Anti-*Ascaris* IgE as a Risk Factor for Asthma Symptoms among 5-Year-Old Children in Rural Bangladesh with Even Decreased *Ascaris* Infection Prevalence

Haruko Takeuchi^a Sayaka Takanashi^b S.M. Tafsir Hasan^c Samar Kumar Hore^d
Sultana Yeasmin^c Shaikh Meshbahuddin Ahmad^e Md Jahangir Alam^e
Masamine Jimba^a Md Alfazal Khan^c Tsutomu Iwata^f

^aDepartment of Community and Global Health, Graduate School of Medicine, The University of Tokyo, Tokyo, Japan; ^bDepartment of Developmental Medical Sciences, Graduate School of Medicine, The University of Tokyo, Tokyo, Japan; ^cNutrition and Clinical Services Division, icddr,b (International Centre for Diarrhoeal Disease Research, Bangladesh), Dhaka, Bangladesh; ^dCentre for Equity and Health System, icddr,b, Dhaka, Bangladesh; ^eInfectious Diseases Division, icddr,b, Dhaka, Bangladesh; ^fDepartment of Education for Childcare, Faculty of Child Studies, Tokyo Kasei University, Sayama, Japan

Keywords

Anti-*Ascaris* IgE · *Ascaris* infection prevalence · Asthma symptoms · Bangladesh · Children

Abstract

Background: Epidemiological studies have indicated that anti-*Ascaris* IgE enhances asthma and allergies under specific conditions although the association between them is still controversial. The association of anti-*Ascaris* IgE with increased asthma symptoms among children from a general population with a mild to moderate *Ascaris* infection prevalence was investigated. **Methods:** A total of 126 children aged 5 years with wheezing during the previous year and 110 children who did not have wheezing were selected randomly from the rural service area of the International Centre for Diarrhoeal Disease Research, Bangladesh. Serum levels of total, anti-*Ascaris*, anti-*Dermatophagoides pteronyssinus*, and anti-cockroach IgEs were tested, and their risks for

wheezing were analyzed. The wheezing children were then classified by hierarchical cluster analysis to investigate the contribution of anti-*Ascaris* IgE to wheezing. **Results:** The anti-*Ascaris* IgE levels in wheezing and never-wheezing children were 1.07 and 0.65 U_A/mL, and it contributed to 11% of wheezing in children. Anti-*Ascaris* IgE was significantly associated with wheezing (odds ratio [OR] per log_e increment: 1.37 [95% CI: 1.01–1.87], $p = 0.046$). The ORs, which were adjusted for sex, parental asthma, pneumonia history, helminth infections, *Haemophilus influenzae* type B combination vaccination, antibiotic use during infancy, and total and specific IgE levels, increased even when only children with more specific symptoms of asthma were included in the analysis. Namely, the ORs for wheezing with sleep disturbance, four or more attacks, and wheezing with speech

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difficulties during the previous 1 year were $OR = 1.44/\log_e$ increment [95% CI: 1.01–2.07], $OR = 1.90/\log_e$ increment [95% CI: 1.11–3.25], and $OR = 1.78/\log_e$ increment [95% CI: 1.01–3.14], respectively. **Conclusions:** The anti-*Ascaris* IgE levels in wheezing and never-wheezing children in the current study significantly decreased concurrently with *Ascaris* infection prevalence compared with their corresponding values in 2001. The contribution of anti-*Ascaris* IgE to wheezing also dropped from 26% in 2001 to 11% in the current study. Despite significant decreases in the levels and the seroprevalence and its contribution to wheezing, anti-*Ascaris* IgE remained significantly associated with increased risk of wheezing. Anti-*Ascaris* IgE significantly increased the risk of wheezing in a general population with a mild to moderate *Ascaris* infection prevalence, suggesting robustness as a risk factor and a possible dose-response relationship.

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Introduction

The main precipitating factors for asthma during childhood are atopy and lower respiratory tract infections [1]. Some helminth infections, especially *Ascaris* species, have also been reported to be associated with an increased risk of wheezing [2–4]. However, the relationship between helminth infection and asthma is controversial. Helminthic infections increased wheezing [2–7], suppressed wheezing [6, 8–10], or no effects are found [11]. Generally, the association between helminths and asthma appears to be more suppressive [6, 8–10].

The prevalence of asthma has increased rapidly in high-income countries since the 1970s with a higher incidence among children living in urban and industrial areas than in those living in rural areas [12–16]. These findings brought about the hypothesis that helminthic infections protect against asthma and allergies by suppressing the host immune response. Some studies have shown that atopy is suppressed by the presence of chronic helminthic infection [17] and that helminths suppress wheezing [6, 8–10]. Among the various helminths, the suppressive function has been reported most in relation to schistosomiasis. *Schistosoma* and filaria seem to be associated with a milder course of asthma [8, 17] by enhancing IL-10 production and activating regulatory T cells [17, 18].

However, the suppressive association has rarely been documented in the context of ascariasis. A systematic review and meta-analysis of 22 studies revealed an association between *Ascaris* infection and wheezing [3]. Another systematic review of studies conducted in Latin America

also revealed a higher risk of asthma and wheezing in patients with *Ascaris* infections [4]. In studies conducted in China and South Africa, *Ascaris* infection was found to increase the risk of airway hyperreactivity (AHR), which is a hallmark clinical symptom of asthma [2, 19]. It was also assumed that mild to moderate *Ascaris* infection accelerated allergic responses to environmental aeroallergens, while moderate to severe infections were protective against asthma.

However, epidemiological studies conducted among 5-year-old rural Bangladeshi children in 2001 and 2016 found neither a protective nor an enhancing association between wheezing and *Ascaris* infection [20, 21]. Remarkably, these studies found that wheezing prevalence decreased significantly from 16.1% in 2001 to 8.7% in 2016, despite a significant decrease in the prevalence of *Ascaris* infection from 76% in 2001 to 18% in 2016. Nevertheless, those studies found that *Ascaris* infection neither protected against wheezing nor increased the risk of wheezing.

In 2001, anti-*Ascaris* immunoglobulin E (IgE) was found to increase the risk of childhood wheezing in a rural area of Bangladesh, where the prevalence of *Ascaris* infection was more than 75% [20]. Furthermore, in 2005, anti-*Ascaris* IgE was found to increase the risk of childhood AHR in the same rural area [22]. Thus, anti-*Ascaris* IgE, but not *Ascaris* infection itself, was a risk factor for wheezing in an area where *Ascaris* infection prevalence was high. This is despite the general assumption that mild to moderate *Ascaris* infection increased allergic responses to environmental aeroallergens and that moderate to severe infections were protective against asthma [8].

In a study conducted in 2001, wheezing participants were classified into three distinct categories, and 26% of wheezing was associated with anti-*Ascaris* IgE [23]. Sixteen percent was due to a history of pneumonia during young childhood, and the other 58% might have been attributable to innate immunity to *Ascaris* infection [23]. These findings further demonstrate that anti-*Ascaris* IgE increases the risk of wheezing. The anti-parasitic effect of IgE antibodies against helminths is thought to be a normal component of host protection during infection [24–28], and these antibodies do not usually play a role in allergic symptoms. However, allergic manifestations have been demonstrated in ascariasis and anisakiasis [26].

These results were later supported by another study showing that anti-*Ascaris* IgE increased the risk of current wheezing in an atopic population with a mild to moderate *Ascaris* infection prevalence [29]. Furthermore, anti-*Ascaris* IgE also increased the risk of current

Fig. 1. Flowchart of the sampling procedure.

wheezing among children in a general population in an area with a moderate to high *Ascaris* infection prevalence [30]. In addition, anti-*Ascaris* IgE increased the risk of wheezing in a population whose mothers received additional caloric intake during pregnancy in an area with a mild to moderate *Ascaris* infection prevalence [31]. However, no study has investigated anti-*Ascaris* IgE as a risk factor for current wheezing in the general population of an area with a mild to moderate *Ascaris* infection prevalence.

Therefore, this study aimed to investigate whether anti-*Ascaris* IgE increases the risk of wheezing in a general population in an area with a mild to moderate *Ascaris* in-

fection prevalence in rural Bangladesh. The study also analyzed the contribution of anti-*Ascaris* IgE to wheezing in children with wheezing.

Methods

Study Site and Participants

This study was conducted from December 2015 to October 2016 in Matlab in rural Bangladesh. The International Centre for Diarrhoeal Disease Research, Bangladesh (icddr,b), has been maintaining a health and demographic surveillance system that consists of regular cross-sectional censuses and the longitudinal registration of vital events since 1966 [32]. The details of the study site and the procedures used for data collection are described else-

where [21]. In brief, 1,800 5-year-old children were randomly selected from all the 67 villages of the icddr,b service area and were asked to participate in the study. A total of 1,658 children responded to the questionnaire of the International Study of Asthma and Allergies in Childhood. We identified 145 (8.7%) children with wheezing and 1,154 (69.8%) children who had never experienced wheezing. For this case-control study, only children with wheezing and those who had never experienced wheezing were included. The remaining 356 children ever experienced wheezing and were excluded from the analysis. After identifying the children with wheezing, we randomly selected an equal number of children as controls who had never experienced wheezing. Then, the contributing factors to wheezing were analyzed on the wheezing children (shown in Fig. 1). Five-year-old children were chosen as the participants since the present study aimed to investigate the effect of anti-*Ascaris* IgE on childhood wheezing and tried to compare the result with our previous study conducted on 5-year-old children in the same area.

Data Collection

Field data were collected as described elsewhere [21]. In brief, written informed consent and information on wheezing, family history of allergy, and socioeconomic status were collected by trained local field research assistants. Wheezing was defined as any episode of wheezing or whistling in the chest 1 year before the interview. Children who had never experienced an episode of wheezing or whistling in the chest were grouped as never-wheezing children. Information on the participants' history of pneumonia was collected from the Matlab Hospital database. Children brought 50 g of stool samples from home, and 7 mL of venous blood was collected from each child at the hospital.

Fresh stool specimens were analyzed as described elsewhere [21]. The FEIA method (Pharmacia KK, Tokyo, Japan) was employed to measure serum IgE levels. The CAP-FEIA System (Pharmacia KK) was used to measure anti-*Dermatophagoides pteronyssinus* (*Der p*), anti-cockroach, and anti-*Ascaris lumbricoides* IgEs.

Statistical Analysis

Data were analyzed using IBM SPSS Statistics, version 26 (IBM Japan, Tokyo, Japan). The sample size was calculated by assuming that wheezing would be experienced by at least 16% of children aged 60–71 months [20]. We calculated that 209 children in each group would provide 80% power to detect a difference in the log serum levels of anti-*Ascaris* IgE of 1.8 U_A/mL (SD 1.5) and 1.4 U_A/mL (SD 1.4) with a two-sided type 1 error of 5%. To achieve this, we had to approach 240 wheezing and 240 never-wheezing children, assuming a 15% rate of refusal or absences. We approached 1,800 individuals with a 20% loss to obtain the required number of wheezing children.

This study was a nested case-control study, and the independent variables were between subjects. First, we compared the prevalence of wheezing and other asthma symptoms reported in 2001 and 2016. After the following descriptive analysis of the wheezing and never-wheezing participants, continuous variables, such as height, total IgE, anti-*Ascaris* IgE, anti-*Der p* IgE, and anti-cockroach IgE, were compared using a *t* test, and categorical variables were compared using a χ^2 test. The significance level for all statistical analyses was set at $p < 0.05$.

Then, we calculated and compared the geometric mean levels of total and specific IgE levels and in the children also with more

specific symptoms of asthma. Then, we calculated the odds ratios (ORs) for wheezing of the independent variables by logistic regression analysis with the wheezing status as the outcome variable. Subsequently, the ORs of total and specific IgE levels were recalculated after excluding children with less specific symptoms of asthma.

Finally, cluster analysis was performed using Ward's hierarchical clustering method. The variables were standardized to equalize the standard deviations of the variables' scales. To compare differences among the clusters, analysis of variance without the following analysis was used for continuous variables, and the χ^2 test was used for categorical variables.

Of the independent variables between the wheezing and never-wheezing participants in the first descriptive analyses, height and weight, total IgE, anti-*Ascaris* IgE, anti-*Der p* IgE, anti-cockroach IgE, and monthly household income were continuous variables, and sex, helminths infections, history of pneumonia, parental asthma, history of vaccination, and antibiotic use during infancy were categorical variables. The distribution of the independent variables in each dependent group was checked to see the normality of the data. The analysis of linearity of the data was not necessary, since the dependent variable was a dichotomous variable. The correlation coefficient of the independent continuous variables was checked to see the collinearity. The IgE data were log transformed since they were skewed to the right or bimodal. The out layers were not excluded from the analysis, and the samples with missing data were excluded from the analysis.

The continuous variables between the two groups were compared using a two-tailed *t* test, and categorical variables were compared using a both-sided χ^2 test or Fisher exact test. The forced entry method was used in the logistic regression models to calculate the ORs for the risk factors. The included covariates were selected when the difference between the two outcome groups was significant ($p < 0.05$) in the first descriptive analyses or known as risk factors for wheezing. The collinearity was assessed by calculating the variance inflation factor.

Results

Anti-Ascaris IgE as a Risk Factor for Wheezing

Blood and stool samples were obtained from 127 (88%) and 114 (79%) wheezing and never-wheezing children, respectively. Whole data were available for 126 wheezing and 110 never-wheezing children, who constituted the study population. The datasets used for the analyses are in the online supplementary Datasets 1–5 (see www.karger.com/doi/10.1159/000521717 for all online suppl. material).

Anti-*Ascaris* IgE significantly increased the risk of wheezing (crude OR = 1.24/log_e increment [95% CI: 1.04–1.47]) (Table 1). The OR for wheezing in relation to anti-*Ascaris* IgE increased when only the children showing more specific symptoms of asthma, such as sleep disturbance, four or more attacks, and speech difficulties during the previous 1 year, were included as wheezers

Table 1. Odds ratios for current wheezing relative to the total, anti-*Ascaris*, and anti-DP IgE including when children with less specific asthma symptoms were excluded

	Wheezers/ nonwheezers	Units of change	Crude odds ratio (95% CI)	<i>p</i> value	<i>N</i>	Adjusted odds ratio (95% CI)	<i>p</i> value
Anti- <i>Ascaris</i> IgE (log _e transformed)							
Current wheezing	126/110	1	1.24 (1.04–1.47)	0.016	231	1.37 (1.01–1.87)	0.046
Sleep disturbance [†]	85/110	1	1.25 (1.04–1.51)	0.018	190	1.44 (1.01–2.07)	0.046
≥4 attacks [‡]	33/110	1	1.36 (1.30–1.80)	0.030	141	1.90 (1.11–3.25)	0.019
Speech disturbance [§]	29/110	1	1.44 (1.06–1.94)	0.018	136	1.78 (1.01–3.14)	0.048
Total IgE (log _e transformed)							
Current wheezing	125/108	1	1.13 (0.90–1.42)	0.311	231	0.86 (0.57–1.29)	0.467
Sleep disturbance [†]	84/108	1	1.14 (0.89–1.47)	0.301	190	0.87 (0.54–1.39)	0.554
≥4 attacks [‡]	33/108	1	1.23 (0.88–1.73)	0.233	141	0.63 (0.33–1.20)	0.160
Speech disturbance [§]	28/108	1	1.39 (0.95–2.04)	0.094	136	0.85 (0.39–1.84)	0.681
Anti-DP IgE (log _e transformed) ^{††}							
Current wheezing	126/110	1	1.03 (0.88–1.20)	0.747	231	0.93 (0.69–1.24)	0.611
Sleep disturbance [†]	85/110	1	1.05 (0.88–1.25)	0.583	190	0.93 (0.65–1.32)	0.673
≥4 attacks [‡]	33/110	1	1.13 (0.90–1.41)	0.302	141	0.90 (0.58–1.39)	0.627
Speech disturbance [§]	29/110	1	1.18 (0.93–1.50)	0.166	136	0.96 (0.57–1.60)	0.868
Anti-cockroach IgE (log _e transformed) ^{††}							
Current wheezing	125/108	1	1.00 (0.86–1.17)	0.959	231	0.98 (0.73–1.33)	0.915
Sleep disturbance [†]	84/108	1	1.01 (0.85–1.19)	0.931	190	0.96 (0.67–1.36)	0.806
≥4 attacks [‡]	33/108	1	1.11 (0.89–1.38)	0.362	141	1.09 (0.68–1.73)	0.723
Speech disturbance [§]	28/110	1	1.14 (0.89–1.44)	0.302	136	1.00 (0.61–1.6)	0.998

IgE, immunoglobulin E; 95% CI, 95% confidence interval; OR, odds ratio; Anti-DP, anti-*Dermatophagoides pteronyssinus*. [†] Individuals without sleep disturbance during the previous 1 year were excluded from the analysis. [‡] Individuals with <4 wheezing attacks during the previous 1 year were excluded from the analysis. [§] Individuals without speech disturbance caused by wheezing during the previous 1 year were excluded from the analysis. ^{||} OR was adjusted for sex, pneumonia, parental asthma, helminth infections, pentavalent vaccine, antibiotic use, total, anti-DP, and anti-cockroach IgE. [¶] OR was adjusted for sex, pneumonia, parental asthma, helminth infections, pentavalent vaccine, antibiotic use, anti-*Ascaris*, anti-DP, and anti-cockroach IgE. ^{††} OR was adjusted for sex, pneumonia, parental asthma, helminth infections, pentavalent vaccine, antibiotic use, total, anti-*Ascaris*, and anti-cockroach IgE. ^{‡‡} OR was adjusted for sex, pneumonia, parental asthma, helminth infections, pentavalent vaccine, antibiotic use, total, anti-*Ascaris*, and anti-DP IgE.

with or without adjustment for covariates (Table 1). In contrast, the OR of total IgE, anti-*Der p* IgE, or anti-cockroach IgE did not show any significant association with wheezing.

Anti-*Ascaris* IgE levels in the wheezing and never-wheezing groups in the present study (1.07 and 0.65 U_A/mL, respectively) dropped significantly ($p < 0.001$ and $p < 0.001$, respectively) compared with those in the corresponding groups in the 2001 study (16.92 and 7.92 U_A/mL, respectively). Similarly, the seroprevalence of anti-*Ascaris* IgE in both groups in the present study (57.1% and 43.6%, respectively) was significantly lower than that in the corresponding groups in the 2001 study (97% and 85.7%, respectively; $p = 0.009$). In addition, the prevalence of *Ascaris* infection in the wheezing and never-wheezing groups, respectively, decreased from 75% and 72% in 2001 to 19% and 10% in the present study.

The characteristics of the study population are shown in Table 2. Eggs of *A. lumbricoides* and *Trichuris trichiura* were detected in 19% and 22%, respectively, of the stool of wheezing children; however, there were no significant differences from the never-wheezing children. Eggs from other helminth species were not detected in the study population. Parental asthma and the use of antibiotics up to the age of 1 year had positive associations with wheezing. In contrast, administration of the *Haemophilus influenzae* type B (Hib) combination vaccine (Shan5TM; Shantha Biotechnics, Telangana, India) in the form of a pentavalent vaccine that included a diphtheria, tetanus, pertussis, hepatitis B, and Hib vaccine was found to be protective against wheezing [33]. These variables were used for subsequent adjustments in the logistic regression models described in the above paragraph. In this study, a history of pneumonia at the age of 2 years or above was not associated with increased wheezing.

Table 2. Description of the characteristics of the population and the variables used for adjustment, stratified by presence of wheezing

		Wheezers, n (%)	Never-wheezers, n (%)	Crude odds ratio	p value
Male/female		63 (50)/63	45 (41)/65	1.44 (0.86–2.42)	0.163
Helminth infection		126	110		
<i>Ascaris</i>	Yes	24 (19)	11 (10)	2.12 (0.99–4.55)	0.055
<i>Trichuris</i>	Yes	28 (22)	17 (15)	1.56 (0.80–3.04)	0.189
History of pneumonia, years at		126	110		
0	Yes	4 (3)	2 (2)	0.57 (0.10–3.15)	0.514
1	Yes	7 (6)	1 (1)	6.41 (0.78–52.96)	0.085
2	Yes	8 (6)	1 (1)	7.39 (0.91–60.05)	0.061
3–4	Yes	7 (6)	0 (0)	–	1.000
Asthmatic father	Yes/total	16/126 (13)	5/110 (5)	3.08 (1.09–8.72)	0.034
Asthmatic mother	Yes/total	22/126 (18)	5/110 (5)	4.49 (1.64–12.30)	0.004
Penta vaccination [†]	Yes/total	107/126 (85)	106/110 (96)	0.21 (0.070–0.65)	0.006
Antibiotics in infancy [‡]	Yes/total	121/126 (97)	98/110 (89)	3.70 (1.16–11.85)	0.027
Monthly household income [§]	BDTk	12,887 (11,362–14,618)	113,467 (11,920–15,230)	0.91 (0.62–1.32)	0.909

BDTk, Bangladeshi taka. [†] Hib combination vaccine, including diphtheria, tetanus and pertussis vaccine, hepatitis B vaccine, and Hib vaccine. [‡] Antibiotics were administered during infancy. [§] Monthly household income (median, interquartile range). ^{||} 1 BDTk = USD 0.012.

Table 3. Geometric mean levels of total, anti-*Ascaris*, anti-DP, and anti-cockroach IgEs

	N	Total IgE, geometric mean (mean ± SD), IU/mL	Anti- <i>Ascaris</i> IgE, geometric mean (mean ± SD), U _A /mL	Anti-DP IgE, geometric mean (mean ± SD), U _A /mL	Anti-cockroach IgE, geometric mean (mean ± SD), U _A /mL
Never-wheezers	110	632 (505–791)	0.65 (0.50–0.86)	0.72 (0.52–0.99)	1.62 (1.15–2.29)
Current wheezers	126	734 (606–890)	1.07 (0.81–1.41)	0.77 (0.58–1.03)	1.64 (1.23–2.20)
Sleep disturbance	85	752 (591–955)	1.13 (0.79–1.62)	0.81 (0.58–1.14)	1.66 (1.16–2.37)
≥4 attacks	33	834 (561–1,240)	1.24 (0.79–1.94)	1.04 (0.57–1.88)	2.25 (1.19–4.25)
Speech difficulty	29	953 (657–1,381)	1.33 (0.89–2.04)	1.11 (0.87–2.05)	2.39 (1.26–4.54)

IgE, immunoglobulin E; SD, standard deviation; Anti-DP, anti-*Dermatophagoides pteronyssinus*.

The distribution of anti-*Ascaris*, anti-*Der p*, and anti-cockroach IgE levels showed two peaks corresponding to the seropositive and seronegative individuals, while the total IgE levels showed almost normal distribution in both the wheezing and never-wheezing groups. The wheezing children were significantly more seropositive (≥ 0.7 U_A/mL) for anti-*Ascaris* IgE than the never-wheezing children (72/54 vs. 48/62; χ^2 test: $p = 0.038$). However, this association was not found in relation to anti-*Der p* or anti-cockroach IgEs (66/60 vs. 53/57, χ^2 test: $p = 0.520$; 89/36 vs. 75/33, χ^2 test: $p = 0.770$, respectively).

Table 3 indicates the geometric mean of total, anti-*Ascaris*, anti-*Der p*, and anti-cockroach IgE levels. The geometric mean anti-*Ascaris* IgE level was significantly higher in the wheezing group than in the never-wheezing group (t test: $p = 0.014$). However, the geometric mean

total IgE level in the wheezing children was not significantly higher than that in the never-wheezing children (t test: $p = 0.554$). No significant differences were observed in the anti-*Der p* and anti-cockroach IgE levels between wheezing and nonwheezing children (t test: $p = 0.748$ and 0.823 , respectively). The geometric mean anti-*Ascaris* IgE levels for wheezing children who had more specific symptoms of asthma (e.g., sleep disturbance, four or more attacks, and speech difficulty) in the preceding 12 months were higher than those for the children who were merely wheezing. However, the differences were not significant (t test: $p = 0.575$, 0.382 , and 0.356 , respectively).

Cluster Analysis

Three clusters were identified in wheezing children using hierarchical cluster analysis. As shown in Figure 2, the

Fig. 2. The mean standardized value of the characteristic variables of the 3 clusters. Three clusters were identified. The vertical axis of this polygonal line graph expresses the mean standardized value of the variables regarding the characteristics of the clusters. The horizontal axis expresses each variable involved in the analysis: a history of pneumonia at zero and 1 year of age, pentavalent vaccina-

tion, *Ascaris* infection intensity, parental asthma, and total and specific IgE levels. Cluster 1 expresses the group with low levels of pneumonia and low anti-*Ascaris* IgE titers, cluster 2 expresses the group with more frequent pneumonia and lower IgE levels, and cluster 3 expresses the group with the highest total, anti-*Ascaris*, anti-*Der p*, and anti-cockroach IgE titers and the least pneumonia.

children in cluster 1 experienced low levels of pneumonia and had the lowest anti-*Ascaris* IgE titers. Cluster 2 experienced pneumonia most frequently and had lower IgE levels. Cluster 3 had the highest total, anti-*Ascaris*, anti-*Der p*, and anti-cockroach IgE titers and experienced pneumonia the least. The total and specific IgE levels, seroprevalence of specific IgEs, prevalence of *Ascaris* infection, and pneumonia history are shown in Table 4. The physical status, family history, vaccination history, and socioeconomic characteristics of the three groups are shown in Table 5.

Cluster 3

Cluster 3 comprised 11% of the participants ($n = 14$). The significant characteristics of this cluster were higher total, anti-*Ascaris*, anti-*Der p*, and anti-cockroach IgE levels. Moreover, the history of pneumonia was lowest when they were aged 0, 1, 2, and 3–4 years. The seroprevalence of anti-*Ascaris* IgE was significantly higher than that of cluster 1, cluster 2, and the never-wheezing chil-

dren (Fisher two-tailed exact test: $p < 0.001$, <0.001 , and <0.001 , respectively). Although the seroprevalence of anti-*Ascaris* IgE in wheezing children was 57%, only 20% of them belonged to this cluster.

Cluster 2

Cluster 2 comprised the smallest cluster ($n = 9$; 7%). Children in this cluster experienced pneumonia most when they were 0, 1, 2, and 3–4 years old. Their monthly household income was the lowest among the three groups, although the difference was not significant.

Cluster 1

Cluster 1 comprised the largest group ($n = 101$; 81%). It consisted of children who experienced pneumonia the least and had the lowest total, anti-*Ascaris*, anti-*Der p*, and anti-cockroach IgE serum levels. The levels were almost equal to those of the never-wheezing children. The seroprevalence of anti-*Ascaris* IgE was significantly lower than that of cluster 3 (Fisher two-tailed exact test: $p <$

Table 4. Comparisons of serum IgE levels, IgE seroprevalence, helminth infections, and pneumonia history among the three groups

	Cluster 1	Cluster 2	Cluster 3	<i>p</i> value
<i>n</i> (% per 114)	101 (81)	9 (7)	14 (11)	
Total IgE (mean±SD), IU/mL	553 (460–666)	1,802 (1,150–2,824)	3,421 (2,360–4,958)	<0.001
Anti- <i>Ascaris</i> IgE (mean±SD), U _A /mL	0.80 (0.61–1.06)	1.77 (0.46–6.86)	7.46 (4.16–13.37)	<0.001
Anti- <i>Der p</i> IgE (mean±SD), U _A /mL	0.57 (0.42–0.76)	1.14 (0.50–2.62)	6.29 (2.91–13.58)	<0.001
Anti-cockroach IgE (mean±SD), U _A /mL	1.15 (0.86–1.55)	3.89 (1.31–11.49)	14.45 (8.86–23.56)	<0.001
Seropositive, <i>n</i> (%)				
Anti- <i>Ascaris</i> IgE	52 (52)	5 (55)	14 (100)	0.003
Anti- <i>Der p</i> IgE	47 (47)	5 (56)	13 (93)	0.005
Anti-cockroach IgE	66 (65)	9 (100)	14 (100)	0.004
Helminth infection (+), <i>n</i> (%)				
<i>Ascaris</i>	17 (17)	3 (33)	4 (29)	0.317
<i>Trichuris</i>	21 (21)	4 (44)	2 (14)	0.199
Pneumonia history (+)				
0 years	0 (0)	4 (44)	0 (0)	<0.001
1 year	3 (3)	3 (33)	1 (7)	0.001
2 years	3 (3)	5 (56)	0 (0)	<0.001
3–4 years	0 (0)	6 (67)	0 (0)	<0.001

IgE, immunoglobulin E; Dp, *Dermatophagoides pteronyssinus*.

Table 5. Comparisons of physical status, family history, vaccination history, and sociodemographic characteristics of the three groups

	Cluster 1	Cluster 2	Cluster 3	<i>p</i> value
<i>N</i> (%)	101 (81)	9 (7)	14 (11)	
Sex (female), <i>n</i> (%)	51 (51)	2 (22)	8 (57)	0.219
Anthropometry				
Height (mean ± SD), cm	107 (106–108)	105 (101–110)	107 (106–108)	0.637
Weight (mean ± SD), kg	16 (16–17)	15 (13–17)	16 (15–17)	0.249
Vaccine coverage, <i>n</i> (%)				
Hib combined	84 (83)	9 (100)	13 (93)	0.276
Parental asthma, yes, <i>n</i> (%)				
Mother	20 (20)	2 (22)	0 (0)	0.179
Father	14 (14)	1 (11)	1 (7)	0.745
Monthly income [†]				
BDTk [‡]	17,742 (13,929–21,556)	12,222 (6,258–18,186)	18,207 (9,364–27,051)	0.679

BDTk, Bangladesh taka. [†] Monthly income (median interquartile range). [‡] 1 BDTk = USD 0.012.

0.001), and there was no significant difference between the wheezing and never-wheezing children (χ^2 test: $p = 0.254$). This group was the least likely to produce total and specific IgEs, and the prevalence of *Ascaris* infection (17%) was the lowest, although the differences between the three groups were not significant.

Discussion

The present study found novel evidence that anti-*Ascaris* IgE significantly increased the risk of wheezing among children aged 5 years from the general population of an area with a mild to moderate *Ascaris* infection prevalence. This is partially consistent with the findings of several other studies that identified anti-*Ascaris* IgE as a

risk factor for wheezing under some specific conditions. One study was conducted in the same region of Bangladesh in 2008, where *Ascaris* infection prevailed in approximately 17.4% of children. In that study, anti-*Ascaris* IgE increased the risk of ever having asthma among children aged 5 years whose mothers received additional caloric intake during pregnancy [31]. Similarly, a study conducted in northeastern Ecuador provided evidence that the risk of wheezing increased when anti-*Ascaris* IgE was present, independent of serum specific IgE levels in both atopic and nonatopic children from the general population [30]. Another study showed that anti-*Ascaris* IgE increased the risk of current wheezing in an atopic population with a mild to moderate *Ascaris* infection prevalence [29]. Combined with these findings, the results of the present study suggest that anti-*Ascaris* IgE increases the risk of wheezing irrespective of *Ascaris* infection prevalence. Anti-*Ascaris* IgE had also been presented to significantly increase the risk of AHR in 5-year-old wheezing children in a high endemic area [21].

Besides, despite the significant drops in the anti-*Ascaris* IgE levels, seroprevalence, and *Ascaris* infection prevalence, the anti-*Ascaris* IgE levels were significantly higher in the wheezing children than nonwheezing children in the current study and still increased the risk of wheezing [19, 20]. Namely, anti-*Ascaris* IgE was proven to robustly increase the risk of wheezing, irrespective of the prevalence of *Ascaris* infection. Contrary to these facts, no significant difference was found in the infection prevalence when stratified by wheezing type in either the 2001 or 2016 study. The prevalence of *Ascaris* infection decreased due to the national deworming program implemented during that period [20].

The OR for wheezing in relation to anti-*Ascaris* IgE was enhanced when only children with more specific symptoms of asthma were included in the analysis. This may indicate that anti-*Ascaris* IgE and wheezing increased in a dose-dependent manner, suggesting a causal relationship. Anti-helminth IgE has been reported to cause Th2 type immunity and mediate the elimination of the worm [24, 25]. Anti-*Ascaris* IgE is thought to play a role in the elimination of the helminth to protect the host against infection. Since allergic manifestations have been described in anisakiasis [28], anti-*Ascaris* IgE may cause allergic reactions likewise [29–31]. The cross-reactivity between *Ascaris* and mite tropomyosin might be partially responsible for this mechanism, although it is entirely unclear how anti-*Ascaris* IgE works in the mechanism underlying the development of wheezing [34–37]. Inhalation of the *Der p* antigen present at high levels in the en-

vironment might result in its combination with anti-*Ascaris* IgE on the mast cell surface in the airways, causing mast cell degranulation and wheezing.

The hierarchical cluster analysis of the wheezing children in the present study found that 11% of the wheezing cases were due to anti-*Ascaris* IgE (Table 5). This group is assumed to have a predisposition to atopy that results in the production of high titers of anti-*Ascaris* IgE when infected with *Ascaris*. In a 2001 study, 26% of wheezing was associated with anti-*Ascaris* IgE [22]. The wheezing children in both the present and previous studies had elevated total and anti-*Ascaris* IgE levels and slightly higher anti-*Der p* and anti-cockroach IgE titers than those of the never-wheezing children. Cluster 3 may resemble a multisensitized atopic wheezing cluster, as identified in other studies [38]. However, cluster 3 does not simply indicate an atopic population, since anti-*Der p* and anti-cockroach IgEs did not increase or decrease the risk of wheezing in the participants of the present study.

Cluster 2 was characterized by a high prevalence of pneumonia. This group may correspond to cluster 3 in the 2001 study and to a group of asthma patients who presented with wheezing, before the age of 7 years, which later resolved as proposed by Stein et al. [39]. Cluster 1 was the largest cluster in which we could not find any specific characteristics using the classification method based on total and specific levels of IgE, parental asthma, *Ascaris* infection prevalence, vaccination history, and pneumonia history. This group might correspond to the non-atopic wheezers presented by Stein et al. [39]. Specific risk factors which characterize this group might include some other precipitating factors such as air pollution, indoor pollution, or scabies, infection by *Sarcoptes*. *Ascaris* migrates through the lungs during maturation, and eosinophilic lung disease develops [40, 41]. Type 2 innate lymphoid cells, in addition to Th2 immunity, due to these factors might cause wheezing.

The present study had several strengths. First, the ORs of this study were adjusted for numerous covariates, such as sex, pneumonia history, parental asthma, helminth infections, Hib combination vaccination, antibiotic use during infancy, and total and specific IgEs, which seemed to affect the results of the analysis [33]. Second, the participants were randomly selected from the general population, even though the study was conducted in a low- to middle-income country. Finally, the response rate exceeds 80%.

There are also some limitations. First, we used a questionnaire to diagnose asthma rather than AHR. This was because AHR testing is a difficult procedure for children

aged 5 years. Next, we speculated that there must have been differences in the results of the effects of some predictors, such as the history of pneumonia and anti-*Der p* IgE. Although a history of pneumonia at the age of 2 years or above increased the risk of wheezing in the overall population of the first cross-sectional survey [20, 21], this was not confirmed in this case-control study. This was caused by the smaller final sample size than our initial expectation. The unforeseen drop in the prevalence of wheezing may be due to the implementation of the national deworming program and the start of Hib combination vaccination [21, 41].

Conclusions

Anti-*Ascaris* IgE was found to increase the risk of wheezing among children from the general population in rural Bangladesh despite the decreased prevalence of *Ascaris* infection. This indicates the robustness of the risk posed by anti-*Ascaris* IgE irrespective of the prevalence of *Ascaris* infection. The ORs for wheezing were increased when only children with more specific symptoms of asthma were included in the analysis, and 11% of the cases were associated with anti-*Ascaris* IgE, thus providing further support for our conclusions. These imply that *Ascaris* causes a serious public health concern in low- and middle-income countries, since it may participate in childhood wheezing through both acquired and innate immunity and infects 447 million people in impoverished areas worldwide. The role of anti-*Ascaris* IgE, as well as the role of innate immunity, in the underlying mechanism of wheezing development requires further investigation.

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Statement of Ethics

The study protocol PR-15054 was approved by the Ethical Review Committee of icddr,b. The Ethics Committee of Tokyo Kasei University approved the study protocol, Sayama H27-09, and protocol 11018 was approved by the Ethics Committee of the University of Tokyo. This study involved human subjects, and therefore

followed the ethical principles of the Declaration of Helsinki. Written informed consent was obtained from all the participants' legal guardians, and their anonymity was preserved using the methods approved by each ethics committee.

Conflict of Interest Statement

All the authors confirm that there are no conflicts of interest to disclose.

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Author Contributions

H.T., S.T., M.J., M.A.K., and T.I. made substantial contributions to the study planning. All authors contributed to the study design and protocol writing. S.M.T.H., S.K.H., S.Y., S.M.A., M.J.A., and M.A.K. contributed substantially to data collection. H.T., S.T., S.M.T.H., M.J., M.A.K., and T.I. contributed substantially to data analysis and interpretation of the results. All authors were involved in drafting and revising the manuscript. All authors read and approved the final submitted version of the manuscript.

Data Availability Statement

All data generated or analyzed during this study are included in the online supplementary files of this article. Further enquiries can be directed to the corresponding author.

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