Helminth infections and allergies in Ghana
Amoah, A.S.

Citation
Amoah, A. S. (2014, November 11). *Helminth infections and allergies in Ghana*. Retrieved from https://hdl.handle.net/1887/29660

Version:	Corrected Publisher's Version
License:	Licence agreement concerning inclusion of doctoral thesis in the Institutional Repository of the University of Leiden
Downloaded from:	https://hdl.handle.net/1887/29660

Note: To cite this publication please use the final published version (if applicable).

Cover Page

Universiteit Leiden

Leiden University Repository

The handle http://hdl.handle.net/1887/29660 holds various files of this Leiden University dissertation

Author: Amoah, Abena Serwaa
Title: Helminth infections and allergies in Ghana
Issue Date: 2014-11-11

chapter 2

Schistosome infection is negatively associated with mite atopy, but not wheeze and asthma in Ghanaian schoolchildren

Benedicta B. Obeng[1,2]*, Abena S. Amoah[1,2]*, Irene A. Larbi[2], Dziedzom K. de Souza[2], Hae-Won Uh[3], Montserrat Fernández-Rivas[4], Ronald van Ree[5], Laura C Rodrigues[6], Daniel A. Boakye[2] , Maria Yazdanbakhsh[1], Franca C. Hartgers[1]

Affiliations:

[1]Department of Parasitology, Leiden University Medical Center, Leiden, The Netherlands
[2]Department of Parasitology, Noguchi Memorial Institute for Medical Research, Accra, Ghana
[3]Department of Medical Statistics and Bioinformatics, Leiden University Medical Center, Leiden, The Netherlands
[4]Department of Allergy, Hospital Clinico San Carlos, Madrid, Spain
[5]Department of Experimental Immunology and Department of Otorhinolaryngology, Academic Medical Center, Amsterdam University, Amsterdam, The Netherlands
[6]Department of Infectious Disease Epidemiology, Faculty of Epidemiology and Population Health, London School of Hygiene and Tropical Medicine, London, United Kingdom

*Shared first authorship

- Clinical and Experimental Allergy 2014 Jul; 44(7):965-75 -

Abstract

Background: Epidemiological evidence suggests that helminth infection and rural living are inversely associated with allergic disorders.

Objective: To investigate the effect of helminth infections and urban versus rural residence on allergy in schoolchildren from Ghana.

Methods: In a cross-sectional study of 1385 children from urban high socioeconomic status (SES), urban low SES and rural schools, associations between body mass index (BMI), allergen-specific IgE (sIgE), parasitic infections and allergy outcomes were analysed. Allergy outcomes were skin prick test (SPT) reactivity, reported current wheeze and asthma.

Results: Helminth infections were found predominantly among rural subjects and the most common were hookworm (9.9%) and Schistosoma species (9.5%). Being overweight was highest among urban high SES (14.6%) compared to urban low SES (5.5%) and rural children (8.6%). The prevalence of SPT reactivity to any allergen was 18.3% and this was highest among rural children (21.4%) followed by urban high SES (20.2%) and urban low SES (10.5%) children. Overall, SPT reactivity to mite (12%) was most common. Wheeze and asthma were reported by 7.9% and 8.3% respectively. In multivariable analyses, factors associated with mite SPT were BMI (aOR 2.43, 95% CI 1.28 - 4.60, p=0.007), schistosome infection (aOR 0.15, 95% CI 0.05-0.41), and mite sIgE (aOR 7.40, 95% CI 5.62 - 9.73, $p < 0.001$) but not area. However, the association between mite IgE and SPT differed by area and was strongest among urban high SES children (aOR = 15.58, 95% CI 7.05-34.43, $p < 0.001$). Compared to rural, urban low SES area was negatively associated with current wheeze (aOR 0.41, 95% CI 0.20-0.83, p=0.013). Both mite sIgE and mite SPT were significantly associated with current wheeze and asthma.

Conclusion and clinical relevance: Infection with Schistosoma appeared to protect against mite SPT reactivity. This needs to be confirmed in future studies, preferably in a longitudinal design where schistosome infections are treated and allergic reactions re-assessed.

Keywords

Africa, allergy, asthma, atopy, body mass index, cockroach, helminth, mite, rural, *Schistosoma*, urban, wheeze

Abbreviations

aOR: Adjusted odds ratio
BMI: body mass index
CI: Confidence interval
cOR: Crude odds ratio
EIB: Exercise induced bronchoconstriction
IQR: Interquartile range
OR: Odds ratio
SES: Socioeconomic status
sIgE: Specific immunoglobulin E
SPT: Skin Prick Test

Introduction

Epidemiological studies show urban-rural differences with the general increase in the prevalence of atopy in industrialized and developing countries [1-3]. Changing disease patterns associated with urbanization [4] make it necessary to identify factors involved in the growing prevalence of allergy in developing countries. Differential exposure to environmental factors, including helminth parasites, could explain some of the observed urban-rural differences [5-7]. Within urban populations, emerging trends suggest that while the incidence of allergic diseases increase with improved socioeconomic status [8, 9], poorer clinical outcomes [10] and asthma morbidity [11, 12] may be associated with poverty.

The hygiene hypothesis attributes lower incidence of allergic disorders to more frequent exchange of pathogens. Immunologically, lower exposure to T-helper (Th) 1 inducing infections allows increased Th2 responses, resulting in more allergies in affluent areas. A broader interpretation proposes that exposure to both Th1 and Th2 inducing pathogens develops regulatory responses that dampen allergies [13]. Thus, strong inducers of regulatory responses like helminths may suppress allergic reactions [14, 15].

However, conflicting results from studies in human populations show that the hygiene hypothesis alone does not explain the trends of allergic disease [16]. Recent studies, summarized in Table 1 [8, 17-24], show inconsistent results that include a lack of association between intestinal helminth infections and allergic outcomes in low helminth prevalence settings in Ethiopia [24]; an intensity-dependent inverse association between schistosome infection and atopy in Zimbabwe [17]; and a positive association between anti-*Ascaris* IgE and asthma in urban affluent subjects in Ghana [8]. High-intensity and chronic helminth infections have been suggested as being important for conferring this protective effect against allergic disease [5, 16, 17, 25-27] while lower intensity and acute infections [6, 7, 28] have been linked with exacerbated allergic outcomes. Helminth species and subject age [29] could also account for observed disparities. In addition, other confounders associated with helminth infections could mediate suppression of allergy such as diet [30], nutritional status or body mass index (BMI) [31-34], socioeconomics [8-11], and urban lifestyle [35].

The Greater Accra region (GAR) of Ghana is home to the largest city (Accra) and encompasses rural areas endemic for helminths and malaria. This region has widely varying socioeconomic status, lifestyle, urbanization and exposure to parasites. In this setting, we investigated associations between BMI, urban versus rural living, urban socioeconomic status, and different parasites, on the outcomes of atopic skin reactivity, reported current wheeze and asthma.

Table 1. Helminth prevalence and associations with allergic outcomes

Study	N	Age (years)	Helminth		Allergic Outcome	Association
			Type	Positive		
Ghana Obeng *et al.*,¥	1385	5 to 16	*Schistosoma*	9.50%	SPT HDM	↓
			Trichuris	1.90%	SPT Cockroach	↑
			Ascaris	6.20%	SPT, Wheeze, Asthma	NS
			Hookworm	9.90%	SPT, Wheeze, Asthma	NS
Ghana Stevens *et al.*, 2012[8]	181	9 to 16	*Ascaris* IgE	52.3% $_{cases}$	Asthma	NS
				36.6% $_{controls}$	Asthma $_{Urban\ Affluent}$	↑
Zimbabwe Rujeni *et al.*, 2012[17]	672	Up to 86	*Schistosoma*	45.4%$_{high}$	*Dpt* IgE, SPT *Dpt*	↓
				8.5%$_{low}$		
Ethiopia Amberbir *et al.*, 2011[24]	878	3	Int. Helminth	9%	SPT, Eczema	NS
			Hookworm	4.90%	SPT, Eczema	NS
			Ascaris	4.30%	SPT, Eczema	NS
			Trichuris	0.10%	SPT, Eczema	NS
Ecuador Endara *et al.*, 2010[18]	3901	6 to 16	Int. Helminth	86.20%	SPT	↓
			Ascaris	57.30%	SPT	NS
			Trichuris	81.50%	SPT	↓
			Hookworm	3.90%	SPT	NS
			Onchocerca	>40%	SPT, Eczema	↑
					Wheeze , EIB	NS
Indonesia Supali *et al.*, 2010[19]	442	12 to 76	Int. Helminth	43.70%	SPT	NS
			B. malayi	46.70%	SPT Cockroach	↓
			Trichuris	14.90%	SPT HDM	NS
			Hookworm	24.20%	SPT Grass	NS
			Ascaris	22.40%	SPT Grass	NS
Vietnam Flohr *et al.*, 2010[20]	1487	6 to 17	Hookworm§	65%	SPT *Dpt*	↓
			Ascaris	7%	SPT *Dpt*	NS
South Africa Calvert & Burney, 2010[21]	749	8 to 12	*Ascaris*	34.60%	SPT, SPT *Dpt*	↓
					SPT *Btr*	NS
					EIB	↑
			Trichuris	46%	EIB	NS
Cuba Wordemann *et al.*, 2008[22]	1320	4 to 14	*Ascaris*	10%	AD	↓
			E. vermicularis†‡	22%	AD, AR	↑
			Hookworm‡	3%	AR	↑
					Asthma, SPT	NS
Brazil Rodrigues *et al.*, 2008[23]	1055	Up to 11	*Ascaris*	19.60%	SPT	↓
			Trichuris†‡	12%	SPT	↓
			Hookworm	0.60%	SPT	NS

¥ Current Study; ↓ Negative; ↑ Positive; NS Not significant;
† Current Infection; ‡ Past Infection; § IL 10 – Interleukin 10, Int = Intestinal;
AD – Atopic Dermatitis; AR – Allergic Rhinitis;
HDM – House Dust Mite; Dpt - *Dermatophagoides pteronysinnus*; Btr - *Blomia tropicalis*

Methods

Study population

The study was conducted in the Greater Accra Region of Ghana (population >4,000,000 [36]), in rural communities and the national capital Accra (Figure 1). Participating rural (R) communities endemic for intestinal helminths, *Schistosoma haematobium* and malaria [37] were; Pantang (PA) - Ga East district; Mayera (MA) and Ayikai Doblo (AD) - Ga West district; Anyamam (AN), Goi (GP), Toflokpo (TP), Agbedrafor (AB) and Koluedor (KD) - Dangme East district. Populations (and rural proportions) for Ga East, Ga West and Dangme East districts were 480,000 (18%): >300,000 (19%) and 90,000 (82%) [38] respectively. Urban schools, in the Accra Metropolis (all urban: population >1,800,000), were categorised as urban high (UH) and urban low (UL) to reflect average socioeconomic status (SES) of children attending fee-paying private and government-funded public schools respectively. Jamestown (JT), Immanuel Presbyterian (IP), and Nii Okine (NB) were UL schools, whilst Greenhill (GR) and University Primary (UP) were categorised as UH.

Subject recruitment and ethical approval

Between 2003 and 2006, 4612 schoolchildren were invited to participate in the study; 5 to 16 year old subjects were eventually recruited from thirteen schools in the communities described above. District education offices and school authorities granted permission for research in their districts and schools respectively. Initially conducted in four schools, the study was expanded to include nine additional schools. Parents and guardians agreed to participation of children by signing or thumbprinting an informed consent form after a standardized oral presentation and distribution of information letters by research staff. The Institutional Review Board of the Noguchi Memorial Institute for Medical Research in Ghana granted ethical approval.

BMI measurement

Height and weight were determined by a portable stadiometer and a scale (BS-8001, capacity: 130kg) respectively. Body mass index (BMI) was defined as weight in kilograms divided by the square of height in metres. Using previously published BMI cut-off points by Cole *et al.*, (2000, 2007) [39, 40] obtained from averaging international (2 to 18 years) data, we defined underweight as BMI of <17kg/m2 and overweight as BMI > 25 kg/m2.

Parasitology

Intestinal helminth infections were determined by the Kato-Katz technique on 25 mg sieved faecal sample per subject [41]. For *S. haematobium*, the urine filtration method was employed on 10 ml urine samples collected at mid-day [42] using 12 μm pore, 25 mm diameter nucleopore filters. Each subject provided a single stool and urine sample for these analyses. In a subset of 54 subjects, 3 samples were collected to determine sensitivity and specificity of single stool samples to hookworm infection. Helminth infection was classified

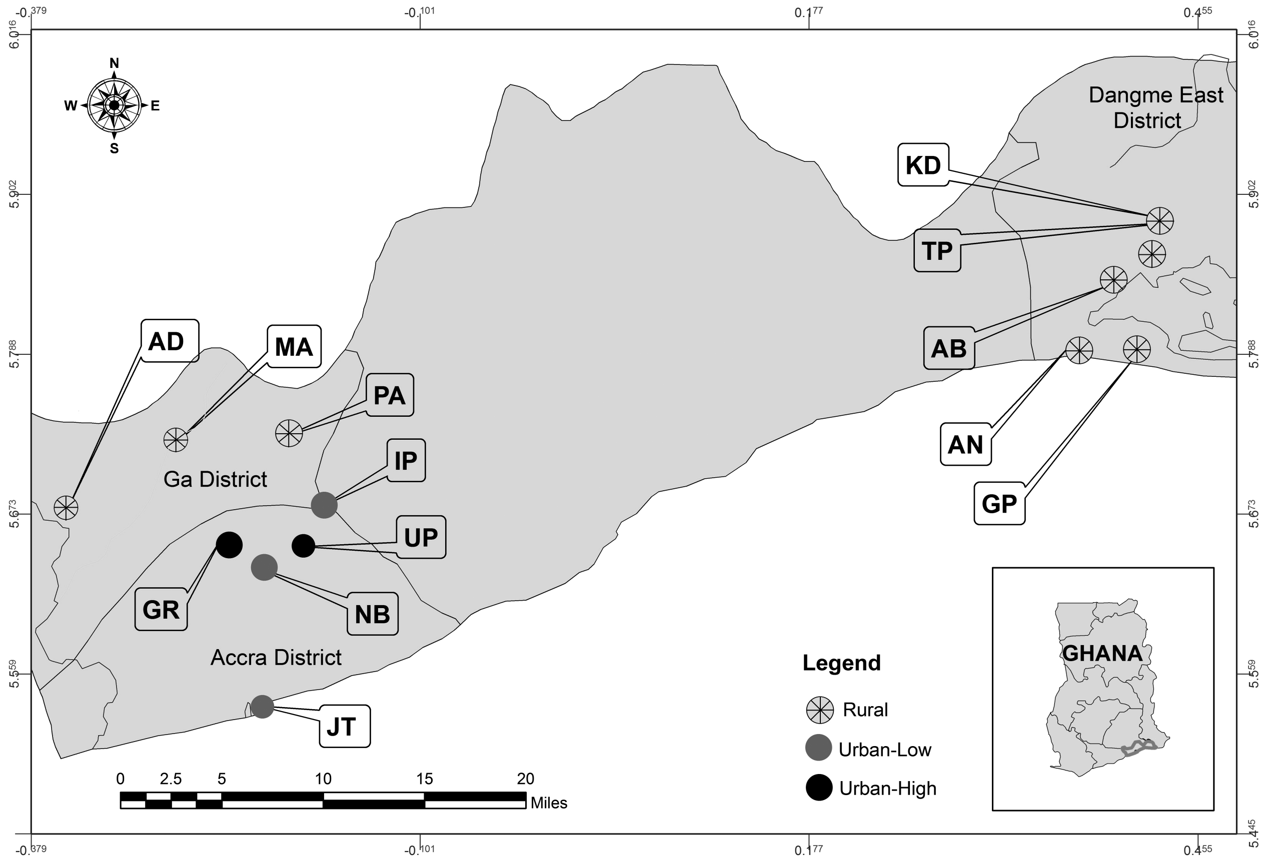

Figure 1: Map of the study area showing urban and rural schools.
Map depicts the distribution of participating schools in the Ga, Accra and Dangme East districts of the Greater Accra region of Ghana.

qualitatively by the presence of eggs and quantitatively by the number of eggs per gram of sample for intestinal helminths and per 10 ml of urine for *S. haematobium* infection. Malaria infection was determined from thick blood smears with Giemsa staining [43].

Atopy - Specific IgE sensitization and skin prick tests (SPT)

Serum allergen-specific immunoglobulin E (sIgE) antibodies to house dust mite (*Dermatophagoides pteronysinnus - Der p),* cockroach (*Blattella germanica - Bla g) and* peanut (*Arachis hypogaea - Ara h*) were measured by the Immuno-CAP™ system (Phadia AB, Uppsala, Sweden). A serum-specific IgE value ≥0.35kU/L was taken as the sensitization cut-off.

Skin tests were performed on the volar part of the left arm using 1mm standardised lancets. Dust mite species, *Dermatophagoides pteronysinnus* (Dpt) and *D. farinae* (Dfe) (HAL Allergy BV, the Netherlands) and peanut (ALK-Abelló, Denmark) were used in the first four schools. In the remaining schools, mixed mite, peanut and newly available cockroach (*Blatella germanica*) allergens (ALK-Abelló) constituted the testing panel. For analysis, a positive response to either Dpt or Dfe in the first four schools was considered as a positive SPT response to mixed mite. Diluent and histamine chloride were used as negative and positive controls, respectively. A skin reaction was assessed after 15 minutes and was considered positive when the average of the longest wheal diameter (D1) and its perpendicular length (D2) was 3 mm for the tested allergen and histamine, while that to the negative control was < 3mm. Any SPT was defined as a positive skin reaction to any of the allergens tested.

Questionnaire assessment of wheeze and asthma

A detailed questionnaire (see thesis appendix) on demographic factors, lifestyle, and socioeconomic factors, as well as on symptoms of asthma and wheeze adapted from the ISAAC Phase II questionnaire [44] was administered to parents or guardians of study participants. To minimize interviewers bias, interviewers were trained to administer questionnaires uniformly though several training sessions and by use of test questionnaires. To assess current wheeze and asthma, parents were asked the following questions:

- "Has this child had wheezing or whistling in the chest in the past 12 months?
- "Has your child ever had asthma?" or "Has a doctor ever diagnosed your child as having asthma?"

Statistical analyses

Statistical analyses included only subjects with complete data for all parasites, BMI, mite SPT and mite sIgE. Preliminary analyses involved testing prevalence differences between UH, UL and R school categories by Pearson's χ^2 at 2 degrees of freedom (4 degrees of freedom for BMI). Mean egg counts in helminth positive subjects were compared by non-parametric Kruskal-Wallis tests between rural and urban subgroups and schools.

To determine if the different manufacturer sources of mite SPT allergens gave similar results, associations between mite IgE and mite SPT were tested for heterogeneity using the inverse variance method in subjects tested with HAL and ALK allergens. Heterogeneity was also tested between urban to rural school categories for the association between IgE and SPT of like allergen.

Associations between area, BMI, parasitic infection and the outcomes of SPT, wheeze and asthma were investigated using multivariable random effects regression models to account for clustering within schools. Models were adjusted for age, sex, and log-transformed sIgE levels as *a priori* confounders. Similar associations were explored for outcomes of reported wheeze and asthma in all subjects. For measures of effect, crude and adjusted ORs and 95%CIs were generated. All statistical tests were considered significant at $p < 0.05$.

To explore the effect of multiple testing, the alpha level of 5% was divided by the total number of tests (Bonferroni correction): variables in each multivariable model and the number of outcomes. This resulted in a corrected alpha level of 5% / (7 x 4) = 0.18% for a model with 7 variables and 4 outcomes.

Statistical analyses were performed using SPSS 16.0 (SPSS Inc.), STATA version 9.2 (StataCorp, Texas, USA) and R version 2.15 (The R Foundation for Statistical Computing) software packages.

Results

Study population

A total of 2331 participants were recruited from 8 rural (n=1347), 3 urban low (n=564) and 2 urban high (n=420) schools with response rates of 68.8%, 44.8%, and 30.1% respectively. Faecal samples were provided by 86.0% (n=2013), urine by 93.2% (n=2182), and blood by 83.8% (n=1961) of the participants for analyses. Skin prick tests were performed in 2018 (86.2%) subjects for mite, 1416 (60.5%) for cockroach and 1907 (81.5%) for peanut. For complete data analyses, 1385 subjects (30% of targeted population and 59% of eligible) with data on parasites, BMI, mite SPT and mite IgE were included in analyses for this study (Figure S1).

Characteristics differed significantly between these subjects when compared to participants excluded due to missing data (n=946). Subjects included in analyses had fewer males (47.9% versus 52.6%, $p < 0.05$) and fewer *Schistosoma* positive subjects (9.5% versus 16.5%, $p < 0.001$) but more urban low (26.4% versus 20.9%, $p < 0.01$), mite SPT positive (12% versus 9%, $p < 0.05$) as well as mite IgE positive (28% versus 18.6%, $p < 0.001$) subjects.

Subject characteristics

Demographics, BMI and parasite infections:

The distributions of basic demographic, BMI, infection characteristics and outcome measures in the complete dataset are summarized in Table 2 by urban SES and rural categories. Subjects from UL schools were significantly older but gender was evenly distributed by category. The prevalence of overweight was highest in UH schools (14.6%) compared to 5.5% and 8.6% in UL and R schools respectively. Intestinal helminths were detected in 23.1% of subjects with 92.2% of these being from the rural area. Hookworm (9.9%) was most common, compared to *Ascaris lumbricoides* (6.2%) and *Trichuris trichiura* (1.9%). Median egg counts [IQR] were *Ascaris* 62 [18-230] eggs per gram (epg); hookworm 12 [3-187] epg and *Trichuris* 7 [3-47] epg. Schistosome infection was detected in 9.5% of subjects (Table 2), the majority of which (85.5 %) were in rural schools. The intensity of infection was low, median [IQR], 21 [4–62] eggs/10 ml urine. Malaria was detected in 24.9% of all subjects (UH 3.8%, UL 6.6% and R 40%).

IgE and SPT to mite, cockroach and peanut:

Subjects with sIgE ≥0.35 kU/L ranged from 6.8% to 55.6% for mite, 4.2% to 58.3% for cockroach and 4.1% to 61.2% for peanut in individual schools. Some individual rural schools (TP) with the highest proportions of parasite infections also reported the highest proportions of atopy (Figures S2 and S3). Overall, positivity for sIgE was highest in the rural areas. Particularly for peanut, many subjects with a positive sIgE response did not have a corresponding positive peanut SPT response (Table 2). A positive SPT to any allergen was seen in 266 subjects (18.3%). Specifically, 12% reacted to mite, 10.1% to cockroach and 1.6% to peanut. There was considerable variation in SPT to mite and cockroach between schools (Figure S3A) with the lowest prevalence in the UL category (Table 2). Any SPT reactivity was comparable between UH and R school categories even though mite was predominant in UH and cockroach in rural schools (Table 2). Mite allergens for SPT from the two manufacturers HAL and ALK were similar: estimates of association between mite IgE and mite SPT were OR = 7.43, 95% CI (4.39 - 12.57) for HAL and OR = 7.37 95%CI (5.65 - 9.62) for ALK (test of heterogeneity p-value= 0.873).

Reported current wheeze and asthma:

Similar to mite and cockroach specific IgE and SPT, current wheeze was least reported in UL schools (4.0%) compared to UH (8.6%) and rural counterparts (9.2%), $p < 0.05$. There were no differences across SES and area categories in reports of asthma (Table 2).

Factors associated with mite and cockroach SPT

There was no evidence of an independent area or SES level association with mite SPT. Being overweight (adjusted OR (aOR) = 2.43, 95% CI 1.28 - 4.60, p=0.007) and schistosome infected (aOR = 0.15, 95% CI 0.05 - 0.41, p <0.001) were both independently associated with mite SPT, but not significant for cockroach SPT. No intestinal helminth infection was associated with mite SPT. Increasing levels of mite specific IgE was strongly associated with mite SPT (aOR = 7.40, 95% CI 5.62 - 9.73, p <0.001) (Table 3). The strongest association between mite IgE and mite SPT was in UH children (aOR = 15.58, 95% CI 7.05 - 34.43,

Table 2. Basic characteristics of subjects by SES and urban-rural categories.

	Category n (%)				
Factor	**UH = 239**	**UL = 366**	**R = 780**	**All = 1385**	**p-value**
Age					
Median [IQR] $	10.6 [8.7-12.1]	11.1 [9.5-12.9]	10.2 [8.7-12]	10.5 [8.9-12.1]	***
Gender					
Males	110 (46.0)	179 (48.9)	374 (47.9)	663 (47.9)	
BMI					
Underweight	8 (3.3)	30 (8.2)	25 (3.2)	63 (4.5)	***
Normal	196 (82.0)	316 (86.3)	688 (88.2)	1200 (86.6)	
Overweight	35 (14.6)	20 (5.5)	67 (8.6)	122 (8.8)	
Helminth					
Hookworm	0 (0)	5 (1.4)	132 (16.9)	137 (9.9)	***
Ascaris spp.	0 (0)	7 (1.9)	79 (10.1)	86 (6.2)	***
Trichuris spp.	1 (0.4)	7 (1.9)	19 (2.4)	27 (1.9)	
Schistosoma spp.	3 (1.3)	16 (4.4)	112 (14.4)	131 (9.5)	***
Any Intestinal Helminth	1 (0.4)	17 (4.6)	212 (27.2)	230 (16.6)	***
Any Helminth	4 (1.7)	32 (8.7)	283 (36.3)	319 (23.1)	***
Malaria	9 (3.8)	24 (6.6)	312 (40)	345 (24.9)	***
sIgE £					
Mite	70 (29.3)	60 (16.4)	258 (33.1)	388 (28.0)	***
Cockroach	68 (30.0)	81 (22.2)	308 (42.1)	457 (34.5)	***
Peanut	26 (10.9)	34 (9.3)	225 (28.8)	285 (20.6)	***
SPT					
Mite	39 (16.3)	33 (9.0)	94 (12.1)	166 (12.0)	*
Cockroach	17 (9.0)	16 (5.5)	78 (12.6)	111 (10.1)	**
Peanut	5 (2.5)	6 (1.6)	10 (1.3)	21 (1.6)	
Any Allergen	40 (20.2)	31 (10.5)	135 (21.4)	206 (18.3)	***
Wheeze	16 (8.6)	11 (4.0)	66 (9.2)	93 (7.9)	*
Asthma	18 (9.7)	25 (9.2)	54 (7.6)	97 (8.3)	

Significant p-value codes: *** < 0.001 ** < 0.01 * < 0.05 for Chi square or Kruskal Wallis$ tests;
£ Allergen sIgE ≥ 0.35kU/L.
UH - urban high, UL - urban low, R - rural;
Any intestinal helminth – Hookworm, *Ascaris*, or *Trichuris*; Any helminth – Any Intestinal helminth or *Schistosoma*

p <0.001), followed by UL (aOR = 10.44, 95% CI 5.60-19.47, p <0.001) and then rural children (aOR = 5.43, 95% CI 3.83 - 7.69, p <0.001), test for heterogeneity p=0.007.

Trichuris was positively associated with cockroach SPT (adjusted OR = 3.73, 95% CI 1.22 – 11.41, p=0.021) as shown in Table 3. Cockroach sIgE was significantly

Table 3. Factors associated with SPT reactivity to mite and cockroach

	Mite				Cockroach			
Factor	**cOR[95% CI]**	**p-value**	**aOR[95% CI]**	**p-value**	**cOR[95% CI]**	**p-value**	**aOR[95% CI]**	**p-value**
Age	1.06 [0.98 - 1.15]	0.105	1.10 [1.00 - 1.21]	0.060	**1.11 [1.00 - 1.22]**	**0.041**	1.07 [0.96 - 1.20]	0.218
Gender - Male	**1.64 [1.18 - 2.29]**	**0.003**	1.47 [0.95 - 2.27]	0.082	1.49 [0.99 - 2.25]	0.054	**1.73 [1.07 - 2.79]**	**0.025**
BMI§								
Underweight	0.43 [0.13 - 1.42]	0.167	0.35 [0.08 - 1.48]	0.152	0.27 [0.04 - 2.00]	0.199	0.18 [0.02 - 1.48]	0.109
Overweight	**1.86 [1.14 - 3.03]**	**0.012**	**2.43 [1.28 - 4.60]**	**0.007**	1.34 [0.71 - 2.53]	0.365	1.69 [0.81 - 3.53]	0.165
Area, SES§								
Urban low	0.60 [0.30 - 1.23]	0.165	0.68 [0.27 - 1.71]	0.409	**0.34 [0.16 - 0.73]**	**0.006**	0.59 [0.29 - 1.20]	0.146
Urban high	1.57 [0.74 - 3.29]	0.247	0.55 [0.20 - 1.53]	0.252	0.79 [0.36 - 1.78]	0.575	1.21 [0.60 - 2.46]	0.591
Malaria	1.09 [0.73- 1.65]	0.666	1.10 [0.65 - 1.86]	0.728	1.29 [0.80 - 2.09]	0.301	1.21 [0.70 - 2.10]	0.500
Trichuris	1.68 [0.60 - 4.71]	0.322	2.26 [0.63 - 8.13]	0.212	**4.14 [1.55 - 11.03]**	**0.005**	**3.73 [1.22 - 11.41]**	**0.021**
Schistosoma	**0.40 [0.18 - 0.91]**	**0.028**	**0.15 [0.05 - 0.41]**	**<0.001**	0.78 [0.32 - 1.88]	0.577	0.49 [0.18 - 1.29]	0.147
Specific IgE	**6.76 [5.23 - 8.75]**	**<0.001**	**7.40 [5.62 - 9.73]**	**<0.001**	**5.72 [4.23 - 7.74]**	**<0.001**	**5.94 [4.34 - 8.12]**	**<0.001**

§ Reference categories: normal BMI and rural area. Statistical significance (in bold) is set at $p < 0.05$.
To explore effects of multiple comparisons, p-value is set at < 0.0018.
Adjusted ORs are for mutually adjusted variables (each model has age, gender, BMI, area, individual or composite parasite variables and sIgE).
Specific IgE shows associations with SPT to like allergen.
Hookworm, *Ascaris*, any intestinal helminth and any helminth showed no associations with tested outcomes and are excluded from table for simplicity.

associated with cockroach SPT (OR 5.94, 95% CI 4.34- 8.12, p <0.001), but in contrast to mite atopy, the associations were similar for rural-urban and SES category. Malaria infection was not associated with any SPT allergen. After correcting for multiple comparison, only the effects of *S. haematobium*, and sIgE on SPT of like allergen remained significant at p <0.0018 (Table 3).

Factors associated with reported current wheeze and asthma

The UL school category was independently associated with reported current wheeze compared to rural children (adjusted OR = 0.41, 95% CI 0.20 – 0.83, p=0.013) (Table 4). The UH category was not associated with wheeze or asthma. Mite atopy was positively associated with current wheeze, (SPT, adjusted OR = 3.87, 95% CI 2.33 – 6.41, p <0.001 and IgE, OR = 2.08, 95% CI 1.67 – 2.59, p <0.001). Neither cockroach atopy nor parasite infections were associated with current wheeze.

Age (adjusted OR = 0.87, 95% CI 0.79 – 0.96, p=0.005) and malaria, (adjusted OR = 0.50, 95% CI 0.27 – 0.93, p=0.027) were negatively associated with asthma. Like current wheeze, mite (but not cockroach atopy) was associated with asthma (Table 4). For both wheeze and asthma, only mite atopy remained significantly associated with these outcomes after correction for multiple comparisons.

Discussion

In this study of Ghanaian schoolchildren, we showed that the prevalence of skin test reactivity was not significantly different between urban and rural areas. Despite the similarity in SPT prevalence, the association between mite specific IgE and SPT was strongest in wealthier urban subjects and weakest in the rural. Schistosome infection, common in rural but virtually absent in higher SES urban areas, was negatively associated with mite SPT. In contrast, infection with *Trichuris* showed a positive association with cockroach SPT. Overweight was most prevalent in higher SES urban schools and significantly associated with mite skin reactivity. Mite SPT was strongly associated with reported wheeze and asthma. However, helminth infection, area and being overweight were not associated with wheeze or asthma.

The prevalence of skin test reactivity varied in neighbouring communities, but did not differ significantly between rural and urban areas once covariates were accounted for. Addo-Yobo *et al.* [3] in contrast to our study but in line with studies from South Africa, Congo [45] and Kenya [46], showed a decreasing urban-rural trend with SPT and exercise-induced bronchospasm (EIB) in Kumasi, the second largest city in Ghana. Subjects were similarly categorised by location and socioeconomic status, but the settings are geographically and culturally different from our study. One possibility for the discrepancy could be that the urban-rural classification in general is too simple to address socio-economic and cultural differences important for atopy. Particularly in the Greater Accra setting of our study, pockets of self-driven developments, alteration in

Table 4. Factors associated with current wheeze and asthma

	Current Wheeze				Asthma			
Factor	**cOR[95% CI]**	**p-value**	**aOR[95% CI]**	**p-value**	**cOR[95% CI]**	**p-value**	**aOR[95% CI]**	**p-value**
Age	0.93 [0.85 - 1.02]	0.124	0.91 [0.82 - 1.00]	0.060	**0.89 [0.82 - 0.98]**	**0.017**	**0.87 [0.79 - 0.95]**	**0.005**
Gender	1.50 [0.98 - 2.30]	0.064	1.29 [0.82 -2.01]	0.268	**1.56 [1.02 - 2.38]**	**0.040**	1.43 [0.93 - 2.21]	0.110
BMI §								
Underweight	0.24 [0.03 - 1.77]	0.161	0.29 [0.04 - 2.14]	0.224	1.92 [0.82 - 4.49]	0.132	2.05 [0.85 – 4.91]	0.109
Overweight	0.70 [0.30 - 1.66]	0.422	0.61 [0.25 - 1.47]	0.269	1.40 [0.69 - 2.83]	0.350	1.34 [0.65 - 2.77]	0.429
Area, SES §								
Urban low	**0.41 [0.21 - 0.79]**	**0.008**	**0.41 [0.20 - 0.83]**	**0.013**	1.32 [0.64 - 2.71]	0.450	1.36 [0.62 - 2.95]	0.440
Urban high	0.93 [0.52 - 1.64]	0.791	0.84 [0.45 - 1.58]	0.587	1.36 [0.59 - 3.15]	0.470	1.17 [0.48 - 2.85]	0.732
Malaria	0.83 [0.49 - 1.42]	0.502	0.61 [0.35 - 1.06]	0.079	**0.55 [0.31 - 0.98]**	**0.044**	**0.50 [0.27 - 0.93]**	**0.027**
Trichuris	0.53 [0.07 - 4.03]	0.541	0.40 [0.05 - 3.19]	0.388	0.55 [0.07 - 4.21]	0.563	0.53 [0.07 - 4.29]	0.556
Schistosoma	1.44 [0.75 - 2.77]	0.273	1.54 [0.78 - 2.05]	0.210	1.31 [0.66 - 2.58]	0.436	1.72 [0.84 - 3.53]	0.136
Mite IgE	**2.07 [1.68 - 2.55]**	**<0.001**	**2.08 [1.67 - 2.59]**	**<0.001**	**1.55 [1.25 - 1.91]**	**<0.001**	**1.54 [1.24 - 1.91]**	**<0.001**
Mite SPT	**3.66 [2.25 - 5.97]**	**<0.001**	**3.87 [2.33 - 6.41]**	**<0.001**	**2.52 [1.49 - 4.27]**	**<0.001**	**2.72 [1.58 - 4.70]**	**<0.001**
Cockroach IgE	**1.32 [1.02 - 1.70]**	**0.032**	1.26 [0.97 - 1.64]	0.078	1.00 [0.77 - 1.30]	1.000	1.04 [0.79 - 1.36]	0.782
Cockroach SPT	1.61 [0.83 - 3.13]	0.161	1.51 [0.77 - 2.96]	0.229	1.09 [0.51 - 2.36]	0.818	1.24 [0.57 - 2.74]	0.587

§ Reference categories: normal BMI and rural area. Statistical significance (in bold) is set at $p < 0.05$. To explore the effects of multiple comparisons, p-value is set at < 0.0018. Adjusted ORs are for mutually adjusted variables (each model has age, gender, BMI, area, individual or composite parasite variables and sIgE or SPT).
Hookworm, *Ascaris*, any intestinal helminth and any helminth showed no associations with tested outcomes and are excluded from table for simplicity.

physical and spatial organization, limited access to amenities and changing cultural and ethnic environments, make urban-rural demarcation challenging and present different opportunities for disease [4]. These complexities within one country could also account for some conflicting findings between countries at similar levels of economic growth.

Consistent with findings from animal [14, 47] and epidemiological studies [27, 48], we showed schistosome infection was negatively associated with mite SPT. This has also been shown in Zimbabwe [17] particularly with higher intensity infections. Though we observed a tendency for a negative association between schistosome infection and cockroach SPT, this was not statistically significant. A general suppressory effect of schistosomiasis would be expected on all forms of atopy. Therefore, a lack of association between schistosome infection and cockroach SPT is likely due to reduced statistical power: though a confounder effect is possible.

Similar to some previous studies [18-20, 49], no significant association was observed in our population between hookworm or *Ascaris* and any allergic outcome - possibly due to relatively low infection intensities in our communities. However, higher burdens of *Ascaris*, *Trichuris* [23], and hookworm [20, 49] have been reported to be associated with less atopy. Endara *et al*., (2010) [18] reported a negative association between *Trichuris* and any atopy for both light and heavy intensity infections, with a stronger association in the latter. Conversely, we found a positive association between *Trichuris* and cockroach SPT similar to observations made in Indonesia [19] and among rural Ethiopian subjects [5]. However, within the context of much lower *Trichuris* prevalence and intensity, our findings were less certain - given the wide confidence intervals.

Inconsistent associations between different helminth effects and skin test reactivity could result from unique characteristics of each parasite, intensity and timing of exposure. Low intensity infection with *Trichuris*, an exclusively intestinal helminth, may result in a stronger Th2 response in the face of a weak regulatory response compared to *Schistosoma*, a systemic infection with a strong regulatory characteristic [29]. Acute helminth infections could worsen allergy and be associated with pulmonary inflammation, while chronic worm infections suppress cell-mediated immune responses towards unrelated antigens [14, 50]. However, Feary *et al.,* (2011) [51] showed in a meta-analyses, consistent protective effect by helminths in general or by specific species against allergen skin sensitization or elevated specific IgE.

Immunoglobulin levels were the strongest predictor of skin reactivity to same allergen even though elevated sIgE levels did not always translate into SPT reactivity or clinical outcomes. Additionally, these associations varied with allergen type: mite (but not cockroach) atopy was associated with both wheeze and asthma. Vereecken *et al.,* (2012) [52] have similarly reported associations between IgE, SPT, and asthma while others show a dissociation between atopy and clinical outcomes [18, 24, 53] - a phenomenon attributed to infections. While our data can only speak to active regulation by current infection, programming by early life exposure to helminths [23] could account for some of these observations. The absence of a significant association between cockroach atopy and wheeze or asthma could reflect these complexities or allergen specific factors important for clinical outcome in this study. Possibly, cross-reactivity plays a more important role in IgE sensitization to cockroach than mite allergens, thus accounting for poor association of cockroach with clinical outcomes. For this population, the role of helminth-induced IgE against cross-reactive carbohydrate determinants in peanut sensitization is discussed by Amoah *et al.*, [54].

Despite the association between mite atopy and reported outcomes, the protective effect of *Schistosoma* was not observed with wheeze or asthma. This could be due to a reporting of non-atopic or infection related wheeze in this population. Though earlier findings in Brazil [55], Cuba [18] and Ecuador [52] have also reported no association between helminths and allergic disease, a meta-analysis [26] showed *Ascaris lumbricoides* infection was associated with increased asthma risk, while hookworm was negatively associated with asthma. Possibly, the infection intensity in this population did not lead to observable changes at the clinical level of allergy. Interestingly, the negative association between malaria and asthma observed could result from immunosuppression and elevated IL-10 with malaria infection [56, 57].

Some schools with high helminth prevalence also had the highest prevalence of skin reactivity - an indication that individual, ethnic and lifestyle factors are involved in predisposing to atopy. We found being overweight was significantly associated with mite SPT, similar to urban South Africa [58]. Multiple studies have shown the importance of excess body weight in allergic disease [59-62]. In Ghana, higher BMI has been reported to be associated with urban affluent children in general, and with exercise-induced bronchospasm in urban poor, suburban and rural children [8]. Additionally, allergen exposure could account for high atopy prevalence to both mite and cockroach in rural communities. Cockroach atopy was most prevalent among the rural possibly due to greater exposure as a result of poorer hygiene. The indoor environment – humidity, ventilation and furnishing – is important for cockroach and mite allergen exposure and could vary broadly between individual homes. In addition, the high prevalence of helminth infection in these communities may have led to more frequent self-administered anti-helminthic treatment. Therefore, detected infections may have been recent or not chronic helminth infection postulated to induce down-modulation of allergy [63].

Lower SES urban subjects had the lowest proportions of sIgE sensitization, any skin reactivity and wheeze and remained negatively associated with wheeze after multivariable analyses. Addo-Yobo *et al.*, [3] showed atopy and EIB prevalence in urban areas differed according to SES. In Chile [64, 65], asthma symptoms were more common in subjects with lower socioeconomic status yet overcrowding was associated with less wheeze, atopy and bronchial hyperresponsiveness. Calvert and Burney [21] reported a relationship between possessing consumer items and EIB in South Africa. Additionally, the UL category in our study had the highest proportion of underweight and lowest proportion of overweight subjects. Sub-optimal nutrition in the urban poor, a less sedentary lifestyle, coupled with parasitic infections (including possible heavy intensity infections in early life) could result in reduced immune sensitivity, whilst likely overcrowding would be associated with less hygiene and low rates of allergic outcomes.

A drawback of this study is the cross-sectional design which limits our ability to make inferences on causality and timing of exposure. A major limitation is the overall low response rate particularly in urban versus rural schools, lack of data on non-participants, and incomplete data from participants. The inability to perform non-responder analyses made it impossible to assess the potential bias introduced in the prevalence of atopy and helminths as well as the association between the two factors. While it did not address the bias in prevalence, it was reassuring to find in a stratified analysis that, the observed association between helminths and atopy was independent of urban high schools with high atopy prevalence but no helminths (data not shown). It was taken into consideration that with the number of associations tested; some of the findings might have arisen by chance. Also, some helminth infections may have been missed due to lower sensitivity from using single samples. Even though our analysis showed sensitivity was good for hookworm in single stool samples, this was not assessed for urine samples.

In conclusion, our results suggest that helminth infections, socioeconomic status and lifestyle are important factors in the prevalence of allergic diseases in Ghana. Against the backdrop of rapid urbanization in developing countries, it is crucial for research to recognize and address the potential for increasing allergic disease in these populations.

Acknowledgements

The authors wish to acknowledge Dr. Paul van Rijn (HAL Allergy BV, the Netherlands) and Dr. Domingo Barber (ALK-Abelló, Spain) for providing the SPT material. We thank Professor Michael D. Wilson, Professor Kwabena M. Bosompem, and Ms Yvonne Kruize for assistance with the fieldwork and laboratory analyses. We are also indebted to the community leaders and head teachers of all the schools involved in this study for their invaluable assistance.

Author Contributions

MY, DAB, LCR, RR and FH were involved in the planning and design of the study. BBO, ASA and IAL were involved in subject recruitment, field visits and laboratory analysis of parasitological samples. LCR and HU assisted with epidemiological and statistical issues, DKS worked on the database and GIS components of the study. MF provided expertise on allergens. ASA and BBO conducted the data analysis. BBO wrote the manuscript and all other authors critically reviewed and approved the final version.

Conflict of Interest

The authors have no conflict of interest to declare.

Declaration of Sources of Funding

This study was funded by WOTRO grant # WB 93-443, GLOFAL project (FOOD-CT-2005-517812) and EUROPREVALL project (FOOD-CT-2005-514000) and Wellcome Trust (075791/Z/04/Z). The funding parties had no role in the study design, data collection and analysis, decision to publish, or preparation of the manuscript.

References

1. Weinmayr G, Genuneit J, Nagel G, Bjorksten B, van Hage M, Priftanji A, Cooper P, Rijkjarv MA, von Mutius E, Tsanakas J, Forastiere F, Doekes G, Garrido JB, Suarez-Varela MM, Braback L, Strachan DP, International variations in associations of allergic markers and diseases in children: ISAAC Phase Two. Allergy 2010;65: 766-75.
2. Yemaneberhan H, Bekele Z, Venn A, Lewis S, Parry E, Britton J, Prevalence of wheeze and asthma and relation to atopy in urban and rural Ethiopia. The Lancet 1997;350: 85-90.
3. Addo-Yobo EO, Woodcock A, Allotey A, Baffoe-Bonnie B, Strachan D, Custovic A, Exercise-induced bronchospasm and atopy in Ghana: two surveys ten years apart. PLOS Medicine 2007;4: e70.
4. Agyei-Mensah S, de-Graft Aikins A, Epidemiological transition and the double burden of disease in Accra, Ghana. Journal of Urban Health : bulletin of the New York Academy of Medicine 2010;87: 879-97.
5. Dagoye D, Bekele Z, Woldemichael K, Nida H, Yimam M, Hall A, Venn AJ, Britton JR, Hubbard R, Lewis SA, Wheezing, allergy, and parasite infection in children in urban and rural Ethiopia. American Journal of Respiratory and Critical Care Medicine 2003;167: 1369-73.
6. Palmer LJ, Celedon JC, Weiss ST, Wang B, Fang Z, Xu X, Ascaris lumbricoides infection is associated with increased risk of childhood asthma and atopy in rural China. American Journal of Respiratory and Critical Care Medicine 2002;165: 1489-93.
7. Obihara CC, Beyers N, Gie RP, Hoekstra MO, Fincham JE, Marais BJ, Lombard CJ, Dini LA, Kimpen JL, Respiratory atopic disease, Ascaris-immunoglobulin E and tuberculin testing in urban South African children. Clinical and Experimental Allergy : journal of the British Society for Allergy and Clinical Immunology 2006;36: 640-8.
8. Stevens W, Addo-Yobo E, Roper J, Woodcock A, James H, Platts-Mills T, Custovic A, Differences in both prevalence and titre of specific immunoglobulin E among children with asthma in affluent and poor communities within a large town in Ghana. Clinical and Experimental Allergy : journal of the British Society for Allergy and Clinical Immunology 2011;41: 1587-94.
9. Mercer MJ, Joubert G, Ehrlich RI, Nelson H, Poyser MA, Puterman A, Weinberg EG, Socioeconomic status and prevalence of allergic rhinitis and atopic eczema symptoms in young adolescents. Pediatric Allergy and Immunology : official publication of the European Society of Pediatric Allergy and Immunology 2004;15: 234-41.
10. Poyser MA, Nelson H, Ehrlich RI, Bateman ED, Parnell S, Puterman A, Weinberg E, Socioeconomic deprivation and asthma prevalence and severity in young adolescents. The European Respiratory Journal 2002;19: 892-8.
11. Chen E, Hanson MD, Paterson LQ, Griffin MJ, Walker HA, Miller GE, Socioeconomic status and inflammatory processes in childhood asthma: the role of psychological stress. The Journal of Allergy and Clinical Immunology 2006;117: 1014-20.
12. Bacon SL, Bouchard A, Loucks EB, Lavoie KL, Individual-level socioeconomic status is associated with worse asthma morbidity in patients with asthma. Respiratory research 2009;10: 125.
13. Yazdanbakhsh M, Kremsner PG, van Ree R, Allergy, parasites, and the hygiene hypothesis. Science (New York, NY) 2002;296: 490-4.
14. Smits HH, Hammad H, van Nimwegen M, Soullie T, Willart MA, Lievers E, Kadouch J, Kool M, Kos-van Oosterhoud J, Deelder AM, Lambrecht BN, Yazdanbakhsh M, Protective effect of Schistosoma mansoni infection on allergic airway inflammation

depends on the intensity and chronicity of infection. The Journal of Allergy and Clinical Immunology 2007;120: 932-40.

15. Wilson MS, Taylor MD, Balic A, Finney CA, Lamb JR, Maizels RM, Suppression of allergic airway inflammation by helminth-induced regulatory T cells. The Journal of Experimental Medicine 2005;202: 1199-212.
16. Flohr C, Quinnell RJ, Britton J, Do helminth parasites protect against atopy and allergic disease? Clinical and Experimental Allergy : journal of the British Society for Allergy and Clinical Immunology 2009;39: 20-32.
17. Rujeni N, Nausch N, Bourke CD, Midzi N, Mduluza T, Taylor DW, Mutapi F, Atopy is inversely related to schistosome infection intensity: a comparative study in Zimbabwean villages with distinct levels of Schistosoma haematobium infection. International Archives of Allergy and Immunology 2012;158: 288-98.
18. Endara P, Vaca M, Chico ME, Erazo S, Oviedo G, Quinzo I, Rodriguez A, Lovato R, Moncayo AL, Barreto ML, Rodrigues LC, Cooper PJ, Long-term periodic anthelmintic treatments are associated with increased allergen skin reactivity. Clinical and Experimental Allergy : journal of the British Society for Allergy and Clinical Immunology 2010;40: 1669-77.
19. Supali T, Djuardi Y, Wibowo H, van Ree R, Yazdanbakhsh M, Sartono E, Relationship between different species of helminths and atopy: a study in a population living in helminth-endemic area in Sulawesi, Indonesia. International Archives of Allergy and Immunology 2010;153: 388-94.
20. Flohr C, Tuyen LN, Quinnell RJ, Lewis S, Minh TT, Campbell J, Simmons C, Telford G, Brown A, Hien TT, Farrar J, Williams H, Pritchard DI, Britton J, Reduced helminth burden increases allergen skin sensitization but not clinical allergy: a randomized, double-blind, placebo-controlled trial in Vietnam. Clinical and Experimental Allergy : journal of the British Society for Allergy and Clinical Immunology 2010;40: 131-42.
21. Calvert J, Burney P, Ascaris, atopy, and exercise-induced bronchoconstriction in rural and urban South African children. The Journal of Allergy and Clinical Immunology 2010;125: 100-5 e1-5.
22. Wordemann M, Diaz RJ, Heredia LM, Collado Madurga AM, Ruiz Espinosa A, Prado RC, Millan IA, Escobedo A, Rojas Rivero L, Gryseels B, Gorbea MB, Polman K, Association of atopy, asthma, allergic rhinoconjunctivitis, atopic dermatitis and intestinal helminth infections in Cuban children. Tropical Medicine & International Health : TM & IH 2008;13: 180-6.
23. Rodrigues LC, Newcombe PJ, Cunha SS, Alcantara-Neves NM, Genser B, Cruz AA, Simoes SM, Fiaccone R, Amorim L, Cooper PJ, Barreto ML, Early infection with Trichuris trichiura and allergen skin test reactivity in later childhood. Clinical and Experimental Allergy : journal of the British Society for Allergy and Clinical Immunology 2008;38: 1769-77.
24. Amberbir A, Medhin G, Erku W, Alem A, Simms R, Robinson K, Fogarty A, Britton J, Venn A, Davey G, Effects of Helicobacter pylori, geohelminth infection and selected commensal bacteria on the risk of allergic disease and sensitization in 3-year-old Ethiopian children. Clinical and Experimental Allergy : journal of the British Society for Allergy and Clinical Immunology 2011;41: 1422-30.
25. Davey G, Berhane Y, Duncan P, Aref-Adib G, Britton J, Venn A, Use of acetaminophen and the risk of self-reported allergic symptoms and skin sensitization in Butajira, Ethiopia. The Journal of Allergy and Clinical Immunology 2005;116: 863-8.
26. Leonardi-Bee J, Pritchard D, Britton J, Asthma and current intestinal parasite infection: systematic review and meta-analysis. American Journal of Respiratory and Critical Care Medicine 2006;174: 514-23.
27. Araujo MI, Lopes AA, Medeiros M, Cruz AA, Sousa-Atta L, Sole D, Carvalho EM, Inverse association between skin response to aeroallergens and Schistosoma mansoni infection. International Archives of Allergy and Immunology 2000;123: 145-8.
28. Camara AA, Silva JM, Ferriani VP, Tobias KR, Macedo IS, Padovani MA, Harsi CM, Cardoso MR, Chapman MD, Arruda E, Platts-Mills TA, Arruda LK, Risk factors for wheezing in a subtropical environment: role of respiratory viruses and allergen sensitization. The Journal of Allergy and Clinical Immunology 2004;113: 551-7.
29. 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. British Medical Bulletin 2006;79-80: 203-18.

30. Hooper R, Calvert J, Thompson RL, Deetlefs ME, Burney P, Urban/rural differences in diet and atopy in South Africa. Allergy 2008;63: 425-31.
31. Mitchell EA, Beasley R, Bjorksten B, Crane J, Garcia-Marcos L, Keil U, The association between BMI, vigorous physical activity and television viewing and the risk of symptoms of asthma, rhinoconjunctivitis and eczema in children and adolescents: ISAAC Phase Three. Clinical and Experimental Allergy : journal of the British Society for Allergy and Clinical Immunology 2013;43: 73-84.
32. Scholtens S, Wijga AH, Seidell JC, Brunekreef B, de Jongste JC, Gehring U, Postma DS, Kerkhof M, Smit HA, Overweight and changes in weight status during childhood in relation to asthma symptoms at 8 years of age. The Journal of Allergy and Clinical Immunology 2009;123: 1312-8 e2.
33. Magnusson JO, Kull I, Mai XM, Wickman M, Bergstrom A, Early childhood overweight and asthma and allergic sensitization at 8 years of age. Pediatrics 2012;129: 70-6.
34. Porter M, Wegienka G, Havstad S, Nageotte CG, Johnson CC, Ownby DR, Zoratti EM, Relationship between childhood body mass index and young adult asthma. Annals of Allergy, Asthma and Immunology : official publication of the American College of Allergy, Asthma, and Immunology 2012;109: 408-11 e1.
35. Rodriguez A, Vaca M, Oviedo G, Erazo S, Chico ME, Teles C, Barreto ML, Rodrigues LC, Cooper PJ, Urbanisation is associated with prevalence of childhood asthma in diverse, small rural communities in Ecuador. Thorax 2011;66: 1043-50.
36. Ghana Statistical Service, 2010 Population and Housing Census: Summary Report of Final Results. Accra: Ghana Statistical Service, 2012.
37. Aryeetey ME, Wagatsuma Y, Yeboah G, Asante M, Mensah G, Nkrumah FK, Kojima S, Urinary schistosomiasis in southern Ghana: 1. Prevalence and morbidity assessment in three (defined) rural areas drained by the Densu river. Parasitology international 2000;49: 155-63.
38. Dangme East District Assembly, Dangme East Municipal: Demographic Characteristics. Accra: Ministry of Local Government and Rural Development, 2006.
39. Cole TJ, Flegal KM, Nicholls D, Jackson AA, Body mass index cut offs to define thinness in children and adolescents: international survey. BMJ (Clinical research ed) 2007;335: 194.
40. Cole TJ, Bellizzi MC, Flegal KM, Dietz WH, Establishing a standard definition for child overweight and obesity worldwide: international survey. BMJ (Clinical research ed) 2000;320: 1240-3.
41. Katz N, Grinbaum E, Chaves A, Zicker F, Pellegrino J, Clinical trials with oxamniquine, by oral route, in schistosomiasis mansoni. Revista do Instituto de Medicina Tropical de Sao Paulo 1976;18: 371-7.
42. Peters PA, Mahmoud AA, Warren KS, Ouma JH, Siongok TK, Field studies of a rapid, accurate means of quantifying Schistosoma haematobium eggs in urine samples. Bulletin of the World Health Organization 1976;54: 159-62.
43. Trape JF, Rapid evaluation of malaria parasite density and standardization of thick smear examination for epidemiological investigations. Transactions of the Royal Society of Tropical Medicine and Hygiene 1985;79: 181-4.
44. ISAAC Steering Committee, ISAAC Tools: The University of Auckland, 1998.
45. Nyembue TD, Jorissen M, Hellings PW, Muyunga C, Kayembe JM, Prevalence and determinants of allergic diseases in a Congolese population. International Forum of Allergy & Rhinology 2012;2: 285-93.
46. Ng'ang'a LW, Odhiambo JA, Mungai MW, Gicheha CM, Nderitu P, Maingi B, Macklem PT, Becklake MR, Prevalence of exercise induced bronchospasm in Kenyan school children: an urban-rural comparison. Thorax 1998;53: 919-26.
47. Cardoso LS, Oliveira SC, Goes AM, Oliveira RR, Pacifico LG, Marinho FV, Fonseca CT, Cardoso FC, Carvalho EM, Araujo MI, Schistosoma mansoni antigens modulate the allergic response in a murine model of ovalbumin-induced airway inflammation. Clinical and Experimental Immunology 2010;160: 266-74.
48. van den Biggelaar AH, van Ree R, Rodrigues LC, Lell B, Deelder AM, Kremsner PG, Yazdanbakhsh M, Decreased atopy

in children infected with Schistosoma haematobium: a role for parasite-induced interleukin-10. The Lancet 2000;356: 1723-7.

49. Flohr C, Tuyen LN, Lewis S, Quinnell R, Minh TT, Liem HT, Campbell J, Pritchard D, Hien TT, Farrar J, Williams H, Britton J, Poor sanitation and helminth infection protect against skin sensitization in Vietnamese children: A cross-sectional study. The Journal of Allergy and Clinical Immunology 2006;118: 1305-11.
50. Hartgers FC, Obeng BB, Kruize YC, Duijvestein M, de Breij A, Amoah A, Larbi IA, van Ree R, Wilson MD, Rodrigues LC, Boakye DA, Yazdanbakhsh M, Lower expression of TLR2 and SOCS-3 is associated with Schistosoma haematobium infection and with lower risk for allergic reactivity in children living in a rural area in Ghana. PLOS Neglected Tropical Diseases 2008;2: e227.
51. Feary J, Britton J, Leonardi-Bee J, Atopy and current intestinal parasite infection: a systematic review and meta-analysis. Allergy 2011;66: 569-78.
52. Vereecken K, Kanobana K, Wordemann M, Junco Diaz R, Menocal Heredia L, Ruiz Espinosa A, Nunez FA, Rojas Rivero L, Bonet Gorbea M, Polman K, Associations between atopic markers in asthma and intestinal helminth infections in Cuban schoolchildren. Pediatric Allergy and Immunology : official publication of the European Society of Pediatric Allergy and Immunology 2012;23: 332-8.
53. Weinmayr G, Weiland SK, Bjorksten B, Brunekreef B, Buchele G, Cookson WO, Garcia-Marcos L, Gotua M, Gratziou C, van Hage M, von Mutius E, Riikjarv MA, Rzehak P, Stein RT, Strachan DP, Tsanakas J, Wickens K, Wong GW, Atopic sensitization and the international variation of asthma symptom prevalence in children. American Journal of Respiratory and Critical Care Medicine 2007;176: 565-74.
54. Amoah AS, Obeng BB, Larbi IA, Versteeg SA, Aryeetey Y, Akkerdaas JH, Zuidmeer L, Lidholm J, Fernandez-Rivas M, Hartgers FC, Boakye DA, van Ree R, Yazdanbakhsh M, Peanut-specific IgE antibodies in asymptomatic Ghanaian children possibly caused by carbohydrate determinant cross-reactivity. The Journal of Allergy and Clinical Immunology 2013;132: 639-47.
55. Alcantara-Neves NM, Veiga RV, Dattoli VC, Fiaccone RL, Esquivel R, Cruz AA, Cooper PJ, Rodrigues LC, Barreto ML, The effect of single and multiple infections on atopy and wheezing in children. The Journal of Allergy and Clinical Immunology 2012;129: 359-67, 67.e1-3.
56. Lell B, Borrmann S, Yazdanbakhsh M, Kremsner PG, Atopy and malaria. Wiener Klinische Wochenschrift 2001;113: 927-9.
57. Ayimba E, Hegewald J, Segbena AY, Gantin RG, Lechner CJ, Agosssou A, Banla M, Soboslay PT, Proinflammatory and regulatory cytokines and chemokines in infants with uncomplicated and severe Plasmodium falciparum malaria. Clinical and Experimental Immunology 2011;166: 218-26.
58. Calvert J, Burney P, Effect of body mass on exercise-induced bronchospasm and atopy in African children. The Journal of Allergy and Clinical Immunology 2005;116: 773-9.
59. Mahut B, Beydon N, Delclaux C, Overweight is not a comorbidity factor during childhood asthma: the GrowthOb study. The European Respiratory Journal 2012;39: 1120-6.
60. Cibella F, Cuttitta G, La Grutta S, Melis MR, Bucchieri S, Viegi G, A cross-sectional study assessing the relationship between BMI, asthma, atopy, and eNO among schoolchildren. Annals of Allergy, Asthma and Immunology : official publication of the American College of Allergy, Asthma, and Immunology 2011;107: 330-6.
61. Visness CM, London SJ, Daniels JL, Kaufman JS, Yeatts KB, Siega-Riz AM, Liu AH, Calatroni A, Zeldin DC, Association of obesity with IgE levels and allergy symptoms in children and adolescents: results from the National Health and Nutrition Examination Survey 2005-2006. The Journal of Allergy and Clinical Immunology 2009;123: 1163-9, 69.e1-4.
62. Visness CM, London SJ, Daniels JL, Kaufman JS, Yeatts KB, Siega-Riz AM, Calatroni A, Zeldin DC, Association of childhood obesity with atopic and nonatopic asthma: results from the National Health and Nutrition Examination Survey 1999-2006. The Journal of Asthma : official journal of the Association for the Care of Asthma 2010;47: 822-9.
63. Lynch NR, Hagel I, Perez M, Di Prisco MC, Lopez R, Alvarez N, Effect of anthelmintic

treatment on the allergic reactivity of children in a tropical slum. The Journal of Allergy and Clinical Immunology 1993;92: 404-11.

64. Farfel A, Tirosh A, Derazne E, Garty BZ, Afek A, Association between socioeconomic status and the prevalence of asthma. Annals of Allergy, Asthma and Immunology : official publication of the American College of Allergy, Asthma, and Immunology 2010;104: 490-5.

65. Corvalan C, Amigo H, Bustos P, Rona RJ, Socioeconomic risk factors for asthma in Chilean young adults. American Journal of Public Health 2005;95: 1375-81.

Supplementary material

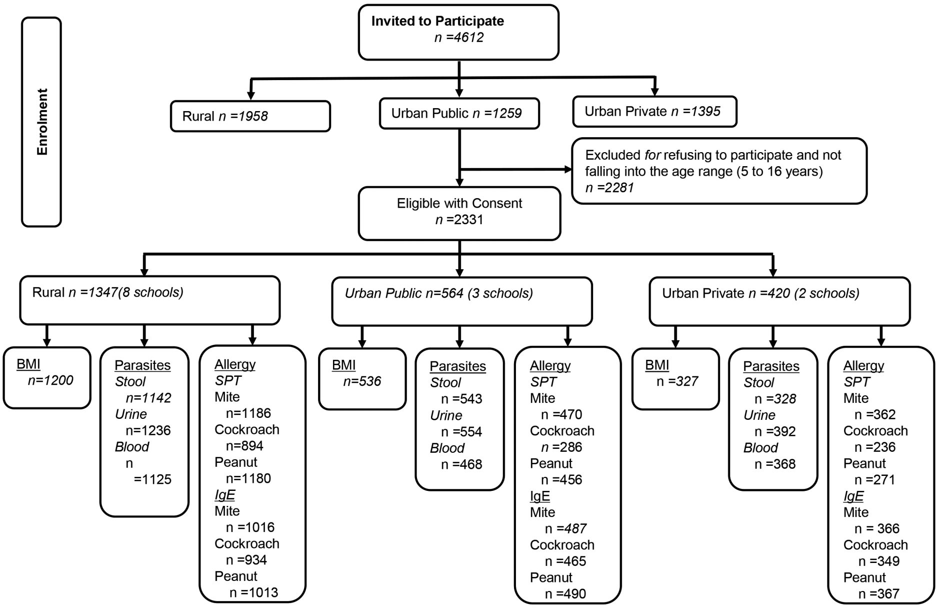

Figure S1: Study Flow Diagram

Study flow diagram detailing the number of participants targeted and the number who enrolled. Also shown is the breakdown of participants by the study parameters collected. Response rates were highest among rural schools (68.8%) and lowest in urban high SES schools (30.1%).

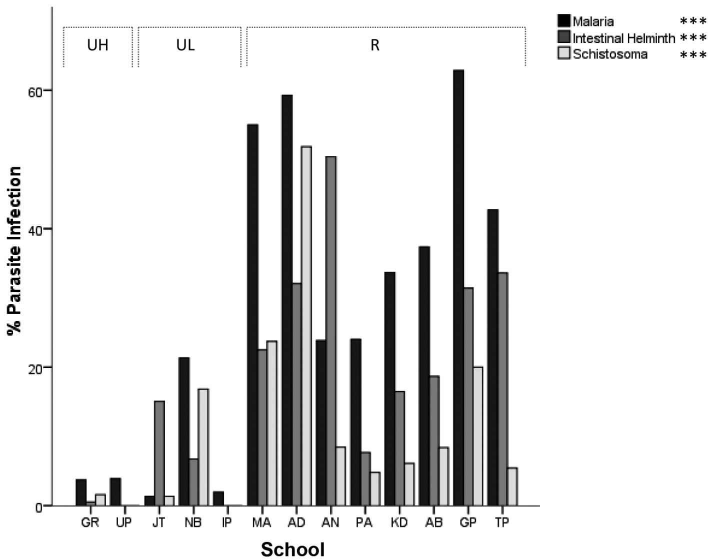

Figure S2: Parasite infection prevalence by school

Parasite infection rates in UH (Urban High), UL (Urban Low) and R (Rural) Schools. Bars represent the percentage positive rates.

P-values were calculated for χ^2 tests, * p<0.05, ** p<0.01, *** p<0.001.

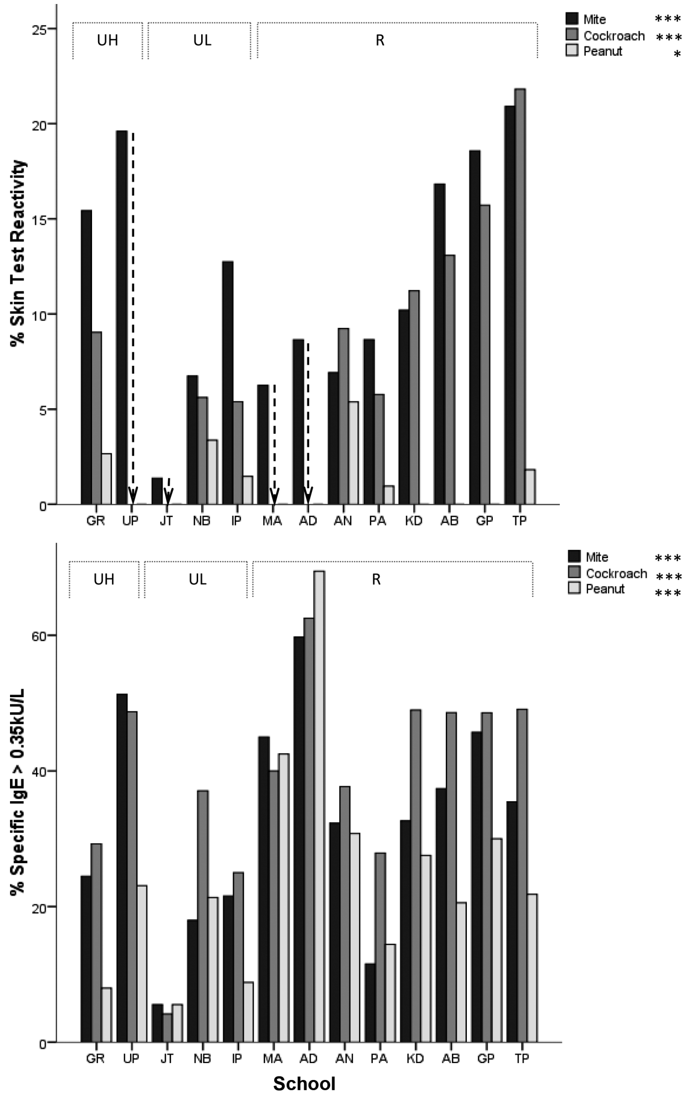

Figure S3: Atopy prevalence by school

Skin test reactivity and allergen specific IgE sensitization rates in UH (Urban-High), UL (Urban-Low) and R (Rural) Schools. Bars represent the percentage positive rates. Dashed arrows show schools for which cockroach skin test reactivity was not determined.

P-values were calculated for χ^2 tests, * p<0.05, ** p<0.01, *** p<0.001.

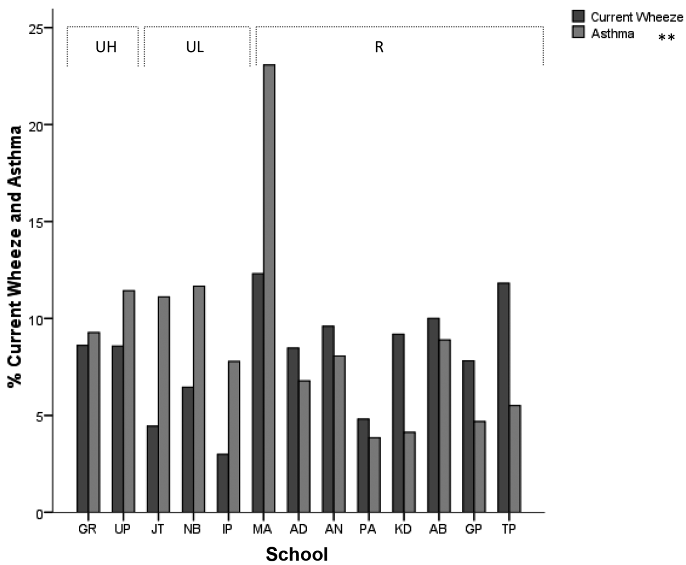

Figure S4: Reported current wheeze and asthma prevalence by school
Reported current wheeze and asthma rates in UH (Urban-High), UL (Urban-Low) and R (Rural) Schools. Bars represent the percentage positive rates.
P-values were calculated for χ^2 tests, * p<0.05, ** p<0.01, *** p<0.001.