Impact of early life geohelminths on wheeze, asthma, and atopy in Ecuadorian children at 8 years.

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Abstract

Background: Early-life exposures to geohelminths may protect against the development of wheeze/asthma and atopy. Objective: Study effect of maternal geohelminths and infections in children during the first 5 years of life on atopy, wheeze/asthma, and airways reactivity/inflammation at 8 years. Methods: Birth cohort of 2,404 neonates followed to 8 years in rural Ecuador. Data on wheeze/asthma were collected by questionnaire and atopy by skin prick test (SPT) reactivity to 9 allergens. We measured airways reactivity to bronchodilator, fractional exhaled nitric oxide (FeNO), and nasal eosinophilia. Stool samples were examined for geohelminths by microscopy. Results: 1,933 (80.4%) children were evaluated at 8 years. Geohelminths were detected in 45.8% of mothers and in 45.5% of children to 5 years. Frequencies of outcomes at 8 years were: wheeze (6.6%), asthma between 5 and 8 years (7.9%), SPT (14.7%), airways reactivity (10%), and elevated FeNO (10.3%) and nasal eosinophilia (9.2%). Any maternal geohelminth was associated with reduced prevalence of SPT (OR 0.72). Childhood T. trichiura infections were associated with reduced wheeze (OR 0.57) but greater parasite burdens with A. lumbricoides were associated with increased wheeze (OR 2.83) and asthma (OR 2.60). Associations between maternal geohelminths and wheeze/asthma were modified by atopy. Parasite-specific effects on wheeze/asthma and airways reactivity and inflammation were observed in non-atopic children. Conclusions: Our data provide novel evidence for persistent effects of in utero geohelminth exposures on childhood atopy but highlight the complex nature of the relationship between geohelminths and the airways. Registered as an observational study (ISRCTN41239086).

Introduction

Asthma is the most common chronic disease of childhood in high-income countries and is estimated to affect 358 millions worldwide.[1] Asthma is increasing in prevalence in many low and middle-income countries (LMICs).[2] Temporal trends of increasing asthma prevalence in LMICs are considered to be related to urbanization and loss of protective exposures associated with rural residence.[3]

Recent years have seen increasing urbanization in LMICs, accompanied by reductions in poverty, improved access to basic services, and transformation of the living environment.[3] Under such circumstances, the intensity of microbial exposures in early childhood is likely to have declined, affecting the maturation and regulation of the immune system and risk of inflammatory diseases including asthma.[4,5]

Geohelminths (caused by *Ascaris lumbricoides* , *Trichuris truchiura* , and hookworm) infect over 1 billion humans worldwide[6] and are most prevalent among children living in conditions of poverty in tropical regions of LMICs. Geohelminths cause chronic infections that are associated with modulation of host Th2 inflammatory mechanisms.[7] The tight regulation of Th2 inflammatory responses may modulate inflammation associated with allergy and asthma. It has been suggested that the lower prevalence of asthma symptoms observed in rural compared to urban populations in tropical regions of LMICs[3] is explained by the immune modulatory effects of endemic geohelminth infections.[5]

Epidemiological studies of the relationship between geohelminths and allergic inflammation (measured by atopy) or asthma have shown conflicting findings in cross-sectional analyses and intervention studies done largely in schoolchildren.[8-14] We hypothesized that *in utero* or early childhood exposures to geohelminths protect against the development of atopy and asthma in later childhood. To test this hypothesis, we followed an Ecuadorian cohort from birth to 8 years of age in an area of high endemicity. Previous analyses from the cohort showed a protective effect of maternal geohelminths on atopy to mite allergens at 3 years.[15]At 5 years, we observed that maternal infections increased the risk of wheeze while childhood infections protected against wheeze and atopy to perennial aeroallergens, and raised the possibility that childhood infections might modulate wheeze through non-allergic mechanisms.[16]

To understand better the effects of early childhood geohelminth exposures on wheeze, asthma and atopy, and whether the previously observed effects persist, we report findings at 8 years including novel measurements of airways inflammation and reactivity to provide further insights on effects of geohelminths on non-atopic wheezing illness and asthma.

Methods

Study design, setting, and participants

A prospective study from birth was done in the District of Quininde in Esmeraldas Province, Ecuador, as described.[17] The District serves a population of approximately 150,000 with limited access to basic services. The economy in the District is based largely on agricultural activities. Neonates were recruited at a public hospital in the District between November 2005 and December 2009. Follow-up evaluations and sample collections were done at 13 months and 2, 3, 5, and 8 years of age.

Study procedures

A questionnaire was used to collect data on socio-demographic factors, family history of allergy, and home environment by interview of the child's mother around the time of the child's birth. Questionnaires were repeated periodically to collect data on wheeze and asthma symptoms. Wheeze was defined as any episode of parentally-reported wheeze during the previous 12 months at 8 years. Asthma was defined as parentally reported wheeze during the previous 3 years plus one or both of parentally reported wheeze up to 5 years and a doctor diagnosis of asthma ever.

Stool samples to detect geohelminths were collected from mothers before birth of the child, and from children periodically from birth. Samples were examined using of a combination of methods: saline mounts, modified Kato-Katz, formol-ether concentration, and carbon-coproculture methods.[18] A positive sample was defined by the presence of at least one egg or larva from any of the four detection methods. *A. lumbricoides* and *T. trichiura* infection intensities were expressed as eggs per gram (epg) of faeces using results of Kato-Katz.

Spirometry was done at 8 years using a Microloop spirometer (CareFusion, UK) before and after 200 ug of salbutamol administered through a spacer. A positive test for bronchial hyperreactivity was an increase in FEV_1 of [?]12%. Fractional exhaled nitric oxide was measured in parts per billion using NObreath (Bedfont Scientific, UK) at a flow rate of 50 ml/sec. Nasal wash samples were collected at 8 years using a standardized protocol as described.[19]

Allergic sensitization was measured by SPTs with 9 allergen extracts (Greer laboratories, Lenoir, North Carolina, USA): house dust mites (*Dermatophagoides pteronyssinus/Dermatophagoides farinae* mix), American

cockroach (*Periplaneta americana*), cat, dog, grass pollen (9 southern grass mix), fungi (New stock fungi mix), egg, milk, and peanut, with positive histamine and negative saline controls. A positive reaction was defined as a mean wheal diameter at least 3 mm greater than the saline control 15 min after pricking the allergen onto the forearm with lancets. Positive SPT was defined as a positive reaction to any of the allergens tested.

Statistical analysis

Follow-up was initially planned for 5 years but later extended to 8 to allow more reliable diagnosis of asthma and testing of airways function and inflammation. To measure effects of geohelminths on asthma prevalence with >80% power at significance level of 0.05, we estimated that we would need to follow-up 1,725 children to detect a difference in asthma prevalence of [?]6% with infection rates of 50% among mothers and 35% among children. Primary exposures were maternal and childhood geohelminth infections and primary outcomes were wheeze, asthma, and SPT to any allergen. Exploratory analyses addressed the effects of geohelminth species and infection intensities on primary outcomes, and effects of geohelminths on airways reactivity, and markers of airways inflammation. Univariable and multivariable logistic regression were used to estimate associations. Potential confounders to be considered in the analyses are shown in Table 1. Urban-rural residence was defined by geographic boundaries. A socio-economic status (SES) index was created using principal components analysis of 7 socio-economic variables as described.[15] A conservative analytic approach was used for all adjusted analyses in which potential confounders included were those with P<0.05 in univariable analyses for any of the primary outcomes. Trends across infection intensity categories were evaluated using Cochran–Armitage test for trend**.** All statistical analyses were done using Stata 11 (Statacorp, College Station, Tex).

Ethical considerations

Study protocols were approved by ethics committees in Ecuador (Hospital Pedro Vicente Maldonado, Universidad San Francisco de Quito, and Universidad Internacional del Ecuador) and UK (London School of Hygiene and Tropical Medicine). The study is registered as an observational study (ISRCTN41239086). Informed written consent was obtained from the child's mother and minor assent was obtained from the child at 8 years. Anthelmintic treatment was provided to mothers and children with positive stools for geohelminths as recommended.[20]

Results

Cohort participants

Analyses at 8 years of age were done using data from 1,933 (80.4%) children of 2,404 newborns initially recruited and for whom complete data were available on primary exposures and outcomes (Figure 1). Frequencies of potential confounders for children included in and excluded from the analysis were generally similar (Supplementary Table 1).

Frequencies of exposures and outcomes

Almost half (45.8%) the children had an infected mother (*A. lumbricoides* 27.6%, T. *trichiura* 28.9%, hookworm 5.6%, and*S. stercoralis* 4.0%). Geohelminth infections during the first 5 years of life were observed in 45.5% of the 1,933 children analysed at 8 years, most frequently with *A. lumbricoides* (36.3%) and*T. trichiura* (25.5%). Other infections were hookworm (1.1%),*S. stercoralis* (1.5%) and *Hymenolepis* spp. (4.2%). Geometric mean infection intensities at 5 years among infected children were 1,162 eggs per gram (epg) for *A. lumbricoides* and 227 epg for *T. trichiura* . Maternal and childhood geohelminth infections were strongly associated; of 1,933 children, 35.5% neither maternal nor childhood infections, 19.0% had maternal geohelminths only, 18.7% had childhood infections only, and 26.8% had both (P<0.001). At least one episode of wheeze from birth to 8 years of age was reported for 38.0% of children. Prevalence of wheeze at 8 years was 6.6% and asthma between 5 and 8 years was 7.9%. The prevalence of SPT at 8 years was 14.7%: *D. pteronyssinus/farinae* 10.7%, cockroach 5.3%, mixed fungi 0.3%, dog 0.1%, cat 0.2%, mixed grasses 1.1%, peanut 0.3%, milk 0.1%, and egg 0.1%. Bronchial hyper-responsiveness was observed in 10%

of children while evidence of airways inflammation measured by elevated levels of FeNO (>35 ppb) and nasal eosinophilia (>5%) was observed in 10.3% and 9.2%, respectively.

Effects of geohelminths on primary outcomes

Before adjustment for potential non-helminth confounders, both maternal geohelminths and childhood geohelmiths were associated with a significant reduction in SPT positivity, but not with wheeze or asthma (Table 1). The univariate association of maternal helminths with atopy (OR 0.69) was not attenuated by adjustment for non-helminth confounders (OR 0.68, 95% CI 0.52-0.89, P=0.004) and remained little changed by further adjustment for childhood geohelminths (OR 0.72, Table 2 and Figure 2). The unadjusted association of childhood geohelminths with atopy was of similar magnitude (OR 0.75) and was barely altered by adjustment for non-helminth confounders (OR 0.77, 95% CI 0.59-1.01, P=0.054) but became somewhat weaker with further adjusted for maternal helminths (OR 0.82, Table 2 and Figure 2).

Effects of geohelminth infection, parasite species and parasite burden on primary outcomes.

Geohelminth infections to 13 months, 2 and 3 years of age were not significantly associated with primary outcomes (supplementary Table 2). Maternal geohelminth parasite species or parasite burden were not associated with primary outcomes (Table 3). The presence of any *T. trichiura* infection within the first 5 years of life was associated with a reduced prevalence of wheeze (adj. OR 0.57, 95% CI 0.35-0.94, P=0.029), while moderate to heavy parasite burdens with *A. lumbricoides* at 5 years were associated with an increased risk of wheeze (adj. OR 2.83, 95% CI 1.13-7.13, P=0.027) and asthma (adj. OR 2.60, 95% CI 1.13-6.00, P=0.025).

Effects of geohelminth infection chronicity and age of first infection on primary outcomes

Chronic exposures to childhood geohelminth infections were assessed as repeated infections in childhood (i.e., 0, 1, and >=2 documented infections with *A. lumbricoides* or *T. trichiura*during the first 5 years of life), and as cumulative burdens for*A. lumbricoides* or *T. trichiura* during the first 5 years of life. None of these had significant effects on outcomes (data not shown). Maternal geohelminths were strongly associated with childhood infections (adj. OR 2.70, 95% CI 2.23-3.22, P<0.001). To try and separate maternal/childhood geohelminth effects, we did a four-group analysis of combinations of maternal and childhood geohelminth infections (mother-/child-, mother+/child, mother-/child+, and mother+child+). Significant effects were observed on SPT for the mother+/child+ group (vs. mother-/child-, adj. OR 0.58, 95% CI 0.41-0.83, P=0.003).

Effects of geohelminths on secondary outcomes

There were no significant associations of geohelminths with airways reactivity, FeNO and nasal eosinophilia (Figure 2 and supplementary Table 3). When considering parasite species and burden, elevated FeNO was associated with moderate/heavy parasite burdens with *A. lumbricoides* both in mothers (vs. uninfected, adj. OR 2.19, 1.23-3.90, P=0.008) and children at 5 years (vs. uninfected, adj. OR 2.27, 1,10-4.70, P=0.027). After co-adjusting for maternal and child infection intensities, only the maternal effect remained significant (vs. uninfected, adj. OR 2.20, 95% CI 1.16-4.19, P=0.016).

Effects of geohelminths on outcomes stratified by SPT

SPT reactivity was strongly associated with wheeze (adj. 4.13, 95% CI 2.80-6.08, P<0.001) and asthma (adj. OR 2.32, 95% CI 1.57-3.42, P<0.001). We explored if effects of geohelminths on outcomes might vary by SPT (Figure 2 and Table 4). Although interactions were seen for SPT on geohelminth-outcome associations, they were not highly significant. However, overall associations between maternal geohelminths and wheeze/asthma were positive among atopic but negative among non-atopic children.

Comparisons of effects of maternal and childhood geohelminths by SPT

Effects of maternal and childhood geohelminths, species-specific effects, and effects by parasite burden are shown in Table 4 and supplementary Tables 4 and 5. Maternal geohelminths were positively associated with

wheeze (adj. OR 1.73), an effect that appeared to be explained by maternal *T. trichiura* infections (adj. OR 1.78), while a maternal effect on asthma was associated with moderate to heavy infection intensities with *A. lumbricoides* (vs. uninfected, adj. 2.11) (Table 4). To separate contrasting effects of maternal vs. childhood *T. trichiura* on wheeze in non-atopic children, we did a 4-group analysis by strata of maternal/child *T. trichiura*infection using maternal-/child- as reference group: we observed that mother+/child- children had an elevated risk of wheeze (adj. OR 2.27, 95% CI 1.31-3.92, P=0.003), an effect that was abolished by childhood infections (mother+/child+, adj. OR 0.78, 95% CI 0.33-1.83, P=0.573). Neither any maternal nor any childhood geohelminth infections were associated with airways reactivity, elevated FeNO, or nasal eosinophilia irrespective of atopy (supplementary Table 4). Analyses by parasite species and burden showed effects among non-atopic children (supplementary Table 5): 1) light infection intensities with *T. trichiura* in mothers were positively (vs. uninfected, adj. OR 1.56, 95% CI 1.05-2.01, P=0.028) but childhood *T. trichiura* infections inversely (adj. OR 0.62, 95% CI 0.40-0.96, P=0.031) associated with airways reactivity; 2) childhood *A. lumbricoides* (adj. OR 1.61, 95% CI 1.07-2.42, P=0.021) and moderate/heavy infection intensities with *A. lumbricoides* in mothers (vs. uninfected, adj. OR 2.89, 95% CI 1.53-5.49, P=0.001) were positively associated with elevated FeNO; and 3) nasal eosinophilia was associated with moderate/heavy infections with *A. lumbricoides* in mothers (vs. uninfected, adj. OR 2.27, 95% CI 1.00-5.12, P=0.049).

Discussion

We have tested the hypothesis that early life exposures to geohelminths - through an infected mother during pregnancy or early childhood, or both - protect against wheeze/asthma and atopy at school-age. To do this, we did a birth cohort study to measure the effects of maternal and early childhood geohelminths on the development of atopy (measured as SPT), wheeze/asthma, and airways reactivity and inflammation at 8 years. Our findings indicate that maternal geohelminths have persistent effects on childhood SPT but that this effect was strongest among children of infected mothers who also acquired infections during early childhood. A maternal effect on increased wheeze and airways inflammation was seen among non-atopic children, the dominant phenotype in non-affluent societies.[21,22] The maternal effect on SPT was not associated with a specific parasite species, while that on wheeze appeared to be mediated by *T. trichiura* infection. In contrast, early childhood *T. trichiura* protected against wheeze irrespective of atopy.

There are few previous longitudinal analyses of the effects of early geohelminth infections on development of allergy, and none of these have adequately addressed effects of maternal or childhood geohelminths on asthma or atopy: 1) a birth cohort in Ethiopia that did not measure maternal gehelminths and in which the prevalence of geohelminths (<4%) in early childhood was too low to explore effects on allergy at 5 years;[23] and 2) a longitudinal study in Brazil, with no data on maternal geohelminths, showed that *T. trichiura* infections in early childhood, particularly at higher parasite burdens were associate with a reduced risk of SPT in later childhood.[24] To our knowledge, the only other study to show effects of maternal geohelminths on allergy-related outcomes was an observational analysis in Uganda showing maternal hookworm to be associated with a reduced risk of eczema in children to 5 years.[25]

Previous cross-sectional studies have provided evidence that childhood geohelminths might protect against wheeze/asthma: 1) a study in Ethiopia in 1-4 year olds showed a negative association between *A. lumbricoides* infection and wheeze;[26] 2) a study among schoolchildren in a rural region in Ecuador showed an inverse association between heavy infections with *T. trichiura* and atopic wheeze[27] - most previous cross-sectional studies, however, showed no effects of *T. trichiura* on asthma symptoms;[8,21,28,29] and 3) three separate studies in Ethiopia showed an inverse relationship between hookworm infection and asthma symptoms.[8] With respect to *A. lumbricoides* infections in school-age children, several studies have shown a positive association between *A. lumbricoides* infection or allergic sensitization to *Ascaris* antigens and asthma symptoms[8,28,30,31] and airways reactivity,[29,31,32] an effect that was strongest in non-atopics.[28] Our data showed positive associations between greater parasite burdens with *A. lumbricoides* in mothers and risk of asthma and markers of airways inflammation in non-atopic children, while *A. lumbricoides* in children was associated with elevated FeNO.

Our observation that maternal infections protect against atopy are consistent with observations of inverse associations between geohelminths and SPT from cross-sectional studies of schoolchildren.[21,24,33] A protective

effect of maternal geohelminth (against mite) was present from 3 years of age.[15,16] Childhood infections appeared to protect against SPT to perennial allergens from 5 years,[16] but the observation that the effect was only seen among children with infected mothers irrespective of childhood infection status, indicate the maternal effect is key. Maternal geohelminths were strongly associated with childhood infections to 5 years of age – reflecting a shared risk of infection in the household environment – a child growing up in a household where one or more family members are infected, is at greater risk of infection.[34] The previous observation from Brazil showing a protective effect of early life *T. trichiura* infections against SPT at school age[24]could have been mediated by maternal infections which were not measured but with which early childhood infections are likely to be strongly associated. A maternally-mediated effect on SPT could explain two previous observations from Ecuador: 1) bimonthly anthelmintic treatments in schoolchildren showed no treatment effect on allergen SPT;[10] and 2) community mass drug administrations with the broad-spectrum anthelmintic, ivermectin, over 15 years for the elimination of onchocerciasis, was associated with an increase in SPT prevalence in schoolchildren. Long-term ivermectin started before most children were born, likely resulted in reduced geohelminth infections in mothers.[35]

We have shown previously in this population that newborns of mothers infected with *A. lumbricoides* have evidence of sensitization of CD4+ T cells to *Ascaris* antigens.[36] The same is likely to be true for *T. trichiura* that, although purely enteric, has an intimate relationship with the mucosal immune system.[7] Certainly, geohelminth antigens are present in the blood[37] of infected mothers and can cross the placenta to sensitize the foetus. Immunological sensitization of the foetus could increase or decrease immune responsiveness. Decreased responsiveness could be associated with tolerization to parasite allergens including those that are cross-reactive with aeroallergens. Extensive cross-reactivity has been demonstrated between helminth parasites and aeroallergens such mite allergens,[38] and such cross-reactivity can mediate cross-sensitization in immediate hypersensitivity skin reactions in murine models.[39]The suppressive effect of maternal geohelminths on SPT, particularly to mite allergens, in children could occur through tolerization to cross-reactive allergens.

The effect of maternal *T. trichiura* on childhood wheeze likely occurs through a distinct non-allergic mechanism, perhaps through the interconnected mucosal immune system.[40] The evolutionary significance of such an effect resulting in increased mucosal responsiveness could, for example, increase chemical signals leading to airways reactivity and wheeze symptoms (as observed here) but the same signals in the gut might enhance peristalsis and expulsion of parasites. The type of immune response generated in the foetus likely will be affected by a number of factors such as host genetics, the 'intensity' of exposure, and geohelminth parasite species and could be parasite antigen-specific. The maternal effect of *T. trichiura*was evident only among children of infected mothers who did not acquire *T. trichiura* infections during the first 5 years of life. The acquisition of childhood *T. trichiura* abrogated the maternal effect indicating that *in utero* effects could be modified by exposure during childhood, presumably by modulation of the same mechanisms.

Asthma is a highly heterogeneous disease for which several phenotypes and endotypes have been described.[41] Numerous traits have been described for asthma (e.g. airflow limitation and airway inflammation) caused by distinct causal mechanisms.[42]Similarly, geohelminths are a diverse group of parasites with distinct life cycles and niches within (and outside) the intestine in humans. The human host has developed a wide variety of inflammatory mechanisms, primarily mediated by Th2 cytokines, with which to kill and expel geohelminths.[7] Balanced parasitism requires an accommodation between host and parasite to allow parasites to survive without severely debilitating their host. Such an accommodation includes the modulation of host anti-parasite Th2 responses.[7]Our observations of parasite species-specific effects on different 'traits' (e.g. wheeze symptoms, airways reactivity and elevated FeNO) likely reflects this complex interaction.

Strengths of the study include prospective design with follow-up from birth, stool data on maternal geohelminths during pregnancy, and collection of large number of sociodemographic and lifestyle variables allowing us to control for potential confounders. Potential biases were reduced by using objective measures of geohelminth infections, performing all evaluations blind to the child's exposure status, and high retention in the cohort to 8 years (˜80%). Repeated exposure measures for childhood geohelminths during the first 5 years of life provided more precise estimates of infection rates but children with positive stools were treated

thus reducing prevalence and parasite burdens.

Conclusions

Evidence of a protective effect of STH parasites against allergy in children remains fragmentary and inconsistent. Our data indicate that maternal geohelminths protect children from the development of allergen SPT but increase the risk of wheeze, and airways reactivity and inflammation. The latter effects were attributable to specific parasite species. Childhood *T. trichiura* appeared to protect against wheeze. Overall, our findings indicate that *in utero* exposures to geohelminths through maternal infections may have long-lasting effects on allergic inflammation and airways disease. These effects extended to school age and, depending on the specific trait being measured, could be modified by childhood infections, parasite species, and atopy.

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Variable	Overall	Wheeze	Wheeze	Wheeze	Asthma	Asthma	Asthma	SPT to any allergen	SPT to any allergen
	n (%)	%	OR (95% CI)	P value	%	OR (95% CI)	P value	%	OR (95% CI)

Variable	Overall	Wheeze	Wheeze	Wheeze	Asthma	Asthma	Asthma	SPT to any allergen	SPT to any allergen
Any maternal geo-helminth No Yes	1048 (54.2) 885 (45.8)	6.2 7.0	1 1.14 (0.79-1.63)	0.478	8.2 7.6	1 0.92 (0.66-1.28)	0.606	16.8 12.2	1 **0.69 (0.53-0.89)**
Any child-hood geo-helminths No Yes	1054 (54.5) 879 (45.5)	6.7 6.4	1 0.94 (0.66-1.35)	0.747	8.4 7.4	1 0.88 (0.63-1.22)	0.439	16.4 13.0	1 **0.75 (0.59-0.98)**
Maternal age (yrs) [?]20 21-29 [?]30	501 (25.9) 929 (48.1) 503 (26.0)	5.8 6.6 7.4	1 1.14 (0.72-1.80) 1.29 (0.78-2.14)	0.564 0.317	6.6 8.7 7.8	1 1.35 (0.89-2.06) 1.19 (0.74-1.93	0.157 0.474	13.8 14.0 16.9	1 (0.74-1.40) 1.27 (0.90-1.80)
Maternal ethnicity Afro-Ecuadorian Non-Afro-Ecuadorian	508 (26.3) 1425 (73.7)	7.7 6.2	1 0.79 (0.54-1.17)	0.242	11.8 6.5	1 **0.52 (0.37-0.73)**	**<0.001**	14.8 14.7	1 0.99 (0.75-1.32)
Maternal educa-tional level Il-literate Com-plete pri-mary Com-plete Secondary	293 (15.2) 1133 (58.6) 507 (26.2)	5.5 6.2 8.2	1 1.14 (0.65-1.99) 1.52 (0.84-2.77)	0.646 0.167	8.2 7.3 9.1	1 0.89 (0.55-1.42) 1.12 (0.67-1.87)	0.616 0.671	14.7 13.7 17.0	1 0.92 (0.64-1.33) 1.19 (0.89-1.77)
Area of residence Urban Rural	1346 (69.6) 587 (30.4)	7.4 4.6	1 **0.60 (0.39-0.93)**	**0.022**	9.4 4.6	1 **0.47 (0.30-0.72)**	**<0.001**	15.5 13.0	1 0.81 (0.61-1.08)
Sex Male Female	984 (50.9) 949 (49.1)	7.9 5.2	1 **0.63 (0.44-0.91)**	**0.015**	8.7 7.1	1 0.79 (0.57-1.11)	0.172	16.5 12.9	1 **0.75 (0.58-0.96)**

Variable	Overall	Wheeze	Wheeze	Wheeze	Asthma	Asthma	Asthma	SPT to any allergen	SPT to any allergen
Socioeconomic status§ 1 2 3	642 (33.2) 638 (33.0) 653 (33.8)	6.2 5.6 7.8	1 0.90 (0.57-1.43) 1.28 (0.83-1.96)	0.656 0.267	7.8 7.2 8.7	1 0.92 (0.61-1.40) 1.13 (0.76-1.68)	0.695 0.539	13.4 14.0 16.7	1 1.05 (0.76-1.44) 1.30 (0.95-1.76)
Birth order 1st 2nd-4th [?]5th	490 (25.4) 1063 (55.0) 380 (19.6)	6.1 7.3 5.0	1 1.21 (0.79-1.88) 0.81 (0.45-1.46)	0.382 0.477	5.5 9.4 6.8	1 **1.78 (1.15-2.76)** 1.26 (0.72-2.20)	**0.010 0.416**	16.7 13.5 15.5	1 1.05 (0.76-1.44) 1.30 (0.95-1.76)
Maternal allergy No Yes	1830 (95.4) 89 (4.6)	6.3 12.4	1 **2.10 (1.09-4.06)**	**0.027**	7.7 14.6	1 **2.06 (1.12-3.81)**	**0.020**	14.4 18.0	1 1.30 (0.75-2.27)
Household over-crowding¶ [?]3 >3	1086 (56.2) 847 (43.8)	6.5 6.6	1 1.01 (0.70-1.45)	0.948	7.6 8.4	1 1.12 (0.80-1.56)	0.502	15.6 13.6	1 0.85 (0.66-1.10)
Pets inside house No Yes	1438 (74.4) 495 (25.6)	6.3 7.3	1 1.16 (0.78-1.73)	0.465	7.7 8.7	1 1.15 (0.79-1.66)	0.461	14.5 15.2	1 1.05 (0.79-1.40)
Large farm animals++ No Yes	1294 (66.9) 639 (33.1)	7.0 5.8	1 0.82 (0.55-1.22)	0.331	8.0 7.7	1 0.95 (0.67-1.35)	0.778	15.8 12.5	1 0.76 (0.58-1.01)
Pneumonia to 13 months No Yes	1758 (95.3) 90 (4.7)	6.6 5.6	1 0.83 (0.33-2.09)	0.697	7.9 7.8	1 0.99 (0.45-2.18)	0.980	15.0 7.8	1 0.48 (0.22-1.04)

Table 1. Frequencies of maternal and childhood geohelminth infections to 5 years of age and potential confounders and associations with wheeze and allergen skin test (SPT) reactivity to any allergen at 8 years and asthma between 5 and 8 years.

SPT – allergen skin prick test reactivity to any of 9 allergens. Odds ratios (OR) and 95% confidence intervals (95% CI) for univariable associations were estimated using logistic regression. P<0.05 are shown in bold. Ethnicity 'other' represents: 1,417 Mestizo/8 Indigenous. Numbers of missing values (brackets) were: maternal allergy (14) and pneumonia during first 13 months (85). §Socioeconomic status (SES) represents tertiles of z scores obtained using a factor analysis with 1 representing the lowest and 3 the highest SES. ¶ Household overcrowding is defined as the number of people living in the household per sleeping room. ++ Any of cows, pigs, mules, donkeys, and horses. Other helminths: mother (*S. stercoralis* , 4.0%;*Hymenolepis* spp., 0.5%); child (hookworm, 1.1%; *S. stercoralis* , 1.5%; *Hymenolepis* spp., 4.2%).

Variable	Wheeze	Wheeze	Asthma	Asthma	SPT to any allergen	SPT to any allergen
	OR (95%CI)	P value	OR (95% CI)	p-value	OR (95% CI)	p-value
Any maternal geohelminth No Yes	1 1.11 (0.76-1.62)	0.594	1 0.81 (0.57-1.16)	0.254	1 **0.72 (0.55-0.94)**	**0.018**
Any childhood geohelminth No Yes	1 0.94 (0.64-1.39)	0.740	1 0.84 (0.58-1.20)	0.329	1 0.82 (0.62-1.08)	0.157
Maternal allergy No Yes	1 **2.24 (1.14-4.39)**	**0.019**	1 **2.07 (1.10-3.88)**	**0.024**	1 1.37 (0.78-2.41)	0.274
Maternal ethnicity Afro-Ecuadorian Non-Afro-Ecuadorian	1 0.88 (0.58-1.34)	0.551	1 **0.55 (0.38-0.79)**	**0.001**	1 0.95 (0.70-1.28)	0.725
Area of residence Urban Rural	1 **0.60 (0.38-0.97)**	**0.035**	1 **0.46 (0.29-0.73)**	**0.001**	1 0.84 (0.62-1.14)	0.269
Sex Male Female	1 **0.60 (0.41-0.88)**	**0.009**	1 0.78 (0.55-1.09)	0.149	1 **0.70 (0.54-0.90)**	**0.006**
Birth order 1^{st} 2^{nd} – 4^{th} [?]5^{th}	1 1.20 (0.77-1.87) 0.78 (0.43-1.45)	0.416 0.440	1 **1.87 (1.19-2.92)** 1.26 (0.71-2.24)	**0.006** 0.438	1 0.81 (0.60-1.09) 1.02 (0.70-1.48)	0.168 0.930
Large farm animals++ No Yes	1 0.91 (0.60-1.38)	0.650	1 1.17 (0.80-1.71)	0.431	1 0.81 (0.60-1.09)	0.171

Table 2. Adjusted analyses for associations between maternal and childhood geohelminth infections to 5 years of age or potential confounders and associations with wheeze and allergen skin prick test (SPT) reactivity to any allergen at 8 years and asthma between 5 and 8 years. SPT – allergen skin prick test reactivity to any of 9 allergens. Odds ratios (OR) and 95% confidence intervals (95% CI) were estimated using logistic regression and adjusted for all variables shown. P<0.05 are shown in bold.

Variable	Overall
	n (%)
Maternal geohelminth *A. lumbricoides* No Yes Intensity Uninfected	1400 (72.4) 533 (27.6) 1485 (77.5) 33
Light	
Moderate/heavy *T. trichiura* No Yes Intensity Uninfected	
Light	
Moderate/heavy Hookworm No Yes	
Child geohelminths *to* 5 yr *A. lumbricoides* No Yes *T. trichiura* No Yes	1223 (63.7) 697 (36.3) 1430 (74.5) 49
Child geohelminths *at* 5 yr Any No Yes *A. lumbricoides* No Yes Intensity Uninfected	1197 (74.8) 404 (25.2) 1358 (84.8) 24
Light	
Moderate/heavy *T. trichiura* No Yes Intensity Uninfected	
Light Moderate/heavy	

Table 3. Adjusted analyses for associations between geohelminth parasite species and parasite burdens in mothers and in children up to and at 5 years of age, and wheeze and allergen skin test (SPT) reactivity to any allergen at 8 years and asthma between 5 and 8 years

SPT – allergen skin prick test reactivity to any of 9 allergens. Odds ratios (OR) and 95% confidence intervals (95% CI) were estimated using logistic regression and adjusted for maternal allergy and ethnicity, area of residence, sex, birth order, contact with large farm animals, geohelminths in the child to 5 years (for maternal geohelminths), maternal geohelminths (for childhood geohelminths), and presence of other species of geohelminth parasite. Infection intensity categories are: *A. lumbricoides* , light (<5,000 epg), moderate/heavy (>5,000 epg); *T. trichuira* , light (<1000 epg), moderate/heavy (>1,000 epg). P<0.05 are shown in bold. Denominators for geohelminth prevalence versus intensity results differ because it was not possible to do Kato-Katz examinations on all samples where sample volume was insufficient. P<0.05 are shown in bold.

Variable	Wheeze
	SPT- OR (95% CI)
Maternal geohelminths Any No Yes *A. lumbricoides* No Yes Intensity Uninfected	1 **1.73 (1.06-2.83)** 1 1.12 (0.67-1.88
Light	
Moderate/heavy *T. trichiura* No Yes Intensity Uninfected	
Light	
Moderate/heavy	
Child geohelminths to 5 yr Any No Yes *A. lumbricoides* No Yes *T. trichiura* No Yes	1 0.89 (0.55-1.45) 1 1.24 (0.75-2.06) 1

Table 4. Adjusted analyses for associations between geohelminths, geohelminth parasite species and parasite burdens in mothers and children and wheeze/asthma in later childhood, stratified by presence (+) and absence (-) of allergen skin prick test reactivity.

SPT – allergen skin prick test reactivity to any of 9 allergens (SPT+, n=1649; SPT-, n=284). Odds ratios (OR) and 95% confidence intervals (95% CI) were estimated using logistic regression and adjusted for maternal allergy and ethnicity, area of residence, sex, birth order, contact with large farm animals, geohelminths in the child to 5 years (for maternal geohelminths), maternal geohelminths (for childhood geohelminths), and presence of other species of geohelminth parasite. P<0.05 are shown in bold. Interaction P values are shown for adjusted models but did not differ markedly from those of unadjusted models. Infection intensity categories are: *A. lumbricoides* , light (<5,000 epg), moderate/heavy (>5,000 epg);*T. trichuira* , light (<1000 epg), moderate/heavy (>1,000 epg). Denominators for geohelminth prevalence versus intensity results differ because it was not possible to do Kato-Katz examinations on all samples where sample volume was insufficient. P<0.05 are shown in bold.

Figure legends

Figure 1. Participant flow through follow-up to 8 years of age and those included and excluded from the analysis. FU- follow-up.

Figure 2. Associations between maternal and childhood geohelminths and study outcomes among all children and stratified by atopy.

Shown are adjusted ORs and 95% CIs. SPT – allergen skin prick test reactivity; Air React. – airways reactivity; FeNO – fractional exhaled nitric oxide; Nas eosin – nasal eosinophilia.

Figure 1

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Figure 2