P* for trend 0.076). These findings suggest that repeated *Ascaris* infection is a risk factor for asthma and atopy in rural Bangladeshi children. Further analysis is required to examine the mechanism of developing asthma and atopy in relation to helminth infection.
Key words: pediatric asthma, atopy, IgE, helminth infection, Bangladesh

Introduction

Asthma and allergic diseases pose a serious public health problem in many middle and low-income countries. Asthma is a chronic inflammatory disease of the airways and one of the most common diseases of childhood. Atopy is an increased level of immunoglobulin against common allergens as well as house dust mites. Currently, asthma affects 300 million people worldwide [1]. There are estimates that lifetime prevalence of atopic dermatitis in school children in Western countries is now in the range of 10% to 20%. In the United States, the prevalence of asthma rose from 30.7 per 1000 in 1980 to 53.8 per 1000 in 1993–1994 [2]. In Western countries, serial prevalence studies have shown an increasing trend toward childhood asthma and airway hyper-responsiveness [3]. It has been established that the cause of allergic diseases is multifactorial: the main factors related to the development of allergic diseases having been identified as allergen exposure, pollutant exposure, endotoxin exposure, immunizations, diet, genetic predisposition, and exposure to parasites and viruses [4]. There is now increasing interest in whether helminth infections cause or protect against asthma and atopy. It is estimated that 2 billion people worldwide are currently infected with the intestinal parasites *Ascaris lumbricoids*, *Trichuris trichiura*, hookworm, and *Strongyloides stercor-*

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alis [5]. As many as 150,000 deaths per annum (at an incidence rate of 0.015%) may be attributed to intestinal helminthiasis [6]. Previous studies mentioned the multidimensional relationship between helminth infections and asthma and atopy [7]. However, the association between helminth infection and childhood asthma and atopy remains controversial [8]. In the present study, we examined the effects of helminth infections on asthma and atopy among children in Bangladesh, where repeated helminth infections occur and the prevalence of asthma and atopy is increasing [9, 10].

Materials and Methods

Study area and population

This cross-sectional study was nested into a large-scale randomized clinical trial of nutrition interventions in pregnancy, namely the Maternal and Infant Nutrition Intervention in Matlab (MINIMat) Trial (trial registration: isrctn.org Identifier: ISRCTN16581394) [11]. Matlab is a rural region of Bangladesh located about 50 km southeast of the capital city of Dhaka [12]. The International Centre for Diarrhoeal Disease Research, Bangladesh (icddr,b), has been running a health and demographic surveillance system (HDSS) in the area since 1966 that covers a population of about 220,000. Community health research workers visit every household on a monthly basis to update information on demographic events such as, marriage, pregnancy, birth, death and in- and out-migration, as well as to collect information on the morbidity of children below five years of age and women of childbearing age. Socioeconomic information, including education and household assets, is also recorded by periodic censuses [12]. The details of the MINIMat study have been described elsewhere [11].

Study subjects and design

The 4,436 mothers in the MINIMat trial were followed during pregnancy when data on the socioeconomic status (SES) and morbidity of mothers were collected. Information about their children's gestational age, birth weight, birth length, head circumference and chest circumference were also collected at delivery. Out of these, a total of 3,625 singleton babies were born. A total of 2,745 children were eligible for a follow-up assessment when they reached 4.5 years of age. To reduce the burden of repeated examinations on each child in the multi-component 4.5 year follow-up study, the children were split into two groups according to the year of birth (Group A, May 2002 to April 2003; Group B May 2003 to April 2004). The study component of immunity, asthma and allergic diseases was included in the protocol using Group B children. There was no significant statistical difference between the two groups with regard to basic characteristics of age, sex and socioeconomic status.

Laboratory procedures

Stools from the participants were collected in the morning for helminth egg counts by routine microscopy examination. After the count, the numbers of eggs was categorized into low < 5/high power field (hpf), moderate 5–10/hpf and high > 10/hpf. The stools were also grouped as positive or negative according to the presence or absence of eggs. Moreover, 5 ml of venous blood was collected to measure specific IgE against *Ascaris* and house dust mites (*Dermatophagoides pteronyssinus*) in the serum. The serum samples were separated and kept at −70°C until analysis. Specific IgE levels against *Ascaris* and house dust mites were measured by the CAP-FEIA method (Phadia AB, Uppsala, Sweden). Both anti-*Ascaris* IgE and anti-DP IgE ≥ 0.70 UA/ml were evaluated according to the guidelines provided by the manufacturer. Immediate hypersensitivity reaction was determined by a skin prick test using a mite allergen (*Dermatophagoides pteronyssinus*) following standard procedures (Torii Pharmaceutical Co. Ltd. Tokyo, Japan). Skin reactions were recorded at 20 minutes as the average of the maximum wheel diameters. A swelling size equal to or greater than 5 mm in diameter was considered positive. Atopy was defined as IgE against dust mites ≥ 0.70 UA/ml and/or the presence of positive skin prick test.

Questionnaires

Ever asthma, ever wheezing and current wheezing were identified using the International Study on Asthma and Allergies in Childhood (ISAAC) questionnaire [13]. Ever-asthma was defined as asthma symptoms at any point in the past. Ever-wheezing was defined as wheezing symptoms at any point in the past, while current wheezing was defined as wheezing symptoms within the last 12 months. The written ISAAC questionnaires were translated into Bangla, the national and local language, following the ISAAC protocol. A trained expert in both Bangla and English translated the original ISAAC questionnaire. Then it was back-translated into English by another bilingual expert. The translated questionnaire was pretested and necessary modifications made before data collection. The ISAAC questionnaires were administered by trained interviewers.

Ethical considerations

This study was approved by the research review com-

mittees and ethical review committees of both icddr,b and Uppsala University. Written informed consent was obtained from each child's legal guardian prior to participation in the study.

Statistical analysis

Data were categorized and analyzed using SPSS statistics version 21.0 (IBM Corporation, NY, USA). Descriptive statistics were performed for age, sex, asthma information, specific IgE, skin prick test, history of diarrhea and parasite egg counts. Values were expressed as means, standard deviation (SD), number (n) and percentage (%). Socio-economic status (SES) was estimated using a wealth index based on information about household assets and the related principal component analysis, producing a weighted score [14]. Scores were categorized into quintiles, with category 1 representing the poorest and category 5 the richest. Anti-*Ascaris* IgE was classified into quartiles based on the distribution of the study participants. The linear trends of association with ever-asthma, positive anti-DP IgE and positive skin prick test were tested by an ordinal variable for quartiles of anti-*Ascaris* IgE titre. Univariate logistic regression was performed to examine the association between anti-*Ascaris* IgE, *A. lumbricoids* egg count, *T. trichiura* egg count and asthma and atopy. Finally, multivariate logistic regressions were performed including the available covariates (sex, socioeconomic status, family history of asthma, history of diarrhea, and intervention trial arm) for asthma and atopy. The associations were estimated as odds ratios (ORs) together with a 95% confidence interval (95% CI). Two-sided p-values less than 0.05 were considered statistically significant.

Results

The mean age of children in this cross-sectional study was 54.4 months (range 53.5–60.8 months). Boys accounted for 52.6% of the sample. Ever-asthma was found in 18.0% of the children, current wheezing in 19.7% and ever-wheezing in 45.2%. Family history of asthma was positive for 22.0% of the children. Anti-*Ascaris* IgE was positive for 69.7% and anti-DP IgE was positive for 44.3%. The skin prick test with mite antigen (*Dermatophagoides pteronyssinus*) was positive in 15.7% of the study children (Table 1). To see the intensity of active helminth infection, we considered the details of the helminth egg counts: *A. lumbricoids* egg in stool was 17.4% positive, *T. trichiura* was 17.5% positive, hookworm was scarce at only 0.4% positive. Categorizing the egg counts as low, moderate and high, we found a low level of *Ascaris* eggs in 11.0% of stools, a moderate level of eggs in 5.8%, but a high level in only 0.6%. Most of the *Trichuris* egg counts were at a low level (16.9%), very few at a moderate level (0.6%), and none at a high level (Table 2).

Univariate logistic regression analysis revealed that anti-*Ascaris* IgE was significantly associated with ever-asthma (OR = 1.93, 95% CI: 1.23–2.04, highest compared with lowest quartile; *P* for trend 0.007) but was not associated with either current wheezing (OR = 1.40, 95% CI: 0.91–2.17; highest compared with lowest quartile) or ever-wheezing (OR = 1.04, 95% CI: 0.73–1.48; highest compared with lowest quartile). We also analyzed the

Table 1. Number of cells in pSC or aSC macrochatatete cell lineage from 0–6 h APF

Characteristics	n	%
Age of the children (month)	54.4 ± 0.7*	
Sex (boys)	480	52.6
Ever asthma	164	18.0
Ever wheezing	412	45.2
Current wheezing	180	19.7
Family history of asthma (positive)	201	22.0
Anti-*Ascaris* IgE (positive)	636	69.7
Anti-DP IgE (positive)	404	44.3
Mite antigen skin prick test (positive)	139	15.7
History of diarrhea in last 15 days (yes)	123	13.5
Socioeconomic status (calculated by asset score)		
Poor	148	16.2
Below Middle	187	20.5
Middle	180	19.7
Upper Middle	195	21.4
Wealthy	202	22.1

*Mean ± SD

Table 2. Helminth egg counts of the study participants (n = 890)

	n	%
A. lumbricoids eggs in stool (positive)	155	17.4
Low (< 5/hpf*)	98	11.0
Moderate (5–10/hpf)	52	5.8
High (> 10/hpf)	5	0.6
T. trichiura eggs in stool (positive)	156	17.5
Low (< 5/hpf)	151	16.9
Moderate (5–10/hpf)	5	0.6
High (> 10/hpf)	0	0.0
Hookworm eggs in stool (positive)	4	0.4
Few (< 5/hpf)	3	0.3
Moderate (5–10/hpf)	1	0.1
High (> 10/hpf)	0	0.0

*High power field

Table 3. Unadjusted and adjusted odds ratio with 95% CI interval for ever asthma with sex, stool egg count and anti-*Ascaris* IgE

	Ever asthma			
	Yes n (%)	No n (%)	Unadjusted OR (95% CI)	Adjusted* OR (95% CI)
Sex (n = 912)				
Boys	96 (20.0)	384 (80.0)	1.34 (0.95–1.88)	1.42 (0.98–2.07)
Girls	68 (15.7)	364 (84.3)	1	1
A .lumbricoids eggs (n = 890)				
Positive	24 (15.4)	132 (84.6)	0.78 (0.48–1.25)	0.79 (0.47–1.33)
Negative	139(18.9)	595 (81.1)	1	1
T. trichiura eggs (n = 890)				
Positive	29 (18.6)	127 (81.4)	1.02 (0.65–1.60)	1.19 (0.73–1.94)
Negative	134(18.3)	600 (81.7)	1	1
Anti-*Ascaris* IgE (n = 912)**				
Positive	125(19.7)	511 (80.3)	1.49 (1.00–2.20)	1.34 (0.86–2.09)
Negative	39 (14.1)	237 (85.9)	1	1
Anti-*Ascaris* IgE (n = 912)				
			P for trend = 0.007	*P* for trend = 0.016
4th quartile	55 (24.1)	173 (75.9)	1.93 (1.23–3.04)	1.86 (1.14–3.04)
3rd quartile	39 (17.2)	188 (82.8)	1.26 (0.78–2.04)	1.24 (0.74–2.08)
2nd quartile	31 (17.1)	150 (82.9)	1.25 (0.75–2.10)	1.18 (0.68–2.04)
1st quartile	39 (14.1)	237 (85.9)	1	1

*Adjusted for sex, socioeconomic status, family history of asthma, history of diarrhea and intervention trial arm
**Anti-*Ascaris* IgE ≥ 0.70 UA/ml considered as positive

relationship between ever-asthma and anti-Ascaris IgE as positive/negative and found a significant association in univariate analysis (OR = 1.49, 95% CI: 1.00–2.20) but not after adjustment in multivariate analysis (OR = 1.34, 95% CI: 0.86–2.09). *A. lumbricoids* and *T. trichiura* egg count in stools did not show any significant association with ever-asthma (OR = 0.78, 95% CI: 0.48–1.25) and (OR = 1.02, 95% CI: 0.65–1.60). In the multivariate model, after adjustment for available co-variables, anti-*Ascaris* IgE remained significantly associated with ever-asthma (OR = 1.86, 95% CI: 1.1–3.04, highest compared with lowest quartile; *P* for trend 0.016) (Table 3). We also looked for a correlation between stool egg count and anti-*Ascaris* IgE but did not find any such influence (*P* = 0.21).

We found that anti-*Ascaris* IgE was significantly associated with anti-DP IgE (OR = 9.93, 95% CI: 6.57–15.00, highest compared with lowest quartile; *P* for trend < 0.001) in the univariate model. After adjustment for available co-variables, the relationship between anti-*Ascaris* IgE and anti-DP IgE remained significant (OR = 9.89, 95% CI: 6.52–15.00, highest compared with lowest quartile; *P* for trend < 0.001). We analyzed the relationship between positive anti-DP IgE and anti-*Ascaris* IgE as positive/negative and found it to be significant both in univariate (OR = 5.34, 95% CI: 3.80–7.50) and multivariate (OR = 5.33, 95% CI: 3.77–7.53) analysis. We also found that, compared with girls, boys were significantly associated with anti-DP IgE both in univariate (OR = 1.52, 95% CI: 1.17–1.98) and multivariate analysis (OR = 1.48, 95% CI: 1.11–1.96) (Table 4).

We further tested the association between *Ascaris* egg count and anti-*Ascaris* IgE using the skin prick test and observed that *Ascaris* egg count was not significant in either univariate or multivariate analysis (OR = 1.00, 95% CI: 0.62–1.61) and (OR = 1.01, 95% CI: 0.62–1.65). However, anti-*Ascaris* IgE was significant both in univariate (OR = 1.68, 95% CI: 1.01–2.79, highest compared with lowest quartile, *P* for trend 0.079) and multivariate analysis (OR = 1.69, 95% CI: 1.01–2.81, highest compared with lowest quartile, *P* for trend 0.076). The skin prick test was also significant when tested for an association with anti-*Ascaris* IgE (OR = 1.63, 95% CI: 1.06–2.50) as positive/negative (Table 5). In addition, anti-DP IgE and skin prick test had no significant association with ever-asthma (OR = 0.91, 95% CI: 0.51–1.61) and OR = 1.37, 96% CI: 0.83–2.28).

Table 4. Unadjusted and adjusted odds ratio with 95% CI interval for anti-DP IgE with sex, stool egg count and anti-*Ascaris* IgE

	Positive anti-DP IgE*			
	Yes n (%)	No n (%)	Unadjusted OR (95% CI)	Adjusted** OR (95% CI)
Sex (n = 912)				
Boys	236 (49.2)	244 (50.8)	1.52 (1.17–1.98)	1.48 (1.11–1.96)
Girls	168 (38.9)	264 (61.1)	1	1
A. lumbricoids eggs (n = 890)				
Positive	64 (41.0)	92 (59.0)	0.84 (0.59–1.20)	0.92 (0.63–1.35)
Negative	332 (45.2)	402 (54.8)	1	1
T. trichiura eggs (n = 890)				
Positive	65 (41.7)	91 (58.3)	0.87 (0.62–1.24)	0.96 (0.66–1.41)
Negative	330 (45.0)	404 (55.0)	1	1
Anti-*Ascaris* IgE (n = 912)***				
Positive	352 (55.3)	284 (44.7)	5.34 (3.80–7.50)	5.33 (3.77–7.53)
Negative	52 (18.8)	224 (81.2)	1	1
Anti-*Ascaris* IgE (n = 912)				
			P for trend = <0.001	*P* for trend = <0.001
4th quartile	159 (69.7)	69 (30.3)	9.93 (6.57–15.00)	9.89 (6.52–15.00)
3rd quartile	108 (47.6)	119 (52.4)	3.91 (2.62–5.82)	3.90 (2.61–5.82)
2nd quartile	85 (47.0)	96 (53.0)	3.81 (2.51–5.80)	3.92 (2.57–5.99)
1st quartile	52 (18.8)	224 (81.2)	1	1

*Anti-DP IgE ≥ 0.70 UA/ml considered as positive
**Adjusted for sex, socioeconomic status, family history of asthma, history of diarrhea and intervention trial arm
***Anti-*Ascaris* IgE ≥ 0.70 UA/ml considered as positive

Discussion

The results of the present study implicated anti-*Ascaris* IgE as a possible risk factor for asthma and atopy in children. In particular, ever-asthma and atopy were positively associated with elevated serum anti-*Ascaris* IgE, a finding similar to that of a previous study [15]. But stool egg counts did not show any association. Elevated serum IgE due to helminthes does not necessarily indicate an active infection because it reflects both current and past infections [16, 17], suggesting that repeated *Ascaris* infection, rather than only the presence of infection, is a risk factor for asthma and atopy in children.

Exactly a century ago, Herrick recognized for the first time that helminth infections can trigger asthma [18]. After his work in the early 20th century, very little attention was paid to the potential links between endoparasitic infestations and asthma until the 1970s. Many authors emphasized that further evidence is needed to either support or disprove the hypothesis that parasites cause asthma and allergies. A more recent systematic review and meta-analysis of 30 cross-sectional studies found that an inverse relationship exists between asthma and geohelminths for hookworm infection and that this effect was related to infection intensity. However, *A. lumbricoids* appeared to increase asthma risk while *T. trichiura* exerted no significant effect [19], which is similar to our findings. In our study area, helminth infections are endemic but the intensity of infections is not particularly high, indicating that low intensity but continuous presence of infection might cause allergic sensitization. In our study, we found that ever-asthma was associated with *Ascaris* infection but not current wheezing or ever-wheezing. It is postulated that wheezing symptoms may be misinterpreted by parents. Wheezing in children is usually related to lower respiratory tract infections which are caused by common respiratory viral or bacterial infections. Asthma, on the other hand, is a more specific disease and is usually diagnosed by medical personnel.

Previous studies have provided conflicting evidence showing that helminth infection inhibits [20, 21], causes [22] or is unrelated to asthma and atopic diseases [23, 24]. The role of anti-*Ascaris* IgE in the development of asthma is not clear. One possible explanation for the relationship is that elevated anti-*Ascaris* IgE levels are associated with larval migration after re-infection, as *Ascaris* migrates

Table 5. Unadjusted and adjusted odds ratio with 95% CI interval for skin prick test for *D. pteronyssinus* antigen with sex, stool egg count and anti-*Ascaris* IgE

	Positive skin prick test*			
	Yes n (%)	No n (%)	Unadjusted OR (95% CI)	Adjusted** OR (95% CI)
Sex (n = 886)				
Boys	75 (16.2)	389 (83.8)	1.08 (0.75–1.55)	1.08 (0.75–1.55)
Girls	64 (15.2)	358 (84.8)	1	1
A. lumbricoids eggs (n = 769)				
Positive	11 (15.5)	604 (84.5)	1.00 (0.62–1.62)	1.01 (0.62–1.65)
Negative	24 (15.6)	130 (84.4)	1	1
T. trichiura eggs (n = 869)				
Positive	114 (15.9)	603 (84.1)	0.89 (0.55–1.47)	0.89 (0.54–1.50)
Negative	22 (14.5)	130 (85.5)	1	1
Anti-*Ascaris* IgE (n = 886)***				
Positive	108 (17.5)	510 (82.5)	1.62 (1.05–2.48)	1.63 (1.06–2.50)
Negative	31 (11.6)	237 (88.4)	1	1
Anti-*Ascaris* IgE (n = 886)				
			P for trend = 0.079	*P* for trend = 0.076
4th quartile	40 (18.0)	182 (82.0)	1.68 (1.01–2.79)	1.69 (1.01–2.81)
3rd quartile	35 (16.1)	183 (83.9)	1.46 (0.87–2.46)	1.46 (0.89–2.46)
2nd quartile	33 (18.5)	145 (81.5)	1.74 (1.02–2.96)	1.76 (1.03–2.30)
1st quartile	31 (11.6)	237 (88.4)	1	1

*Skin prick test ≥ 5 mm considered as positive
**Adjusted for sex, socioeconomic status, family history of asthma, history of diarrhea and intervention trial arm
***Anti-*Ascaris* IgE ≥ 0.70 UA/ml considered as positive

through the lungs during maturation and causes pulmonary infiltrates of Th2 immunity and episodic airway obstruction associated with wheezing [25]. It is postulated that, due to the high prevalence of infection in this area, repeated *Ascaris* infections and larval migration could increase the risk of asthma symptoms. Another explanation is that anti-*Ascaris* IgE acts as IgE specific to common inhaled aero-antigens directly triggering mast cell activation [26]. This finding is supported by two other studies in which researchers who handled helminthes suffered from wheezing [27] and fisherman who suffered from wheezing caused by IgE-mediated hypersensitivity to common earthworms [28]. The third explanation is that the higher anti-*Ascaris* IgE levels in the wheezing group simply mean that atopic children produce more anti-*Ascaris* IgE in response to *Ascaris* infection [29]. Another explanation is that the human immune response to helminth infections is associated with elevated levels of IgE, tissue eosinophilia and mastocytosis and the presence of CD4+ T cells that preferentially produce IL-4, IL-5 and IL-13 [30]. Still another explanation is that parasites in the tissues stimulate a strong localized Th2 response, characterized by an eosinophil-rich inflammatory infiltrate [31].

Our study confirms the existence of an association between atopy and repeated *Ascaris* infections shown by anti-*Ascaris* IgE. The intriguing "Hygiene Theory" suggests that exposure to infection, or lack of exposure, produces the conditions necessary for the development of atopy. On the one hand, because of a lack of bacterial infections (or lack of viral infections because of immunization), lower levels of IL-12 result in decreased Th1 stimulation which allows the bias of the immune system toward Th2 to be realized. On the other hand, exposure to parasites may influence Th1 dominance [32, 33]. In 2001, the World Health Assembly passed a resolution urging member states to control the morbidity of soil–transmitted helminth infections through large-scale use of anthelmintic drugs among school-aged children in less developed countries [34]. Following that resolution, deworming medication was initiated in the study area once in every six month interval among children from two to five years old in 2004. Some earlier studies indicated that deworming may increase the IgE level, but a recent study from Cuba indicated that deworming is not a risk factor for atopic disea-

ses [35].

We found that boys were more prone than girls to increased anti-DP IgE, which is similar to a previous report [36]. Population-based large birth cohort studies showed that total IgE and allergen-specific IgE concentrations varied by gender [36, 37]. The observation that total IgE increases more rapidly in boys than in girls during early childhood was consistent with the many previous reports of a higher prevalence of asthma among boys than girls prior to puberty [38]. These gender differences in IgE development were related to the differences in risk factors associated with asthma and differences in the prevalence of asthma between boys and girls.

In our study, the results of the skin prick test were related to anti-*Ascaris* IgE but not to helminth egg count. The relationship of the test results to helminth infections may depend on the intensity and chronicity of helminth infections. Thus the lack of an effect of present worm infection on skin prick test reactivity in our study and those of Palmer et al. [22] and Cooper et al. [24] might be due to the lower intensity as compared to that in which skin prick test reactivity was inversely related to helminthiases [32]. The suppression of skin prick test reactivity to aeroallergens by geohelminth infection is postulated on the basis of a biological model in which helminth infections actively inhibit allergic effector responses including immediate hypersensitivity [33]. In our study there was no association between asthma and atopy, which is similar to a previous Latin American study on children and adolescents in underprivileged populations [39], indicating that the asthma in our study area is the non-atopic type. The association between asthma and atopic sensitization increases with economic development, a trend evidenced in industrialized countries. In the ISAAC Phase II study, the population fraction of asthma attributable (PAF) to atopy measured by the skin prick test was 41% in affluent countries but only 20% in non-affluent countries among children aged 8–12 years [40]. Moreover, in the two Latin American study centers included in the ISAAC Phase II study (Pichincha Province, Ecuador and Uruguaiana, Brazil), only 11% of asthma was attributable to atopy measured by the skin prick test, while a study of children of the same age living in rural Esmeraldas Province in Ecuador showed that only 2.4% of asthma was attributable to atopy measured by the same test. The weak association between asthma and atopy in those populations could be explained by the attenuation of atopy or Th2-mediated allergic responses by environmental factors including chronic helminth infections [41].

One strength of the current cross-sectional study is the relatively large sample size, which promotes confidence in the results reported. Other strengths are the compact age group of study participants and the unique demographic and socioeconomic statuses. However, there were also several limitations to the present study. First, it was a cross-sectional study; therefore, the data did not provide direct information as to whether *Ascaris* infection is a cause of the development of asthma and atopy. Second, we could not do lung function tests, which provide stronger evidence for the diagnosis of asthma. Another weakness is that we used a questionnaire based on the ISAAC to diagnose wheezing and asthma. The term wheezing is often misinterpreted by parents and this may produce overestimation or underestimation of the symptoms; however, asthma is a more specific disease and usually diagnosed by medical personnel. On the other hand, the ISAAC questionnaire has been used worldwide and has reportedly provided a reliable estimation of the prevalence of asthma in children 2–6 years of age.

In conclusion, our data suggest that *Ascaris* infection is a risk factor for asthma and atopy in rural Bangladeshi children. Further analysis is required to examine the exact mechanism of high levels of anti-*Ascaris* IgE and asthma and atopic responses in countries with low-income populations, repeated helminth infections and an increasing prevalence of asthma and atopic disease.

Conflicts of Interest

The authors declare no conflicts of interest.

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References

1. Braman SS. The global burden of asthma. Chest 2006; 130: 4S–12S.
2. Greer FR, Sicherer SH, Burks AW; American Academy of Pediatrics Commitee on Nutrition, American Academy of Pediatrics Section on Allergy and Immunology. Effects of early nutritional interventions on the development of atopic disease in infants and children: the role of maternal dietary restriction, breastfeeding, timing of introduction of complementary foods, and hydrolyzed formulas. Pediatrics 2008; 121: 183–191.
3. Thygarajan A, Burks AW. American Academy of Pediatrics recommendations on the effects of early nutritional interventions on the development of atopic disease. Curr Opin Pediatr 2008; 20: 698–702.
4. Matsui EC, Matsui W. Higher serum folate levels are associated with a lower risk of atopy and wheeze. J Allergy Clin Immunol 2009; 123: 1253–1259.
5. Santos AB, Rocha GM, Oliver C, Ferriani VP, Lima RC, Palma MS, Sales VS, Aalberse RC, Chapman MD, Arruda LK. Cross-reactive IgE antibody responses to tropomyosins from *Ascaris lumbricoids* and cockroach. J Allergy Clin Immunol 2008; 121: 1040–1046.
6. Bundy DAP. New initiatives in the control of helminths. Trans Roy Soc Trop Med Hyg 1990; 84: 467–468.
7. Takeuchi H, Zaman K, Takahashi J, Yunus M, Chowdhury HR, Arifeen SE, Baqui A, Wakai S, Iwata T. High titre of anti-*Ascaris* immunoglobulin E associated with bronchial asthma symptoms in 5-year-old rural Bangladeshi children. Clin Exp Allergy 2008; 38: 276–282.
8. Pinelli E, Willers SM, Hoek D, Smit HA, Kortbeek LM, Hoekstra M, de Jongste J, van Knapen F, Postma D, Kerkhof M, Aalberse R, van der Giessen JW, Brunekreef B. Prevalence of antibodies against *Ascaris* and its association with allergic manifestations in 4-year-old children in The Netherlands: the PIAMA birth cohort study. Eur J Clin Microbiol Infect Dis 2009; 28: 1327–1334.
9. Hasan MR, Kabir AR, Mahmud AM, Rahman F, Hossain MA, Bennoor KS, Amin MR, Rahman MM. Self-reported asthma symptoms in children and adult of Bangladesh: findings of the National Asthma Control Study. Int J Epidemiol 2002; 31: 483–488.
10. Zaman K, Takeuchi H, Md Yunus, El Arifeen S, Chowdhury HR, Baqui AH, Wakai S, Iwata T. Asthma in rural Bangladeshi children. Indian J Pediatr 2007; 74: 539–543.
11. Persson LÅ, Arifeen SE, Ekström EC, Rasmussen KM, Frongillo EA, Yunus M; MINIMat Study Team. Effects of Prenatal Micronutrient and Early Food Supplementation on Maternal Hemoglobin, Birth Weight, and Infant Mortality among Children in Bangladesh: the MINIMat randomized trial. JAMA 2012; 307: 2050–2059.
12. Razzaque A, Streatfield PK. INDEPTH Monograph: Volume 1 Part C. Matlab DSS, Bangladesh: 2000.
13. Leung R, Wong G, Lau J, Ho A, Chan JK, Choy D, Douglass C, Lai CK. Prevalence of asthma and allergy in Hong Kong schoolchildren: an ISAAC study. Eur Respir J 1997; 10: 354–360.
14. Davidson RG, Shea R, Kiersten J, Pande RP, Adam W. Socioeconomic Differences in Health, Nutrition, and Population in Bangladesh. May 2000; Available from: http://siteresources.worldbank.org/INTPAH/Resources/Publications/Country-Reports/bangladesh.pdf. (Downloaded on March 2012).
15. Rîpă C, Bahnea RG, Cojocaru I, Luca MC, Ivan A, Luca M. Atopic diseases and intestinal helminth infections. Rev Med Chir Soc Med Nat Iasi 2010; 114: 1017–1021.
16. Dold S, Heinrich J, Wichmann HE, Wjst M. *Ascaris*-specific IgE and allergic sensitization in a cohort of school children in former East Germany. J Allergy Clin Immunol 1998; 102: 414–420.
17. Obihara CC, Beyers N, Gie RP, Hoekstra MO, Fincham JE, Marais BJ, Lombard CJ, Dini LA, Kimpen JL. Respiratory atopic disease, *Ascaris*-immunoglobulin E and tuberculin testing in urban South African children. Clin Exp Allergy 2006; 36: 640–648.
18. Herrick WW. Experimental eosinophilia with an extract of an animal parasite: its relation to anaphylaxis and certain clinical features. Arch Intern Med 1913; 11: 163–186.
19. Leonardi-Bee J, Pritchard D, Britton J. Asthma and current intestinal parasites infection. Systematic review and meta-analysis. Am J Respir Crit Care Med 2006; 174: 514–523.
20. Scrivener S, Yemaneberhan H, Zebenigus M, Tilahun D, Girma S, Ali S, McElroy P, Custovic A, Woodcock A, Pritchard D, Venn A, Britton J. Independent effects of intestinal parasite infection and domestic allergen exposure on risk of wheeze in Ethiopia: a nested case-control study. Lancet 2001; 358: 1493–1499.
21. Cooper PJ, Chico ME, Rodrigues LC, Strachan DP, Anderson HR, Rodriguez EA, Gaus DP, Griffin GE. Risk factors for atopy among school children in a rural area of Latin America. Clin Exp Allergy 2004; 34: 845–852.
22. Palmer LJ, Celedon JC, Weiss ST, Wang B, Fang Z, Xu X. *A lumbricoids* infection is associated with increased risk of childhood asthma and atopy in rural China. Am J Respir Crit Care Med 2002; 165: 1489–1493.
23. Sharghi N, Schantz PM, Caramico L, Ballas K, Teague B, Hotez PJ. Environmental exposure to Toxocara as a possible risk factor for asthma: a clinical-based case-control study. Clin Infect Dis 2001; 32: 111–116.
24. Cooper PJ, Chico ME, Vaca MG, Moncayo AL, Bland JM, Mafla E, Sanchez F, Rodrigues LC, Strachan DP, Griffn GE. Effect of albendazole treatments on the prevalence of atopy in children living in communities endemic for geohelminths parasites: a cluster randomized trial. Lancet 2006; 367: 1598–1603.
25. Spillman RK. Pulmonary *Ascariasis* in tropical communities. Am J Trop Med Hyg 1975; 24: 791–800.
26. Lynch NR, Isturiz G, Sanchez Y, Perez M, Martinez A,

Caster M. Bronchial challenge of tropical asthmatics with *Ascaris lumbricoids*. J Investing Allergol Clin Immunol 1992; 2: 97–105.
27. Jarrett EE, Miller HR. Production and activities of IgE in helminth infection. Prog Allergy 1982; 32: 178–233.
28. Porcel SL, Camara C, Rodriguez A, Fletes C, Jiménez S, Rodríguez E, Alvarado M, Hernández J, Pereira L, Cobo R. IgE mediated hypersensitivity to common earthworm. Characterization of allergies involved. Allergy and Clinical Immunology International. J World Allergy Org 2005; 17: 246–248.
29. Lynch NR, Hagel IA, Palenque ME, Di Prisco MC, Escudero JE, Corao LA, Sandia JA, Ferreira LJ, Botto C, Perez M, Le Souef PN. Relationship between helminth infection and IgE response in atopic and non-atopic children in a tropical environment. J Allergy Clin Immunol 1998; 101: 217–221.
30. Fallon PG, Mangan NE. Suppression of Th2-type allergic reactions by helminth reactions. Nature Rev Immunol 2007; 7: 220–230.
31. Wilson MS, Mentink-Kane MM, Pesce JT, Ramalingam TR, Thompson R, Wynn TA. Immunopathology of schistosomiasis. Immunol Cell Biol 2007; 85: 148–154.
32. Perzanowski MS, Ng'ang'a LW, Carter MC, Odhiambo J, Ngari P, Vaughan JW, Chapman MD, Kennedy MW, Platts-Mills TA. Atopy, asthma, and antibodies to Ascaris among rural and urban children in Kenya. J Pediatr 2002; 140: 582–588.
33. van den Biggelaar AH, Lopuhaa C, van Ree R, van der Zee JS, Jans J, Hoek A, Migombet B, Borrmann S, Luckner D, Kremsner PG, Yazdanbakhsh M. The prevalence of parasite infestation and house dust mite sensitization in Gabonese school children. Int Arch Allergy Immunol 2001; 126: 231–238.
34. Horton J. Global anthelmintic chemotherapy programs: learning from history. Trends Parasitol 2003; 19: 405–409.
35. van der Werff SD, Twisk JW, Wördemann M, Campos Ponce M, Díaz R, Junco Núñez FA, Rojas Rivero L, Bonet Gorbea M, Polman K. Deworming is not a risk factor for the development of atopic diseases: a longitudinal study in Cuban school children. Clin Exp Allergy 2013; 43: 665–671.
36. Johnson CC, Peterson EL, Ownby DR. Gender differences in total and allergen-specific immunoglobulin E (IgE) concentrations in a population-based cohort from birth to age four years. Am J Epidemiol 1998; 147: 1145–1152.
37. Nickel R, Illi S, Lau S, Sommerfeld C, Bergmann R, Kamin W, Forster J, Schuster A, Niggemann B, Wahn U; German Multicenter Allergy Study Group (MAS-90). Variability of total serum immunoglobulin E levels from birth to the age of 10 years. A prospective evaluation in a large birth cohort (German Multicenter Allergy Study). Clin Exp Allergy 2005; 35: 619–623.
38. Rancière F, Nikasinovic L, Bousquet J, Momas I. Onset and persistence of respiratory/allergic symptoms in preschoolers: new insights from the PARIS birth cohort. Allergy 2013; 68(9): 1158–1167.
39. Pereira MU, Sly PD, Pitrez PM, Jones MH, Escouto D, Dias AC, Weiland SK, Stein RT. Nonatopic asthma is associated with helminth infections and bronchiolitis in poor children. Eur Respir J 2007; 29: 1154–1160.
40. Weinmayr G, Weiland SK, Björkstén B, Brunekreef B, Büchele G, Cookson WO, Garcia-Marcos L, Gotua M, Gratziou C, van Hage M, von Mutius E, Riikjärv MA, Rzehak P, Stein RT, Strachan DP, Tsanakas J, Wickens K, Wong GW; ISAAC Phase Two Study Group. Atopic sensitization and the international variation of asthma symptom prevalence in children. Am J Respir Crit Care Med 2007; 176: 565–574.
41. Moncayo AL, Vaca M, Oviedo G, Workman LJ, Chico ME, Platts-Mills TA, Rodrigues LC, Barreto ML, Cooper PJ. Effects of geohelminth infection and age on the associations between allergen-specific IgE, skin test reactivity and wheeze: a case-control study. Clin Exp Allergy 2013; 43: 60–72.