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ORIGINAL ARTICLE

Reduced helminth burden increases allergen skin sensitization but not clinical allergy: a randomized, double-blind, placebo-controlled trial in Vietnam

C. Flohr[1,2], L. N. Tuyen[3], R. J. Quinnell[4], S. Lewis[5], T. T. Minh[3], J. Campbell[2], C. Simmons[2], G. Telford[6], A. Brown[6], T. T. Hien[7], J. Farrar[2], H. Williams[8], D. I. Pritchard[6] and J. Britton[5]

[1]Centre for Population Studies, Institute of Clinical Research, University of Nottingham, Nottingham, UK, [2]Oxford University Clinical Research Unit, Hospital for Tropical Diseases, Ho Chi Minh City, Vietnam, [3]Khanh Hoa Provincial Center for Malaria and Filariasis Control, Nha Trang City, Vietnam, [4]Institute of Integrative and Comparative Biology, Faculty of Biological Sciences, University of Leeds, Leeds, UK, [5]Division of Epidemiology and Public Health, University of Nottingham, Nottingham, UK, [6]School of Pharmacy, University of Nottingham, Nottingham, UK, [7]Hospital for Tropical Diseases, Ho Chi Minh City, Vietnam and [8]Centre for Evidence-Based Dermatology, University of Nottingham, Nottingham, UK

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Summary

Background: Observational evidence suggests that infection with helminths protects against allergic disease and allergen skin sensitization. It is postulated that such effects are mediated by helminth-induced cytokine responses, in particular IL-10.

Objective: We tested this hypothesis in a rural area of central Vietnam where hookworm infection is endemic.

Methods: One thousand five hundred and sixty-six schoolchildren aged 6–17 were randomly allocated to receive either anti-helminthic therapy or a placebo at 0, 3, 6, and 9 months. We compared changes in the prevalence of exercise-induced bronchoconstriction, allergen skin sensitization, flexural eczema on skin examination, questionnaire-reported allergic disease (wheeze and rhinitis symptoms), and immunological parameters (hookworm-induced IFN-γ, IL-5, IL-10) between 0 and 12 months.

Results: One thousand four hundred and eighty-seven children (95% of these randomized) completed the study. The most common helminth infections were hookworm (65%) and *Ascaris lumbricoides* (7%). There was no effect of the therapy on the primary outcome, exercise-induced bronchoconstriction (within-participant mean percent fall in peak flow from baseline after anti-helminthic treatment 2.25 (SD 7.3) vs. placebo 2.19 (SD 7.8, $P = 0.9$), or on the prevalence of questionnaire-reported wheeze [adjusted odds ratio (OR) = 1.16, 95% confidence interval (CI) 0.35–3.82, $P = 0.8$] and rhinitis (adjusted OR = 1.39, 0.89–2.15, $P = 0.1$), or flexural dermatitis on skin examination (adjusted OR = 1.15, 0.39–3.45, $P = 0.8$). However, anti-helminthic therapy was associated with a significantly higher allergen skin sensitization risk (adjusted OR = 1.31, 1.02–1.67, $P = 0.03$). This effect was particularly strong for children infected with *A. lumbricoides* at baseline (adjusted OR = 4.90, 1.48–16.19, $P = 0.009$). Allergen skin sensitization was inversely related to hookworm-specific IL-10 at baseline (adjusted OR = 0.76, 0.59–0.99, $P = 0.04$). No cytokine tested, including IL-10, changed significantly after the anti-helminthic therapy compared with the placebo.

Conclusion: A significant reduction in worm burden over a 12-month period in helminth-infected children increases the risk of allergen skin sensitization but not of clinical allergic disease. The effect on skin sensitization could not be fully explained by any of the immunological parameters tested.

Correspondence:
Dr Carsten Flohr, Centre for Population Studies, Institute of Clinical Research, University of Nottingham, Nottingham NG7 3RD, UK. E-mail: carsten.flohr@nottingham.ac.uk

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Introduction

Allergic diseases are rare in rural areas of developing nations compared with urbanized populations and more affluent settings [1–4]. Many cross-sectional studies from developing countries suggest that this urban–rural gradient for allergic disease may be partly due to a higher prevalence of helminth infection in rural areas [5–11]. We have previously suggested that infection by helminth parasites with a systemic migration phase in the human host may be important in this respect [6], and in a recent systematic review and meta-analysis, we reported that hookworm infection appears to be particularly relevant in explaining a reduced risk of asthma [12]. Helminth infections induce T-helper type 2 (Th2) responses, which are host protective, and there is increasing evidence that helminths can immunomodulate these responses to aid their own survival [13]. Such immunomodulation may also affect immune responses directed against environmental allergens, providing a degree of protection against allergic disease. Control of helminth infection is an important public health intervention, given the pathology that such infections cause [14]. However, if this hypothesis is correct, eradicating such parasitic infections would be expected to increase the risk and severity of asthma and other allergic diseases.

Two previous studies, one an observational study built into a helminth control programme in 375 Venezuelan children and the other an open-label randomized trial of 317 children in Gabon, have reported evidence of an increase in allergen skin sensitization following anti-helminthic therapy, and it has been suggested that such changes are mediated by helminth-induced anti-inflammatory cytokines such as IL-10 [15, 16]. However, a clinical improvement of established asthma was reported following de-worming in a study of 89 Venezuelan children and adults with asthma [17], while a cluster-randomized trial of helminth therapy in a much larger sample of Ecuadorian children was recently reported to show no effect on atopy or allergic disease [18].

The predominant parasite infections in these study populations were *Ascaris lumbricoides* and *Trichuris trichiura*. It is therefore important to test this hypothesis in a population in which the predominant pathogen is hookworm. We now report the first individually randomized, double-blind, placebo-controlled trial of the effect of anti-helminthic therapy on markers of asthma, eczema, and allergen skin sensitization. The study was conducted in children living in a rural area of Vietnam, where hookworm (predominantly *Necator americanus*) is the main gut parasite (H. T. Kim, N. T. V. Hoa, and R. Marchand, unpublished data).

Methods

Study population, study design, and epidemiological tools

In May 2005, we invited all 1707 primary and secondary schoolchildren in grades 1–8 in four communes in the Khanh Son district, Khanh Hoa province, in central Vietnam to take part in a baseline survey. The methods and results of this baseline survey have already been reported elsewhere [19]. In brief, children were visited at home by local health care workers, specifically trained for the project. The purpose of the study was explained to parents in detail with the support of information leaflets. Field workers requested parental consent and gave each child a sample container in which they were to return a fresh fecal sample the next morning. In school the next day, the stool sample was collected and information was gathered by a questionnaire-led interview from a parent by the same field workers on the children's age, sex, ethnic group, and other demographic and lifestyle factors, previous helminth infection, and anti-helminthic treatment, as well as symptoms of allergic disease. The questions on asthma and rhinitis symptoms were taken from the International Study of Asthma and Allergies in Childhood (ISAAC) Phase Two questionnaires [20], translated into Vietnamese, pilot tested among 254 volunteers, and back-translated into English to ensure an accurate translation of key terms. Exercise-induced bronchoconstriction was measured in all children as the percentage change in the best of three measures of peak expiratory flow (PEF) measured with Mini-Wright peak flow meters (Clement Clarke, Harlow, UK) immediately before and 5 min after a 6-min period of free outdoor running at jogging pace [21]. Children ran at approximately the same speed throughout. The pulse rate was measured for 15 s immediately after completion of the exercise as an indicator of exercise intensity, and for the exercise test to be valid, the pulse rate had to be either 170 beats/min or 85% maximum for age, whichever was greater [21, 22]. All children were also examined for flexural eczema around the eyes, the neck, in front of the elbows, behind the knees, and in front of the ankles by the same two field workers, who had been trained in accordance with the ISAAC Phase Two study manual for flexural eczema [23]. In all children with a flexural rash, the diagnosis of eczema was confirmed by the principal investigator (C.F.), a trained paediatrician and dermatologist. Allergen skin prick testing to house dust mites (HDM) (*Dermatophagoides pteronyssinus* and *Dermatophagoides farinae*) and American cockroach (*Periplaneta americana*; all ALK-Abelló, Round Rock, TX, USA), and to normal saline (negative) and histamine 10 mg/mL (positive) controls (both Merck, supplied by Diagenics, Newark, UK) was performed by the same two field workers throughout the study, both at baseline and after anti-helminthic treatment, using the standardized

ISAAC Phase Two study protocol [20]. A positive skin prick test (SPT) was defined as a weal diameter at least 3 mm greater than the saline control. All investigators and field workers were blind to treatment allocation. The allergens used had previously been identified as the most prevalent in the local population in a pilot study using a broader range of allergens in 100 volunteers. Stool samples were analysed within a maximum of 6 h from collection using McMaster salt flotation to determine helminth status qualitatively and quantitatively in terms of eggs per gram (epg) of feces [24]. The minimum sensitivity of the method was 50 epg. The laboratory technicians conducting the analysis were blind to treatment allocation.

Immunology

We had study permission to take blood only from secondary schoolchildren, and 244 out of a total of 284 consented to venesection. Only those who were hookworm infected at baseline also provided a further sample of venous blood at 12 months ($n = 142$). Within 4 h after venesection, 1.5 mL of whole blood was diluted 1 : 4 with RPMI 1640 culture medium (Sigma, Poole, UK), supplemented with penicillin (100 U/mL), streptomycin (100 μg/mL), and 2 mM L-glutamine (all Sigma). One millilitre diluted whole blood was cultured for 48 h in 48-well plates at 37 °C with 5% CO_2, in the presence of *N. americanus* excretory-secretory antigen (25 μg/mL) or phytohaemagglutinin (10 μg/mL, Sigma) [25]. The antigen was tested for endotoxin using a commercial kit (E-TOXATE, Sigma) and no endotoxin was detected. The *N. americanus* antigen concentration had been previously optimized during a pilot study in the same population. A third well was left unstimulated. After 48 h, the supernatant was removed and stored at −80 °C. Cytokine production (IFN-γ, IL-5, IL-10) was detected by ELISA using matched antibody pairs and recombinant human cytokines (Pharmingen, BD Biosciences, Poole, UK) and expressed as picogram per millilitre, after subtraction of background (no antigen) levels [26]. Background cytokine levels were consistently low, with only 0.5% (IL-5) or 1.5% (IFN-γ, IL-10) of individuals with detectable cytokine in no antigen wells. The lower detection limits for IL-10, IL-5, and IFN-γ were 8, 8, and 4 pg/mL, respectively.

Serology. IgE assays were performed by ELISA. Assay plates were coated overnight at 4 °C with 50 μL capture antigens at 1 μg/mL in 0.1 M carbonate/bicarbonate (pH 9.6). Antigens used were affinity-purified Der p 1 (NA-DP1, Indoor Biotechnologies, Warminster, UK) and Der f 1 (NA-DF1, Indoor Biotechnologies) and recombinant Blag1 (RP-BG1, Indoor Biotechnologies). A laboratory preparation of an *N. americanus*-excretory/secretory (ES) product at 2.5 μg/mL was used to coat plates for *Necator*-specific responses [27]. For total IgE, plates were coated with 50 μL of 2 μg/mL capture antibody (Pharmingen, 555894). Plates were blocked for 1 h at room temperature with 100 μL tris-buffered saline (TBS) containing 5% Marvel® (Premier Foods, St. Albans, UK) milk powder. Plates were washed three times with 300 μL TBS containing 0.05% Tween 20 (TBS/tween) and incubated with 50 μL plasma diluted 1 : 20 in TBS/Tween plus 5% milk powder at 4 °C overnight. Plates were washed and incubated for 2 h at room temperature with 50 μL of 0.5 μg/mL anti-IgE/Biotin (Pharmingen, 555858) per well. After a further wash, the plates were incubated for 30 min at room temperature with 50 μL 1 : 1000 streptavidin/peroxidase (Pharmingen, 554066). Signal was detected by incubating with 100 μL tetramethyl benzidine substrate (Sigma, T3405) in 0.1 M acetate buffer pH 6.0, and colour development was stopped by the addition of 20 μL 2 M H_2SO_4. Total IgE samples were compared with a WHO IgE reference standard (75/502), and results were expressed in International Units per millilitre. Specific IgE responses were expressed in arbitrary units (AU/mL) relative to in-house positive reference samples.

Study intervention

Directly after the initial baseline survey, consenting children were randomized to receive either anti-helminthic therapy immediately and then at 3, 6, and 9 months, or an identical-looking placebo control. The treatment was with single-dose oral mebendazole 500 mg (Phardazone®) or a matching placebo treatment (both Pharbaco, Hanoi, Vietnam), because Phardazone® was for the time being used by the World Health Organization-led national helminth control programme in Vietnam in areas where hookworm is the main geohelminth parasite [28]. Randomization was stratified by school. Tablets were packaged in a concealed envelope by the Clinical Trials Unit at the Oxford University Clinical Research Unit, Hospital for Tropical Diseases, Ho Chi Minh City, and marked only with a letter code, following a computer-generated randomization sequence, to ensure complete blinding of all field workers and participants. The tablets were first chewed and then swallowed under direct observation.

In accordance with the study protocol, we further collected a stool sample 14 days after the first dose of mebendazole from all 309 children attending primary school in one of the four study communes to check treatment efficacy. The analysis was performed by the trial statistician (S. L.) in Nottingham with no involvement of the study team in Vietnam (C. F., L. N. T., T. T. H., T. T. M., C. S., J. C., J. F.), and the latter had no access to the randomization sequence. We found that single-dose mebendazole had been much less effective than expected, and therefore carried out a separate brief randomized, placebo-controlled trial in an adult population in the same geographical area, comparing mebendazole 500 mg triple

dose with single- and triple-dose albendazole 400 mg. As reported elsewhere, this investigation demonstrated conclusively that albendazole 400 mg daily for 3 days was the most efficacious treatment, and this regimen was therefore adopted for the remaining three treatment rounds at 3, 6, and 9 months [28]. An identical-looking placebo was used throughout. At 12 months, the initial baseline measures were repeated in all children.

Sample size, data management, and analysis

The primary outcome was a within-person change in exercise-induced bronchospasm between baseline and at 12 months, and this outcome was compared between treatment groups. We determined *a priori* from an available estimate of the standard deviation for a fall in peak expiratory flow rate (PEFR) of 6% (study by Burr et al. [29]) that 1341 children (670 per group) would provide 86% power to detect a difference of 1% in the fall in PEFR between the intervention and the control group, and over 99% power to detect a difference of 2% between groups ($\alpha = 5\%$). For our secondary outcomes of prevalence change in SPT positivity, flexural eczema, and questionnaire-derived wheeze and rhinitis at 12 months, using the same assumptions, for an outcome with a prevalence of around 10% (such as sensitization to *D. pteronyssinus*), this sample size would provide 90% power to detect an increase to 16% prevalence. For outcomes with a prevalence of around 5%, such as 'wheeze', the study would have 92% power to look for an increase to a prevalence of 10%.

All trial data were double entered using SPSS Data Entry Station 4.0 and then analysed in SPSS version 14.0. The data were analysed on an intention-to-treat basis. The primary outcome of change in exercise-induced bronchospasm (percent fall in PEFR after exercise) between baseline and 12 months was normally distributed, and compared between the intervention and the control group by analysis of covariance. Those with missing data on the primary outcome were excluded from this analysis, but we performed a sensitivity analysis, using an imputation method assuming no change in PEFR (percent reduction after exercise) between the baseline and the end of the study in those with missing data on the primary outcome.

Secondary outcomes (skin test responses) and other binary variables were analysed by logistic regression, fitting the effect of treatment group. The randomization sequence was not broken until after completion of the primary analysis. Treatment efficacy was calculated as a loss of any helminth infection (hookworm, *A. lumbricoides*, or *T. trichiura*) at 12 months' follow-up in the treatment compared with the placebo group.

Our previously published cross-sectional survey, conducted in the same population, suggested a stronger protective effect for *A. lumbricoides* on allergen skin sensitization than for hookworm [19]. In addition, the relationship between hookworm and allergen skin sensitization was infection intensity-related. In a *post hoc* analysis, we therefore tested for interaction between the type of geohelminth infection as well as the infection intensity (epg) and treatment allocation (anti-helminthic therapy vs. placebo) on all study outcomes. Where a significant interaction was present, a subgroup analysis was conducted within those with specific infection at baseline.

At baseline, we examined whether any immune response was associated with allergen skin sensitization, using logistic regression. Immunological responses were right-skewed and therefore analysed after log-transformation. Responses below the assay detection limit were assigned a value of half the detection limit before log transformation. A low proportion of individuals produced detectable IgE responses to allergen, and these were therefore analysed as binary (responders vs. non-responders) rather than continuous variables. For the intervention study, we compared the effect of treatment on immune responses. Because immune response data were highly skewed, these data were analysed as rank data using Mann–Whitney *U*-tests.

Ethics approval for the study and for the assessment of the effectiveness of different treatment regimens was granted by the Scientific Committee of Khanh Hoa Provincial Health Service, Nha Trang City, Vietnam, and the University of Nottingham Research Ethics Committee. The ethics approval required us to not only treat the children in the treatment arm with anti-helminthic therapy but also all children who had been in the placebo group, after completion of the study. Had we not conducted this trial, none of the children in Khanh Son district would have received any anti-helminthic therapy, because the district is not part of the WHO helminth control campaign in Vietnam. The study is reported in coherence with the CONSORT guidelines for the reporting of clinical trials and registered at http://www.controlled-trials.com/ISRCTN71058787.

Results

Participant flow is shown in Fig. 1. A total of 1566 primary and secondary schoolchildren aged 6–17 (mean age 8.6) took part in the baseline survey. Hookworm was the most prevalent helminth infection, being present in 64.8% (1015/1566) of children (mean epg 286, range 0–7300). *A. lumbricoides* was present in 7.0% (109/1566) of children (mean epg 63, range 0–13 850), and 5.4% (84/1566) of children had infection with both these helminths. All children and their parents consented to be randomized to one of the two treatment groups: 794 to active treatment and 772 to placebo. A total of 1487 (95%) children completed the study. Non-participation

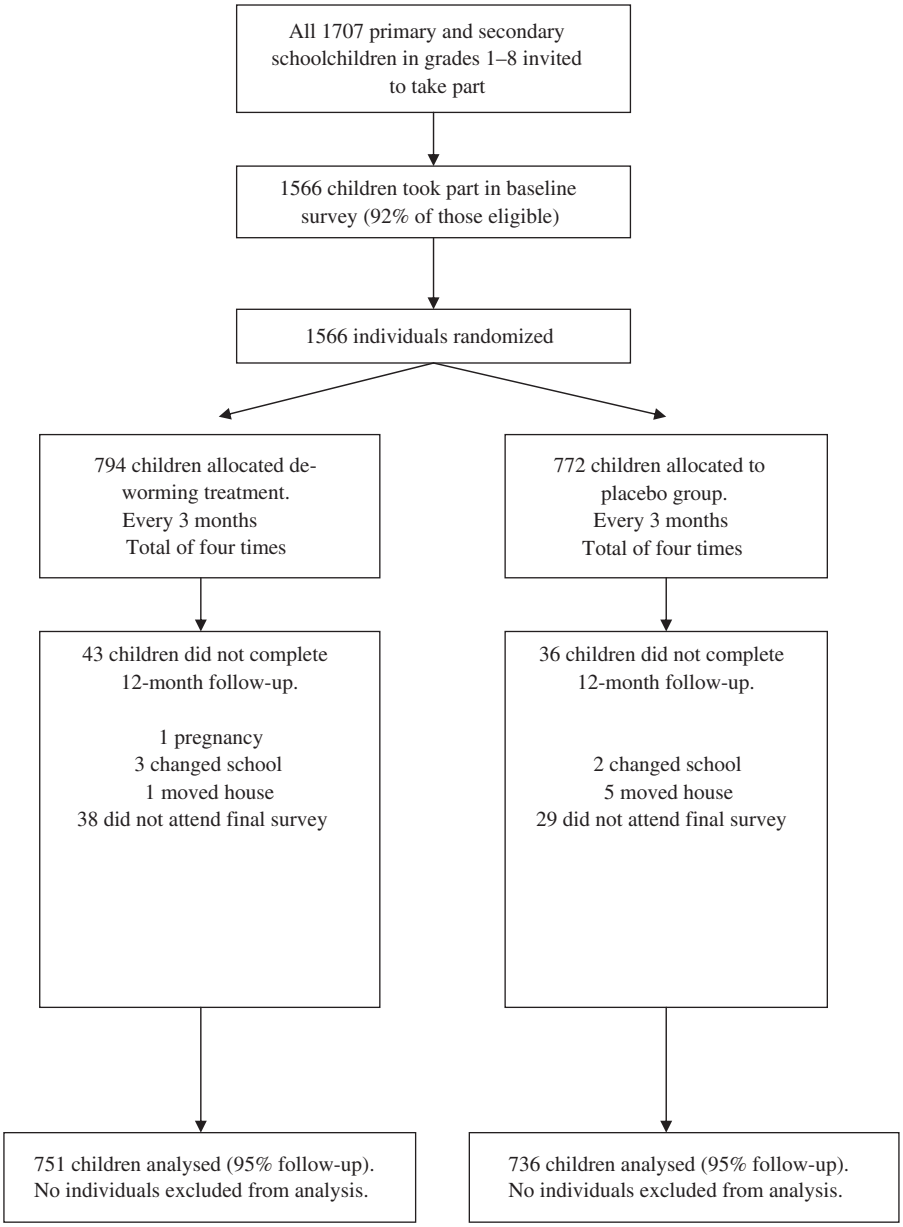

Fig. 1. Trial flow diagram.

(comprising of all those who did not provide complete data at 12 months) was similar in both treatment groups. The baseline characteristics of the two study groups were similar, except for the prevalence of allergen skin sensitization, which was slightly higher in the active treatment group (34.4%) than in the placebo group (32.1%), and a slight difference in ethnicity between the two groups (Table 1).

The supervising field workers reported that 96% of children received all doses of their allocated treatment under direct observation at 0, 3, 6, and 9 months. Adherence to the treatment protocol was similar in both study groups. While availability of over-the-counter anti-helminthics is extremely limited in Khanh Son, we recognized that some parents might have treated their children for gut worms at their own initiative and therefore asked parents at follow-up whether any extra anti-helminthics had been given during the study period. The parents of two children, both in the active treatment group, said that they had initiated treatment. Reported sighting of passed adult worms at 12 months' follow-up was uncommon and similar in the treatment (6.8%, 51/751) and placebo groups (6.5%, 48/736). There was no significant difference in the frequency of reported side effects between study groups, the most common being headache (9.3% active treatment vs. 7.5% placebo), abdominal pain (7.6% active treatment vs. 8.4% placebo), and a skin rash (0.9% active treatment vs. 1.0% placebo). At 12 months'

Table 1. Baseline characteristics

Characteristic	Anti-helminthic treatment, *N* (%)	Placebo, *N* (%)
Total	794 (50.7)	772 (49.3)
Sex		
Male	375 (47.2)	372 (48.2)
Female	419 (52.8)	400 (51.8)
Age group		
6–8	466 (58.7)	449 (58.2)
9–11	250 (31.5)	250 (32.4)
12+	78 (9.8)	73 (9.5)
Area (communes)		
Thanh Son	185 (23.3)	185 (24.0)
Son Lam	291 (36.6)	278 (36.0)
Son Binh	214 (27.0)	204 (26.4)
Ba Cum Nam	104 (13.1)	105 (13.6)
Ethnic group		
Raclay (local ethnic minority)	623 (78.5)	635 (82.5)
Kinh (ethnic Vietnamese)	159 (20.0)	126 (16.4)
Other	12 (1.5)	9 (1.2)
Parental education		
Illiterate	196 (24.7)	193 (25.0)
Primary	406 (51.1)	395 (51.2)
Secondary or higher	192 (24.2)	184 (23.8)
Hookworm (stool)		
Yes	515 (64.9)	500 (64.8)
No	279 (35.1)	272 (35.2)
Ascaris lumbricoides (stool)		
Yes	55 (6.9)	54 (7.0)
No	739 (93.1)	718 (93.0)
Trichuris trichiura (stool)		
Yes	6 (0.8)	6 (0.8)
No	788 (98.2)	766 (99.2)
Hookworm eggs/gram feces		
Mean (range)	248 (0–5400)	325 (0–7300)
Ascaris eggs/gram feces		
Mean (range)	39 (0–10 150)	88 (0–13 850)
Malaria (smear)		
Yes	9 (1.1)	7 (0.9)
No	785 (98.9)	765 (99.1)
Exercise-induced bronchospasm (% fall in PEFR after exercise)	−2.4 (SD 7.1)	−2.3 (SD 7.6)
Wheeze ever (questionnaire)	40 (5.0)	36 (4.7)
Rhinitis ever (questionnaire)	146 (18.4)	138 (17.9)
Flexural dermatitis (skin examination)	4 (0.5)	3 (0.4)
Skin sensitization		
Any allergen	273 (34.4)	248 (32.1)
Dermatophagoides pteronyssinus	65 (8.2)	43 (5.6)
Dermatophagoides farinae	95 (12.0)	78 (10.1)
Cockroach	232 (29.2)	197 (25.5)

follow-up, there was a marked decline in helminth prevalence in the treatment group (any helminth from 67.4% at baseline to 9.2% after treatment; hookworm from 64.9% to 8.9%), compared with a slight change in the placebo group (any helminth from 66.4% to 50.4%; hookworm from 64.8% to 49.6%), and a highly significant effect of treatment vs. placebo on prevalence at 12 months of any helminth [odds ratio (OR) = 0.10, 0.08–0.13, $P < 0.001$] or hookworm (OR = 0.10, 0.08–0.13, $P < 0.001$). The effects of treatment on *A. lumbricoides* prevalence at 12 months were less marked (OR = 0.49, 0.12–1.96, $P = 0.30$), as *A. lumbricoides* prevalence declined in both treatment (from 6.9% to 0.4%) and placebo (from 7.0% to 0.8%) groups. There was an 86% reduction in the mean hookworm epg at 12 months in the treatment compared with the placebo group, and a smaller reduction of 32% in the mean *A. lumbricoides* epg.

There was a 2.25% (SD 7.3) and 2.19% (SD 7.8) worsening in the exercise-induced peak flow reduction in the active and placebo groups between the baseline and the 12-month assessments. After adjustment for the difference in the prevalence of sensitization between treatment groups at baseline, the difference between treatment groups was 0.06% [95% confidence interval (CI) −0.71 to 0.83, $P = 0.9$, Table 2]. The results of the sensitivity analysis were very similar [mean change (SD) in the intervention group 2.13 (7.13), control group 2.07 (7.64), adjusted mean difference 0.06%, 95% CI −0.68 to 0.79]. This, together with the fact that the few children lost to follow-up (5%, 86/1566) were equally distributed between treatment and placebo groups, suggests that the missing values did not appreciably affect our results.

The risk of allergen skin sensitization to any of the three allergens tested was significantly increased after 12 months of active relative to placebo therapy, both in the univariate analysis (OR = 1.28, 1.03–1.60, $P = 0.03$) and after an adjustment for baseline atopy prevalence (adjusted OR = 1.31, 1.02–1.67, $P = 0.03$). Sensitization risk was also increased to individual allergens, although not significantly so after adjustment for baseline sensitization prevalence (*D. pteronyssinus*-adjusted OR = 1.26, 0.81–1.95, $P = 0.31$; *D. farinae*-adjusted OR = 1.45, 0.98–2.15, $P = 0.06$; Cockroach-adjusted OR = 1.26, 0.96–1.66, $P = 0.09$). There were non-significant risk increases at 12 months in the treatment group relative to placebo for the other secondary outcomes questionnaire-derived wheeze (adjusted OR = 1.16, 0.35–3.82, $P = 0.8$), rhinitis (adjusted OR = 1.39, 0.89–2.15, $P = 0.1$), and flexural dermatitis on physical examination (adjusted OR = 1.15, 0.39–3.45, $P = 0.8$). None of these effect estimates were appreciably altered by additional adjustment for ethnic group.

There was no significant interaction between hookworm infection status or infection intensity at baseline and treatment effects on any of the study outcomes. In the *post hoc* analysis, however, there was a significant interaction between *A. lumbricoides* infection at baseline and the effect of treatment on allergen skin sensitization ($P = 0.04$), such that the risk of sensitization to any allergen at 12 months in those who had received

Table 2. Effect of anti-helminthic treatment on study outcomes at 12 months' follow-up

	Anti-helminthic treatment, *N* (%)	Placebo, *N* (%)	Size of effect*	*P*-value
Total	751 (50.5)	736 (49.5)	–	–
			Mean difference (95% CI)	
Change in exercise-induced bronchospasm (%) mean (SD)	2.25 (7.3)	2.19 (7.8)	+0.06 (–0.71, 0.83)	0.9
			Odds ratio (95% CI)	
Wheeze since start of treatment (questionnaire)	6 (0.8)	5 (0.7)	1.16 (0.35–3.82)	0.8
Rhinitis since start of treatment (questionnaire)	50 (6.7)	36 (4.9)	1.39 (0.89–2.15)	0.1
Flexural dermatitis since start of treatment (skin examination)	7 (0.9)	6 (0.8)	1.15 (0.39–3.45)	0.8
Skin sensitization				
Any allergen	251 (33.4)	207 (28.1)	1.31 (1.02, 1.67)	0.03
Children infected with A. lumbricoides at baseline				
Total	52 (50.5)	51 (49.5)	–	–
Skin sensitization				
Any allergen	20 (38.5)	9 (17.6)	4.90 (1.48–16.19)	0.009

*Adjusted for the observed difference between groups in allergic sensitization at baseline.
CI, confidence interval.

Table 3. Univariate and adjusted odds ratios for relationship between allergen skin sensitization and immunological parameters at baseline ($n = 244$, secondary schoolchildren)

Immunological factors*	Median (IQR, pg/mL)	% responders	Univariate OR (95% CI)	*P*-value	Adjusted OR† (95% CI)	*P*-value
IL-10 ES	0 (0–28)	50%	0.78 (0.61–0.99)	0.03	0.76 (0.59–0.99)	0.04
IL-10 PHA	835 (449–1244)	100%	0.88 (0.62–1.26)	0.5	–	
IL-5 ES	0 (0–0)	16%	0.92 (0.68–1.26)	0.6	–	
IL-5 PHA	168 (32–380)	79%	1.04 (0.91–1.19)	0.5	–	
IFN-γ ES	0 (0–0)	16%	1.11 (0.87–1.43)	0.4	–	
IFN-γ PHA	8827 (6869–11 807)	100%	0.95 (0.68–1.32)	0.8	–	
IgE-total	235 (123–486)	91%	1.47 (1.15–1.89)	0.002	1.63 (1.25–2.13)	<0.001

*Odds ratios are based on $\log_e$-transformed data.
†Odds ratios mutually adjusted and also adjusted for ethnic group, including all variables with $p < 0.05$ in the final model.
OR, odds ratio; IQR, inter-quartile range; CI, confidence interval; IU, International Units; PHA, phytohaemagglutinin; ES, *Necator americanus* excretory/secretory.

anti-helminthic treatment compared with placebo was more marked in those with *Ascaris* infection at baseline (adjusted OR = 4.90, 1.48–16.19, $P = 0.009$), most of whom were also infected with hookworm (77%, 79/103). Adjusting these results for hookworm infection intensity did not alter the risk estimate appreciably. Numbers were too small to calculate risk estimates for individual allergens and for those infected with *Ascaris* alone. No significant risk increases were seen for any of the other primary and secondary outcomes, and there were no interactions with *A. lumbricoides* baseline infection intensity.

Of the subgroup of 244 secondary schoolchildren who were also tested for immunological parameters, at baseline, 62.7% (153/244) were infected with hookworm and 2.5% (6/244) with *A. lumbricoides*. This subgroup of children was aged 9–16 (mean age 11.5) and similar to the overall sample with regard to sex and ethnic group (data not shown). In the univariate analysis, skin sensitization was significantly positively associated with total IgE (Table 3), and there was a similar trend for specific IgE responses [*D. pternonyssinus* = 2.36 (0.94–5.95), $P = 0.07$; *D. farinae* = 1.83 (1.06–3.16), $P = 0.03$; Cockroach = 1.26 (0.74–2.16), $P = 0.4$; *N. americanus* = 1.73 (1.00–2.99), $P = 0.05$]. In addition, skin sensitization was inversely related to levels of hookworm-induced IL-10, but not hookworm-induced IFN-γ or IL-5, or non-specific cytokine responses to mitogen (Table 3). In the final adjusted model, only IL-10 (adjusted OR = 0.76, 0.59–0.99, $P = 0.04$) and total IgE (adjusted OR = 1.63, 1.25–2.13), $P < 0.001$) remained as significant predictors of skin sensitization, with ethnic group as a significant confounder (Table 3). Inclusion of age, sex, and area as *a priori* confounders did not change the risk estimates.

At 12 months, there was a highly significant reduction in hookworm prevalence in this subgroup of children in the treatment compared with the placebo group

Table 4. Effect of anti-helminthic treatment on immunological parameters at 12 months in all secondary schoolchildren who were hookworm-infected at baseline

	Anti-helminthic treatment			Placebo		
	N	Median (IQR)	% responders	*N*	Median (IQR)	% responders
IL-10 ES (pg/ml)	79	0 (0–9)[a]	25	63	0 (0–21)	38
IL-5 ES (pg/ml)	79	0 (0–0)	14	63	0 (0–0)	23
IFN-γ ES (pg/ml)	79	0 (0–5)	25	63	0 (0–9)	32
Total IgE (IU/ml)	77	168 (97–365)[b]	82	58	240 (138–607)	95

[a]$P = 0.09$ (Mann–Whitney *U*-test).

[b]$P = 0.03$ (Mann–Whitney *U*-test).

ES, *Necator americanus* excretory/secretory antigen-induced; IQR, inter-quartile range.

(OR = 0.11, 0.05–0.23, $P < 0.001$), with a 78% reduction in hookworm epg. With regard to the effect of anti-helminthic treatment on immunological responses, there was a trend towards lower IL-10 responses in the treatment compared with the placebo group ($P = 0.09$), and a significant reduction in the levels of total IgE ($P = 0.03$, Table 4). The numbers of *A. lumbricoides*-infected individuals were too small (2.5%, 6/244) to examine *A. lumbricoides*-induced cytokine responses.

Discussion

This study shows that 3-monthly anti-helminthic therapy over a 12-month period had no significant effect on the primary endpoint of exercise-induced asthma and the secondary clinical outcomes of wheeze, rhinitis, and flexural dermatitis. However, the treatment was associated with an increased risk of allergen skin sensitization. Because anti-helminthic drugs do not cause skin prick positivity to environmental allergens, this finding is consistent with the hypothesis that helminth infection has a direct immunomodulatory effect on allergen skin test responses. *Post hoc* analysis indicates that this effect was particularly strong in children infected with *A. lumbricoides*. None of the immunological responses examined could explain the increased risk of skin sensitization as a consequence of loss of helminth infection, although hookworm-induced IL-10 responses were negatively associated with skin sensitization at baseline and there was a trend, albeit non-significant, towards a decrease following anti-helminthic treatment.

This is, to date, the largest individually randomized intervention study on the potential links between helminth infection and allergic disease, and the only such study conducted in a geographical area where hookworm predominates. Participation and adherence to the trial protocol were high, with 95% of the participants completing the study. Blinding is likely to have been successful for the majority of children, because hookworm was the main gut worm infection, and, in contrast to *A. lumbricoides*, adult hookworms passed in the feces after therapy are small and unlikely to be seen. There was no evidence of a difference between the treatment and the placebo groups in the proportion of children reporting sightings of worms after treatment. We used validated and objective outcomes assessed by researchers blinded to intervention status. Because we used a population-based sample of all schoolchildren enrolled in grades 1–8 in one well-defined geographical area, and because over 95% of children there are known to attend school, our findings are likely to be generally representative of the target population.

Although mebendazole was being used for the national helminth control programme in Vietnam, we found, to our surprise, that the first treatment with single-dose mebendazole 500 mg as per the study protocol was not superior to the placebo, and we therefore had to change the trial regimen to albendazole 400 mg daily for 3 consecutive days. As a result, those in the active treatment group received efficacious anti-helminthic therapy for only 9 rather than the originally intended 12 months. We were unable, for logistic and funding reasons, to extend the follow-up to compensate for this. It is possible that the increase in SPT positivity in the treatment group would have been greater had we used albendazole from the start or, indeed, given anti-helminthic treatment for longer than 12 months. On the other hand, our study protocol ensured that treatment efficacy was assessed after the first treatment round, and the change from mebendazole to albendazole is likely to have contributed to the validity of our findings. Unexpectedly, we found a reduction in *A. lumbricoides* infection not only in the treatment but also in the placebo group. This probably reflects natural year-on-year variation in *Ascaris* worm burden in the study population, as adult worms only have a lifespan of around 12 months. At the same time, children in the placebo group would still have been infected with hookworm.

It is unlikely that other, unmeasured infectious or parasitic diseases confounded the study results. Geohelminths and malaria, which we tested for, are the prevalent endoparasitic 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 (National Institute of Malariology, Parasitology, and Entomology (NIMPE), Hanoi, unpublished internal report, March 2006). To date, no case of HIV-1 or 2 has been detected in Khanh Son, despite routine screening of all people who seek medical treatment in the district's only hospital.

Cooper et al. suggested in a recent cluster-randomized trial in Ecuadorian children that regular anti-helminthic treatment does not increase clinical allergic disease, namely exercise-induced bronchospasm, wheeze, rhinitis, and flexural eczema [18]. This is in keeping with our primary outcome result. In both studies, the lack of an association between loss of exposure to helminths and clinical allergic disease may be due to a lack of causality or because helminth eradication was incomplete and/or short-lived in an environment where most children's immune systems are repeatedly exposed to gut worm infection from early in life.

Early priming of the infant's immune system both *in utero* and postnatally may also be important for protection from clinical allergic disease, as suggested by a small randomized, double-blind, placebo-controlled trial comparing the risk of eczema development in 103 Ugandan infants whose mothers had been allocated to either single-dose anti-helminthic treatment or placebo during the second or the third trimester [30]. Children whose mothers had received anti-helminthic treatment rather than placebo showed a more than twofold increase in cumulative eczema risk up to the age of 15 months, although this effect was not statistically significant, possibly because of a small sample size. In view of this partly conflicting evidence, it will be important to explore the effect of early loss of helminth infection on clinical allergic disease further through carefully conducted birth cohort and intervention studies.

As for allergen skin sensitization, a protective effect of host invasive parasites has been reported in several cross-sectional studies [5, 11, 31–36]. Our previously reported cross-sectional analysis in the same study population indicated independent protective effects on SPT responses of *A. lumbricoides* (adjusted OR = 0.28, 0.10–0.78) and hookworm infection (adjusted OR = 0.61, 0.39–0.96) [19]. In the intervention study, as in cross-sectional analysis at baseline, the protective effect was particularly strong for children harbouring infection with *A. lumbricoides*, most of whom were also infected with hookworm. Statistical analysis suggested that hookworm infection intensity did not confound this effect.

On the basis of data from a series of studies conducted in Venezuela, it has also been argued that a low-intensity chronic helminth infection might increase the risk of allergic disease, while a high-intensity infection acts protectively [37–40]. However, we found a significant increase in SPT positivity following a loss of gut worm infection in a population where the majority of children have, according to WHO criteria, a low-intensity helminth infection (mean hookworm epg 286 and mean *A. lumbricoides* epg 63) [41]. This suggests that loss of helminth infection, even if the infection intensity is relatively low, may have a direct positive effect on allergen SPT reactivity.

An endoparasite-induced modulation of host immune responses has been noted previously [42]. For instance, the saliva of *N. americanus* contains molecules that protect the worm against an attack from the host immune system while feeding in the small intestine, such as substances that bind to host complement components, control tissue eosinophilia, and can induce apoptosis in activated T cells [13]. In addition, elevated levels of anti-inflammatory host cytokines, such as IL-10, have been described in cross-sectional studies in association with hookworm, *A. lumbricoides,* and schistosomiasis infections [43–46]. While the exact mechanisms of IL-10-induced anti-inflammatory action are currently uncertain, parasite-induced regulatory T cells have been argued to play a pivotal role in the increased expression of IL-10 and seem to be a part of the regulatory network that provides a subtle immunological balance between the host and the parasite [47, 48]. An elevation of IL-10 is likely to have a systemic anti-inflammatory action but may also affect mast cells in the skin, which would influence allergen skin reactivity. For instance, IL-10 is known to inhibit the expression of high-affinity FcεRI receptors on mast cells and mast cell activation, which could lead to reduced mast cell degranulation and histamine release [49, 50].

Two cross-sectional studies have investigated the role of helminth-induced cytokine responses and their effect on SPT responses: one conducted among 520 Gabonese schoolchildren predominantly infected with schistosomiasis and the other a study in 80 Ecuadorian children with *Ascaris* infection [35, 51]. The former study found a significant reduction in skin-test reactivity associated with schistosome-induced IL-10, while the latter study found no significant association between the level of *A. lumbricoides*-induced IL-10 or IL-10^+ T cells and skin test responses. The results of the Gabon study and our cross-sectional analysis are consistent with a role for IL-10 in mediating helminth effects on skin-test responses. However, the reduction in hookworm-induced IL-10 post-treatment missed conventional statistical significance.

There are a number of possible explanations for this observation: IL-10 may not be the most important marker of regulatory responses, and may not sensitively reflect potential changes in parasite-induced regulatory T cell populations after treatment. Moreover, we examined changes in immunological responses only in a subsample of schoolchildren and the statistical power may therefore have been too low to detect subtle changes in immune responses. In addition, as observed by others,

helminth-induced cytokine responses measured with whole-blood cultures tend to be lower than cytokine responses determined from separated peripheral blood mononuclear cells, and this might have contributed to a reduced assay sensitivity [52]. It is also possible that, as regulatory T cells are primed from early on in life, helminth-induced regulatory responses may not be altered by a relatively short-lived reduction in worm burden, particularly in the presence of continued exposure to infective worm larvae. In view of the strong effect on SPT responses after anti-helminthic treatment in those infected with *A. lumbricoides* at baseline, it would have been desirable to also study *Ascaris*-induced immune responses. This was not possible due to the very small number of *Ascaris*-infected children among those who were studied immunologically (2.5%, 6/244). However, *N. americanus* and *A. lumbricoides* show significant antigenic cross-reactivity [53]; hence, similar patterns of cytokine responses might be expected.

The relation between helminth infection and allergy warrants further investigation [41]. Our study, which provides independent evidence of an increased risk of allergen sensitization after a significant reduction in gut worm burden from a substantive individually randomized, double-blind, placebo-controlled trial, demonstrates that helminth infection and allergic sensitization are likely to be interrelated. However, we are still lacking a clear immunological mechanism to explain this. Indeed, measuring systemic cytokine and serological changes, as in our and other studies [34], may be inadequate to shed further light on the relationship between helminths and allergic sensitization of the skin. Future work should therefore concentrate on unravelling what happens at the level of the skin mast cell/FcεRI/specific allergen/histamine interface as a consequence of the presence or absence of helminth infection. After all, we are seeking an explanation for a helminth-induced change in immediate skin hypersensitivity. While we did not observe any differences in histamine SPT responses between worm-infected and uninfected individuals both at baseline and after treatment (data not shown), excluding histamine desensitization as a possible explanation of our findings, another plausible mechanism is a parasite-induced alteration in skin mast cell or FcεRI IgE receptor density. In addition, it is possible that either total IgE or parasite-specific IgE compete with allergen-specific IgE on skin mast cells, especially if such helminth-induced phenomena do not lead to a proportional up-regulation in allergen-specific IgE. Furthermore, allergen-specific IgG_4 may either block skin mast cell degranulation or IgE antibody-facilitated allergen presentation [54]. While the importance of such studies appears to be obvious, skin biopsies would have to be taken, something unlikely to be considered ethical in a paediatric study population such as ours. Nevertheless, further investigation of the mechanisms underlying the association between SPT responses and helminth infection is important, as it has the potential to provide insights into the pathogenesis and therapy of asthma and other allergic diseases. Such efforts will also help us to further understand part of our human co-evolution with helminth parasites, a relationship that is marked by significant morbidity associated with severe infection, but that may well be mutually beneficial below a certain threshold of infection intensity through a parasite-induced reduction in host allergic tissue inflammation, concomitantly leading to prolonged parasite survival.

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