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Amoah, A.S.

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Author: Amoah, Abena Serwaa

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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. Larbi², Dziedzom K. de Souza², Hae-Won Uh³, Montserrat Fernández-Rivas⁴, Ronald van Ree⁵, Laura C Rodrigues⁶, Daniel A. Boakye², Maria Yazdanbakhsh¹, Franca C. Hartgers¹

Affiliations:

¹Department of Parasitology, Leiden University Medical Center, Leiden, The Netherlands

²Department of Parasitology, Noguchi Memorial Institute for Medical Research, Accra, Ghana

³Department of Medical Statistics and Bioinformatics, Leiden University Medical Center, Leiden, The Netherlands

⁴Department of Allergy, Hospital Clinico San Carlos, Madrid, Spain

⁵Department of Experimental Immunology and Department of Otorhinolaryngology, Academic Medical Center, Amsterdam University, Amsterdam, The Netherlands

⁶Department of Infectious Disease Epidemiology, Faculty of Epidemiology and Population Health, London School of Hygiene and Tropical Medicine, London, United Kingdom

*Shared first authorship

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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

slgE: 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	<i>Schistosoma</i>	9.50%	SPT HDM	↓
			<i>Trichuris</i>	1.90%	SPT Cockroach	↑
			<i>Ascaris</i>	6.20%	SPT, Wheeze, Asthma	NS
			Hookworm	9.90%	SPT, Wheeze, Asthma	NS
Ghana Stevens et al., 2012 ^[8]	181	9 to 16	<i>Ascaris</i> IgE	52.3% ^{cases}	Asthma	NS
				36.6% ^{controls}	Asthma Urban Affluent	↑
Zimbabwe Rujeni et al., 2012 ^[17]	672	Up to 86	<i>Schistosoma</i>	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
			<i>Ascaris</i>	4.30%	SPT, Eczema	NS
			<i>Trichuris</i>	0.10%	SPT, Eczema	NS
Ecuador Endara et al., 2010 ^[18]	3901	6 to 16	Int. Helminth	86.20%	SPT	↓
			<i>Ascaris</i>	57.30%	SPT	NS
			<i>Trichuris</i>	81.50%	SPT	↓
			Hookworm	3.90%	SPT	NS
			<i>Onchocerca</i>	>40%	SPT, Eczema Wheeze, EIB	↑ NS
Indonesia Supali et al., 2010 ^[19]	442	12 to 76	Int. Helminth	43.70%	SPT	NS
			<i>B. malayi</i>	46.70%	SPT Cockroach	↓
			<i>Trichuris</i>	14.90%	SPT HDM	NS
			Hookworm	24.20%	SPT Grass	NS
			<i>Ascaris</i>	22.40%	SPT Grass	NS
Vietnam Flohr et al., 2010 ^[20]	1487	6 to 17	Hookworm [§]	65%	SPT Dpt	↓
			<i>Ascaris</i>	7%	SPT Dpt	NS
South Africa Calvert & Burney, 2010 ^[21]	749	8 to 12	<i>Ascaris</i>	34.60%	SPT, SPT Dpt	↓
					SPT Btr	NS
					EIB	↑
Cuba Wordemann et al., 2008 ^[22]	1320	4 to 14	<i>Trichuris</i>	46%	EIB	NS
			<i>Ascaris</i>	10%	AD	↓
			<i>E. vermicularis</i> ^{†‡}	22%	AD, AR	↑
			Hookworm [†]	3%	AR	↑
Brazil Rodrigues et al., 2008 ^[23]	1055	Up to 11	<i>Ascaris</i>	19.60%	Asthma, SPT	NS
					SPT	↓
			<i>Trichuris</i> ^{†‡}	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 pteronyssinus*; 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

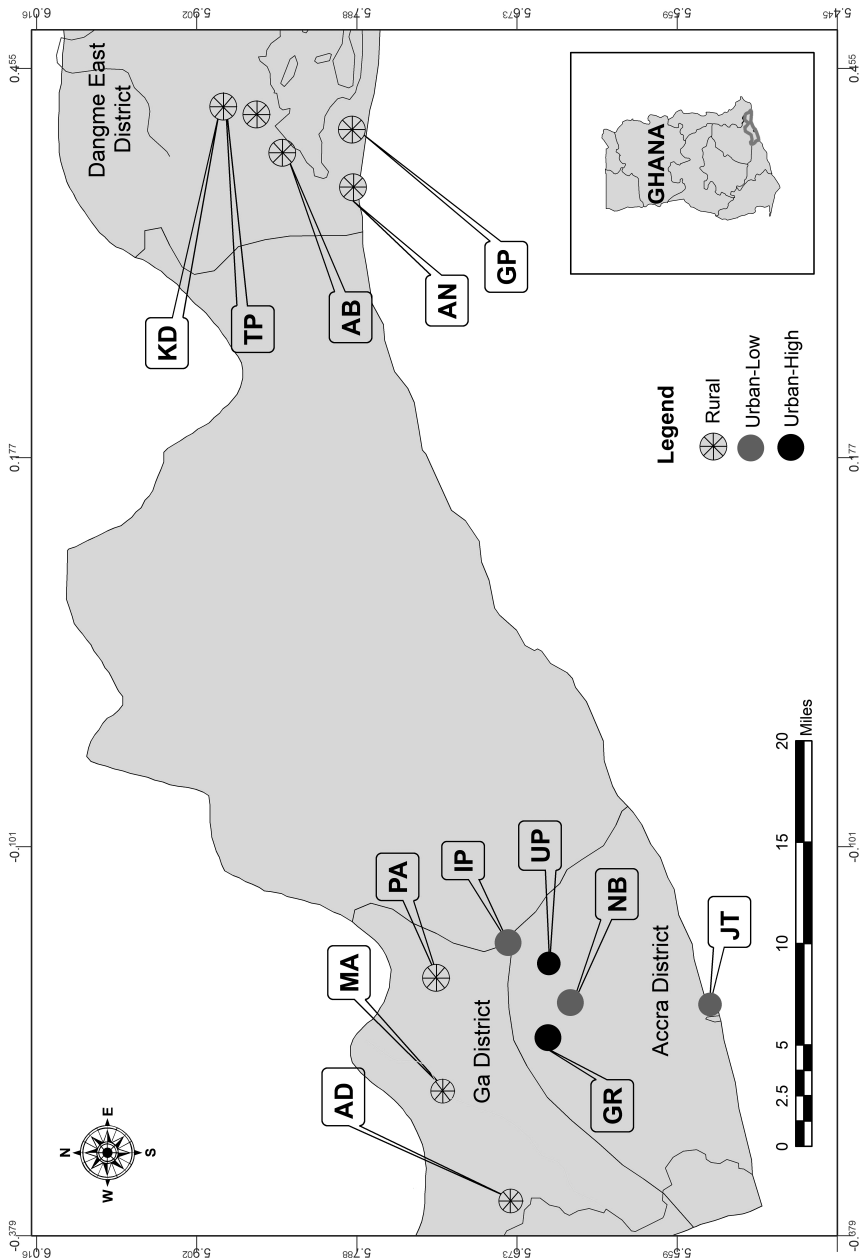


Figure 1: Map of the study area showing urban and rural schools. Map depicts the distribution of participating schools in the Ga, Accra and Dangme East districts of the Greater Accra region of Ghana.

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 pteronyssinus* - *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 ≥ 0.35 kU/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 pteronyssinus* (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 (*Blattella 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 through 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 slgE 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 $5\% / (7 \times 4) = 0.18\%$ 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.

Factor	Category n (%)				p-value
	UH = 239	UL = 366	R = 780	All = 1385	
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)	***
<i>Ascaris</i> spp.	0 (0)	7 (1.9)	79 (10.1)	86 (6.2)	***
<i>Trichuris</i> spp.	1 (0.4)	7 (1.9)	19 (2.4)	27 (1.9)	
<i>Schistosoma</i> 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)	***
slgE [‡]					
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 slgE ≥ 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 slgE was significantly

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

Factor	Mite			Cockroach		
	cOR[95% CI]	p-value	aOR[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
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
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
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
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
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
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
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
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
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

[§]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

Factor	Current Wheeze			Asthma		
	cOR[95% CI]	p-value	aOR[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
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
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
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
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
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
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
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
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
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
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
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
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

[§] 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-analysis, 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.

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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.

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Supplementary material

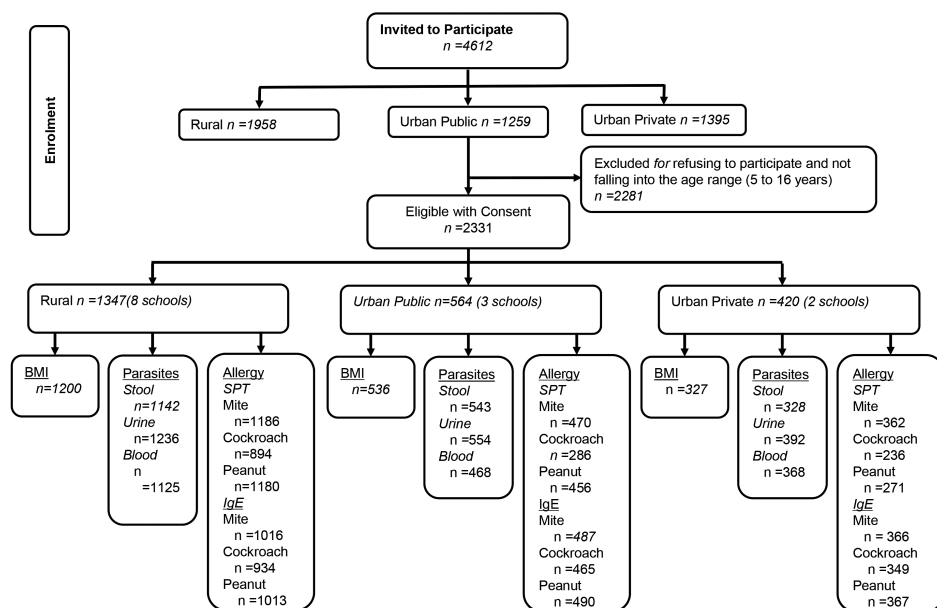


Figure S1: Study Flow Diagram

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%).

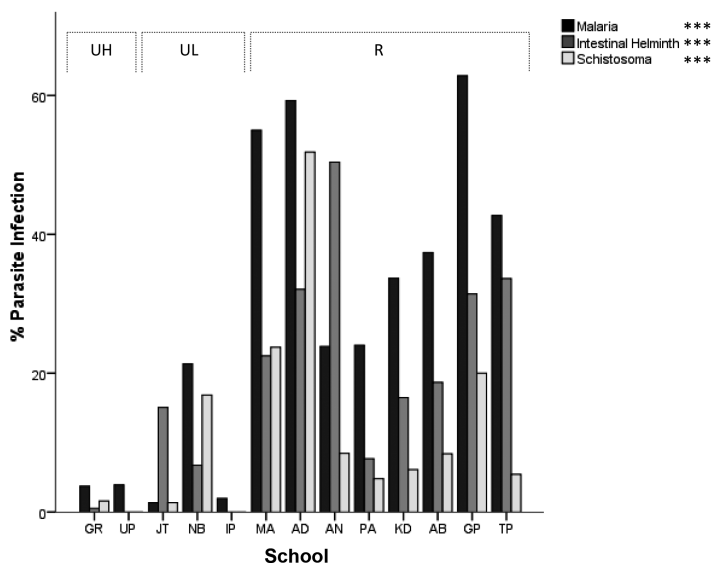


Figure S2: Parasite infection prevalence by school

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, * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$.

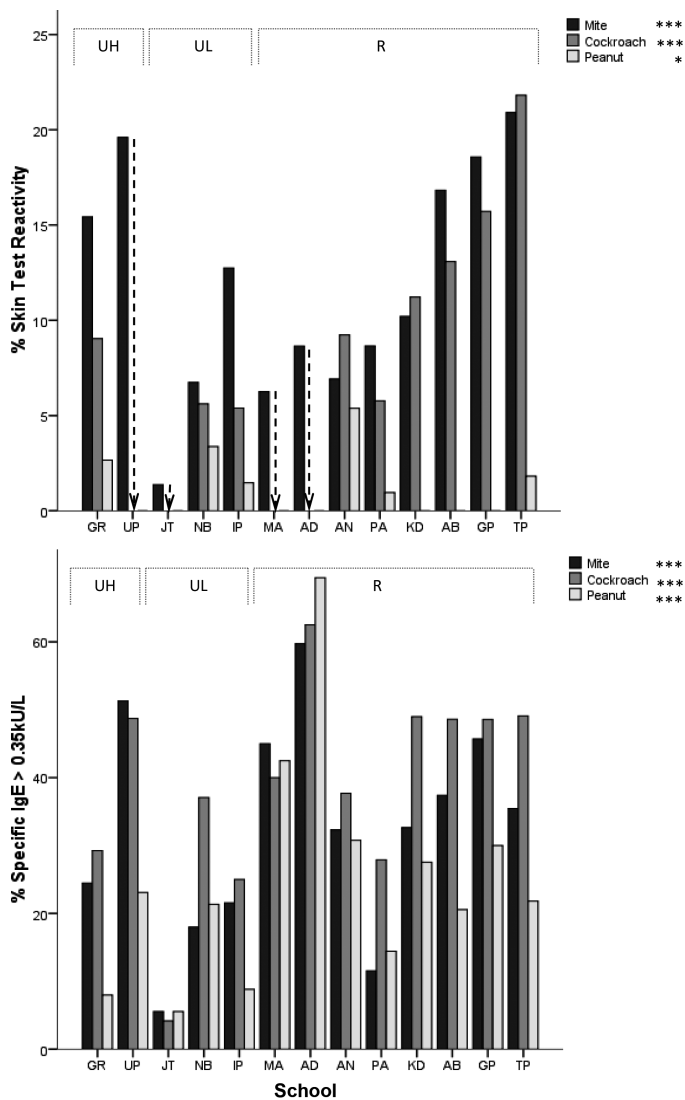


Figure S3: Atopy prevalence by school

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, * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$.

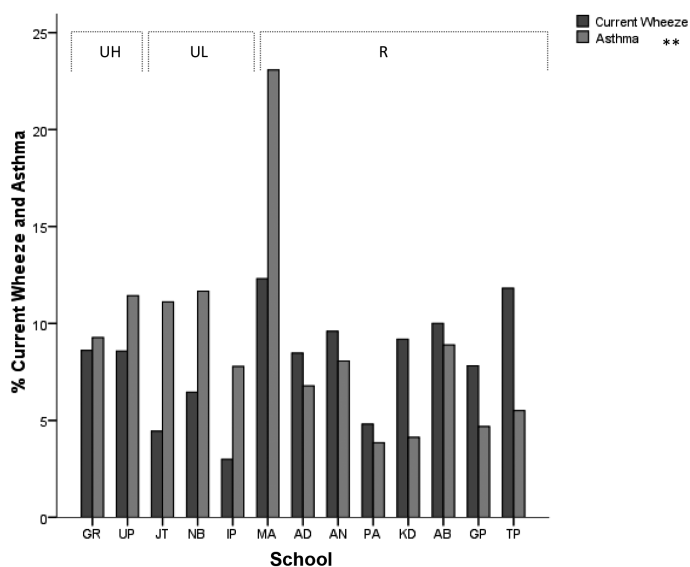


Figure S4: Reported current wheeze and asthma prevalence by school

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, * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$.

