

ORIGINAL PAPER

Early infection with *Trichuris trichiura* and allergen skin test reactivity in later childhood

L. C. Rodrigues*, P. J. Newcombe*, S. S. Cunha†, N. M. Alcantara-Neves‡, B. Genser†, A. A. Cruz§, S. M. Simoes†, R. Fiaccone¶, L. Amorim¶, P. J. Cooper|| and M. L. Barreto†, SCAALA (Social Change, Asthma and Allergy in Latin America)

*London School of Hygiene and Tropical Medicine, London WC1E 7HT, UK, †Instituto de Saude Coletiva, Universidade Federal da Bahia, Salvador, Brazil, ‡Instituto de Ciências da Saúde, Universidade Federal da Bahia, Salvador, Brazil, §ProAR Faculdade de Medicina, Universidade Federal da Bahia, Salvador, Brazil, ¶Department of Statistics, Universidade Federal da Bahia, Salvador, Brazil and ||Instituto de Microbiologia, Universidad San Francisco de Quito, Quito, Ecuador and Centre for Infection, St George's University of London, London, UK

Clinical and Experimental Allergy

Summary

Background Allergic diseases cause a large and increasing burden in developed countries and in urban centres in middle-income countries. The causes of this increase are unknown and, currently, there are no interventions to prevent the development of allergic diseases. The 'hygiene hypothesis' has tried to explain the increase through a reduction in the frequency of childhood infections causing a failure to program the immune system for adequate immune regulation. Intestinal helminth parasites are prevalent in childhood in developing countries and are associated with a lower prevalence of allergen skin test reactivity and asthma.

Objectives To investigate whether children who had intestinal helminth infections during early childhood have a lower prevalence of allergen skin test reactivity later in childhood.

Methods We re-visited a population of 1055 children from whom stool samples had been collected for detection of intestinal helminth infections for another study, and collected new stool samples and performed allergen skin prick testing. Information on potential confounding variables was collected.

Results Children with heavy infections with *Trichuris trichiura* in early childhood had a significantly reduced prevalence of allergen skin test reactivity in later childhood, even in the absence of *T. trichiura* infection at the time of skin testing in later childhood.

Conclusion Early heavy infections with *T. trichiura* may protect against the development of allergen skin test reactivity in later childhood. Novel treatments to program immune-regulation in early childhood in a way that mimics the effects of early infections with *T. trichiura* may offer new strategies for the prevention of allergic disease.

Keywords allergen skin test reactivity, asthma, atopy, immune programming, intestinal helminths, rhinitis, *Trichuris trichiura*

Submitted 16 December 2007; **revised** 5 March 2008; **accepted** 1 April 2008

Correspondence:

Prof. Laura C Rodrigues MD, MSc, PhD,
London School of Hygiene and Tropical
Medicine, London WC1E 7HT, UK.
E-mail: laura.rodrigues@ishtm.ac.uk

Introduction

The increase in asthma and other allergic diseases over recent decades in western countries and in urban areas of some middle-income countries have been attributed partly to an increase in allergic sensitization. Atopy and allergic diseases are caused by interactions between environmental and genetic factors. The increases in allergy have occurred over a short time period and are likely, therefore, to be caused by changes in environmental rather than genetic factors. Although health programmes

to control the severity of asthma can reduce mortality and hospitalizations associated with asthma, primary prevention of asthma remains elusive and the results of allergen avoidance trials to prevent allergic sensitization in children with a family history of asthma have been disappointing [1].

Causal environmental exposures for atopy are likely to be present in early childhood and to be mediated by immunological mechanisms [2]. The prevailing current theory to explain the increase in allergy, the hygiene hypothesis, proposes that the increase in allergy is caused

by changes in immune regulation during early childhood, as a consequence of reduced chronic exposures to microbes and their products [3–5]. Intestinal helminth infections are of particular interest because they have strong immune regulatory effects on their hosts [6], continue to be highly prevalent in developing countries, are estimated to infect a quarter of humans worldwide, and first occur in infancy [7].

Previous studies have provided evidence that the prevalence of atopy (measured by allergen skin test reactivity) and allergic diseases may be reduced in areas that are endemic for intestinal helminths [8], and that these effects may be dose-dependent (i.e. heavy infection intensities have stronger inhibitory effects than light intensities) and vary between different intestinal helminth parasites [9].

The original hygiene hypothesis was explained in terms of early infections promoting T helper type 1 (Th1)-biased immunity that was considered to be protective against the T helper type 2 (Th2) responses associated with allergy [3]. The finding that Th2-inducing helminth infections were also associated with a reduced risk of allergy led to a re-working of the hygiene hypothesis to explain the protection afforded by childhood infectious diseases in terms of the promotion of immune regulation by chronic microbial and parasitic exposures [3, 4]. Most data in support of a protective role of helminths against allergy are derived from cross-sectional studies of schoolage children that cannot distinguish the effects of current from past infections [3, 10]. Distinguishing the allergy-regulatory effects of early vs. late infections is important because the mechanisms by which early and late infections mediate their effects are likely to be different and could explain the contradictory findings of intervention studies of anthelmintic treatment in which some studies have shown an allergy-enhancing effect of treatment [10, 11] while another study showed no effect [12]. Current (i.e. later childhood) infections may induce active anti-allergic-regulatory mechanisms that decrease the risk of atopy and the effect may be reversible by anthelmintic treatment (removal of the immunologic stimulus). In contrast, early helminth infections may program non-reversible or longer lasting regulatory mechanisms. Because individuals with high helminth infection intensities are likely to maintain high infections intensities in the long term (and are likely also to become re-infected with high parasite burdens after treatment [13]), the reduced risk of allergy associated with current helminth infections observed in cross-sectional studies is also consistent with the protective effect occurring in early life and may be limited to a time window during early childhood.

Here, we report findings from a prospective study of children in the city of Salvador in Brazil that investigated the effects of intestinal helminth infections in early vs. late childhood on allergen skin test reactivity measured in late childhood. The data provide evidence for a protective

effect of early heavy infections with *Trichuris trichiura* against the development of allergen skin test reactivity that was not dependent on the presence of *T. trichiura* infection in later childhood.

Methods

Study population

The study was conducted in Salvador, Northeastern Brazil, a city with a population of 2.5 million, and where the prevalence of reported wheezing within the previous 12 months in school children aged 12–13 years is very high (27.1%) [14]. The design of this study has been reported elsewhere [15]. Briefly, as part of a prospective cohort study investigating the health impact of installation of a sewage system in marginal neighborhoods in the city of Salvador [16], data were collected from children born between 1994 and 2001 who lived in 'sentinel' areas scattered through the city. Data were collected between 1997 and 2003 (baseline) and included stool examinations for intestinal helminth infections, demographic and social data collected by questionnaire, and observation of the home environment [17]. The children were resurveyed to investigate the effect of helminth infection on allergen skin reactivity in 2005 at which time stool samples were examined, data were collected on relevant risk factors for allergic disease, and skin prick testing to a range of allergens was performed.

Stool examinations and definition of helminth infection groups

At baseline and for the 2005 survey, two stool samples were collected two days apart. Stool samples were analysed by sedimentation [18] and the Kato-Katz technique [19] for the presence and number of helminth eggs (*T. trichiura*, *Ascaris lumbricoides*, hookworm, and *Schistosoma mansoni*). Appropriate treatment was given to all children with helminth infections. Children were examined only at these two observation times, and no data are available on infections occurring at other times. There are no mass treatment programmes for intestinal helminths in Salvador, but of course individual children may have been diagnosed and treated in the health services during the interval between surveys. Because only one child was infected with *S. mansoni* at baseline, this parasite was not considered further in this analysis. Children were classified by the presence of at least one intestinal helminth infection, and by the presence and intensity of infection with *T. trichiura* and *A. lumbricoides*, at each time point. Initially, three categories of infection intensity with *A. lumbricoides* and *T. trichiura* were considered: absence of infection, and infection with intensities above or below the geometric mean of eggs/gram of stools (not infected,

light, and heavy infections). The geometric mean infection intensity for *T. trichiura* was 432 eggs per gram of stool (epg) (range: 24–54, 480 epg), and for 5760 epg (range: 24–285, 792) *A. lumbricoides*. A geometric mean cutoff was used because the previously proposed thresholds for infection intensities have been defined in relation to clinical disease risk [14] and such thresholds are unlikely to be relevant to immunologic effects occurring in infancy. Exploratory analyses of the data did not suggest a better cutoff point (data not shown). The effect on skin test reactivity was greater in heavy compared with light infections (Table 2), and the groups, 'no infection' and 'light infection', were combined for further analyses. Because of small numbers, hookworm infection was analysed by the presence of infection but not by intensity.

Allergen skin test reactivity

Skin prick testing was performed for seven allergens identified as relevant during a previous survey: *Dermatophagoides pteronyssinus*, *Blomia tropicalis*, *Blattella germanica*, *Periplaneta americana*, dog and cat epithelia, and fungi (*Aspergillus amstelodami*, *Aspergillus fumigatus*, *Aspergillus niger*, *Aspergillus terreus*, *Penicillium brevicompactum*, *Penicillium expansum*, *Penicillium notatum*, *Penicillium roqueforti*, *Cladosporium fulvum*, and *Cladosporium herbarum*), using kits from ALK-Abello (São Paulo, Brazil). Food allergens were not investigated. Saline and histamine were used as negative and positive controls, respectively. Allergens and controls were placed onto the volar side of the right forearm of each child and pricked with ALK lancets following the manufacturer's instructions. After 15 min, the reaction sizes were recorded by measuring the two perpendicular diameters of the weal at each puncture site (excluding flare and pseudo-pods). Reactions were considered to be positive if the mean of the two perpendicular readings was at least 3 mm greater than the saline control. Individuals with at least one positive reaction were classified as positive. Children receiving anti-allergic medications at the time of skin testing were asked to stop medication and were evaluated after discontinuation of treatment.

Main variables of interest and confounding variables

The main variables of interest in this analysis were intestinal helminth infections defined by the presence and intensity of infection with *T. trichiurus* and *A. lumbricoides*, and the presence of infection with hookworm in early childhood, and allergen skin test reactivity in later childhood. Other relevant data considered in the analysis included: for the child during the first survey – sex, ethnicity, parental history of asthma, living with older siblings aged under 5 years, nursery school attendance, duration of breastfeeding, and the proportion of

days with diarrhoea (as a proportion of the days observed); for the mother during the first survey – educational level and hygiene behaviour (based on observation during a home visit); for the child's home environment during the first survey – type of housing, whether the street where the house is located is paved or not; and excreta disposal classified as 'good' (sewage system, septic tank with maintenance); 'intermediate' (hole in the ground or septic tank without maintenance) and 'bad' (open air); and for the child at the second survey – the presence and intensity of helminth infections. Area variables that were also considered in the analysis included the proportion of houses linked to a sewage system and without daily garbage collection.

Statistical analysis

We undertook a complete-case analysis because 1055 children (> 94% of children with skin test, and with stool examination for both periods) had complete data for the study variables.

To examine the association between helminth infection and skin test reactivity, we first estimated the effect of each intestinal helminth infection on allergen skin reactivity, followed by the presence and intensity of infection separately for stool collected during the two surveys. To explore whether the effects of *T. trichiurus* and *A. lumbricoides* were independent, we examined the effect of infection with no helminths, each helminth, and combined infection (combined no infection and light infection vs. heavy infection strata) separately for early and late infections.

Because we wished to investigate specifically the role of early helminth infection in allergen skin test reactivity, and because *T. trichiurus* was the only early infection with a significant effect on skin reactivity, further analysis was undertaken to investigate whether this effect was independent of potential confounding factors in a logistic regression model. The associations between all variables and allergen skin test reactivity were investigated initially using a multivariate logistic regression model that adjusted for all variables that were statistically significant (at the 5% level). Backwards stepwise selection was then carried out and variables with $P > 0.05$ were excluded from the model. Because we were interested primarily in the association between helminth infection in early childhood (i.e. at the baseline survey) and allergen skin test reactivity, any variable observed to change the effect estimates for early-life parasite infection by more than 10% was left in the model, regardless of significance.

Infected individuals tend to remain infected for the lifetime of the helminth and have a high probability of re-infection after treatment. To assess whether the lower prevalence of skin test reactivity among children with high-intensity *T. trichiura* infections in early childhood

was likely to be caused by the early infection, rather than by the late infections (i.e. infection at the second survey conducted at the time of allergen skin testing), the final model was repeated including a variable that combined early and later childhood data on *T. trichiura* infection (also classified according to intensity categories) for the 1036 children for whom data were available from the two surveys. This was done for *T. trichiura* only (rather than for both *T. trichiura* and *A. lumbricoides*) because the limited number of infected individuals did not allow further stratification. We tested whether the effect of early infection with *T. trichiurus* remained significant after controlling for late infection with *A. lumbricoides* in a separate logistic model that included those two variables only. We explored the effect of early infections with *T. trichiura* on the number of positive skin tests by examining the association between the number of positive skin tests (0, 1, ≥ 2) and *T. trichiura* infection (no/light infection vs. heavy infection). We also explored whether the association between early helminth infection and allergen skin test reactivity was modified by parental history of asthma, using the Wald test for interaction.

For all the above analyses, hypothesis testing was conducted using Wald tests. For variables with more than one level of exposure, two types of *P*-values are presented: individual *P*-values comparing each level of exposure against a reference category, and a joint *P*-value that investigates whether at least one of the categories in the variable is significant. All multivariate models were fitted with random intercepts at the micro-area level, to adjust for a potential effect of clustering. All analyses were performed using STATA (StataCorp. 2005. Stata Statistical Software: Release 9. College Station, TX: StataCorp LP). Ethical approval was granted by Instituto de Saude Coletiva at Federal University of Bahia, National Commission on Ethics in Research (CONEP), Brazil, and the London School of Hygiene and Tropical Medicine. The parents or guardians of all participants gave written informed consent. The study was conducted through the SCAALA (Social Change, Asthma and Allergy in Latin America) initiative, funded by the Wellcome trust grant no.072405/Z/03/Z. BG is also supported by the Fundação de Amparo à Pesquisa do Estado da Bahia, Brazil (FAPESB), contract no. 1181 8050587. The funders had no role in the analysis or writing of the paper.

Results

The mean age at the time of the first survey was 2.3 years (range: 1 month–4.6 years) and that of the second survey was 7.1 (range: 4.4–11.3 years). The median time between the first ('early childhood') and the second ('later childhood') survey was 4.4 years (inter-quartile range: 4.3–4.7 years). The baseline characteristics of the cohort at the time of the first early childhood survey stratified by

allergen skin test reactivity are shown in Table 1. The results of stool examinations by age in early and later childhood for *A. lumbricoides* and *T. trichiura* are shown in Fig. 1.

The prevalence of intestinal helminth infections in early and later childhood in skin test-positive and -negative children is shown in Table 2. In early childhood, infections with both *A. lumbricoides* (OR 0.63, 95% CI 0.44–0.90, *P*=0.01) and *T. trichiura* (OR 0.58, 95% CI 0.37–0.92, *P*=0.02) were inversely associated with skin test reactivity. Heavy infections (*A. lumbricoides*, OR 0.50, 95% CI 0.30–0.844, *P*=0.009; *T. trichiura*, OR 0.29, 95% CI 0.13–0.64, *P*=0.002) reduced the prevalence of allergen skin test reactivity; light infections also reduced skin test reactivity but not significantly and the magnitude of the effects was smaller. Only five children were infected with hookworm in early childhood and although children with hookworm infections had a lower prevalence of skin test reactivity this was not statistically significant. In later childhood, significant inverse associations were observed between skin test reactivity and heavy infections with *A. lumbricoides* (OR 0.39, 95% CI 0.21–0.72, *P*=0.002) and *T. trichiura* (OR 0.56, 95% CI 0.32–0.98, *P*=0.04).

Table 3 shows the effects of infection with *A. lumbricoides* and *T. trichiura* alone and in combination (grouped into no/light and heavy infection) separately for early and later childhood infections. Heavy infections with *A. lumbricoides* in early childhood were not statistically significantly associated with allergen skin test reactivity after controlling for *T. trichiura* infection; and heavy infections with both *A. lumbricoides* and *T. trichiura* in early childhood appeared to have similar effects on allergen skin reactivity as infection with *T. trichiura* alone. Heavy infections with *A. lumbricoides* in late childhood significantly reduced the prevalence of allergen skin reactivity alone or in combination with *T. trichiura*. In later childhood, infections with *T. trichiura* were not significantly associated with allergen skin test reactivity after controlling for the effects of *A. lumbricoides*.

Table 4 shows the results of a multivariate analysis to investigate whether the inverse association between heavy infection with *T. trichiura* in early childhood and skin test reactivity in later childhood remains after controlling for potential early childhood confounders, and whether it remains among children without *T. trichiura* infection at the time of allergen skin testing (i.e. later childhood). Compared with children with no or light *T. trichiura* infections in early and later childhood, the risk of allergen skin test reactivity was dramatically reduced (OR 0.18, 95% CI 0.04–0.78, *P*=0.022) among children with heavy *T. trichiura* infections in both early and later childhood, and importantly, it remained statistically significant among children with heavy *T. trichiura* infection in early childhood but not later childhood (i.e. at the time of the skin testing) (OR = 0.35, 95% CI 0.13–0.94, *P*=0.04). There

Table 1. Distribution of potential confounding variables in the study population

		No skin reactivity to aeroallergens (<i>n</i> = 745)		Skin reactivity to aeroallergens (<i>n</i> = 310)		
Variable	Category	<i>n</i>	%	<i>n</i>	%	Joint <i>P</i> -value
Individual characteristics						
Sex	Male	380	51.0	187	60.3	0.007
	Female	365	49.0	123	39.7	
Ethnicity	Mixed Race	486	65.2	214	69.0	0.2728
	White	65	8.7	30	9.7	
	Black	194	26.0	66	21.3	
Mother or father has a history of asthma	Neither	643	86.3	263	84.8	0.4638
	One or both	102	13.7	47	15.2	
Number of older siblings	One or more	527	70.7	250	80.6	0.0007
	None	218	29.3	60	19.4	
Attends nursery school	No	729	97.9	297	95.8	0.0661
	Yes	16	2.1	13	4.2	
Duration of reported breast feeding	0–2 months	477	64.0	173	55.8	0.0201
	More than 2 months	268	36.0	137	44.2	
Percentage of days with diarrhoea	None	217	29.1	83	26.8	0.6313
	0.2–1%	182	24.4	71	22.9	
	> 1%–2.7%	170	22.8	81	26.1	
	2.7%	176	23.6	75	24.2	
Characteristics of the mother						
Mothers education	Illiterate/primary	412	55.3	160	51.6	0.3334
	Secondary or more	333	44.7	150	48.4	
Hygiene behaviour	Intermediate	310	41.6	145	46.8	0.1089
	Bad	164	22.0	61	19.7	
	Good	271	36.4	104	33.5	
Characteristics of the home						
Type	House	671	90.1	286	92.3	0.0665
	Apartment	13	1.7	9	2.9	
	Shack	16	2.1	7	2.3	
	Room	45	6.0	8	2.6	
Paving in the street	No	401	53.8	156	50.3	0.1622
	Yes	344	46.2	154	49.7	
Excreta disposal	Sewage/septic tank	470	63.1	190	61.3	0.1165
	Hole in the ground	99	13.3	57	18.4	
	Open air	176	23.6	63	20.3	
Proportion of census sectors in the area with daily rubbish collection	0–33%	470	63.1	171	55.2	0.4049
	33%–66%	77	10.3	35	11.3	
	Over 66%	198	26.6	104	33.5	
Proportion of census sectors with no homes linked to sewage system	0–50%	568	76.2	209	67.4	0.0633
	50%–75%	93	12.5	51	16.5	
	Over 75%	84	11.3	50	16.1	

was no evidence of effect modification by a family history of allergic diseases on skin test reactivity nor on the association between early and later childhood *T. trichiura* infections and allergen skin test reactivity. Analyses of the effects of early infections with *T. trichiura* and late infections with *A. lumbricoides* on skin test reactivity indicated that early *T. trichiura* infections remained significantly inversely associated with allergen skin reactivity after controlling for late infection with *A. lumbricoides* (OR = 0.44, 95% CI 0.2–0.8, *P* = 0.01).

Heavy infection *T. trichiura* reduced the prevalence of one positive skin test against one allergen to less than half (OR = 0.43, 95% CI 0.11–1.20) and against two or more allergens to one-fifth (OR = 0.20, 95% CI 0.004–0.64). The effect of early *T. trichiura* infection on allergen skin test reactivity was not dependent on the inter-survey interval: among children who were surveyed < 7 years apart, the OR for the association between heavy *T. trichiura* infection and allergen skin test reactivity was 0.30 (95% CI 0.12–0.80, *P* = 0.016) while for the 251 children surveyed

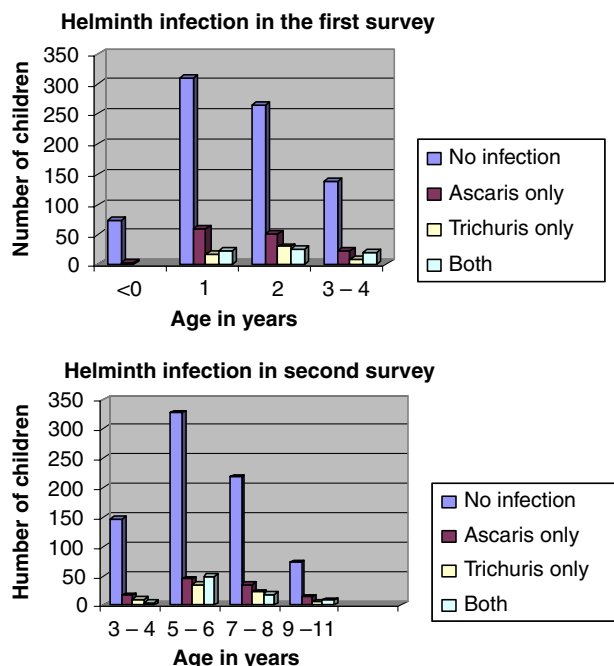


Fig. 1. Number of children with *Ascaris lumbricoides*, *Trichuris trichiura*, none or both in the first and the second survey, by age at testing.

seven or more years apart, the OR was 0.22 (95% CI 0.05–0.99, $P = 0.049$).

Discussion

Our data provide evidence that children infected with *T. trichiura* in early childhood (on average 2 years of age) have a lower prevalence of allergen skin test reactivity in later childhood, and that this effect is independent of later infections with the same helminth. The study provides new evidence that infections occurring in early childhood, in this case with an intestinal helminth parasite, may affect the risk of allergen skin test reactivity in later childhood. Allergic disease is a growing public health problem in urban centres in Latin America (ISAAC, 1998; [20]) and the environmental causes of this disease 'epidemic' are unclear. The data from this study may suggest that modifications in hygiene, which result in dramatic declines in diarrhoea morbidity and parasitic infections [16], may be a contributing factor to this epidemiologic phenomenon. We also identified high skin test reactivity in males and in children with a parental history of asthma (consistent with the literature) and of breastfeeding. This is a complex variable, and a more detailed analysis will be published in a separate paper.

The important strengths of the study are: (1) this study was a large prospective cohort study that collected data on helminth infections in early and later childhood, and measured allergen skin test reactivity in later childhood allowing

us to explore separately the effects of helminth infections occurring in early life and later childhood, and (2) we were able to control for *T. trichiura* infection in later childhood and for other important socio-demographic and environmental confounding factors. Possible study limitations are: (1) we have no data on allergen exposure in early childhood, and there is the possibility that infection with *T. trichiura* was a marker for lower allergen exposure; (2) because we made use of data collected for different purposes, there was a range of ages at the surveys, and in the interval between surveys; and (3) we have no data on helminth infections between surveys and on treatments occurring during this period. However, given the possible limitations, this study is, to our knowledge, the largest and most detailed prospective investigation of the effects of early helminth infections on the risk of allergen skin test reactivity in later childhood.

We determined the presence of allergen sensitization using skin prick testing rather than specific IgE in sera. Unlike industrialized countries, where allergen-specific IgE and allergen skin test responses are closely associated, among populations exposed to helminth infections, levels of allergen-specific IgE are not closely associated with the results of allergen skin testing [21–23]. This may result from the effect of helminths themselves, and for this reason, many epidemiological studies that have investigated allergen sensitization in a setting of endemic helminth infections have used allergen skin test reactivity as the main outcome in preference to serologic evaluations [11, 12, 24, 25].

There was no statistically significant effect of hookworm in early childhood on allergen reactivity in later childhood although the direction of the effect was as predicted; this may be due to insufficient study power, given the small number of children infected with hookworm. There was an association between early infection with *A. lumbricoides* and allergen reactivity but this effect was lost after controlling for *T. trichiura* infections. There was an inverse association between current *A. lumbricoides* and allergen reactivity, consistent with some previous studies [12, 25–29].

The prevalence of skin test reactivity in later childhood was significantly lower among children who had had heavy infections with *T. trichiura* in early childhood compared with those children who had not. *Trichuris trichiura* infection is estimated to infect 800 million people worldwide and the prevalence of and intensity of infection peak within the first decade of life in most endemic areas. Transmission is by ingestion of embryonated eggs that hatch into larvae in the small intestine and then migrate to the large intestine where they mature as adults. The parasite is restricted to the intestinal tract throughout its life cycle in humans but the adult has intimate contact with the intestinal mucosa and submucosa in which it embeds its thread-like tail. It may thus appear surprising to find an effect of *T. trichiura* in early childhood on allergen skin

Table 2. Number and proportion of children by the presence and severity of infection with *Trichuris trichiura*, *Ascaris lumbricoides*, hookworm, and any helminth infection in early and late childhood, in children with and without allergen skin test reactivity, with OR for atopy, given helminth infection and 95% CI intervals

Helminth infection			No skin reactivity to aeroallergens		Skin reactivity to aeroallergens		OR	95% CI	P	Joint Wald P
			n	%	n	%				
Early childhood (n = 1055)			745	70.6	310	29.4				
<i>A. lumbricoides</i>										
Presence	No		584	78.4	264	85.2	1	–	–	0.023
	Yes		161	21.6	46	14.8	0.63	(0.44, 0.90)	0.012	
Intensity	No infection		584	78.4	264	85.2	1	–	–	
	Light		77	10.3	27	8.7	0.78	(0.49, 1.23)	0.281	
	Heavy		84	11.3	19	6.1	0.50	(0.30, 0.84)	0.009	
<i>T. trichiura</i>										
Presence	No	1055	644	86.4	284	91.6	1	–	–	0.010
	Yes		101	13.6	26	8.4	0.58	(0.37, 0.92)	0.020	
Intensity	No infection		644	86.4	284	91.6	1	–	–	
	Light		46	6.2	19	6.1	0.94	(0.54, 1.63)	0.816	
	Heavy		55	7.4	7	2.3	0.29	(0.13, 0.64)	0.002	
Hookworm										
Presence	No	1055	740	99.3	309	99.7	1	–	–	–
	Yes		5	0.7	1	0.3	0.48	(0.06, 4.12)	0.502	
Any										
Presence	No	1055	539	72.3	250	80.6	1	–	–	–
	Yes		206	27.7	60	19.4	0.63	(0.45, 0.87)	0.005	
Late childhood (n = 1038)										
<i>A. lumbricoides</i> Intensity										0.008
	No infection		584	79.9	259	84.4	1	–	–	
	Light		72	9.8	35	11.4	1.10	(0.71, 1.68)	0.675	
	Heavy		75	10.3	13	4.2	0.39	(0.21, 0.72)	0.002	
<i>T. trichiura</i> Intensity										0.078
	No infection	1036	607	83.3	272	88.6	1	–	–	
	Light		58	8.0	19	6.2	0.73	(0.43, 1.25)	0.253	
	Heavy		64	8.8	16	5.2	0.56	(0.32, 0.98)	0.043	
Hookworm presence										–
	No	1038	723	98.9	304	99.0	1	–	–	
	Yes		8	1.1	3	1.0	0.89	(0.24, 3.38)	0.866	
Any above Presence										–
	No	1036	523	71.7	235	76.5	1	–	–	
	Yes		206	28.3	72	23.5	0.78	(0.57, 1.06)	0.111	

Table 3. Association between allergen skin reactivity to aeroallergens and presence and intensity of infection with *Trichuris trichiura* and *Ascaris lumbricoides* in early and later childhood adjusted for clustering

	No skin reactivity to aeroallergens	Skin reactivity to aeroallergens	OR (95% CI)	P-value
Early childhood				
No infection or mild	626	285	1	
<i>A. lumbricoides</i> heavy only	49	15	0.71 (0.39–1.30)	0.269
<i>T. trichiura</i> heavy only	22	3	0.29 (0.08–0.98)	0.047
<i>T. trichiura</i> + <i>A. lumbricoides</i> heavy	32	4	0.27 (0.01–0.80)	0.017
Late childhood				
No infection or mild	624	285	1	
<i>A. lumbricoides</i> heavy only	41	6	0.31 (0.13–0.74)	0.008
<i>T. trichiura</i> heavy only	30	9	0.68 (0.31–1.48)	0.327
<i>T. trichiura</i> + <i>A. lumbricoides</i> heavy	34	7	0.40 (0.17–0.94)	0.036

Table 4. Association between allergen skin reactivity and presence and intensity of infection with *Trichuris trichiura* in early and later childhood adjusted for potential confounders in early childhood

Study variables			N	Skin reactivity to aeroallergens (%)	OR* (95%CI)	P value	Joint Wald P
<i>T. trichiura</i> Infection at:	First survey (early childhood)	Second survey (late childhood)					
	No or light infection	No or light infection	920	286 (31.1)	1		0.022
	No or light infection	Heavy infection	55	14 (25.5)	0.77 (0.40–1.48)	0.438	
	Heavy infection	No or light infection	36	5 (13.9)	0.35 (0.13–0.94)	0.038	
	Heavy infection	Heavy infection	25	2 (8.0)	0.18 (0.04–0.78)	0.022	
Breastfeeding		0–2 months	639	171 (26.8)	1		–
		2+ months	397	136 (34.3)	1.41 (1.05–1.89)	0.022	
Number of older siblings		None	766	247 (32.3)	1		–
		1+	270	60 (22.2)	0.65 (0.46–0.91)	0.011	
Sex		Male	567	187 (33.0)	1		–
		Female	488	123 (25.2)	0.68 (0.52–0.91)	0.008	
Age in years at skin test		4–5 years	296	81 (27.4)	1		0.751
		6–8 years	440	127 (28.9)	1.11 (0.79–1.57)	0.535	
		8–12 years	300	99 (33.0)	1.15 (0.79–1.67)	0.481	

*Random effect model with sentinel area as cluster, adjusted for age at skin test and all variables in the model. Average number of children per cluster was 44.0. ICC = 0.04, and the χ^2 test of ICC = 0 has $P = 0.002$.

test reactivity because the parasite is restricted to the intestine, and helminth parasites previously reported to have inverse associations with allergy (although all measured current infections only) have a systemic phase of larval migration (e.g. Schistosome parasites [24, 30], *A. lumbricoides* [27], and hookworm [31]). However, the association with *T. trichiura* is biologically plausible: experimental animal models have demonstrated the suppression of allergic inflammation in infections with exclusively lumen-dwelling helminth parasites [32], and there is evidence that infections with the porcine whipworm, *Trichuris suis* ova, may provide symptomatic relief in people with inflammatory bowel disease [33].

The mechanism by which heavy infections with *T. trichiura* occurring in infancy may reduce the prevalence of allergen skin test positivity later in childhood is of great interest. *T. trichiura* infections may induce populations of regulatory cells (e.g. CD4+CD25+FoxP3+ regulatory T cells) that are able to suppress allergic effector responses at distal sites (i.e. mast cell activation in the skin) or may induce a regulatory environment mediated through anti-allergic cytokines such as IL-10 as has been suggested to occur during *Schistosoma haematobium* infection [23]. Our data are consistent with a programming effect of *T. trichiura* on the immune system and provide further evidence in support of studies that have shown that the protective effects of environmental exposures (such as pets [34] and farming [35]) are strongest if they occur during the first years of life.

The effect of combined early and later childhood infections was greater than early or late infections alone (although, maybe because of small numbers, this was not

statistically significant). If this is confirmed, it may indicate that more than one regulatory mechanism may be present – early infections may program the immune response for less allergy while later infections may mediate their effects through active regulation, and both mechanisms may be active among children with both early and later childhood infections.

In conclusion, our data from a prospective cohort study in Salvador, Brazil, provide novel evidence that early infections with the intestinal helminth, *T. trichiura*, are associated with a reduced risk of allergen skin test reactivity later in life. Recommendations for further research include the investigation of a potential programming effect in different geographic settings that are endemic for different helminth parasite, definition of the age of susceptibility to the programming effect and identification of the specific immunologic mechanisms involved. Our findings provide important evidence that environmental exposures occurring in early life can have substantive effects on later risk of allergy and if confirmed in further experimental and field studies indicate that interventions that mimic the anti-allergic effects of *T. trichiura* could be developed and administered during the first years of life to prevent the later development of atopy and allergic diseases.

References

- 1 Romei I, Boner AL. Possible reasons for lack of effect of allergen avoidance in atopy-prone infants and sensitive asthmatic patients. *Clin Rev Allergy Immunol* 2005; 28:59–71.

- 2 Calder PC, Krauss-Etschmann S, de Jong EC *et al.* Early nutrition and immunity – progress and perspectives. *Br J Nutr* 2006; **96**:774–90.
- 3 Wills-Karp M, Santeliz J, Karp CL. The germless theory of allergic disease: revisiting the hygiene hypothesis. *Nat Rev Immunol* 2001; **1**:69–75.
- 4 Yazdanbakhsh M, Kreamsner PG, van Ree R. Allergy, parasites, and the hygiene hypothesis. *Science* 2002; **296**:490–4.
- 5 Braun-Fahrlander C, Riedler J, Herz U *et al.* Environmental exposure to endotoxin and its relation to asthma in school-age children. *N Engl J Med* 2002; **347**:869–77.
- 6 Maizels RM, Balic A, Gomez-Escobar N *et al.* Helminth parasites—masters of regulation. *Immunol Rev* 2004; **201**:89–116.
- 7 de Silva NR, Brooker S, Hotez PJ *et al.* Soil-transmitted helminth infections: updating the global picture. *Trends Parasitol* 2003; **19**:547–51.
- 8 Lynch NR, Lopez R, Isturiz G *et al.* Allergic reactivity and helminthic infection in Amerindians of the Amazon Basin. *Int Arch Allergy Appl Immunol* 1983; **72**:369–72.
- 9 Leonardi-Bee J, Pritchard D, Britton J. Asthma and current intestinal parasite infection: systematic review and meta-analysis. *Am J Respir Crit Care Med* 2006; **174**:514–23.
- 10 Lynch NR, Hagel I, Perez M *et al.* Effect of anthelmintic treatment on the allergic reactivity of children in a tropical slum. *J Allergy Clin Immunol* 1993; **92**:404–11.
- 11 van den Biggelaar AH, Rodrigues LC, van Ree R *et al.* Long-term treatment of intestinal helminths increases mite skin-test reactivity in Gabonese schoolchildren. *J Infect Dis* 2004; **189**:892–900.
- 12 Cooper PJ, Chico ME, Vaca MG *et al.* Effect of albendazole treatments on the prevalence of atopy in children living in communities endemic for geohelminth parasites: a cluster-randomised trial. *Lancet* 2006; **367**:1598–603.
- 13 Quinnell RJ, Griffin J, Nowell MA *et al.* Predisposition to hookworm infection in Papua New Guinea. *Trans R Soc Trop Med Hyg* 2001; **95**:139–42.
- 14 WHO. Prevention and control of intestinal parasitic infections. Geneva: WHO, Technical Report Series, n., 1987. 749.
- 15 Barreto ML, Cunha SS, Alcantara-Neves N *et al.* Risk factors and immunological pathways for asthma and other allergic diseases in children: background and methodology of a longitudinal study in a large urban center in Northeastern Brazil (Salvador-SCAALA study). *BMC Pulmonary Med* 2006; **6**:15.
- 16 Barreto ML, Genser B, Strina A *et al.* Effect of city-wide sanitation programme on reduction in rate of childhood diarrhoea in northeast Brazil: assessment by two cohort studies. *Lancet* 2007; **370**:1622–8.
- 17 Strina A, Cairncross S, Barreto ML *et al.* Childhood diarrhea and observed hygiene behavior in Salvador, Brazil. *Am J Epidemiol* 2003; **157**:1032–8.
- 18 Hoffman WA, Pons JA, Janer JL. The sedimentation concentration method in *Schistosomiasis mansoni*. *Puerto Rico J Public Health* 1934; **9**:281–98.
- 19 Katz N, Chaves A, Pellegrino J. A simple device for quantitative stool thick-smear technique in *Schistosomiasis mansoni*. *Rev Inst Med Trop Sao Paulo* 1972; **14**:397–400.
- 20 Asher MI, Montefort S, Björkstén B *et al.* ISAAC Phase Three Study Group. Worldwide time trends in the prevalence of symptoms of asthma allergic rhinoconjunctivitis, and eczema in childhood: ISAAC Phases One and Three repeat multicountry cross-sectional surveys. *Lancet* 2006; **368**:733–43.
- 21 Perzanowski MS, Ng'ang'a LW, Carter MC *et al.* Atopy, asthma, and antibodies to *Ascaris* among rural and urban children in Kenya. *J Pediatr* 2002; **140**:582–8.
- 22 Cooper PJ. The potential impact of early exposures to geohelminth infections on the development of atopy. *Clin Rev Allergy Immunol* 2004; **26**:5–14.
- 23 van den Biggelaar AH, Lopuhaa C, van Ree R *et al.* The prevalence of parasite infestation and house dust mite sensitization in Gabonese schoolchildren. *Int Arch Allergy Immunol* 2001; **126**:231–8.
- 24 van den Biggelaar AH, van Ree R, Rodrigues LC *et al.* Decreased atopy in children infected with *Schistosoma haematobium*: a role for parasite-induced interleukin-10. *Lancet* 2000; **356**:1723–7.
- 25 Flohr C, Tuyen LN, Lewis S *et al.* Poor sanitation and helminth infection protect against skin sensitization in Vietnamese children: a cross-sectional study. *J Allergy Clin Immunol* 2006; **118**:1305–11.
- 26 Cooper PJ, Barreto ML, Rodrigues LC. Human allergy and geohelminth infections: a review of the literature and a proposed conceptual model to guide the investigation of possible causal associations. *Br Med Bull* 2006; **79**:80:203–18.
- 27 Cooper PJ, Chico ME, Rodrigues LC *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.
- 28 Lynch NR, Hagel IA, Palenque ME *et al.* Relationship between helminthic infection and IgE response in atopic and nonatopic children in a tropical environment. *J Allergy Clin Immunol* 1998; **101** (2 part 1):217–21.
- 29 Moncayo AL, Cooper PJ. Geohelminth infections: impact on allergic diseases. *Int J Biochem Cell Biol* 2006; **38**:1031–5.
- 30 Araujo MI, Lopes AA, Medeiros M *et al.* Inverse association between skin response to aeroallergens and *Schistosoma mansoni* infection. *Int Arch Allergy Immunol* 2000; **123**:145–8.
- 31 Scrivener S, Yemaneberhan H, Zebenigus M *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.
- 32 Wilson MS, Taylor MD, Balic A *et al.* Suppression of allergic airway inflammation by helminth-induced regulatory T cells. *J Exp Med* 2005; **202**:1199–212.
- 33 Summers RW, Elliott DE, Urban JF Jr. *et al.* *Trichuris suis* therapy in Crohn's disease. *Gut* 2005; **54**:87–90.
- 34 Ownby DR, Johnson CC. Does exposure to dogs and cats in the first year of life influence the development of allergic sensitization? *Curr Opin Allergy Clin Immunol* 2003; **3**: 517–22.
- 35 Riedler J, Braun-Fahrlander C, Eder W *et al.* Exposure to farming in early life and development of asthma and allergy: a cross-sectional survey. *Lancet* 2001; **358**:1129–33.