

Poor sanitation and helminth infection protect against skin sensitization in Vietnamese children: A cross-sectional study

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Background: Geohelminth infection and poor hygiene may be protective against allergic sensitization.

Objective: To determine whether current helminth infection is associated with a reduced prevalence of allergen skin test sensitization in a Southeast Asian population of children with a high prevalence of hookworm infection.

Methods: A total of 1742 Vietnamese schoolchildren were invited to take part in a cross-sectional survey. Allergen skin sensitization to house dust mites (*Dermatophagoides pteronyssinus* and *Dermatophagoides farinae*) and American cockroach (*Periplaneta americana*) were measured and stool samples for qualitative and quantitative geohelminth estimation collected.

Results: A total of 1601 children age 6 to 18 participated. Sensitization to dust mites was present in 14.4% and to cockroach in 27.6% of children. In a mutually adjusted model, the risk of sensitization to dust mites was reduced in those with higher hookworm burden (adjusted odds ratio [OR] for 350+ vs no eggs per gram, 0.61; 95% CI, 0.39-0.96) and with *Ascaris* infection (adjusted OR, 0.28; 0.10-0.78), and increased in those using flush toilets (adjusted OR for flush toilet vs none/bush/pit, 2.51; 1.00-6.28). In contrast, sensitization to cockroach was not independently related to geohelminth infection but was increased in those regularly drinking piped or well water rather than from a stream (adjusted OR, 1.33; 1.02-1.75).

Conclusion: Geohelminth infection, sanitation, and water supply influence the risk of allergic sensitization in Vietnamese children. This is consistent with a protective effect against allergy by geohelminth or other gastrointestinal infection. **Clinical implications:** If the inverse relationship between geohelminth infection, poor sanitation, and allergic sensitization proves to be causal, drugs derived from parasite products may help to alleviate clinical allergic disease. (J Allergy Clin Immunol ■■■■;■■■:■■■-■■■.)

Key words: Atopy, helminths, hygiene hypothesis, epidemiology

Allergic diseases are rare in developing nations, such as Vietnam, but are more common in areas of urbanization.¹⁻⁴ In this context, the potential role of endoparasitic exposure has been debated for years. Some but not all cross-sectional studies suggest that the relatively high prevalence of allergic disease in urban areas of developing countries may be explained by a reduced exposure to soil-transmitted helminths.^{5,6} Similar observations have been made with regard to allergic sensitization.⁷⁻¹¹ The only study that has so far examined the effect of sanitation (toilet facilities and drinking water) on atopy found weakly protective but statistically nonsignificant associations with poor hygiene.⁸

As part of a wider study of the links between allergic disease and geohelminth infection in Khanh Son district, Khanh Hoa Province, central Vietnam, we now report a cross-sectional study of the association between gut worm infection and other sanitation influences on the prevalence of allergic sensitization in a Vietnamese population of children living a simple subsistence lifestyle and in whom the prevalence of hookworm infection is high. To our knowledge, this is the first such study in a geographic area where hookworm predominates.

METHODS

We invited all 1742 primary and secondary schoolchildren from 4 neighboring rural communes in Khanh Hoa province, central Vietnam, to take part in a cross-sectional survey. On the day before the survey, the children were visited by local health care workers, specifically trained for the project, to explain the purpose of the

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Abbreviations used

epg: Eggs per gram feces

HDM: House dust mite

OR: Odds ratio

SPT: Skin prick test

study, to gain parental consent, and also to give children a container that was to be brought into school with a fresh stool sample the next morning. The next day, the sample was collected and information gathered on children's age, sex, ethnic group, and other demographic and lifestyle factors, including number of siblings, parental smoking, housing style, symptoms of allergic disease, current medication, toilet facilities, drinking water supply, animals in the home, fuel use, and previous geohelminth and malaria infections. The English questionnaire was initially translated into Vietnamese, then pilot-tested among 254 volunteers, and finally back-translated into English to ensure accurate translation of key terms. We measured allergic skin sensitization to 2 house dust mites (HDMs; *Dermatophagoides pteronyssinus* and *Dermatophagoides farinae*) and to American cockroach (*Periplaneta americana*; ALK manufactured, supplied by Thalassa Medical Ltd, Hong Kong) with normal saline (negative) and histamine 1.7 mg/mL (positive) controls (all Merck, supplied by Diagenics, Newark, United Kingdom). The allergens used were identified as being the most prevalent locally from a pilot study using a broader range of allergens in 100 volunteers. We used the International Study of Asthma and Allergies in Childhood (ISAAC) skin prick test (SPT) protocol, which defines SPT positivity as a skin wheal diameter of at least 3 mm greater than the saline control.¹² Fresh stool samples were analyzed within a maximum of 6 hours post-collection to determine geohelminth status qualitatively and in terms of eggs per gram feces (epg), using McMaster salt flotation.¹³ Furthermore, all children were tested for carriage of malaria parasites with a standard thick blood smear read by an expert malaria microscopist at the Khanh Hoa Provincial Centre for Malaria and Filariasis Control.

All data were double-entered using SPSS Data Entry Station 4.0 and then analyzed by logistic regression in SPSS version 12.1 (SPSS Inc., Chicago, Ill). In our multivariate models, we included age, sex, and area as a priori confounders, and initially all those variables that were significant in univariate analysis. We retained in the model those for which there was significant heterogeneity across categories or trend through ordered categories, taking $P < .05$ as statistically significant. In the case of collinear variables, we fitted each in the absence of the other, and retained in the model the variable with the strongest effect. Because the risk factor analysis yielded slightly different patterns of association for HDMs (combined sensitization for either *D pteronyssinus* or *D farinae*) and cockroach sensitization, we have presented results for these 2 allergens separately.

Our sample size was determined by the needs of a parasite eradication trial being performed in this population, but a retrospective power calculation based on 1601 participants, of which 14% were sensitive to dust mite, indicates that we had more than 80% power to detect an odds ratio (OR) of 0.66 for the protective effect of an exposure such as hookworm, which occurs in 65% of the population.

Ethics approval for the study was granted by the University of Nottingham Research Ethics Committee and the Scientific Committee of Khanh Hoa Provincial Health Service, Vietnam.

RESULTS

Data were collected from 1601 participants with a mean age of 8.7 (range, 6–18) years, of whom 47.7% were male,

and accounting for 92% of those eligible to participate. The majority of participants (79.5%) belonged to the Raclay ethnic group; 19.1% were from ethnic Vietnamese (Kinh) families, and the remaining 1.4% were from mixed backgrounds.

Allergic sensitization

A total of 230 (14.4%) children had a positive SPT to either *D pteronyssinus* or *D farinae*, whereas 442 (27.6%) were sensitized to American cockroach. Only 6 (0.4%) children reported a history of doctor-diagnosed asthma, and 4 of the children with asthma were atopic—that is, they had at least 1 positive SPT. Two (0.1%) children had physician-diagnosed eczema. One child with eczema reported a positive family history, and none of the participating children were taking antihistamines or corticosteroids at the time of the survey.

Demographic factors

In univariate analysis, sensitivity to HDM, but not to cockroach, increased with age, and was more common, though not significantly so, in girls (Table I). Allergic sensitization was generally more common in ethnic Vietnamese and least common in those of Raclay descent. Higher levels of parental education and ownership of goods (a marker of socioeconomic status) were both positively associated with sensitization. There was no association between sensitization and number of older siblings.

Household exposures

We examined a number of other exposures previously shown to be associated with allergic sensitization, including breast-feeding, parental smoking, insecticide use, crowding, housing style (roof, walls, floor), sleeping mattress, cooking fuel, and animal ownership (Table II). In univariate analysis, sensitization was less common in those exposed to smoking in the household, and in those using wood for fuel. Sensitization was more common in those using gas as a domestic fuel, and with exposure to animals, especially cat. However, none of these exposures were independently related to sensitization to dust mite or cockroach in multivariate analysis.

Associations with infections, parasites, and poor hygiene

Hookworm infection was identified in 65% of children and *Ascaris* in 7%. Infection intensity for both geohelminths was relatively low (hookworm epg: range, 0–7300, mean epg, 432; *Ascaris* epg: range, 0–13,850, mean epg, 93). Other geohelminth infections, such as *Trichuris*, were rare (1% or less) and therefore not analyzed further. In univariate analysis, hookworm and *Ascaris* infection were both associated with a reduced risk of sensitization to dust mite and to cockroach, which for hookworm was intensity-related (Table III). Allergen sensitization was more common in those with more modern toilet facilities, and in those with a piped drinking water supply. Past malaria infection was protective against both HDM and cockroach sensitization, whereas current malaria increased

TABLE I. Demographic and socioeconomic factors

	N (%)	Der p 1 or Der f 1-sensitive (N [%])	Crude OR (95% CI)	P value	Cockroach-sensitive (N [%])	Crude OR (95% CI)	P value
Total	1601	230 (14.4)	—	—	442 (27.6)	—	—
Age (y)							
6-7	540 (33.7)	73 (13.5)	1*	<.001*	156 (28.9)	1	.6
8-9	585 (36.5)	66 (11.3)	0.81 (0.57-1.16)*	P_{trend} *	144 (24.6)	0.80 (0.62-1.05)	P_{trend}
10-11	290 (18.1)	40 (13.8)	1.02 (0.68-1.55)*		85 (29.3)	1.02 (0.75-1.40)	
12+	186 (11.6)	51 (27.4)	2.42 (1.61-3.63)*		57 (30.6)	1.09 (0.76-1.56)	
Sex							
Male	764 (47.7)	100 (13.1)	1	.2	196 (25.7)	1	.09
Female	837 (52.3)	130 (15.5)	1.22 (0.92-1.62)		246 (29.4)	1.21 (0.97-1.50)	
Ownership of goods							
None	403 (25.2)	40 (9.9)	1*	.001*	110 (27.3)	1	.09
1 or 2 items	745 (46.5)	108 (14.5)	1.54 (1.05-2.26)*	P_{trend} *	186 (25.0)	0.89 (0.67-1.17)	P_{trend}
3 or more items	453 (28.3)	82 (18.1)	2.01 (1.34-3.01)*		146 (32.2)	1.27 (0.94-1.70)	
Ethnicity							
Raelay	1271 (79.5)	148 (11.6)	1*	<.001*	294 (23.1)	1*	<.001*
Vietnamese (Kinh)	305 (19.1)	77 (25.2)	2.56 (1.88-3.49)*		139 (45.6)	2.78 (2.15-3.61)*	
Other	23 (1.4)	5 (21.7)	2.11 (0.77-5.76)*		9 (39.1)	2.14 (0.92-4.99)*	
Parental education							
Illiterate	393 (24.5)	43 (10.9)	1*	<.001*	80 (20.4)	1*	<.001*
Primary	819 (51.2)	103 (12.6)	1.17 (0.80-1.71)*	P_{trend}	215 (26.3)	1.39 (1.04-1.86)*	P_{trend}
Higher	389 (24.3)	84 (21.6)	2.24 (1.51-3.34)*		147 (37.8)	2.38 (1.73-3.27)*	
Area							
Thanh Son	370 (23.1)	42 (11.4)	1*	<.001*	88 (23.8)	1*	.003*
Son Lam	604 (37.7)	116 (19.2)	1.86 (1.27-2.71)*		177 (29.3)	1.33 (0.99-1.79)*	
Son Binh	418 (26.1)	49 (11.7)	1.04 (0.67-1.61)*		135 (32.3)	1.53 (1.12-2.09)*	
Ba Cum Nam	209 (13.1)	23 (11.0)	0.97 (0.56-1.65)*		42 (20.1)	0.81 (0.53-1.22)*	
Older siblings (N)							
0	417 (26.0)	61 (14.6)	1	.3	121 (29.0)	1	.3
1	340 (21.2)	53 (15.6)	1.08 (0.72-1.61)	P_{trend}	95 (27.9)	0.95 (0.69-1.30)	P_{trend}
2 or 3	530 (33.1)	81 (15.3)	1.05 (0.73-1.51)		145 (27.4)	0.92 (0.69-1.23)	
4 or more	314 (19.6)	35 (11.1)	0.73 (0.47-1.14)		81 (25.8)	0.85 (0.61-1.18)	

*Significance at the 5% level.

sensitization risk, but this did not reach conventional statistical significance. Both effects were not retained in the final logistic regression model. Anthelmintic treatment within the past 6 months had no effect on any of these estimates (data not shown). As for other measures related to infections, we also collected data on childhood vaccinations, previous tuberculosis and measles, and past antibiotic use. Among these variables, only BCG vaccination showed a significant and weakly protective effect against HDM sensitization in univariate analysis (Table III), but this was not retained in the final logistic regression model.

Multivariate analysis

In a multivariate model, hookworm burden and *Ascaris* infection were the strongest independent predictors of sensitivity to HDM. The effect of hookworm infection was significantly intensity-related, and those in the highest category of hookworm egg counts (350+) had a 39% reduction in risk of sensitivity to HDM (adjusted OR, 0.61; 0.39-0.96; $P_{trend} = .03$; Table IV). Those with *Ascaris* infection had a 72% reduction in risk (adjusted OR, 0.28; 0.10-0.78; Table IV), but there were too few infected to determine whether this effect was related to infection

intensity. There was also a significant effect of having better toilet facilities (adjusted OR comparing a flush toilet with none, 2.51; 1.00-6.28), but with no independent effect of drinking water source. Sensitivity to HDMs was independently related to ethnicity ($P = .02$). These effects were not appreciably changed by adjustment for parental education or ownership of goods.

Cockroach sensitization was increased in individuals who had regular access to well or piped drinking water, the adjusted OR relative to those drinking from a stream being 1.33 (1.02-1.75; Table V). The protective effect of *Ascaris* infection was of borderline statistical significance in this model (adjusted OR, 0.66; 0.35-1.05). Cockroach sensitization was also independently related to ethnicity ($P < .001$; Table V), but there was no significant effect of having better toilet facilities.

DISCUSSION

This study has demonstrated independent protective effects of low-intensity hookworm and *Ascaris* infections, markers of poor sanitation, and of ethnic group on allergic

TABLE II. Exposures in the household

	N (%)	Der p 1 or Der f 1-sensitive (N [%])	Crude OR (95% CI)	P value	Cockroach-sensitive (N [%])	Crude OR (95% CI)	P value
Smoking in the household (N)							
0	263 (16.4)	48 (18.3)	1*	.003*	74 (28.1)	1	.003*
1	600 (37.5)	96 (16.0)	0.85 (0.58-1.25)*	P_{trend}	202 (33.7)	1.30 (0.94-1.78)	P_{trend}
2 or more	738 (46.1)	86 (11.7)	0.59 (0.40-0.87)*		166 (22.5)	0.74 (0.54-1.02)	
Cooking fuel							
Wood							
No	112 (7.0)	31 (27.7)	1*	<.001*	52 (46.4)	1*	<.001*
Yes	1489 (93.0)	199 (13.4)	0.40 (0.26-0.63)*		390 (26.2)	0.41 (0.28-0.60)*	
Gas							
No	1498 (93.6)	199 (13.3)	1*	<.001*	394 (26.3)	1*	<.001*
Yes	103 (6.4)	31 (30.1)	2.81 (1.80-4.39)*		48 (46.6)	2.45 (1.63-3.66)*	
Animal ownership							
Cat							
No	1288 (80.4)	172 (13.4)	1*	.02*	338 (26.2)	1*	.01*
Yes	313 (19.6)	58 (18.5)	1.48 (1.06-2.05)*		104 (33.2)	1.40 (1.07-1.83)*	
Dog							
No	493 (30.8)	55 (11.2)	1*	.01*	135 (27.4)	1	.8
Yes	1108 (69.2)	175 (15.8)	1.49 (1.08-2.06)*		307 (27.7)	1.02 (0.80-1.29)	
Chicken/duck							
No	638 (39.9)	74 (11.6)	1*	.01*	179 (28.1)	1	.7
Yes	963 (60.1)	156 (16.2)	1.47 (1.10-1.98)*		263 (27.3)	0.96 (0.77-1.21)	
Water buffalo							
No	379 (23.7)	59 (15.6)	1	.4	119 (31.4)	1	.06
Yes	1222 (76.3)	171 (14.0)	0.88 (0.64-1.22)		323 (26.4)	0.79 (0.61-1.01)	
Pig							
No	1113 (69.5)	159 (14.3)	1	.9	316 (28.4)	1	.3
Yes	488 (30.5)	71 (14.5)	1.02 (0.76-1.38)		126 (25.8)	0.88 (0.69-1.12)	

*Significance at the 5% level.

sensitization to dust mites, and effects of drinking water supply and ethnic group on sensitization to cockroach. Overall, these findings support the hypothesis that gastrointestinal infection with either geohelminths or other microorganisms protects against allergy (Fig 1).

It is possible that some of the observed geohelminth effects were reduced by misclassification of infection, because large participant numbers only allowed collection of 1 stool sample, and a few low worm egg counts may therefore have been missed.

However, we confirmed in a separate validation study that the sensitivity of McMaster salt flotation in the field had adequate sensitivity and was comparable to the gold standard laboratory method, formol-ether sedimentation.¹⁴

It may also be that low study power obscured some effects, because the estimates of effect of hookworm infection intensity, *Ascaris* infection, toilet facilities, and drinking water source were all broadly similar for sensitization to both allergens, but were more significant for dust mites. At the same time, it is unlikely that other, unmeasured infectious or parasitic diseases confounded study results. Geohelminths and malaria, which we tested for, are the prevalent endoparasite infections in our study area. There is no schistosomiasis in Vietnam, and recent surveys conducted by the World Health Organization in our study area did not find any cases of lymphatic filariasis

(unpublished internal report National Institute of Malariology, Parasitology, and Entomology, Hanoi, March 2006). To date, no case of HIV-1 or HIV-2 has been detected in Khanh Son, despite routine screening of all people who seek medical treatment in the district's only hospital.

A number of previous studies found a protective effect of geohelminth infection and infection burden (epg) on allergic sensitization.⁷⁻¹¹ Where a distinction between types of geohelminths was made in the analysis, the effect sizes for *Ascaris* and hookworm infection on atopy were in keeping with the ones demonstrated here. One previous study found a negative association between the incidence of falciparum malaria and skin sensitization to HDM in a small cohort of Gabonese children followed over a 5-year period, but did not present risk estimates adjusted for coinfection with other parasitic diseases, such as geohelminth infection.¹⁵ In our study, the negative association between a history of past malaria and allergic sensitization disappeared following mutual adjustment, whereas there was a statistically nonsignificant positive effect on cockroach sensitization seen with current infection. The cross-sectional design of our study allowed us neither to examine the role of repeated malaria infection on allergic sensitization nor to investigate the effect of malaria type and infection intensity, but the relationship between malaria infection (past and present) and allergic disease certainly warrants further investigation, especially in view of the host

TABLE III. Infections, parasites, and hygiene

	N (%)	Der p 1 or Der f 1-sensitive (N [%])	Crude OR (95% CI)	P value	Cockroach-sensitive (N [%])	Crude OR (95% CI)	P value
Hookworm							
No	566 (35.4)	108 (19.1)	1*	<.001*	175 (30.9)	1*	.03*
Yes	1035 (64.6)	122 (11.8)	0.57 (0.43-0.75)*		267 (25.8)	0.78 (0.62-0.97)*	
Hookworm egg							
Low (50-199)	409 (25.5)	56 (13.7)	0.67 (0.47-0.96)*	<.001 <i>P</i> _{trend} *	110 (26.9)	0.82 (0.62-1.09)*	.006 <i>P</i> _{trend} *
Medium (200-349)	228 (14.2)	27 (11.8)	0.57 (0.36-0.90)*		71 (31.1)	1.01 (0.72-1.41)*	
High (350+)	398 (24.9)	39 (9.8)	0.46 (0.31-0.68)*		86 (21.6)	0.62 (0.46-0.83)*	
Ascaris							
No	1492 (93.2)	226 (15.1)	1*	.001*	421 (28.2)	1*	.04*
Yes	109 (6.8)	4 (3.7)	0.21 (0.08-0.59)*		21 (19.3)	0.61 (0.37-0.99)*	
Malaria infection (past)							
No	535 (33.4)	97 (18.1)	1*	.002*	173 (32.3)	1*	.003*
Yes	1066 (66.6)	133 (12.5)	0.64 (0.48-0.86)*		269 (25.2)	0.71 (0.56-0.89)*	
Malaria infection (current)							
No	1585 (99.0)	227 (14.3)	1	.6	434 (27.4)	1	.05
Yes	16 (1.0)	3 (18.8)	1.38 (0.39-4.88)		8 (50.0)	2.65 (0.99-7.11)	
BCG vaccination (with scar)							
No	336 (21.0)	60 (17.9)	1*	.04*	94 (28.0)	1	.9
Yes	1265 (79.0)	170 (13.4)	0.71 (0.52-0.99)*		348 (27.5)	0.98 (0.75-1.28)	
Toilet facilities							
None/bush/pit	1580 (98.7)	221 (14.0)	1*	.001*	429 (27.2)	1*	.001*
Flush toilet	21 (1.3)	9 (42.9)	4.61 (1.92-11.07)*		13 (61.9)	4.36 (1.80-10.59)*	
Drinking water source							
Stream	655 (40.9)	79 (12.1)	1*	.03*	142 (21.7)	1*	<.001*
Well or piped	946 (59.1)	151 (16.0)	1.39 (1.03-1.86)*		300 (31.7)	1.68 (1.33-2.11)*	

*Significance at the 5% level.

TABLE IV. Multivariate model for dust mite sensitivity

	Mutually adjusted OR (95% CI)*	P value
Ethnic group		
Ra clay	1	
Kinh	1.73 (1.17-2.55)	.02
Other	1.57 (0.56-4.39)	
Hookworm		
None	1	
1-199	0.79 (0.54-1.16)	.03 (<i>P</i> _{trend})
200-349	0.75 (0.46-1.23)	
350+	0.61 (0.39-0.96)	
Ascaris		
No	1	
Yes	0.28 (0.10-0.78)	.01
Toilet facilities		
None/bush/pit	1	
Flush toilet	2.51 (1.00-6.28)	.05

*The results are adjusted for age, sex, and area as *a priori* confounders.

immunomodulatory mechanisms associated with malaria.¹⁶ As has been speculated for geohelminth infections and atopy, there may well be different effects on allergic sensitization seen with different types of malaria parasites, also depending on the timing and intensity of infection.^{17,18}

TABLE V. Multivariate model for cockroach sensitivity

	Mutually adjusted OR (95% CI)*	P value
Ethnic group		
Ra clay	1	
Kinh	2.89 (2.12-3.94)	<.001
Other	2.04 (0.87-4.81)	
Drinking water source		
Stream	1	
Well or piped	1.33 (1.02-1.75)	.04

*The results are adjusted for age, sex, and area as *a priori* confounders.

A protective effect of endoparasitic infections against allergic disease and atopy is biologically plausible on the basis of known immunomodulatory mechanisms induced by the endoparasite, often resulting in a downregulation of host immune defenses, both locally in the gut and during host invasion.¹⁹ For instance, parasite-induced T-cell cytokines, such as IL-10, possibly mediated via regulatory T cells, may provide a protective immunomodulatory network against SPT positivity and allergy.^{16,17,20,21} Such an effect of IL-10 has been suggested in cross-sectional studies for combined infection with *Schistosoma mansoni* and *Ascaris lumbricoides* in Brazil and *Schistosoma*



FIG 1. Improved hygiene and sanitation as well as ethnicity increased the risk of allergic sensitization in Vietnamese children, while hookworm and *Ascaris lumbricoides* infections had an independent protective effect.

haematobium in Gabon, but remains to be demonstrated for hookworm.^{22,23} We are therefore planning to perform serologic analyses on this study population.

The opposite may also hold true. Host immune responses against geohelminths are probably among human beings' oldest allergic reactions, and in evolutionary terms, a genetic predisposition toward strong T_H2 -mediated immune responses may have helped to clear parasitic infestations more efficiently, making atopic individuals less likely to be chronically infected and thus enhancing overall chances of survival.²⁴ A recent study conducted in Ecuador demonstrated that children with at least 1 positive SPT to common environmental allergens had stronger T_H2 -cytokine responses (IL-4, IL-5) and basophil histamine release induced by *A lumbricoides*-specific antigens than nonatopic children. The strongest T_H2 cytokine responses were seen in individuals with allergy who were free of infection.²⁵

It has been suggested that a high microbial burden postnatally may be essential in steering the infant's T_H2 -dominated immune system toward more balanced immune responses. In this context, regulatory T cells may play a pivotal role, and such an effect may well be mediated by essentially nonpathogenic microbial exposure, for instance at the level of the gut-associated lymphoid tissues.^{21,26} This would explain the effects we found with poor sanitation and is also in keeping with our recent findings in Ethiopia, where those using river or spring water had a lower risk of childhood eczema than those with access to piped water.²⁷ Furthermore, we did not find any evidence in our study population to suggest that a particular type of infection, such as previous TB or measles, was associated with allergic sensitization.

We saw a strong and uniform effect of ethnicity on allergic sensitization. Effects of ethnicity on allergic sensitization have been described before, and these may be a result of genetic traits, which contribute to strong T_H2 responses to geohelminths and/or environmental allergens.²⁸ Alternatively, ethnic group may operate as a surrogate marker of socioeconomic status, reflecting subtle differences in lifestyle between ethnic groups not captured by our measure of ownership of goods.

In conclusion, our study indicates that poor sanitation and infection with hookworm or *Ascaris*, along with ethnic origin, independently protect against allergic sensitization, particularly to HDM. Only a carefully designed intervention study with regular administration of an antihelminthic agent will be able to prove or disprove whether

this effect is primarily a result of a geohelminth-induced suppression of atopic host responses. Indeed, although 2 small preliminary intervention studies in Venezuela and Gabon have suggested that this may well be the case, these findings were not confirmed in a very recent cluster-randomized trial with regular antihelminthic treatment conducted over a 12-month period among Ecuadorian children in an area where *Ascaris* and *Trichuris* predominate.²⁹⁻³¹ The negative finding of the latter study may be explained by the relatively short period of deworming. It may also be that hookworm rather than *Ascaris* or *Trichuris* is the main agent that suppresses allergic sensitization. We are ourselves in the process of performing a similar randomized, placebo-controlled eradication trial in this Vietnamese population in which hookworm is the main gut parasite.

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REFERENCES

1. International Study of Asthma and Allergies in Childhood (ISAAC) Steering Committee. Worldwide variation in prevalence of symptoms of asthma, allergic rhinoconjunctivitis, and atopic eczema: ISAAC. *Lancet* 1998;351:1225-32.
2. Nga NN, Chai S, Binh TT, Redding G, Takaro T, Checkoway H. ISAAC-based asthma and atopic symptoms among Hanoi schoolchildren. *Pediatr Allergy Immunol* 2003;14:272-9.
3. Addo Yobo EOD, Custovic A, Taggart SCO, Asafo-Agyei AP, Woodcock A. Exercise-induced bronchospasm in Ghana: differences in prevalence between urban and rural schoolchildren. *Thorax* 1997;52:161-5.
4. Yemaneberhan H, Flohr C, Lewis SA, Bekele Z, Parry E, Williams HC, et al. Prevalence and associated factors of atopic dermatitis symptoms in rural and urban Ethiopia. *Clin Exp Allergy* 2004;34:779-85.
5. Scrivener S, Yemaneberhan H, Zebeignus M, Tilahun D, Girma S, Ali S, et al. Independent effects of intestinal parasite infection and domestic allergen exposure on risk of wheeze in Ethiopia: a nested case-control study. *Lancet* 2001;358:1493-9.
6. Dagoye D, Bekele Z, Woldemichael K, Nida H, Yimam M, Hall A, et al. Wheezing, allergy, and parasite infection in children in urban and rural Ethiopia. *Am J Respir Crit Care Med* 2003;167:1369-73.
7. Hagel I, Lynch NR, Perez M, Di Prisco MC, Lopez R, Rojas E. Modulation of the allergic reactivity of slum children by helminthic infection. *Parasite Immunol* 1993;15:311-5.
8. Cooper PJ, Chico ME, Rodrigues LC, Strachan DP, Anderson HR, Rodriguez EA, et al. Risk factors for atopy among school children in a rural area of Latin America. *Clin Exp Allergy* 2004;34:845-52.
9. Cooper PJ, Chico ME, Rodrigues LC, Ordonez M, Strachan D, Griffin GE, et al. Reduced risk of atopy among school-age children infected with geohelminth parasites in a rural area of the tropics. *J Allergy Clin Immunol* 2003;111:995-1000.
10. Cooper PJ, Chico ME, Bland M, Griffin GE, Nutman TB. Allergic symptoms, atopy, and geohelminth infections in a rural area of Ecuador. *Am J Respir Crit Care Med* 2003;168:313-7.
11. Nyan OA, Walraven GEL, Banya WAS, Milligan P, Van der Sande M, Ceesay SM, et al. Atopy, intestinal helminth infection and total serum

- IgE in rural and urban adult Gambian communities. *Clin Exp Allergy* 2001;31:1672-8.
12. International Study of Asthma and Allergies in childhood. Child contact modules. Module 3.1: examination for flexural dermatitis. Available at: <http://isaac.auckland.ac.nz/Phasetwo/Modules/ChilCont/ChilFrame.html>. Accessed September 21, 2006.
 13. The RVC/FAO guide to veterinary diagnostic pParasitology. McMaster egg counting technique: purpose. Available at: <http://www.rvc.ac.uk/Review/Parasitology/EggCount/Purpose.htm>. Accessed September 21, 2006.
 14. Flohr C, Tuyen LN, Lewis S, Minh TT, Campbell C, Britton J, et al. Low efficacy of mebendazole against hookworm in Vietnam: two randomised controlled trials. *Am J Trop Med Hyg* 2006. In press.
 15. Lell B, Borrmann S, Yazdanbakhsh M, Kremsner PG. Atopy and malaria. *Wien Klin Wochenschr* 2001;113:927-9.
 16. Riley EM, Wahl S, Perkins DJ, Schofield L. Regulating immunity to malaria. *Parasite Immunol* 2006;28:35-49.
 17. Yazdanbakhsh M, Kremsner PG, Van Ree R. Allergy, parasites, and the hygiene hypothesis. *Science* 2002;296:490-4.
 18. Cooper PJ. Intestinal worms and human allergy. *Parasite Immunol* 2004;26:455-67.
 19. Pritchard DI, Brown A. Is *Necator americanus* approaching a mutualistic symbiotic relationship with humans? *Trends Parasitol* 2001;17:169-72.
 20. Flohr C. Dirt, worms and atopic dermatitis. *Br J Dermatol* 2003;148:871-7.
 21. Chatila TA. Role of regulatory T cells in human diseases. *J Allergy Clin Immunol* 2005;116:949-59.
 22. Van den Biggelaar AHJ, Van Ree R, Rodrigues LC, Lell B, Deelder AM, Kemsner PG, et al. Decreased atopy in children infected with *Schistosoma haematobium*: a role for parasite-induced interleukin-10. *Lancet* 2000;356:1723-7.
 23. Araujo MIA, Hoppe B, Medeiros M, Alcantara L, Almeida MC, Schrieffer A, et al. Impaired T helper 2 response to aeroallergen in helminth-infected patients with asthma. *J Infect Dis* 2004;190:1797-803.
 24. Pritchard DI. Atopy and helminth parasites. *Int J Parasitol* 1993;23:167-8.
 25. Cooper PJ, Chico ME, Sandoval, Nutman TB. Atopic phenotype is an important determinant of immunoglobulin E-mediated inflammation and expression of T helper cell type 2 cytokines to *Ascaris* antigens in children exposed to ascariasis. *J Infect Dis* 2004;190:1338-46.
 26. Flohr C, Pascoe D, Williams HC. Atopic dermatitis and the "hygiene hypothesis": too clean to be true? *Br J Dermatol* 2005;152:202-16.
 27. Haileamlak A, Dagoye D, Williams H, Venn AJ, Hubbard R, Britton J, et al. Early life risk factors for atopic dermatitis in Ethiopian children. *J Allergy Clin Immunol* 2005;115:370-6.
 28. Blumenthal MN, Ober C, Beaty TH, Bleecker ER, Langefeld CD, King RA, et al. Genome scan for loci linked to mite sensitivity: the Collaborative Study on the Genetics of Asthma (CSGA). *Genes Immun* 2004;5:226-31.
 29. Lynch NR, Hagel I, Perez M, Di Prisco MC, Lopez R, Alvarez N. Effect of antihelminthic treatment on the allergic reactivity of children in a tropical slum. *J Allergy Clin Immunol* 1993;92:404-11.
 30. Van den Biggelaar AHJ, Rodrigues LC, van Ree R, van der Zee JS, Hoeksma-Kruize YCM, Souverein JHM, et al. Long-term treatment of intestinal helminths increases mite skin-test reactivity in Gabonese school-children. *J Infect Dis* 2004;189:892-900.
 31. Cooper PJ, Chico ME, Vaca MG, Moncayo AL, Bland JM, Mafla E, et al. Effect of albendazole treatment on the prevalence of atopy in children living in communities endemic for geohelminth parasites: a cluster-randomised trial. *Lancet* 2006;367:1598-603.