

ORIGINAL ARTICLE

WILEY

Epidemiology, Genetics & Prevention

Hygienic conditions influence sensitization to *Blomia tropicalis* allergenic components: Results from the FRAAT birth cohort

Josefina Zakzuk^{1,2}  | Dilia Mercado^{1,2} | Adriana Bornacelly^{1,2} | Jorge Sánchez^{1,2,3} | Velky Ahumada¹ | Nathalie Acevedo^{1,2} | Luis Caraballo^{1,2} 

¹Institute for Immunological Research, University of Cartagena, Cartagena, Colombia

²Foundation for the Development of Medical and Biological Sciences (Fundemeb), Cartagena, Colombia

³Group of Clinical and Experimental Allergy, University of Antioquia, Medellín, Colombia

Correspondence

Luis Caraballo, Institute for Immunological Research, University of Cartagena, Cartagena de Indias, Colombia.

Email: lcaraballo@unicartagena.edu.co

Funding information

This study was funded by the Colombian Government (Colciencias), Grants 325-2006 and 515-2012.

Edited by: Jon Genuneit

Abstract

Background: In tropical zones, perennial exposure to house dust mite (HDM) allergens and helminth infections is present. Studying the impact of these conditions on the inception and evolution of allergic diseases is necessary to have an accurate view of their natural history. We aimed to evaluate the dynamics of genuine sensitization to *Blomia tropicalis* and *Ascaris* in children from the FRAAT birth cohort and the effects of helminth infection, environmental HDM allergen levels, and sociodemographic factors.

Methods: Children were followed up to 6 years old. Specific IgE to recombinant allergens from *B. tropicalis* (Blo t 5 and Blo t 12) and *Ascaris* spp (Asc I 3, Asc I 13 and Asc s 1) was measured by ELISA at different time points. Allergen levels were measured in dust when children were 6 months old.

Results: IgE sensitization increased over time up to 3 years old. Correlation among the specific IgE levels to *B. tropicalis* and *Ascaris* components is poor at year 1, but coefficients are high and significant (Spearman's rho coefficients >0.70) at year 6. Unhygienic conditions increased the odds of sensitization to *B. tropicalis* allergenic components. Blo t 5 levels were lower in the poorest. IgE response to Blo t 5 and Blo t 12 was less intense in children with high exposure to Blo t 5 (levels >80th percentile).

Conclusion: In this tropical community, the pattern of childhood IgE sensitization is different from that in developing countries and is influenced by the hygienic conditions.

KEYWORDS

allergy, *Ascaris*, birth cohort, *Blomia tropicalis*, FRAAT study, IgE, sensitization, the tropics

1 | INTRODUCTION

The global allergy epidemic also affects tropical countries where prevalence rates are as high as in temperate countries. Several risk factors for allergic diseases in the tropics are similar to those found in other latitudes, but important exceptions justify particular analyses. One striking epidemiologic difference is the perennial inhalation of

house dust mite (HDM) allergens and frequent helminth infections. The impact of this context on the inception and evolution of allergic diseases should be explored to have an accurate view of their natural history. HDM sensitization is one of the most important risk factors for asthma in the tropics,^{1,2} *Blomia tropicalis* and *Dermatophagoides pteronyssinus* being the most prevalent mite species.³ Follow-up of children from the FRAAT birth cohort study,⁴ representing a Latin

American urban community, showed that sensitization to HDM begins early in life (about a third of children at 3 years old); in addition, the helminth *Ascaris spp.* was also an important sensitizer. Poverty and unhygienic living conditions were identified as risk factors for *Blomia* and *Ascaris* sensitization⁵; however, as ascariasis is associated with poor sanitation, it is possible that the results had been confounded by immunologic cross-reactivity between allergens from these sources.⁶ Therefore, the use of specific markers of sensitization could be useful to analyze the evolution of mite and *Ascaris* IgE response and the individual risk factors for sensitization. In this study, we evaluated the IgE responses to purified allergens of *B. tropicalis* (Blo t 5 and Blo t 12) and *Ascaris spp.* (Asc s 1, Asc l 3, and Asc l 13). Blo t 5 and Blo t 12 are HDM specific markers, the latter specific of *B. tropicalis* sensitization.^{7,8} Asc s 1 is a fatty acid binding protein only found in nematodes, not cross-reactive with allergens found in mite extracts.⁶ Asc l 3 (tropomyosin) and Asc s 13 (glutathione S-transferase) are *Ascaris* components, cross-reactive with homologous proteins from mites.^{9,10} Using these components, we aimed to evaluate the dynamics of genuine sensitization to *B. tropicalis* and *Ascaris* in children from the FRAAT birth cohort during the first 6 years of life and to evaluate the effects of helminth infection, environmental HDM allergen levels, and sociodemographic factors.

2 | METHODS

2.1 | Study design and characteristics of the population

The general design, objectives, and methods, including setting and eligibility criteria for participants of the FRAAT birth cohort, have been described elsewhere.⁴ It was conducted in Cartagena de Indias, a city located in the North Caribbean coast of Colombia, where most inhabitants are poor according to governmental indexes.¹¹ The Ethic Committee of the Fundación Santa Fe de Bogotá, Colombia (CCEI-282-206), approved the study. Written informed consent detailing all procedures to be carried out on children was signed by parents.

2.2 | Subjects and serum samples

Serum samples were collected for an initial study describing the evolution of IgE response to HDM and *Ascaris* extracts during childhood.⁵ When enough volume remained, serum samples were used in the present study wherein specific IgE to recombinant purified allergens from *B. tropicalis* and *Ascaris spp.* was determined at four time points during the first 6 years of life.

2.3 | Recombinant allergens

All recombinant allergens were produced in *E. coli* under an isopropyl β -D-1-thiogalactopyranoside (IPTG)-inducible promoter. Asc s 1, also named ABA-1 (UniProt accession number: Q06811) was produced as a GST fusion protein (the plasmid was kindly provided by M.W.

Kennedy, University of Glasgow, United Kingdom), cleaved and purified accordingly.⁶ Recombinant allergens Asc l 3 (ACN32322.1), Asc l 13 (P46436), Blo t 5 (O96870), and Blo t 12.0101 (Q17282) were produced as His-tagged proteins and purified using Ni-NTA resin (Qiagen, Hilden, Germany). By using sequence-specific primers, Blo t 5 was amplified from a cDNA library of *B. tropicalis* constructed from local mites isolated in urban homes from Cartagena, Colombia, cloned into a pET100 vector and expressed in BL21 (DE3) strain. Details about the production of the other allergens are published elsewhere.^{9,10,12}

2.4 | Quantification of total and specific IgE

Total IgE and specific IgE antibody levels to *B. tropicalis*, *D. pteronyssinus* and to the nematode *Ascaris spp.* were determined using ImmunoCAP[®] system according to the manufacturer's instructions (Thermo Fisher Scientific, Phadia AB, Uppsala, Sweden). Total IgE levels were reported as kU/L and specific IgE levels as kU_A/L. The cutoff to define sensitization to the extracts was 0.35 kU/L. Specific IgE was measured by ELISA as previously described^{7,10,13,14} and reported in optical density (O.D.) units. Cut-offs to determine sensitization to the recombinant allergens were evaluated by using receiver operating characteristic (ROC) curve analysis, using *B. tropicalis* or *Ascaris spp.* sensitization status (defined by ImmunoCAP results) as gold standards.

2.5 | Allergen levels in dust mattress

Dust sample collection from child mattress was done when six months old as described elsewhere.⁴ Levels of Blo t 5, Der p 1, Bla g 1, and Feld 1 (representing *B. tropicalis*, *D. pteronyssinus*, cockroach, and cat sources, respectively) were determined with commercially available kits (Indoor Biotechnologies, Charlottesville, USA) according to their instructions. Allergen levels were expressed as ng/gr of dust, except for Bla g 1 which was reported in UI/gr of dust.

2.6 | Statistical analysis

A more detailed description of statistical analyses is found in Data S1. Since specific IgE values are not normally distributed, non-parametric tests were used for data analysis. Correlation was evaluated by the Spearman correlation test. For longitudinal analysis of dependent variables (specific IgE), linear mixed models (LMMs) were used to deal with missing data.^{15,16} Univariate and multivariate binary logistic regressions were used to evaluate the influence of sociodemographic conditions, maternal sensitization, and maternal allergic phenotypes on child sensitization at 6 years old. The main outcome was sensitization to *B. tropicalis* components (defined by a positive test to Blo t 5 and/or Blo t 12). A composite index¹⁷ was derived to summarize the effect of different variables related to hygienic conditions in a single variable. Predictors identified in the univariate analyses to be potentially associated with the

TABLE 1 Frequency of sensitization to allergenic sources at age 6 years in children of the FRAAT birth cohort study

Allergen	Positive result n (%)
IgE seroprevalence (N = 226)	
<i>Blomia tropicalis</i>	92 (40.7)
<i>Ascaris</i> spp.	88 (38.9)
<i>P americana</i>	47 (20.8)
<i>Dermatophagoides pteronyssinus</i>	36 (15.9)
Egg	3 (4.4)
Milk	7 (3.1)
Cat dander	7 (3.1)
Dog dander	5 (2.2)
Skin prick test reactivity (N = 244)	
<i>Blomia tropicalis</i>	23 (9.4)
<i>Dermatophagoides pteronyssinus</i>	20 (8.2)
<i>P americana</i>	12 (4.9)
Cat dander	3 (1.2)
Dog dander	3 (1.2)

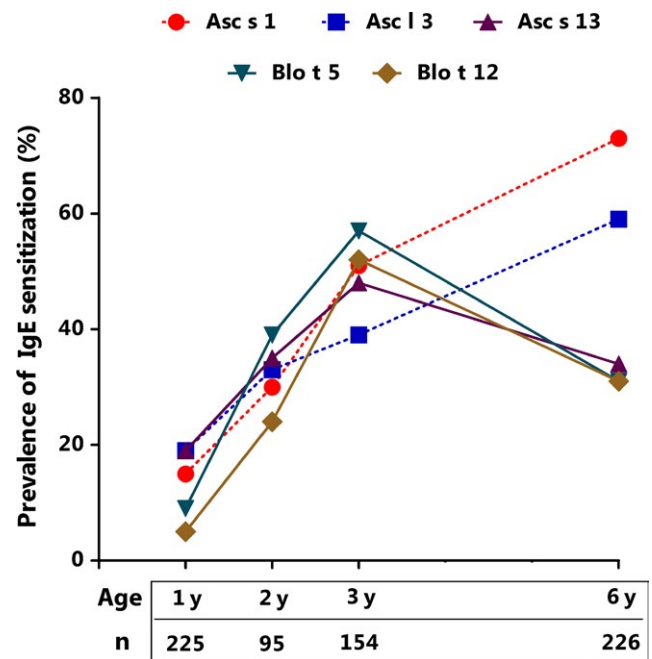
outcome (P -value <0.15) were included in the subsequent multivariate model. Gender was included in all multivariate models as a potential confounder.

The associations of specific IgE levels as a continuous variable with Blo t 5 allergen levels were modeled by using quantile regression in STATA 13.0. Regression models were adjusted by gender and potential confounders associated with HDM sensitization. Inter-quantile tests using the command *iqreg* were used to compare the distribution of the lowest and highest quantiles of specific IgE (Q1=0.25 versus Q3=0.75).

3 | RESULTS

3.1 | Subjects

Two hundred and forty-six children remained active in the FRAAT birth cohort study at 6 years of age (75%). Baseline demographic features of children included in these analyses are not different to the entire cohort (Table S1). The frequency of IgE sensitization to diverse allergenic extracts at age 6 years is shown in Table 1. Description of the sample population with available serology at different ages is shown in Table S2. Most children had at least two IgE determinations for each molecular component at different timepoints ($n = 235$, 82%); 139 (48%) for at least three and 39 (14%) had measurements for the four timepoints. Information about helminth infections was only recovered from 106 children at 6 years old. *Ascaris lumbricoides* infection was found in 10 cases (2.8%), *Trichuris trichiura* in 10 (2.8%), and hookworm in one children. The prevalence of wheezing symptoms that required emergency room visits for treatment at 6 years of age was 5.64% ($n = 14$), while the prevalence of rhinitis was 7.2% ($n = 18$).

**FIGURE 1** Evolution of IgE responses to *Ascaris* and *Blomia tropicalis* allergens from birth to age 6 years. Prevalence of IgE sensitization to the five allergens among children from the FRAAT cohort by age at follow-up is shown [Colour figure can be viewed at wileyonlinelibrary.com]

3.2 | The patterns of sensitization to *Ascaris* and *Blomia tropicalis* components change with age

IgE antibody reactivity to *Ascaris* and HDM specific markers at age 1, 2, 3, and 6 years is presented in Figure 1. At age 1 year, frequencies of IgE sensitization were significantly higher for the components of *Ascaris* compared with those of *B. tropicalis* ($P < 0.0001$). At age 3 years, Blo t 5 and Blo t 12 were the most frequent sensitizers, which changes at age 6 years when Asc l 3 and Asc s 1 became the most common. For all the components, frequencies of IgE sensitization increased over time up to 3 years old. At age 6 years, IgE sensitization to HDM allergens and Asc s 13 significantly decreased while to Asc s 1 and Asc l 3 continued increasing (Figure 1).

After 1 year, new sensitizations were observed for all allergens, mainly for Asc s 1 and Asc l 3 (Figure 2A). In addition, transient sensitization was also observed, mainly for Blo t 5, Blo t 12, and Asc l 13 at 3 years (Figure 2B). Therefore, two sensitization patterns were evident: 1) for Asc s 1 and Asc l 3, high *de novo* sensitization and low transient sensitization rates during all time-points and 2) for the HDM allergens and Asc s 13, a peak of *de novo* sensitization at 3 years, followed by around 25% of desensitization rates at 6 years.

Correlation analyses revealed that at age 1 year, the correlation coefficients of IgE levels to *B. tropicalis* and *Ascaris* components were weak or non-significant (Figure 3, see also details in Figure S1) while the correlation coefficients of specific IgE levels to Blo t 12 and Blo t 5 were moderate. At age 6 years, the correlation coefficients of specific IgE levels to *B. tropicalis* and *Ascaris* components were very high (>0.70) even though these allergens were not related or cross-reactive.

FIGURE 2 De novo sensitization and sensitization rates at different ages during follow-up. Frequency rates of new sensitizations (A) and desensitizations (B) are shown at three time-points: the first measurement at year 1, an intermediate at year 3 or year 2 (in case of a missing value at year 3), and the last at year 6. Desensitization is calculated for years 2-3 and 6 and is defined as sensitized case who is not IgE-positive in the next measurement [Colour figure can be viewed at wileyonlinelibrary.com]

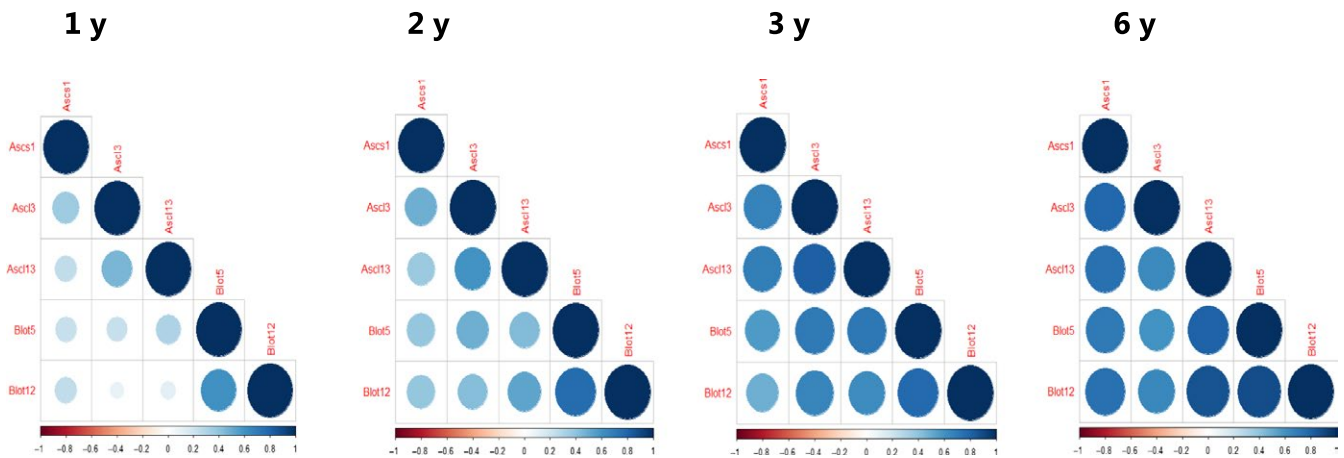
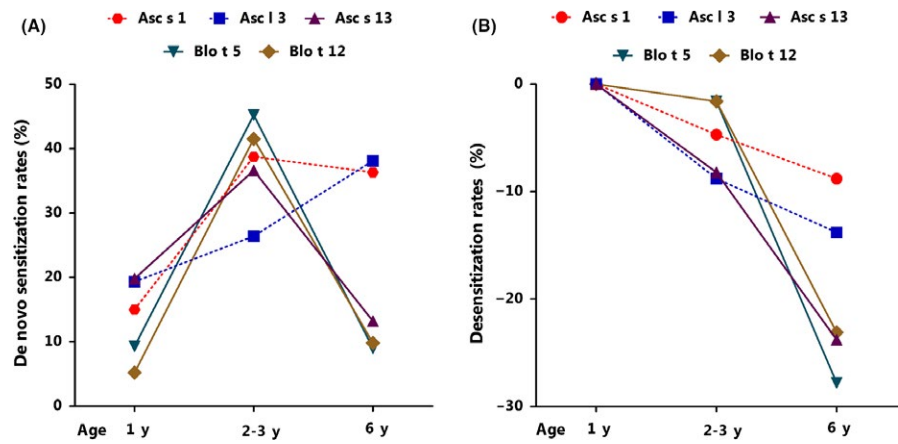


FIGURE 3 Correlation between specific IgE levels to *Ascaris* and *Blomia tropicalis* components. Spearman's correlation coefficients among IgE levels to the six purified allergens were plotted by using the *corplot* package implemented in R.³³ Positive correlations are displayed in blue. Color intensity and the size of the circle are proportional to the correlation coefficients [Colour figure can be viewed at wileyonlinelibrary.com]

3.3 | IgE sensitization to *B. tropicalis* allergens is associated with unhygienic conditions

The exploratory univariate analyses about the effect of different predictors on sensitization to individual components (Table S3) led to identify that different housing features (mostly associated with hygiene) are potentially associated with Blo t 12 or Blo t 5 sensitization and, some of them, with the *Ascaris* GST (Asc l 13). By multivariate logistic regression, it was found that maternal HDM sensitization (aOR: 2.31; 95% CI: 1.28 – 4.17) and hygienic conditions (aOR: 0.47; 95% CI: 0.25 – 0.85) are independently associated with sensitization to any of the *B. tropicalis* components (Table 2). Maternal HDM sensitization (aOR: 2.08; 95% CI: 1.09 – 3.97) and hygienic conditions (aOR: 0.54; 95% CI: 0.29 – 0.99, $p = 0.46$) also kept associated with *Ascaris* GST in the multivariate analyses (Table S4).

Interestingly, among those with stool examination, recent *Ascaris* infection was also associated with this outcome (aOR: 5.8, 95% CI: 1.1–32.1, $P = 0.04$). Asc s 1-sensitized children showed longitudinally higher specific IgE values to Blo t 5 (estimate 0.02 95% CI: 0.01–0.03, $P < 0.0001$) and Blo t 12 (estimate 0.06 95% CI: 0.04–0.06 OD, $P < 0.0001$) than non-sensitized (Figure 4).

3.4 | High environmental levels of Blo t 5 are associated with lower IgE to *B. tropicalis* allergens

The concentrations of Der p 1, Blo t 5, Bla g 1, and Fel d 1 were measured in dust samples collected from child mattresses at 6 months after birth (Table S5). Belonging to the poorest, socioeconomic stratum (SES) was associated with lower levels of Blo t 5 ($P < 0.001$). Children with high exposure to Blo t 5 (≥ 80 th percentile) had significantly lower IgE levels to Blo t 5, Blo t 12, *B. tropicalis*, *Ascaris*, and Asc l 13, but not to Asc s 1 or Asc l 3 (Table 3). There were no differences in specific IgE to the mentioned allergens in regard to Der p 1 high exposure (Table S6). By inter-quantile regression, there was significant inverse association between Blo t 5 levels and specific IgE to Blo t 5 ($P = 0.04$) or Blo t 12 ($P = 0.019$); see complete results of the model in Table S7.

4 | DISCUSSION

In the tropics, the IgE response to HDM is stronger than in other latitudes, which could be explained by genetic, environmental, and

	Crude OR (95% CI)	P-value	aOR (95% CI)	P-value
Gender	1.01 (0.64 – 1.90)	0.74	1.17 (0.65-2.10)	0.60
"Good" hygienic conditions ^a	0.43 (0.24 – 0.78)	0.005	0.47 (0.25-0.85)	0.01
Maternal sensitization to HDM	2.57 (1.45 – 4.57)	0.001	2.31 (1.28-4.17)	0.005
Dog at home	1.51 (0.87 – 2.64)	0.15	1.48 (0.82-2.66)	0.19

^aDefined by a score >2 in the composite index that evaluate hygienic conditions (toilet at home, connection to sewage system, access to tap water, floor type, and house building material).

TABLE 2 Sociodemographic factors associated with sensitization to *Blomia tropicalis* components (Blo t 5 or Blo t 12)

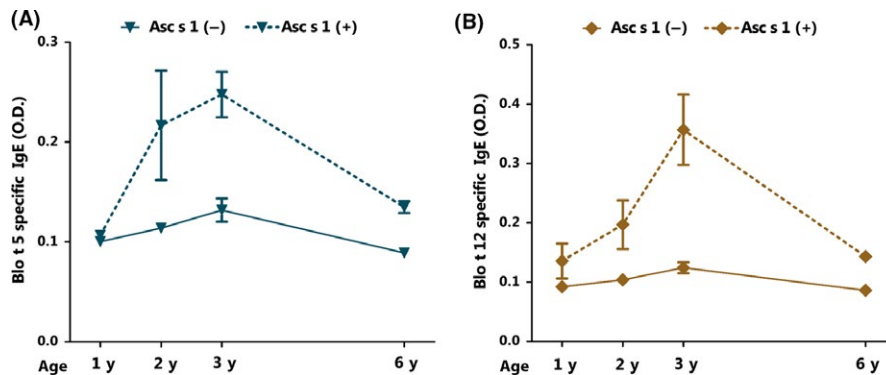


FIGURE 4 Differences in the dynamics of IgE response to Blo t 5 and Blo t 12, according to Ascaris s 1 sensitization. Graph lines representing mean IgE levels at the different time points. Error bars represent SEM [Colour figure can be viewed at wileyonlinelibrary.com]

Allergen	Blo t 5 at 6 months						P-value
	< 80th percentile			≥ 80 th percentile			
	gMean	SD	N	gMean	SD	N	
<i>Blomia tropicalis</i>	0.28	3.22	166	0.15	6.07	43	0.05
<i>Ascaris spp</i>	0.26	5.11	166	0.11	1.02	43	0.02
<i>Dermatophagoides pteronyssinus</i>	0.10	1.59	166	0.13	13.71	43	0.74
Cow milk	0.04	0.08	166	0.05	0.18	43	0.64
Egg	0.05	0.45	166	0.05	0.10	43	0.69
Cat	0.02	0.08	166	0.02	0.27	43	0.91
Dog	0.04	0.12	166	0.04	0.19	43	0.62
<i>P americana</i>	0.09	1.84	166	0.09	3.40	43	0.50
Asc I 13	0.15	0.19	166	0.13	0.11	43	0.03
Asc s 1	0.22	0.22	166	0.20	0.18	43	0.37
Asc I 3	0.16	0.27	166	0.15	0.08	43	0.24
Blo t 5	0.12	0.08	166	0.10	0.04	43	0.01
Blo t 12	0.12	0.09	166	0.10	0.06	43	0.01

Significant associations are highlighted in bold.

TABLE 3 Allergen Specific IgE according to Blo t 5 mattress levels

sociodemographic factors.² In this study, extending the analyses of our birth cohort up to age 6 years, we found high rates of IgE against *B. tropicalis* and *A. lumbricoides*. Having previously reported that these sources are cross-reactive,⁶ which could bias the diagnosis of sensitization, here, we studied the IgE response by using molecular components, finding that HDM and *Ascaris* allergens had different dynamics and peaks of sensitization during the first 6 years of life

and confirming that they are distinct sensitizers. As expected, this strategy also helped us to better analyze the relative importance of the effects of hygiene and poverty on the specific IgE responses.

By using specific markers, we can ascertain that the high rate of sensitization to *B. tropicalis* in children does not obey to cross-reactivity with *Ascaris*.⁶ In other studies in our population, we have observed that *Ascaris* and *B. tropicalis* specific IgE levels were highly

correlated and identified environmental factors associated with sensitization to both sources.⁵ By using non-cross-reactive allergens, we observed that specific IgE responses to *B. tropicalis* (ie, Blo t 12 and Blo t 5) and to *Ascaris* (Asc s 1) were poorly correlated in the first year of life, but at the end of follow-up, this correlation was high, as observed in allergic adults.¹⁸ A way to interpret these results is that the frequent exposition to both sources in tropical environments led to co-sensitization.

Our results show that sensitization to specific markers of *B. tropicalis* was inversely associated with poor hygienic conditions. An explanation for this relationship may be due to the effects of helminthic exposure on atopic responses,¹⁹ since there is a well-known association between ascariasis and sanitation. We had found similar associations with *B. tropicalis* and *Ascaris* extract sensitization at 2-3 years of age.⁵ The use of specific markers in the present study suggests that associations with *B. tropicalis* were not due to the bias of cross-reactive allergens.^{6,14} In spite of the limited sample size number of children with stool examinations, it was observed that *Ascaris* infection was associated with sensitization to Blo t 5/Blo t 12, supporting the idea that helminthiasis boost type 2 responses against sources different to their own antigens.²⁰⁻²² Greater Blo t 5/Blo t 12 specific IgE values in Asc s 1-sensitized children is also in accordance with a non-specific boosting phenomenon. However, this “boosting” effect on atopic responses may be transient. For the HDM allergens, there was a peak of sensitization at 3 years, with levels markedly higher than that of adults in the same population^{7,23} that declined later in life, a pattern previously found for the GST allergen of *A. lumbricoides* specific IgE response.¹⁰ In contrast, IgE response to Asc s 1 and Asc l 3 increased over time and incident sensitizations accumulated and finished at the end of follow-up, with most children sensitized to any of them (80%). It has been shown that helminth IgE responses persist for long time even in absence of infection,²⁴ and then, data of positive IgE to *Ascaris* reflect current plus past infected cases. It is possible that Asc s 1 and Asc l 3 are protective immunity targets due to their great bioavailability that facilitate exposure. Actually, Asc s 1 has been previously associated with natural immunity to *Ascaris* infection.²⁵

Dwellings of the poorest strata had lower environmental levels of *B. tropicalis*, which was independently and inversely associated with sensitization to its allergens (Figure S2). The increased risk of sensitization under bad sanitary conditions was detected for *B. tropicalis* allergens and may be influenced by the low concentration of allergens found in the environment, which in turn was negatively associated with IgE responses to allergens. In agreement with Baqueiro *et al*, we detected lower concentrations of mite allergens in the poorest stratum²⁶ and this could be due to the existence of more open houses among the poorest neighborhood.²⁷ But we also found that the lower the Blo t 5 levels, the higher the IgE to *B. tropicalis* allergens, in contrast to data suggesting that high exposure to HDM is a risk factor for sensitization.²⁸ In fact, this is a controversial issue and results differ among populations.²⁹ Many reports have been found a “bell shape” where very low or high allergen levels protect from sensitization.^{30,31} Others have found, as our data, an

inverse relationship between sensitization and high exposure to mite allergen.³²

One limitation of our study is that the number of cases of asthma and rhinitis was so low that precludes a robust analysis of the clinical impact of sensitizations. Follow-up of this cohort up to older ages, where clinical definition of atopic phenotypes will be more feasible, will permit to obtain important conclusions about this topic.

In summary, by defining sensitization with specific markers, we found that in a tropical environment, unhygienic conditions increase the odds to become sensitized against allergens of *B. tropicalis*, probably by the boosting of type 2 responses that induce helminth infections. The influence of hygiene conditions and poverty on environmental HDM allergen levels should be further investigated.

ACKNOWLEDGMENTS

We thank all the children and families that kindly participated in this study and to the assistant nurse Mileidys Avila for her skillful support with child venipuncture. This study was funded by the Colombian Government (Colciencias), Grants 325-2006 and 515-2012.

CONFLICT OF INTEREST

The authors declare no conflicts of interest.

ORCID

Josefina Zakzuk  <https://orcid.org/0000-0002-6007-5376>

Luis Caraballo  <https://orcid.org/0000-0002-5204-1109>

REFERENCES

- Caraballo L, Puerta L, Fernandez-Caldas E, Lockey RF, Martinez B. Sensitization to mite allergens and acute asthma in a tropical environment. *J Invest Allergol Clin Immunol*. 1998;8(5):281-284.
- Caraballo L, Zakzuk J, Lee BW, et al. Particularities of allergy in the Tropics. *World Allergy Organ J*. 2016;9:20.
- Fernandez-Caldas E, Puerta L, Mercado D, Lockey RF, Caraballo LR. Mite fauna, Der p I, Der f I and *Blomia tropicalis* allergen levels in a tropical environment. *Clin Exp Allergy*. 1993;23(4):292-297.
- Acevedo N, Sanchez J, Zakzuk J, et al. Particular characteristics of allergic symptoms in tropical environments: follow up to 24 months in the FRAAT birth cohort study. *BMC Pulm Med*. 2012;12:13.
- Zakzuk J, Acevedo N, Cifuentes L, et al. Early life IgE responses in children living in the tropics: a prospective analysis. *Pediatr Allergy Immunol*. 2013;24(8):788-797.
- Acevedo N, Sanchez J, Erler A, et al. IgE cross-reactivity between *Ascaris* and domestic mite allergens: the role of tropomyosin and the nematode polypeptide ABA-1. *Allergy*. 2009;64(11):1635-1643.
- Zakzuk J, Jimenez S, Cheong N, et al. Immunological characterization of a Blo t 12 isoallergen: identification of immunoglobulin E epitopes. *Clin Exp Allergy*. 2009;39(4):608-616.
- Carvalho Kdos A, de Melo-Neto OP, Magalhaes FB, et al. *Blomia tropicalis* Blo t 5 and Blo t 21 recombinant allergens might confer higher specificity to serodiagnostic assays than whole mite extract. *BMC Immunol*. 2013;14:11.

9. Acevedo N, Erler A, Briza P, Puccio F, Ferreira F, Caraballo L. Allergenicity of *Ascaris lumbricoides* tropomyosin and IgE sensitization among asthmatic patients in a tropical environment. *Int Arch Allergy Immunol*. 2009;154(3):195–206.
10. Acevedo N, Mohr J, Zakzuk J, et al. Proteomic and immunochemical characterization of glutathione transferase as a new allergen of the nematode *Ascaris lumbricoides*. *PLoS ONE*. 2013;8(11):e78353.
11. Rueda de Vivero F, Espinosa A. Will today's poor be tomorrow's poor? Determinants of the poverty and magnitude of the vulnerability in. *Cartagena de Indias. Ed. Tecnológica de Bolívar*. 2008.
12. Zakzuk J, Benedetti I, Fernandez-Caldas E, Caraballo L. The influence of chitin on the immune response to the house dust mite allergen Blo T 12. *Int Arch Allergy Immunol*. 2014;163(2):119–129.
13. Acevedo N, Mercado D, Vergara C, et al. Association between total immunoglobulin E and antibody responses to naturally acquired *Ascaris lumbricoides* infection and polymorphisms of immune system-related LIG4, TNFSF13B and IRS2 genes. *Clin Exp Immunol*. 2009;157(2):282–290.
14. Acevedo N, Erler A, Briza P, Puccio F, Ferreira F, Caraballo L. Allergenicity of *Ascaris lumbricoides* tropomyosin and IgE sensitization among asthmatic patients in a tropical environment. *International Arch Allergy Immunol*. 2010;154(3):195–206.
15. West BT. Analyzing longitudinal data with the linear mixed models procedure in SPSS. *Eval Health Prof*. 2009;32(3):207–228.
16. Cnaan A, Laird NM, Slasor P. Using the general linear mixed model to analyse unbalanced repeated measures and longitudinal data. *Stat Med*. 1997;16(20):2349–2380.
17. Thakur N, Martin M, Castellanos E, et al. Socioeconomic status and asthma control in African American youth in SAGE II. *J Asthma*. 2014;51(7):720–728.
18. Buendia E, Zakzuk J, Mercado D, Alvarez A, Caraballo L. The IgE response to *Ascaris* molecular components is associated with clinical indicators of asthma severity. *World Allergy Organ J*. 2015;8(1):8.
19. Caraballo L, Buendía E. Human ascariasis increases the allergic response and allergic symptoms. *Curr Trop Med Rep*. 2015;2:224–232.
20. Marretta J, Casey FB. Effect of *Ascaris suum* and other adjuvants on the potentiation of the IgE response in guinea-pigs. *Immunology*. 1979;37(3):609–613.
21. Lee TD, McGibbon A. Potentiation of IgE responses to third-party antigens mediated by *Ascaris suum* soluble products. *Int Arch Allergy Immunol*. 1993;102(2):185–190.
22. Caraballo L, Acevedo N. New allergens of relevance in tropical regions: The impact of *ascaris lumbricoides* infections. *World Allergy Organ J*. 2011;4(5):77–84.
23. Jiménez S, Puerta L, Mendoza D, Chua KY, Mercado D, Caraballo L. IgE antibody responses to recombinant allergens of *Blomia tropicalis* and *dermatophagoides pteronyssinus* in a tropical environment. *Allergy Clin Immunol Int - J World Allergy Org*. 2007;19(6):233–238.
24. Mitre E, Nutman TB. IgE memory: persistence of antigen-specific IgE responses years after treatment of human filarial infections. *J Allergy Clin Immunol*. 2006;117(4):939–945.
25. McSharry C, Xia Y, Holland CV, Kennedy MW. Natural immunity to *Ascaris lumbricoides* associated with immunoglobulin E antibody to ABA-1 allergen and inflammation indicators in children. *Infect Immun*. 1999;67(2):484–489.
26. Baqueiro T, Carvalho FM, Rios CF, dos Santos NM, Alcantara-Neves NM, Medical SG. Dust mite species and allergen concentrations in beds of individuals belonging to different urban socioeconomic groups in Brazil. *J Asthma*. 2006;43(2):101–105.
27. Hagel I, Cabrera M, Hurtado MA, et al. Infection by *Ascaris lumbricoides* and bronchial hyper reactivity: an outstanding association in Venezuelan school children from endemic areas. *Acta Trop*. 2007;103(3):231–241.
28. Wahn U, Lau S, Bergmann R, et al. Indoor allergen exposure is a risk factor for sensitization during the first three years of life. *J Allergy Clin Immunol*. 1997;99(6 Pt 1):763–769.
29. Calderon MA, Linneberg A, Kleine-Tebbe J, Hernandez Fernandez de Rojas D, Virchow JC, et al. Respiratory allergy caused by house dust mites: What do we really know? *J Allergy Clin Immunol*. 2015;136(1): 38–48.
30. Tovey ER, Almqvist C, Li Q, Crisafulli D, Marks GB. Nonlinear relationship of mite allergen exposure to mite sensitization and asthma in a birth cohort. *Journal Allergy Clin Immunol*. 2008;122(1):pp. 114–118, 118 e111–115.
31. Torrent M, Sunyer J, Munoz L, et al. Early-life domestic aeroallergen exposure and IgE sensitization at age 4 years. *J Allergy Clin Immunol*. 2006;118(3):742–748.
32. Dharmage S, Bailey M, Raven J, et al. Current indoor allergen levels of fungi and cats, but not house dust mites, influence allergy and asthma in adults with high dust mite exposure. *Am J Respir Crit Care Med*. 2001;164(1):65–71.
33. Wei T, Simko V. R package "corrplot": Visualization of a Correlation Matrix (Version 0.84). 2017 [cited] Available from: <https://github.com/taiyun/corrplot>.

SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section at the end of the article.

How to cite this article: Zakzuk J, Mercado D, Bornacelly A, et al. Hygienic conditions influence sensitization to *Blomia tropicalis* allergenic components: Results from the FRAAT birth cohort. *Pediatr Allergy Immunol*. 2019;30:172–178. <https://doi.org/10.1111/pai.13004>

Supporting information

STATISTICAL ANALYSIS

All analyses were performed using IBM® SPSS® Statistics version 20.0 (Chicago, IL, USA). Frequency rates and their 95% confidence intervals (CI) were obtained with Epidat 3.1 (Xunta de Galicia, PAO/WHO). Geometric mean and its standard deviation (SD) were reported for specific IgE and allergen levels. Specific IgE values were not normally distributed; thus, non-parametric tests were used for data analysis: Mann-Whitney *U* or Kruskal Wallis test for comparison of continuous variables between 2 or more groups, respectively, and Wilcoxon rank test in the case of paired variables. For non-parametric data, Test for trend across ordered groups were analyzed with *nptrend* command in STATA 13.0. Correlation analyses were done by Spearman correlation test. Differences between proportions were analyzed by Pearson chi-squared test. For longitudinal analysis of dependent variables (specific IgE), linear mixed models (LMM) were used to deal with missing data [1, 2]. The influence of a factor or a covariate on these outcomes was reported as estimate with 95% confidence intervals (95% CI) and its respective p-value. The indicator of a repeated measure was *age* (in years). Gender and socio-economic status (SES) were added to the model as covariates.

Univariate and multivariate binary logistic regression were used to analyze the relationships of exposures and outcomes. The main outcome was sensitization to *B. tropicalis* (defined by a positive test to Blo t 5 and/or Blo t 12). The predictors included socio-demographic conditions (i.e. socioeconomic stratum, house building features, sewage, tap water, pets at home), maternal HDM- or Ascaris- sensitization and maternal allergic phenotypes (i.e. asthma and rhinitis). Variables associated to the outcome in the univariate analysis ($p < 0,15$) were included in subsequent multivariate models. Gender was included in all multivariate models as a potential confounder. Sensitization to any of the individual components (Blo t 5, Blo t 12 Asc s 1, Asc l 13 and Asc l 3) was also analyzed as separate outcomes and presented in the Supplementary Table E3. This exploratory analysis led us to identify that several predictors related with house characteristics were influencing HDM sensitization. To avoid collinearity among different sanitation indicators in the multivariate model, we derived a composite “housing/hygienic condition index” that summarizes their effect on a single variable [3], summing the scores obtained in each indicator as follows: Toilet at home: yes = 1, no = 0; connected to sewage system: yes = 1, no = 0; access to tap water: yes = 1, no = 0; Floor type: tile floor = 1, other types of floor material (i.e. soil or bare concrete) = 0 and house building material: Brick = 1, other types

(i.e. wood, plastic) = 0. The final score has 6 possible ordinal values (0 to 5). Then, to achieve more power, this index was dichotomized into “poor” (score ≤ 3) versus “better” housing/sanitation conditions (score >3). Crude (OR) and adjusted odds ratios (aOR), 95% confidence intervals (95% CI (OR) and p-values were calculated. A p value below 0.05 was considered significant.

The associations of specific IgE levels as a continuous variable with Blo t 5 allergen levels were modeled by using quantile regression in STATA 13.0. Regression models were adjusted by gender and potential confounders associated with HDM sensitization. Inter-quantile tests using the command *iqreg* were used to compare the distribution of the lowest and highest quantiles of specific IgE (Q1=0.25 versus Q3=0.75). This model is useful to evaluate data with the lack of normal distribution or the presence of outlier observations.

Power was calculated assuming an independent case-control design and expressing the alternative hypothesis as Odds Ratio (ψ). For a given risk factor the calculation is done by setting type I error probability at $\alpha = 0.05$, number of sensitized cases (n), probability of exposure among non-sensitized children (p_0), and the ratio of non-sensitized controls/cases (m) [4]. The present study was powered to detect associations ($>80\%$) between the main outcome (HDM sensitization in children) and predictors such as maternal HDM sensitization and hygienic status, but it was underpowered (41%) to detect the effect of *Ascaris* infection due to the limited sample size.

REFERENCES

1. West, B.T., *Analyzing longitudinal data with the linear mixed models procedure in SPSS*. Eval Health Prof, 2009. **32**(3): p. 207-28.
2. Cnaan, A., N.M. Laird, and P. Slasor, *Using the general linear mixed model to analyse unbalanced repeated measures and longitudinal data*. Stat Med, 1997. **16**(20): p. 2349-80.
3. Thakur, N., et al., *Socioeconomic status and asthma control in African American youth in SAGE II*. J Asthma, 2014. **51**(7): p. 720-8.
4. Dupont, W.D. and W.D. Plummer, Jr., *Power and sample size calculations for studies involving linear regression*. Control Clin Trials, 1998. **19**(6): p. 589-601.

Table E1. Comparison of the current study sample with baseline cohort

	Entire cohort	Followed up at 6y	p-value
	N = 326	N = 246	
	n (%)	n (%)	
Female	165 (50,6)	122 (49,6)	0,52
Sewage	191 (58,6)	144 (58,5)	0,84
Access to tap water (yes vs. no)	290 (89)	220 (89,4)	0,60
Tile floor	90 (27,6)	71 (28, 9)	0,76
Living in brick house	243 (74,5)	187 (76,0)	0,37
SES			
1	257 (78,8)	191 (77,6)	0,40
2	59 (18,1)	45 (18,3)	
3	10 (3,1)	9 (3,7)	
Maternal rhinitis	84 (25,7)	76 (30,9)	0,77
Maternal asthma	21 (6,3)	19 (7,7)	0,30

Table E2. Descriptive information of the study sample with available serology data

Age of sampling	Age in months	N	Allergen extract	Number of positives	Frequency (%)
1y	0.7 ± 1.0	225	Ascaris	0	0.0
			<i>B. tropicalis</i>	2	0.9
2y	17.0 ± 4.8	95	Ascaris	9	9.5
			<i>B. tropicalis</i>	11	11.6
3y	32.0 ± 4.6	154	Ascaris	37	24.0
			<i>B. tropicalis</i>	43	27.9
6y	72.9 ± 1.3	226	Ascaris	88	38.9
			<i>B. tropicalis</i>	93	40.7

Table E3 Predictors for IgE sensitization at age 6 years in the FRAAT birth cohort study: univariate analysis

Predictor	Blo t 5				Blo t 12				Asc l 13 (GST)				Asc l 3				Asc s 1			
	OR	95%CI		Sig.	OR	95%CI		Sig.	OR	95%CI		Sig.	OR	95%CI		Sig.	OR	95%CI		Sig.
Female (vs. Male)	1.10	0.63	1.93	0.73	1.02	0.58	1.78	0.96	0.90	0.52	1.56	0.71	1.60	0.94	2.74	0.08	0.92	0.46	1.83	0.80
Sewage (yes vs. no as REF)	0.36	0.19	0.68	<0.001	0.32	0.17	0.61	<0.001	0.42	0.22	0.80	0.01	0.55	0.28	1.07	0.08	0.63	0.29	1.36	0.24
Access to tap water (yes vs. no as REF)	0.49	0.18	1.33	0.16	0.29	0.11	0.80	0.02	0.33	0.12	0.91	0.03	0.17	0.04	0.78	0.02	0.16	0.02	1.23	0.08
Toilet at home (yes vs. no as REF) ^a	0.34	0.16	0.72	<0.001	0.30	0.14	0.62	<0.001	0.39	0.19	0.83	0.01	0.55	0.25	1.22	0.14	0.55	0.22	1.40	0.21
Tile floor (yes vs. Others as REF) ^b	0.44	0.21	0.92	0.03	0.44	0.21	0.92	0.03	0.66	0.34	1.29	0.22	0.61	0.33	1.12	0.11	0.59	0.31	1.15	0.12
Brick house (vs. Others as REF) ^c	0.63	0.32	1.23	0.18	0.56	0.28	1.09	0.09	0.64	0.33	1.26	0.20	0.75	0.38	1.49	0.42	0.75	0.35	1.63	0.47
Socio-economic status (1, 2 or 3)	0.60	0.32	1.15	0.12	0.36	0.17	0.76	0.01	0.47	0.24	0.91	0.03	0.77	0.45	1.30	0.33	0.94	0.52	1.70	0.83
Ascaris infection in the last year ^d	6.42	1.23	33.35	0.03	6.42	1.23	33.35	0.03	6.42	1.23	33.35	0.03	2.71	0.26	28.37	0.40	1.00	0.09	10.74	1.00
(yes vs. no as REF.)																				
Dog at home (yes vs. no as REF)	1.32	0.74	2.34	0.34	1.44	0.81	2.54	0.21	1.64	0.94	2.89	0.08	0.92	0.54	1.59	0.78	0.70	0.38	1.28	0.25
Maternal sensitization to <i>B. tropicalis</i> (yes vs. no as REF)	0.87	0.48	1.56	0.63	1.23	0.69	2.20	0.48	1.08	0.61	1.92	0.79	0.99	0.57	1.72	0.96	1.23	0.65	2.32	0.53
Maternal sensitization to HDM ^e (yes vs. no as REF)	2.70	1.50	4.86	<0.001	2.70	1.50	4.86	<0.001	2.37	1.33	4.23	<0.001	1.83	1.03	3.25	0.04	1.39	0.73	2.65	0.31
Maternal sensitization to Ascaris spp. (yes vs. no as REF.)	0.93	0.53	1.64	0.81	1.01	0.57	1.79	0.96	1.06	0.60	1.85	0.85	0.88	0.51	1.51	0.64	1.09	0.59	2.00	0.79
Maternal sensitization to ABA-1 (yes vs. no as REF.)	1.80	0.82	3.96	0.14	1.53	0.69	3.39	0.29	2.15	0.99	4.69	0.05	1.42	0.63	3.20	0.40	1.89	0.69	5.20	0.22
Maternal rhinitis (yes vs. no as REF.)	0.78	0.40	1.52	0.47	0.88	0.46	1.68	0.69	0.96	0.51	1.81	0.89	0.99	0.54	1.82	0.98	1.20	0.60	2.40	0.61
Maternal asthma (yes vs. no as REF.)	0.58	0.16	2.14	0.41	0.87	0.26	2.86	0.81	1.11	0.36	3.44	0.86	0.93	0.31	2.78	0.90	1.36	0.37	5.05	0.65

^aversus (vs.) Other methods for excretal disposal, ^bvs. others including bare concrete and soil; ^cvs. Others associated with poverty including wood; ^devaluated in 106 children and ^esensitization defined by recombinant HDM allergens. REF. = reference.

Table E4. Multivariate logistic regression for Asc l 13 (Ascaris GST) sensitization

Predictor	aOR	95%CI (aOR)	p-value
Gender	1,081	0,60 - 1,948	0,796
Maternal sensitization to HDM	2,075	1,082 - 3,98	0,028
Good hygienic conditions	0,535	0,29 - 0,99	0,046
Maternal sensitization to ABA-1	1,449	0,65 - 3,255	0,368

Crude odd ratios for predictors included in the model are shown in Table E3.

Table E5 Allergen levels detected in dust mattress

Allergen (ng/Gr)	Detection rate n (%)	Geometric mean $\pm$ SD	80thPercentile
Der p 1	200 (95.2)	448.1 $\pm$ 1335.6	1.5 μ g/gr
Bla g 1*	164 (78.1)	1.2 $\pm$ 2.8	2.3 UI/gr
Blo t 5	105 (50.2)	60.9 $\pm$ 118.7	70 μ g/gr
Fel d 1	94 (47.4)	33.2 $\pm$ 114.4	30 μ g/gr

*IU/gram of weighted dust.

Table E6 Allergen Specific IgE according to Der p 1 levels in mattresses

Allergen-sIgE	< 80 th percentile			≥ 80 th percentile			p-value
	gMean	SD	N	gMean	SD	N	
<i>B. tropicalis</i>	0,23	4,07	167	0,26	3,47	44	0,69
<i>Ascaris spp</i>	0,20	5,08	167	0,24	1,35	44	0,35
<i>D. pteronyssinus</i>	0,11	7,15	167	0,07	0,63	44	0,15
Cow milk	0,04	0,11	167	0,04	0,09	44	0,90
Egg	0,05	0,45	167	0,05	0,09	44	0,87
Cat	0,02	0,16	167	0,02	0,02	44	0,90
Dog	0,04	0,15	167	0,03	0,04	44	0,15
<i>P. americana</i>	0,10	2,50	167	0,06	0,34	44	0,09
Asc l 13	0,15	0,17	167	0,16	0,19	44	0,15
Asc s 1	0,21	0,22	167	0,20	0,18	44	0,88
Asc l 3	0,16	0,08	167	0,17	0,51	44	0,98
Blo t 5	0,11	0,08	167	0,12	0,06	44	0,48
Blo t 12	0,11	0,08	167	0,12	0,08	44	0,07

Table E7. Relationship between specific IgE levels to Blo t 5 or Blo t 12 with Blo t 5 levels:

Results from the interquartile regression (P75-P25) models

Model	Coefficient	95% CI (coefficient)		p-value
Blo t 5 IgE levels (O.D)				
Gender	0,004	-0,020	0,029	0,71
Maternal HDM sensitization	0,0232	-0,001	0,048	0,06
Toilet at home	-0,0327	-0,106	0,041	0,38
Blo t 5 in dust	-0,00007	-0,0001	-2,8700E-06	0,04
Blo t 12 IgE levels (O.D)				
Gender	0,0054745	0,0160145	0,34	0,733
Maternal HDM sensitization	0,0437413	0,0120703	0,0754123	0,007
Toilet at home	-0,0565678	-0,1552252	0,0420896	0,26
Blo t 5 in dust	-0,0000981	-0,0001799	-0,0000162	0,019
Significant associations in bold				

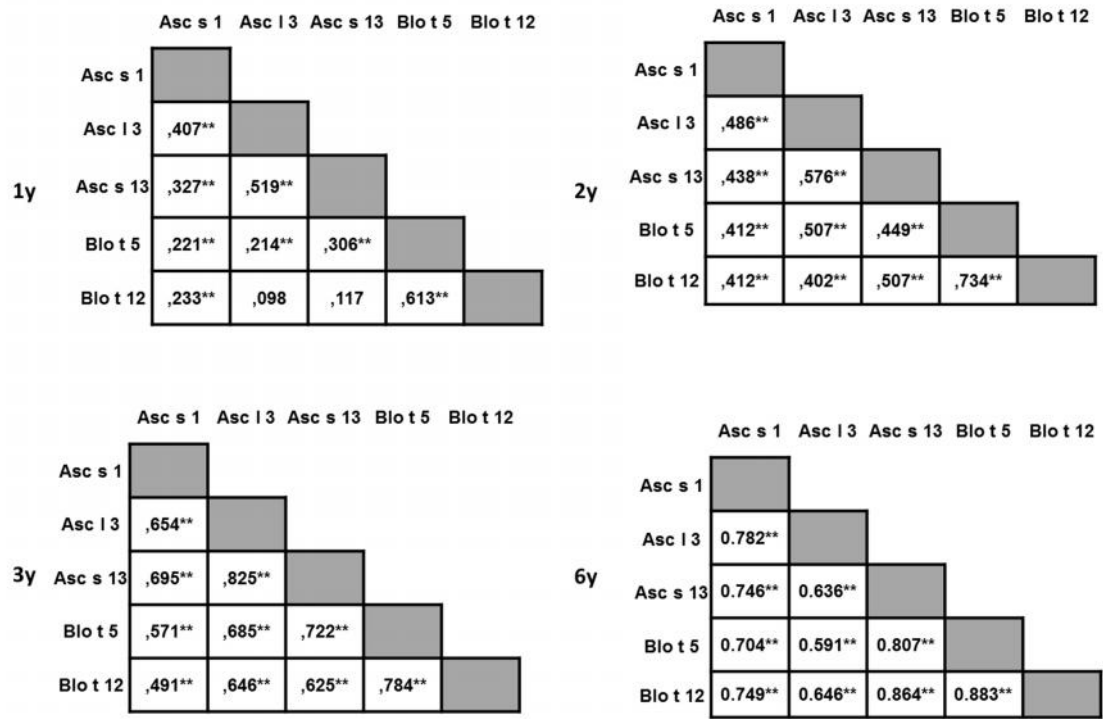
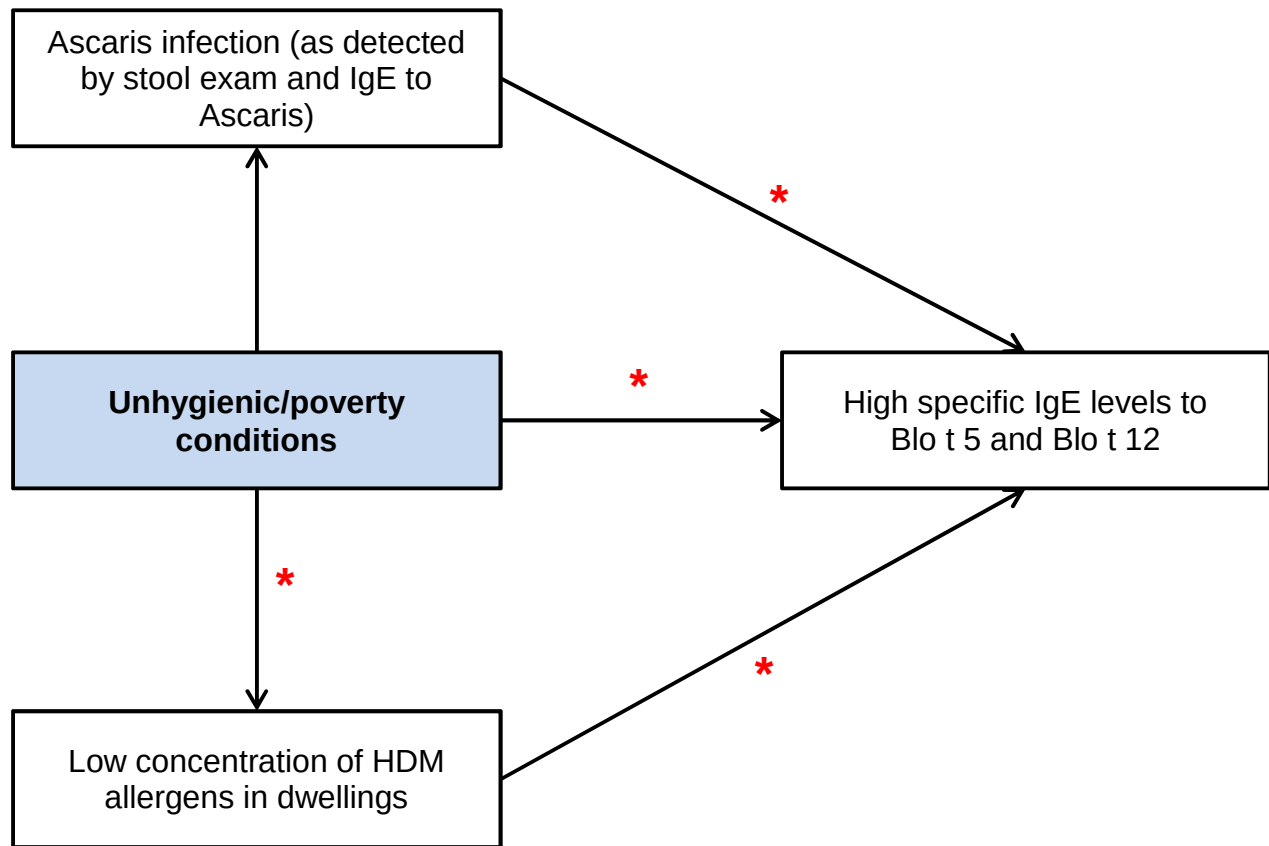


Figure E1. Correlation between specific IgE levels to *Ascaris* and *B. tropicalis* components. Spearman rho coefficients are shown inside boxes. ** p-values < 0.0001.



* Significant associations found in this study

Figure E2. Factors influencing the IgE responses to HDM allergens in children living in the tropics (FRAAT Cohort)

