Allergy, soil-transmitted helminths and their association

van der Werff, S.D.

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Soil-transmitted helminth (re)infection and the development of atopic disease in Cuban schoolchildren

Suzanne D. van der Werff, Jos W.R. Twisk, Maiza Campos Ponce, Raquel Junco Díaz, Fidel A. Núñez, Lázara Rojas Rivero, Mariano Bonet Gorbea, Katja Polman

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Abstract

Background: Longitudinal studies on the relationship of soil-transmitted helminths (STHs) with allergic sensitization and atopic diseases are scarce. Most of these investigated the effect of deworming on atopic conditions. So far, it has not been assessed how changes over time in STH infection status are related to (changes in) atopic conditions and whether these effects are species-specific.

Methods: We followed up 271 Cuban schoolchildren in six-monthly intervals for 24 months. STH infections were diagnosed by stool examination. Asthma, allergic rhinoconjunctivitis, and atopic dermatitis were diagnosed by International Study of Asthma and Allergies in Childhood questionnaire and allergic sensitization by skin prick testing. At each time point, STH positive children were treated with 500 mg mebendazole. Logistic Generalized Estimating Equations models were developed to determine over time the effect of (changes in) STH infection status, influenced by anthelminthic treatment and reinfection, on (changes in) allergic sensitization and atopic diseases.

Results: Becoming (re)infected with *Ascaris lumbricoides* and *Trichuris trichiura* was positively associated (OR 2.39 (95%CI 1.08-5.26) and 2.08 (95%CI 1.03-4.17), respectively) with developing asthma while hookworm was negatively associated (OR 0.86 (95%CI 0.48-1.55)). These associations were similar for allergic rhinoconjunctivitis and atopic dermatitis, and opposite for allergic sensitization.

Conclusion: Our results indicate that STH (re)infection is associated with atopic disease development. Over time species-specific effects exist with *A. lumbricoides* and *T. trichiura* being positively and hookworm negatively associated with atopic disease. A reverse trend was seen for allergic sensitization. The underlying mechanisms of these associations need to be further elucidated.

Introduction

Following the hygiene hypothesis, which postulates that early childhood infections protect against the development of atopic diseases, the association between soil-transmitted helminth (STH) infections and atopic diseases has been the focus of much research (e.g. (1-6)) However, the exact nature of this relationship remains unclear. So far, studies have been mainly cross-sectional, with contradicting results (7, 8). There are some indications that effects of STHs on atopic diseases may be species-specific (9, 10). Prospective intervention studies are a better way to assess causal associations (11), but these remain scarce. They have so far mainly focussed on the impact of anthelminthic treatment on atopic conditions (12-17).

In a cohort of Cuban schoolchildren, we set out to conduct a two-year prospective intervention study, assessing whether and how changes over time in STH infection status are related to (changes in) allergic sensitization and atopic disease status. Specific attention was paid to STH species-specific effects.

Methods

Study design and population

A longitudinal study was performed between December 2003 and May 2006 in primary schoolchildren from San Juan y Martínez (SJM), a municipality in the west of Cuba, and Fomento, a municipality in the center of Cuba, both endemic for STHs (18). From 19 randomly selected primary schools ($N_{children}$=1321) (18), all STH positive children (N=271) were included in the study (measurement P0). This cohort was followed up at six-monthly intervals for 24 months (measurements P1-P4).

Informed written consent was obtained from the parents or guardians of each participating child. The study was approved by the Ethical Committees of the Institute of Tropical Medicine (ITM) in Antwerp, Belgium, the National Institute for Hygiene, Epidemiology and Microbiology (INHEM) and the Pedro Kourí Institute (IPK) of Tropical Medicine in Havana, Cuba.

Infection and treatment

From each child one fresh stool sample was collected and used for one direct smear and two 25 mg Kato Katz examinations (19, 20). Infection with STHs, i.e. *Ascaris lumbricoides*, *Trichuris trichiura*, and hookworm, was defined as the presence of species-specific eggs detected by either of the two methods. At each measurement period, infection and reinfection status were determined and STH positive children received one single dose of mebendazole (500 mg).

Atopic diseases and allergic sensitization

A parent or guardian of each child was interviewed using the standard Spanish version of the International Study of Asthma and Allergies in Childhood (ISAAC) questionnaire (21). ISAAC definitions were used: asthma was defined as an affirmative answer to the second ISAAC core asthma question on current wheeze (22); allergic rhinoconjunctivitis was diagnosed as defined by Strachan (23), and atopic dermatitis as defined by Williams (24), i.e. an affirmative answer to the second and third ISAAC core question on rhinitis or eczema, respectively.

Skin prick testing for allergic sensitization (atopy) was performed using extracts of seven allergens (*Dermatophagoides pteronyssinus*, *D. farinae*, cat dander, mixed tree, mixed grass, *Alternaria alternata*, and cockroach) produced by ALK (Nieuwegein, The Netherlands). Histamine (10 mg/ml) was used as a positive and allergen diluent as a negative control. The extracts and controls were placed on the volar side of the left forearm using separate ALK lancets. Skin response was measured after 15 minutes, considering a wheal of 3 mm or larger in the absence of significant reactivity to the diluent control as a positive reaction. Allergic sensitization was defined as a positive reaction to at least one of the seven applied allergens in the presence of a positive reaction to histamine.

Statistical analysis

All statistical analyses were performed with SPSS Statistics 17.0 for Windows (SPSS Inc., Chicago, IL, USA) and a *P*-value of ≤0.05 was considered as statistically significant.

Due to anthelminthic treatment and (re)infection children's STH infection status will change over time. Different logistic Generalized Estimating Equations (GEE) models with an exchangeable correlation structure were developed to determine over time the effect of (changes in) STH infection status on allergic sensitization and atopic diseases. First, a marginal logistic GEE model was used to assess the longitudinal association between STH infection status (yes/no) and allergic sensitization or atopic disease status (yes/no) (model 1: is being infected with STHs associated with having allergic sensitization or atopic disease?). Second, the model was extended with STH infection status at the previous measurement period to assess the effect of a change in STH infection status on allergic sensitization or atopic disease status (model 2: is becoming (re)infected with STHs associated with having allergic sensitization or atopic disease?).

Finally, this model was extended with allergic sensitization or atopic disease status at the previous measurement period to assess the effect of a change in STH infection status on a change in allergic sensitization or atopic disease status (model 3: is becoming (re)infected with an STH associated with developing allergic sensitization or atopic disease?). Crude and adjusted ORs were calculated for all models. Additional covariates included in the adjusted model, and collected by means of a structured parental questionnaire, were age (continuous), sex, area of residence (rural vs. urban), municipality (SJM vs. Fomento), monthly household income (≤ 250 (≈ 7 euro) pesos/month vs. > 250 pesos/month), family history of atopic diseases (yes/no) and antibiotics use during the child's first year of life (yes/no) (25). These were relevant confounders as they satisfied the change-in-estimate criterion of ≥10% (26).

Table 1. Baseline characteristic of overall study population and by municipality.

	SJM (*N*=108)	**Fomento (*N*=163)**	**Overall (*N*=271)**
Age (years; mean ± SD)	8.0 ± 2.0	8.9 ± 2.1	8.5 ± 2.1
Urban area (% (*N*))	60.2 (65)	25.8 (42)	39.5 (107)
Female gender (% (*N*))	43.5 (47)	36.2 (59)	39.1 (106)
Income (>250 pesos/month; % (*N*))	42.1 (45)	28.2 (46)	33.7 (91)
STH infection (% (*N*))			
A. lumbricoides	52.8 (57)	14.7 (24)	29.9 (81)
T. trichiura	47.2 (51)	52.8 (86)	50.6 (137)
Hookworm	25.9 (28)	57.1 (93)	44.6 (121)
Allergy (% (*N*))			
Asthma	33.3 (36)	16.6 (27)	23.2 (63)
Allergic rhinoconjunctivitis	13.9 (15)	12.3 (20)	12.9 (35)
Atopic dermatitis	7.4 (8)	4.3 (7)	5.5 (15)
Allergic sensitization	10.2 (11)	23.9 (39)	18.5 (50)
Family history of atopic diseases (% (*N*))	46.7 (50)	46.6 (76)	46.7 (126)
Antibiotic use in first life year (% (*N*))	50.0 (54)	38.1 (61)	42.9 (115)

Results

In total 271 children aged 5-13 year were included in the treatment study; 165 boys (60.9%) and 106 girls (39.1%). Ninety-three percent (252/271) of the children still participated in the study after two years. Losses of follow-up were mainly due to migration of children to another municipality. Table 1 shows the baseline data for the overall study population and by municipality.

The GEE models for asthma (Table 2) indicated that children who were infected with *A. lumbricoides* or *T. trichiura* were more likely to have asthma than those who were uninfected (model 1). This effect was even more apparent in children who became (re)infected with these infections; they were more at risk of having asthma (model 2) and even more at risk of developing asthma (model 3) than children who were uninfected. On the other hand, children who over time became infected with hookworm were less likely to have or develop asthma than children who remained uninfected.

Table 2. Frequencies of asthma by STH infection status and results from the logistic GEE models.

	Proportion asthma in*		**Logistic GEE models**			
	non infected children	infected children	**Crude OR (95% CI)**	***P***	**Adjusted OR† (95% CI)**	***P***
Model 1						
A. lumbricoides	13.5%	26.8%	1.59 (0.87-2.89)	0.131	1.39 (0.78-2.46)	0.263
T. trichiura	13.4%	22.1%	1.57 (0.94-2.65)	*0.087*	1.57 (0.83-2.99)	0.168
Hookworm	15.8%	9.6%	0.76 (0.52-1.11)	0.161	0.95 (0.63-1.42)	0.802
Model 2						
A. lumbricoides			2.07 (1.17-3.68)	**0.013**	1.89 (1.03-3.45)	**0.039**
T. trichiura			1.78 (1.06-3.01)	**0.030**	1.76 (0.90-3.42)	*0.096*
Hookworm			0.77 (0.51-1.16)	0.211	0.92 (0.60-1.41)	0.707
Model 3						
A. lumbricoides			2.44 (1.12-5.30)	**0.025**	2.39 (1.08-5.26)	**0.031**
T. trichiura			2.23 (1.18-4.20)	**0.014**	2.08 (1.03-4.17)	**0.040**
Hookworm			0.77 (0.43-1.41)	0.404	0.86 (0.48-1.55)	0.623

Significant associations are given in bold and borderline significant associations in italic.
* After anthelminthic treatment, i.e. children with successful treatment versus children with unsuccessful treatment or reinfection.
† Adjusted for municipality, area, gender, age, income, family history of atopic diseases and antibiotics use in child's first life year.

The GEE models for allergic rhinoconjunctivitis and atopic dermatitis showed similar trends as for asthma, with *T. trichiura* infections being positively and hookworm negatively associated with both atopic diseases. However, *A. lumbricoides* infection appeared to decrease the risk of atopic dermatitis, while increasing the risk of allergic rhinoconjunctivitis (Table 3 and 4).

Table 3. Frequencies of allergic rhinoconjunctivitis by STH infection status and results from the logistic GEE models.

	Proportion asthma in*		**Logistic GEE models**			
	non infected children	infected children	**Crude OR (95% CI)**	***P***	**Adjusted OR† (95% CI)**	***P***
Model 1						
A. lumbricoides	8.9%	13.4%	1.29 (0.68-2.44)	0.438	1.43 (0.72-2.85)	0.310
T. trichiura	9.1%	10.3%	1.16 (0.62-2.16)	0.636	1.28 (0.63-2.61)	0.494
Hookworm	10.3%	5.1%	0.50 (0.23-1.06)	*0.069*	0.51 (0.23-1.13)	*0.097*
Model 2						
A. lumbricoides			1.69 (0.87-3.27)	0.122	1.95 (0.93-4.08)	*0.077*
T. trichiura			1.32 (0.69-2.53)	0.405	1.37 (0.66-2.86)	0.400
Hookworm			0.53 (0.24-1.18)	0.118	0.54 (0.23-1.24)	0.148
Model 3						
A. lumbricoides			1.73 (0.88-3.39)	0.114	2.06 (0.96-4.43)	*0.063*
T. trichiura			1.33 (0.68-2.59)	0.401	1.39 (0.65-2.94)	0.394
Hookworm			0.53 (0.23-1.19)	0.122	0.54 (0.23-1.27)	0.161

Significant associations are given in bold and borderline significant associations in italic.
* After anthelminthic treatment, i.e. children with successful treatment versus children with unsuccessful treatment or reinfection.
† Adjusted for municipality, area, gender, age, income, family history of atopic diseases and antibiotics use in child's first life year.

Although not significant, the logistic GEE model for allergic sensitization indicated a trend opposite to that observed for atopic diseases; children who became (re)infected with *A. lumbricoides* or *T. trichiura* over time were less likely to develop a positive skin prick test than children who remained uninfected, whereas hookworm was positively associated with the development of allergic sensitization (Table 5).

Table 4. Frequencies of atopic dermatitis by STH infection status and results from the logistic GEE models.

	Proportion asthma in*		**Logistic GEE models**			
	non infected children	infected children	**Crude OR (95% CI)**	***P***	**Adjusted OR† (95% CI)**	***P***
Model 1						
A. lumbricoides	6.9%	11.0%	1.16 (0.50-2.67)	0.727	0.72 (0.36-1.45)	0.356
T. trichiura	6.8%	10.3%	1.42 (0.76-2.64)	0.274	1.66 (0.80-3.46)	0.174
Hookworm	8.1%	3.9%	0.54 (0.28-1.03)	*0.062*	0.83 (0.40-1.73)	0.624
Model 2						
A. lumbricoides			1.18 (0.58-2.39)	0.642	0.81 (0.43-1.53)	0.517
T. trichiura			1.48 (0.80-2.75)	0.210	1.73 (0.84-3.54)	0.136
Hookworm			0.62 (0.31-1.22)	0.163	0.87 (0.42-1.79)	0.696
Model 3						
A. lumbricoides			1.07 (0.49-2.33)	0.867	0.78 (0.39-1.57)	0.489
T. trichiura			1.47 (0.77-2.79)	0.239	1.74 (0.84-3.60)	0.135
Hookworm			0.60 (0.27-1.31)	0.197	0.85 (0.40-1.79)	0.664

Significant associations are given in bold and borderline significant associations in italic.
* After anthelminthic treatment, i.e. children with successful treatment versus children with unsuccessful treatment or reinfection.
† Adjusted for municipality, area, gender, age, income, family history of atopic diseases and antibiotics use in child's first life year.

Discussion

Most studies on the association between STH infections and atopic diseases have been cross-sectional and indicated possible species-specific effects (9, 10). Longitudinal studies are scarce and so far have not studied how changes over time in STH infection status are related to changes in allergic sensitization and atopic disease status, nor have they looked into the role of helminth species-specificity in this (12-16, 27). We found that infection with *A. lumbricoides* and *T. trichiura* was positively associated with atopic diseases, and that becoming (re)infected with these STHs was related to developing atopic disease. Hookworm was negatively associated with developing atopic disease. An opposite trend was seen for allergic sensitization in relation to these STH infections.

Table 5. Frequencies of allergic sensitization by STH infection status and results from the logistic GEE models.

	Proportion asthma in*		**Logistic GEE models**			
	non infected children	infected children	**Crude OR (95% CI)**	***P***	**Adjusted OR† (95% CI)**	***P***
Model 1						
A. lumbricoides	26.2%	19.3%	0.68 (0.39-1.21)	0.192	0.66 (0.37-1.19)	0.166
T. trichiura	25.8%	23.9%	0.86 (0.55-1.35)	0.511	0.90 (0.57-1.43)	0.667
Hookworm	24.5%	30.4%	1.24 (0.88-1.76)	0.221	1.30 (0.90-1.87)	0.162
Model 2						
A. lumbricoides			0.82 (0.46-1.46)	0.509	0.75 (0.41-1.38)	0.354
T. trichiura			0.86 (0.54-1.36)	0.522	0.90 (0.56-1.43)	0.650
Hookworm			1.26 (0.87-1.83)	0.222	1.29 (0.87-1.90)	0.201
Model 3						
A. lumbricoides			0.83 (0.47-1.49)	0.533	0.74 (0.39-1.39)	0.351
T. trichiura			0.85 (0.54-1.36)	0.499	0.89 (0.56-1.44)	0.643
Hookworm			1.29 (0.88-1.89)	0.195	1.33 (0.89-2.00)	0.162

Significant associations are given in bold and borderline significant associations in italic.
* After anthelminthic treatment, i.e. children with successful treatment versus children with unsuccessful treatment or reinfection.
† Adjusted for municipality, area, gender, age, income, family history of atopic diseases and antibiotics use in child's first life year.

The species-specific effects we found for asthma are similar to those found by Leonardi-Bee *et al.* (9) in a meta-analysis of cross-sectional studies on asthma and current intestinal parasite infections. No meta-analysis of cross-sectional studies on allergic rhinoconjunctivitis and atopic dermatitis and current STH infection have been performed to date, but generally no clear associations have been observed, except that *A. lumbricoides* might increase allergic rhinoconjunctivitis (8, 28-32). Prospective intervention studies have so far mainly assessed the effect of anthelminthic treatment on atopic disease, not of changes in infection status due to treatment or reinfection. They found either no effect on atopic diseases (15-17, 27) or a decrease in asthma (13, 17). Most of these populations were highly endemic for *A. lumbricoides* and *T. trichiura* (13, 15, 17, 27), except for one which was endemic for hookworm (16).

For allergic sensitization, we also observed STH species-specific effects in our study population, but these were less clear and opposite to those found for atopic diseases. A negative association of *A. lumbricoides* and *T. trichiura* with allergic sensitization was

also found in a meta-analysis of cross-sectional studies on this topic (10). Endara *et al.* (27) investigated species-specific effects in treated and non-treated communities and observed an inverse association of allergic sensitization with *T. trichiura* as well, but no association with *A. lumbricoides* and hookworm. Prospective intervention studies on the effect of anthelminthic treatment on allergic sensitization showed an increase in allergic sensitization, irrespective of whether these populations were highly endemic for *A. lumbricoides* and *T. trichiura* (12, 14) or for hookworm (16). Only one prospective study showed no effect of deworming on allergic sensitization; this study was conducted in an area that was highly endemic for *A. lumbricoides* and *T. trichiura* (15).

The findings of our longitudinal study in Cuba, corroborate those of the two meta-analyses of cross-sectional studies (9, 10), namely that the effect of STH infections -*A. lumbricoides* and *T. trichiura*- on allergic sensitization is opposite to that on asthma, i.e. protective versus risk increasing. Neither Leonardi-Bee and colleagues nor we have an explanation for this apparently rather common phenomenon. Apparently, STH infections affect allergic sensitization and atopic diseases through different processes and further research on the underlying mechanisms is warranted (10).

Apart from helminth species-specificity, timing is considered an important factor in determining the effect of STH infections on atopic diseases. Chronic infections and early childhood infections supposedly down-regulate the immune system, leading to less atopic diseases. Acute or periodic infections and infections later in childhood are believed to have an opposite, detrimental effect on atopic diseases (33). Our results indicate that acute infections (i.e. after treatment) or late childhood infections (i.e. in schoolchildren) may indeed not exert a beneficial effect on the immune system and induce the development of atopic diseases. Similarly, prospective intervention studies on the effect of anthelminthic treatment in schoolchildren suggested a detrimental effect (13) or no effect (15, 16, 27) of late STH infections on atopic disease development.

To our knowledge only one study by Rodrigues *et al.* (34) assessed the aspect of timing and this was for allergic sensitization. They observed a negative association of *T. trichiura* with allergic sensitization for both early and late childhood infections, but with the effect of early childhood infection being the strongest. This confirms the assumption that infections in early childhood have a beneficial effect on atopic conditions (35), but does not correspond with the idea that early and late childhood infections have opposite effects. We also found a negative association of *T. trichiura* with allergic sensitization, but then in late childhood, which does not correspond with the 'theory of timing' either. Moreover, we observed a protective effect of *A. lumbricoides* on allergic sensitization that was even slightly stronger than that of

T. trichiura, which is contrary to the observation of Rodrigues *et al*, who found the effect of *T. trichiura* on allergic sensitization to outweigh the effect of *A. lumbricoides.* In other words, both timing and helminth species specificity appear to play an important role, but the exact details are still not known.

We only measured STH infections later in childhood and therefore cannot measure the effects that early childhood infections might have already exerted on the development of allergic sensitization and atopic diseases. Early and chronic infections, which probably also affected the development of the immune system in Cuban schoolchildren, have been assumed to program the immune system to an irreversible anti-inflammatory phenotype (35). However, if this is the case then infections later in childhood would have no effect on allergic sensitization or atopic disease development. Our results indicate that this phenotype might only be present for allergic sensitization, but not for atopic diseases.

In conclusion, our results confirm the species-specific effects observed in cross-sectional studies, with *A. lumbricoides* and *T. trichiura* being positively associated and hookworm negatively associated with asthma in school-aged children. These effects were most apparent in children who became (re)infected with these infections; they were more at risk of having asthma as well as of developing asthma than children who were uninfected. Similar associations were observed for allergic rhinoconjunctivitis and atopic dermatitis. However, an opposite trend was seen for allergic sensitization. Apparently, the relationship between STH (re)infection and atopic disease development is more complex than assumed and calls for further research on the underlying mechanisms.

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