Independent effects of intestinal parasite infection and domestic allergen exposure on risk of wheeze in Ethiopia: a nested case-control study

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Summary

Background Why asthma is rare in rural subsistence societies is not clear. We tested the hypotheses that the risk of asthma is reduced by intestinal parasites or hepatitis A infection, and increased by exposure to dust-mite allergen or organophosphorus insecticides in urban and rural areas of Jimma, Ethiopia.

Methods From 12 876 individuals who took part in a study of asthma and atopy in urban and rural Jimma in 1996, we identified all who reported wheeze in the previous 12 months, and a random subsample of controls. In 1999, we assessed parasites in faecal samples, Der p 1 levels in bedding, hepatitis A antibodies, serum cholinesterase (a marker of organophosphorus exposure), total and specific serum IgE, and skin sensitisation to *Dermatophagoides pteronyssinus* in 205 cases and 399 controls aged over 16 years. The effects of parasitosis, Der p 1 level, hepatitis A seropositivity, and cholinesterase concentration on risk of wheeze, and the role of IgE and skin sensitisation in these associations, were analysed by multiple logistic regression.

Findings The risk of wheeze was independently reduced by hookworm infection by an odds ratio of 0·48 (95% CI 0·24–0·93, p=0·03), increased in relation to Der p 1 level (odds ratio per quartile 1·26 [1·00–1·59], p=0·05), and was unrelated to hepatitis A seropositivity or cholinesterase concentration. In the urban population, *D pteronyssinus* skin sensitisation was more strongly related to wheeze (9·45 [5·03–17·75]) than in the rural areas (1·95 [0·58–6·61], p for interaction=0·017), where *D pteronyssinus* sensitisation was common, but unrelated to wheeze in the presence of high-intensity parasite infection.

Interpretation High degrees of parasite infection might prevent asthma symptoms in atopic individuals.

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Introduction

Asthma is rare in rural subsistence societies but becomes much more prevalent as populations become urbanised and affluent.[1–6] Although the explanation for this fact is not known, one feature that could be important is the lower prevalence of infection with intestinal parasites in more affluent populations, since parasite infection has been postulated to prevent IgE-mediated allergic disease by blocking effector-cell IgE receptors with parasite-induced specific and polyclonal IgE,[7,8] or by production of the anti-inflammatory cytokine interleukin 10.[9] Other potential explanations include increased exposure to housedust-mite allergen arising from the adoption of housing and bedding styles that favour dust-mite replication,[10,11] or the effect of the more hygienic affluent lifestyle in reducing childhood infections with agents such as hepatitis A virus,[12] resulting in the programming of T helper cells in early life to produce a more allergic phenotype.[13] Exposure to organophosphorus insecticides has also been implicated in asthma aetiology, possibly by increasing muscarinic effects in the airway.[14]

From a previous study in Jimma, Ethiopia, we reported that the prevalence of self-reported wheeze in the previous 12 months was much lower in rural subsistence areas (1·2%) than in the urban population (3·7%).[4] We also noted that allergen skin sensitisation to *Dermatophagoides pteronyssinus*, a strong determinant of wheeze in the urban area, was generally more common and was unrelated to wheeze in the rural population. Various urban lifestyle factors were associated with an increased risk of wheeze or allergen skin sensitisation, including the use of synthetic mattresses and malathion insecticide.[4] We now report a nested case-control study in the same study population with objective measures to test hypotheses relating to the roles of parasite infection and exposure to dust-mite allergen, hepatitis A virus, and organophosphorus insecticides in determining the risk of wheeze and allergen skin sensitisation in individuals aged 16 or over in urban and rural areas of Jimma.

Methods

Study population

In 1996, we obtained cross-sectional data for respiratory symptoms and lifestyle factors in 9844 people in urban households and 3032 in rural households in Jimma.[4] Cases for this study were the 311 individuals who at the time of the 1996 survey were aged 14 or over and reported wheezing in their chest in the previous 12 months. Controls were all those who had not already been identified as cases in a random subsample of 570 people aged 14 or over from the full 1996 study population. This complete random subsample was also used in this study to define the distributions of exposure variables in the Jimma population, and for cross-sectional analyses of risk factors for allergen skin sensitisation. Approval for the study was granted by ethics committees in Jimma and Nottingham.

Design and procedures

Local health-workers contacted eligible cases and controls at home and arranged for a fieldwork team to visit all those who consented to take part during the dry season months of January to June, 1999. At each visit, the person's identity and oral consent were confirmed, the respiratory and lifestyle questionnaire used in 1996 was administered by an interviewer, a venous blood sample was taken, and instructions were given for collection of a faecal sample for parasite estimation. Allergen skin sensitivity to *D pteronyssinus*, cockroach, cat fur, dog dander, aspergillus mould mix, mixed cereals, and grass mix (Biodiagnostics, Upton-upon-Severn, UK) was measured by means of skin-prick lancets (Biodiagnostics). A positive test was defined as an average of two perpendicular wheal diameters, one of which was the maximum measurable diameter of at least 3 mm greater than the saline control response. Dust was sampled from the participant's sleeping area by 2 min suction through an ALK-Abello dust filter (Horsholm, Denmark) by a 1·2 kW vacuum cleaner powered by a petrol generator.

Serum samples were separated within 8 h of collection and stored at –20°C. Faecal samples were analysed for parasite eggs qualitatively and quantitatively in terms of eggs per gram of faeces by formalin-ether sedimentation.[15] Dust samples containing more than 25 mg dust were analysed by a standard ELISA (Indoor Biotechnologies, Chester, UK) for dust mite (Der p 1), cat (Fel d 1), cockroach (Bla g 2), and dog (Can f 1) allergens, quantified as micrograms of allergen per gram of dust. IgE assays were done with a monoclonal mouse antibody to human IgE as capture antibody (clone GE-1, Sigma, Poole, UK).[16] The capture antigen used to assay specific IgE to *Ascaris lumbricoides* was pseudoceolomic fluid prepared by drainage from the parasite; that for *Necator americanus* was a soluble extract of whole worm homogenate; and that for trichuris was derived from excretory/secretory fluid from *Trichuris muris*.[17] Der p 1 antigen was purified from a crude mite extract (Indoor Biotechnologies, Cardiff, UK). Serum cholinesterase was measured as a marker of organophosphorus exposure with an Olympus AU600 analyser (Olympus Diagnostic Systems, Southall, Middlesex, UK). Total hepatitis A antibodies were measured by a qualitative ELISA (bioelisa HAV, Biokit, Barcelona, Spain).

Statistical analysis

Data were analysed with SPSS-pc (version 9). Dependent on the availability of adequate numbers within categories, explanatory variables were analysed in quartiles or tertiles, defined by their distribution in the random population subsample. Serum cholinesterase activities, concentrations of total and specific IgE, and amounts of allergen in dust samples were analysed in quartiles, with dust samples of less than 25 mg dust treated as missing. Stool samples positive for parasite egg per gram were analysed qualitatively as positive or negative, and quantitatively within the positive samples in tertiles of eggs per gram. Hepatitis A seropositivity was analysed as a binary variable. Descriptive analyses of exposure variables were done with Mann-Whitney *U* tests, Kruskal-Wallis tests, χ^2 tests, and Spearman's rank correlation as appropriate.

The effects of intestinal parasite infection, dust allergen exposure, serum cholinesterase, and hepatitis A seropositivity on the relative odds of wheeze were estimated by multiple logistic regression, with adjustment for age (in four age-groups), sex, and urban or rural residence. Separate urban and rural odds ratios, adjusted for age and sex, were estimated if there was a significant difference in effect between the urban and rural populations. The independence of exposures identified as significant was explored by mutual adjustment, and the effects of potential confounding by educational status, occupation type, smoking, vaccination, crowding in the home, housing style, and indoor kerosene use were also explored. We also investigated the role of total and specific IgE and allergen skin sensitisation as possible mechanisms for recorded effects. Since the case definition relied on wheeze status reported in 1996, we also repeated the analyses in the subgroup who replied consistently positively or negatively to wheeze in the previous 12 months in both 1996 and 1999. Relative odds of skin sensitisation to *D pteronyssinus* in relation to intestinal parasitosis and dust allergen exposure were estimated by similar methods in the random subsample.

We aimed to study all 311 available cases from the 1996 study, and a random sample of 570 individuals from the total 1996 study population, of whom we expected about 20 individuals to have been identified as cases and the remaining 550 to be eligible as controls. With the assumptions of an 80% response rate, that all exposures could at least be dichotomised into binary variables, and that the prevalence of exposures would be about 50%, the study had power of at least 80% to detect odds ratios of 1·6 and 90% for odds ratios of 1·7 at 5% significance.

Results

205 cases and 399 controls, 71% of those selected, took part in the study. 7% (60 of 855) of those selected had died, were too ill, or refused to take part, 9% (79 of 855) had moved from the area, and 13% (112 of 855) could not be contacted. Cases were predominantly from the urban area, were older than controls, and the proportion who were male was higher. Urban participants generally had higher educational attainment than those in rural areas. Less than 6% of the population were current smokers (table 1). The random population subsample consisted of all 399 controls and 18 of the cases.

Hepatitis A serology was tested and serum cholinesterase concentrations were measured in 93% (560 of 604) of participants. Hepatitis A seropositivity was equally common in urban and rural areas (89% [380 of 427] and 83% [110 of 133]), and was unrelated to wheeze (odds ratio adjusted for age, sex, urban-rural residence 1·44 [95% CI 0·79–2·61]). Serum cholinesterase concentrations were significantly higher in the urban area than in the rural area and higher in participants with than in those without self-reported insecticide use (Mann-Whitney p=0·006), but they were unrelated to wheeze (adjusted odds ratio per quartile 1·07 [0·90–1·27]).

Faecal samples for parasite estimation were available from 95% (572 of 604) of participants. *T trichiura* was present in 65% (371 of 572) of individuals (table 1), with no difference in prevalence between urban and rural areas, and its presence was unrelated to wheeze (table 2). *A lumbricoides* was present in 40% (228 of 572) of participants and was slightly more common in the rural than the urban area (table 1, p=0·08); it was associated with a reduced risk of wheeze (odds ratio adjusted for age, sex, and urban-rural residence 0·59 [0·40–0·88]) but with no evidence that this effect was intensity-related (table 2). Hookworm (predominantly *N americanus)* was present in 24% (140 of 572) of individuals and was more common in rural than urban areas (table 1); it was associated with a significant reduction in the risk of wheeze (odds ratio adjusted for age, sex, and urban-rural residence 0·48 [0·29–0·82]), which seemed to be related to the intensity of infection (table 2), though not significantly so.

	Cases		Controls	
	Urban (n=190)	Rural (n=15)	Urban (n=277)	Rural (n=122)
Age (years)				
16–30	37 (20%)	6 (40%)	142 (51%)	55 (45%)
31–45	66 (35%)	3 (20%)	67 (24%)	29 (24%)
46–60	54 (28%)	3 (20%)	40 (14%)	29 (24%)
>60	33 (17%)	3 (20%)	28 (10%)	9 (7%)
Men/women	86 (45%)/104 (55%)	11 (73%)/4 (27%)	96 (35%)/181 (65%)	63 (52%)/59 (48%)
Educational attainment*				
Higher education	6 (3%)	0	11 (4%)	1 (1%)
Grades 7–12	59 (31%)	1 (7%)	136 (49%)	9 (7%)
Grades 1–6	59 (31%)	5 (33%)	61 (22%)	33 (27%)
No education	66 (35%)	9 (60%)	69 (25%)	79 (65%)
Current smokers	10 (5%)	1 (7%)	11 (4%)	12 (10%)
Parasites†				
T trichiura	109 (63%)	9 (60%)	182 (69%)	71 (59%)
A lumbricoides	53 (31%)	6 (40%)	112 (42%)	57 (47%)
Hookworm	18 (10%)	6 (40%)	56 (21%)	60 (50%)
Schistosoma mansoni	15 (9%)	0	37 (14%)	1 (1%)
Dust allergens (median [IQR]) μg/g				
Der p 1	3·38 (1·52–9·94)	0·74 (0·42–2·16)	2·32 (1·20–4·94)	0 (0–1·04)
Fel d 1	1·19 (0–4·71)	0 (0–0·27)	0·46 (0–1·92)	0
Bla g 2	0·12 (0–0·26)	0·06 (0–0·16)	0 (0–0·24)	0·19 (0–0·41)
Can f 1	0·45 (0–0·81)	0 (0–0·97)	0·31 (0–0·80)	0·63 (0–1·05)
IgE (median [IQR])				
Total (IU/L)	2086 (802–3776)	3742 (3014–3988)	1942 (615–3608)	3031 (2318–3690)
Anti–trichuris (AU)	573 (254–1079)	799 (502–1272)	617 (270–1284)	531 (278–1267)
Anti–Ascaris (AU)	366 (129–937)	437 (245–1261)	332 (104–887)	479 (188–1053)
Anti–Necator (AU)	350 (91–821)	1336 (508–2976)	348 (104–939)	1230 (498–2257)
Anti–Der P 1 (AU)	82 (26–374)	48 (24–110)	38 (12–93)	40 (22–101)
Allergen skin sensitivity				
Dust mite	75 (40%)	6 (40%)	14 (5%)	25 (21%)
Cockroach	7 (4%)	1 (7%)	3 (1%)	7 (6%)
Aspergillus and mould mix	2 (1%)	1 (7%)	3 (1%)	1 (1%)
Cat	3 (2%)	1 (7%)	2 (1%)	0
Any positive skin test‡	77 (41%)	7 (47%)	24 (9%)	31 (25%)

AU=arbitrary units. *Grades 7–12 are equivalent to secondary school education in the UK; grades 1–6 to primary school education. †Any eggs detected in stool sample. ‡Includes total of five or fewer positive reactions to dog dander, mixed cereals, or mixed grasses. Data are number (%) individuals unless otherwise indicated.

Table 1: **Baseline characteristics of cases and controls and distributions of exposures**

S mansoni infection was present in only 9% of individuals, almost exclusively from one urban district, and was unrelated to the risk of wheeze (table 2). *Strongyloides stercoralis*, *Enterobius vermicularis*, *Taenia* spp, and *Hymenolepis nana* were present in very few individuals and were not analysed further.

Dust samples of 25 mg or more were available for 59% (358 of 604) of participants (50% [234 of 467] urban, 90% [124 of 137] rural). Dust contents of Der p 1 and Fel d 1 were both significantly higher in the urban than in the rural area (table 1) and in users of synthetic rather than natural mattresses (Mann-Whitney p<0·001). The odds of wheeze adjusted for age, sex, and urban-rural residence increased significantly in relation to the amounts of both Der p 1 and Fel d 1 in dust (table 3), and, although after mutual adjustment neither trend was

Parasite	Range (eggs/g)	Cases		Controls		Adjusted odds ratio (95% CI)*	p†
		Urban (n=172)	Rural (n=15)	Urban (n=264)	Rural (n=121)		
Trichuris							
No eggs	0	63 (37%)	6 (40%)	82 (31%)	50 (41%)	1·00	0·41
Tertile 1	<13·51	34 (20%)	6 (40%)	47 (18%)	36 (30%)	0·97 (0·57–1·64)	
Tertile 2	13·51–55·37	35 (20%)	2 (13%)	66 (25%)	21 (17%)	0·68 (0·40–1·14)	
Tertile 3	55·38–2067	40 (23%)	1 (7%)	69 (26%)	14 (12%)	0·75 (0·45–1·26)	
Ascaris							
No eggs	0	119 (69%)	9 (60%)	152 (58%)	64 (53%)	1·00	0·02
Tertile 1	<23·87	14 (8%)	–	44 (17%)	13 (11%)	0·35 (0·18–0·69)	
Tertile 2	23·87–95·18	20 (12%)	3 (20%)	37 (14%)	17 (14%)	0·79 (0·44–1·43)	
Tertile 3	95·19–2408	19 (11%)	3 (20%)	31 (12%)	27 (22%)	0·69 (0·38–1·25)	
Hookworm							
No eggs	0	154 (90%)	9 (60%)	208 (79%)	61 (50%)	1·00	0·04
Tertile 1	<5·79	6 (3%)	3 (20%)	24 (9%)	15 (12%)	0·54 (0·25–1·21)	
Tertile 2	5·80–48·87	10 (6%)	0	18 (7%)	20 (17%)	0·60 (0·28–1·31)	
Tertile 3	48·88–1458	2 (1%)	3 (20%)	14 (5%)	25 (21%)	0·30 (0·11–0·82)	
Schistosoma‡							
No eggs	0	157 (91%)	15 (100%)	227 (86%)	120 (99%)	1·00	0·50
Eggs detected	0·01–109	15 (9%%)	0	37 (14%%)	1(1%%)	0·80 (0·41–1·55)	

*Adjusted for age, sex, and urban or rural residence. †For groups as unordered categories. ‡Number of positive samples too small for division into teritles.

Table 2: **Prevalence of parasite eggs in stool samples in case-control groups, and odds ratios for wheeze**

Allergen category	Range (μg/g)	Cases		Controls		Adjusted odds ratio 95% CI	p†	p trend‡	Mutually adjusted odds ratio (95% CI)*	p†	p trend‡
		Urban (n=94)	Rural (n=14)	Urban (n=140)	Rural (n=110)						
Der p 1											
1	<1·01	15 (16%)	8 (57%)	30 (21%)	82 (75%)	1·00	0·05	0·01	1·00	0·22	0·07
2	1·01–2·17	15 (16%)	3 (21%)	36 (26%)	9 (8%)	1·14 (0·50–2·56)			1·13 (0·50–2·58)		
3	2·18–4·52	22(23%)	2 (14%)	38 (27%)	8 (7%)	1·17 (0·54–2·52)			1·10 (0·50–2·42)		
4	>4·52	42(45%)	1 (7%)	36 (26%)	11 (10%)	2·36 (1·16–4·80)			2·00 (0·94–4·24)		
Fel d 1											
1	<0·45	35 (37%)	12 (86%)	68 (49%)	92 (84%)	1·00	0·06	0·02	1·00	0·26	0·12
2	0·45–0·94	10 (11%)	1 (7%)	26 (19%)	4 (4%)	0·85 (0·37–1·93)			0·79 (0·34–1·82)		
3	0·95–3·88	22 (23%)	1 (7%)	23 (16%)	8 (7%)	2·09 (1·03–4·23)			1·76 (0·84–3·67)		
4	>3·88	27 (29%)	0	23 (16%)	6 (5%)	1·86 (0·93–3·70)			1·48 (0·71–3·06)		
Bla g 2											
1	<0·18	60 (64%)	11 (79%)	94 (67%)	54 (49%)	1·00	0·57	0·28	..	..	
2	0·18–0·29	17 (18%)	1 (7%)	19 (14%)	14 (13%)	1·16 (0·57–2·37)			..	..	
3	0·30–0·57	10 (11%)	1 (7%)	11 (8%)	23 (21%)	0·89 (0·39–2·03)			..	..	
4	>0·57	7 (7%)	1 (7%)	16 (11%)	19 (17%)	0·57 (0·24–1·36)			..	..	
Can f 1											
1	<0·53	54 (57%)	9 (64%)	86 (61%)	47 (43%)	1·00	1·00	0·83	..	..	
2	0·53–0·79	17 (18%)	1 (7%)	19 (14%)	19 (17%)	0·97 (0·48–1·94)			..	..	
3	0·80–1·12	10 (11%)	2 (14%)	13 (9%)	27 (25%)	0·92 (0·42–2·03)			..	..	
4	>1·12	13 (14%)	2 (14%)	22 (16%)	17 (16%)	0·94 (0·46–1·93)			..	..	

*Odds ratio adjusted for age, sex, urban or rural residence, and Der p 1 or Fel d 1 exposure. †for groups as unordered categories. ‡for trend across categories.

Table 3: **Distribution of dust allergens and odds ratios for wheeze**

independently significant, the mutually adjusted effect of Der p 1 was stronger than that of Fel d 1 and remained on the borderline of significance (p=0·07, table 3). The use of synthetic mattresses was associated with increased odds of wheeze (odds ratio adjusted for age, sex, and urban-rural residence 1·80 [0·98–3·28]), which was reduced to 1·49 (0·79–2·81) by adjustment for Der p 1 content. Amounts of Bla g 2 and Can f 1 were significantly higher in the rural than in the urban areas, but neither was associated with wheeze.

In multivariate analysis of the 347 individuals with complete data on hookworm, ascaris, and dust Der p 1 content, the estimated effects of these variables were not appreciably changed by mutual adjustment, though only the effects of hookworm infection (adjusted odds ratio 0·48 [0·24–0·93], p =0·03) and Der p 1 allergen content (adjusted odds ratio per quartile 1·26 [1·00–1·59], p=0·05) were independently significant. Adjustment for educational attainment, occupation, crowding in the home, housing style, vaccination, and smoking had very little effect on these odds ratios. In view of results from our 1996 study,[18] we also explored the effect of adjustment for domestic kerosene use. However, only 17 individuals reported kerosene exposure, and adjustment for this variable had no effect on our findings. Restriction of analysis to the 128 cases and 358 controls with consistent case-control status in both 1996 and 1999 produced similar estimates to those in the full 1999 dataset.

The odds ratio for wheeze adjusted for age and sex in the urban relative to the rural population in participants with complete data on parasites and Der p 1 content was 5·25 (2·77–9·95). Adjustment for Der p 1 content reduced this ratio to 3·95 (1·99–7·84) and adjustment for hookworm infestation lowered it to 4·25 (2·20–8·22), whereas adjustment for the presence of ascaris had little effect (5·24 [2·76–9·97]). With adjustment for all these factors the odds ratio was reduced, but remained significant at 3·33 (1·65–6·73).

Total IgE concentrations were higher in participants living in rural areas than in urban residents (table 1, Mann-Whitney p<0·001), and were positively correlated with the total number of parasite eggs per g in faeces (r=0·18, p<0·001). There was no association between total IgE and wheeze. Concentrations of specific IgE to trichuris were similar in urban and rural areas, whereas concentrations of IgE to necator and ascaris were significantly higher (p<0·001) in the rural areas (table 1); concentrations of all three were significantly correlated with eggs per g of faeces for each respective parasite (p<0·001). There was no significant association between any parasite-specific IgE and wheeze. Concentrations of specific Der p 1 IgE were similar in urban controls, rural controls, and rural cases, but were significantly higher in urban cases (table 1, Kruskal-Wallis p<0·001). Concentrations of Der p 1 IgE were significantly associated with an increased risk of wheeze (adjusted odds ratio per quartile 1·51 [1·27–1·79], p<0·001) and were directly correlated with amounts of Der p 1 in dust samples (r=0·13, p=0·01).

Positive allergen skin tests were recorded in over 40% (77 of 190 and 7 of 15) of urban and rural cases, 25% (31 of 122) of rural controls, and 9% (24 of 277) of urban controls (table 1). 86% (119 of 139) of individuals with any positive allergen skin test were sensitised to *D pteronyssinus* and 13% (18 of 139) to cockroach. Responses to the other allergens were rare. Allergen skin sensitisation to *D pteronyssinus* was associated with a significantly greater risk of wheeze in the urban (odds ratio adjusted for age and sex 9·45 [5·03–17·75]) than in the rural area (1·95 [0·58–6·61], p for interaction 0·017). This difference was not attributable to the higher amounts of Der p 1 in the urban area, since among participants with dust allergen data, the odds ratios adjusted for age and sex for wheeze in relation to a positive *D pteronyssinus* skin test in the urban and rural areas were respectively 5·92 (2·57–13·63) and 2·31 (0·67–7·96), and those after adjustment for Der p 1 exposure were 5·93 (2·55–13·76) and 2·22 (0·64–7·70).

In the random population subsample there was no significant association between *D pteronyssinus* skin sensitivity and total serum IgE or specific IgE to trichuris, ascaris, or necator. Specific IgE to schistosomes was not measured in view of the small number of infected individuals. *D pteronyssinus* skin sensitisation was directly related to specific Der p 1 IgE amounts (odds ratio adjusted for age, sex, and urban-rural residence for a positive skin test per quartile of Der p 1 specific IgE 1·41 [1·03–1·93], p=0·03, table 4). This association seemed

IgE quartiles	Range	*D Pteronyssinus* sensitised		Not *D Pteronyssinus* sensitised		Adjusted odds ratio (95% CI)	p*	p trend†
		Urban (n=18)	Rural (n=25)	Urban (n=254)	Rural (n=99)			
Total IgE (IU/L)								
1	<1020	4 (22%)	1 (4%)	87 (34%)	7 (7%)	1·00	0·52	0·74
2	1020–2606	7 (39%)	7 (28%)	63 (25%)	22 (22%)	2·31 (0·76–7·07)		
3	2607–3663	2 (11%)	11(44%)	46 (18%)	40 (40%)	1·65 (0·52–5·29)		
4	>3663	5 (28%)	6 (24%)	58 (23%)	30 (30%)	1·63 (0·51–5·19)		
Trichuris IgE (AU)								
1	<277	4 (22%)	7 (28%)	65 (26%)	23 (23%)	1·00	0·85	0·55
2	277–595	4 (22%)	7 (28%)	61 (24%)	27 (27%)	1·02 (0·40–2.60)		
3	596–1244	4 (22%)	4 (16%)	65 (26%)	26 (26%)	0·91 (0·33–2·51)		
4	>1244	6 (33%)	7 (28%)	63 (25%)	23 (23%)	1·36 (0·55–3·38)		
Ascaris IgE (AU)								
1	<135	4 (22%)	4 (16%)	76 (30%)	15 (15%)	1·00	0·60	0·23
2	135–373	4 (22%)	4 (16%)	66 (26%)	25 (25%)	1·05 (0·36–3·09)		
3	374–931	5 (28%)	7 (28%)	53 (21%)	34 (34%)	1·17 (0·42–3·21)		
4	>931	5 (28%)	10 (40%)	59 (23%)	25 (25%)	1·78 (0·67–4·73)		
Necator IgE (AU)								
1	<152	4 (22%)	2 (8%)	85 (34%)	8 (8%)	1·00	0·49	0·83
2	152–528	6 (33%)	5 (20%)	72 (28%)	16 (16%)	1·82 (0·62–5·38)		
3	529–1227	5 (28%)	8 (32%)	65 (26%)	21 (21%)	1·64 (0·56–4·85)		
4	>1227	3 (17%)	10 (40%)	32 (13%)	54 (55%)	1.00 (0·31–3·24)		
Der p 1 IgE (AU)								
1	<15·4	4 (22%)	2 (8%)	77 (30%)	16 (16%)	1·00	0·18	0·03
2	15·4–41	0	11 (44%)	56 (22%)	32 (32%)	1·53 (0·51–4·62)		
3	41–99	4 (22%)	6 (24%)	64 (25%)	25 (25%)	1.67 (0·55–5·03)		
4	>99	10 (56%)	6 (24%)	57 (22%)	26 (26%)	2·97 (1·05–8·39)		

AU=arbitrary units. *p value for groups as unordered categories. †p value for trend across categories.

Table 4: **Relation between IgE concentrations and allergen skin sensitisation to *D pteronyssinus* in the random subsample**

stronger in the urban than in the rural population, though not to the point of significance (p for interaction 0·07).

There was no evidence of an association between *D pteronyssinus* skin sensitisation and dust allergen exposure (odds ratio adjusted for age, sex, and urban-rural residence per quartile of exposure 0·97 [0·68–1·39]). There was also no evidence of an association between *D pteronyssinus* skin sensitisation and parasite infection in the urban area, though in the rural area the risk of *D pteronyssinus* skin sensitisation was consistently highest in individuals in the highest tertile of infection intensity, especially for trichuris (table 5).

Almost all urban participants with hookworm infection had egg counts in the first two quartiles (ie, below the median), and in these individuals the relation between *D pteronyssinus* skin sensitisation and wheeze was strong (odds ratio adjusted for age and sex 8·13 [4·24–15·57], p<0·001). Among rural participants with hookworm infection below the median value, *D pteronyssinus* skin sensitisation was also associated with an increased risk of wheeze of borderline significance (odds ratio adjusted for age and sex 3·97 [0·93–16·98], p=0·06) which did not differ from the relation seen in urban participants (p for interaction=0·16). In rural participants with hookworm egg counts above the median value there was no association between *D pteronyssinus* skin sensitisation and wheeze (odds ratio adjusted for age and sex 0·63 [0·03–11·45]). There were too few urban participants with this degree of hookworm infection for us to estimate an equivalent odds ratio. Findings for ascaris and trichuris were broadly similar. When total parasite exposure was analysed in groups based on a pooled estimate of total eggs per g faeces, there was a significant interaction (p=0·05) such that the effect of *D pteronyssinus* sensitisation on the risk of wheeze was greatly decreased with increasing intensity of parasite

Parasite	Range (eggs/g)	Sensitised		Not sensitised		Adjusted odds ratio (95% CI): urban	p*	Adjusted odds ratio (95% CI): rural	p*
		Urban (n=19)	Rural (n=24)	Urban (n=260)	Rural (n=100)				
Trichuris									
No eggs	0	7 (37%)	7 (29%)	80 (31%)	45 (45%)	1·00	0·32	1·00	0·01
Tertile 1	<13·51	2 (11%)	4 (17%)	49 (19%)	33 (33%)	0·50 (0·10–2·62)		0·85 (0·21–3·46)	
Tertile 2	13·51–55·37	2 (11%)	6 (25%)	65 (25%)	15 (15%)	0·29 (0·05–1·51)		2·88 (0·76–10·95)	
Tertile 3	>55·38–2067	8 (42%)	7 (29%)	66 (25%)	7 (7%)	1·16 (0·38–3·55)		8·16 (1·94–34·34)	
Ascaris									
No eggs	0	9 (47%)	11 (46%)	151 (58%)	54 (54%)	1·00	0·22	1·00	0·42
Tertile 1	<23·87	5 (26%)	2 (8%)	41 (16%)	11 (11%)	1·64 (0·48–5·63)		1·27 (0·23–7·07)	
Tertile 2	23·87–95·18	4 (21%)	3 (13%)	36 (14%)	16 (16%)	2·51 (0·66–9·50)		1·19 (0·27–5·31)	
Tertile 3	95·19–2408	1 (5%)	8 (33%)	32 (12%)	19 (19%)	0·24 (0·03–2·15)		2·63 (0·83–8·30)	
Hookworm									
No eggs	0	17 (89%)	9 (38%)	205 (79%)	55 (55%)	1·00	0·85	1·00	0·23
Tertile 1	<5·79	2 (11%)	3 (13%)	22 (9%)	12 (12%)	2·02 (0·40–10·32)		1·02 (0·20–5·15)	
Tertile 2	5·80–48·87	0	5 (21%)	19 (7%)	15 (15%)	··†		2·33 (0·61–8·83)	
Tertile 3	48·88–1458	0	7 (29%)	14 (5%)	18 (18%)	··†		3·41 (0·96–12·10)	
Schistosoma‡									
No eggs	0	16 (84%)	24 (100%)	224 (86%)	99 (99%)	1·00			
Any eggs	0·1–109	3 (16%)	0	36 (14%)	1 (1%)	1·78 (0·45–6·98)	0·41	··†	

*p value for groups as unordered categories. †Available numbers too small to calculate estimates. ‡Number of positive samples too small for division into tertiles.

Table 5: **Relation between parasite eggs per gram and allergen skin sensitisation to *D pteronyssinus* in the random subsample**

Range of total parasite eggs per gram	Adjusted odds ratio for combined urban and rural (95% CI)*	Adjusted odds ratio for urban (95% CI)†	Adjusted odds ratio for rural (95% CI)‡
0	13·87 (4·26–45·13)	27·08 (5·77–127)	··§
1–28	20·96 (4·25–103)	25·41 (3·09–209)	19·89 (0·99–399)
29–124	3·47 (1·38–8·72)	4·07 (1·40–11·84)	2·63 (0·17–39·9)
125–994	2·54 (0·76–8·49)	2·79 (0·64–12·12)	1·61 (0·16–16·75)

p values for interaction between parasite quartile and *D Pteronyssinus* sensitisation: *0·05; †0·03; ‡0·23. §Available number too small to calculate estimate.

Table 6: **Odds ratios for wheeze in relation to *D Pteronyssinus* allergen skin sensitisation, stratified by quartiles of total parasite infection intensity**

infection. There was no evidence of a difference in this relation between urban and rural populations (table 6).

In western blot studies there was no evidence of cross-reactivity between crude dust-mite extract or Der p 1 purified with monoclonal antibody 4C1 and any hookworm allergen.

Discussion

Our results suggest that the increased occurrence of wheeze associated with urbanisation in this population is due partly to a loss of a protective effect from hookworm infection, partly to the effect of increased dust-mite allergen exposure, and partly to unidentified factors. Infection with ascaris was also associated with a reduced risk of wheeze, but this effect was not independently significant. The effect of Der p 1 exposure seems to be mediated through the production of specific IgE, but there was no evidence that IgE mediated the parasite effects. We have also confirmed our previous finding that skin sensitisation to *D pteronyssinus* is common in the rural population but does not generally confer the increased risk of wheeze that is seen in the urban population in Jimma, or indeed in other more affluent populations in other countries. Our data suggest that this dissociation of the usual relation between *D pteronyssinus* sensitisation and wheeze was not attributable to differences in Der p 1 allergen exposure, or to cross-reactivity between Der p 1 and hookworm allergens, but to inhibition of the effect of skin sensitisation on the risk of wheeze by high-intensity parasite infection.

Although our study method relied on the acquisition of objective measures of exposure nearly 3 years after definition of case or control status, restriction of the final multivariate analysis to the subgroup with consistent case and control status in both 1996 and 1999 made very little difference to the main effects identified. This finding suggests that misclassification of case or exposure status did not introduce appreciable bias in our findings. Exposure status in relation to both parasites and domestic dust is in any case likely to be stable between the two studies, since our work in Papua New Guinea has shown that individuals have a significant predisposition to either high or low hookworm burden over several years,[20] and there was also very little change between 1996 and 1999 in the study population in any of the household characteristics associated with high levels of Der p 1 exposure. However, the fact that adequate samples of dust for analysis could be obtained in only half the urban homes, compared with 90% of rural homes, suggests that urban families in particular may have cleaned in preparation for the study visit. If so, we are likely to have underestimated the true effect of dust exposure in the urban population.

Our finding of an inverse association between wheeze and helminth infection is consistent with the hypothesis that parasites protect against wheeze and possibly other allergic disorders.[7,8] However, our analysis of the specific IgE data suggested that this effect is not mediated by an IgE-receptor-blocking mechanism, thus other modulating effects on the immune system are probably involved.[8,9,20] Few participants in our study were infected with schistosomes, but our findings in these individuals do not provide support for the finding that infection with this organism protects against allergen skin sensitisation.[9] Indeed, if anything, allergen skin sensitisation tended to be more common in people with parasite infections. Protection against wheeze was most pronounced with hookworm and to a lesser extent ascaris, both of which have life cycles that have a pulmonary phase. This finding supports the notion that the local immune suppression by parasites that has probably evolved to facilitate the passage of the parasite through host tissues might also suppress local inflammatory responses to allergens.[21] Further direct evidence for this idea comes from laboratory findings that *N americanus* (hookworm) produces a metalloproteinase that digests eotaxin, a protein central to eosinophil migration,[22] and can induce apoptosis in activated human T cells.[23] *N americanus* also secretes calreticulin, which inactivates C1q and subsequent inflammatory responses.[24] These mechanisms result in local down-regulation of the antiparasite type 1 (allergic) response along the path of parasite migration.

The positive association between Der p 1 exposure and wheeze in our study is consistent with reports from other African countries[10,25] and from economically more developed countries.[26] The strong correlation between Der p 1 in dust and use of synthetic mattresses provides further evidence that bedding type may be one important factor in the adoption of a westernised lifestyle that results in increased prevalence of allergic disease. However, the dissociation of the relation between *D pteronyssinus* sensitisation and wheeze in our rural population is unlikely to be a chance finding, since it is consistent in this study population,[4] and has been noted in other studies in less developed countries.[27,28] Our findings suggest that this dissociation of risk is due to the higher degrees of parasite infection in the rural area, and that in the presence of a high parasite load, *D pteronyssinus* sensitisation is both common and benign in relation to wheeze. This finding is consistent with the high frequency of *D pteronyssinus* sensitisation reported among children who become infected with ascaris.[29] Our study does not have the necessary power to show whether this effect is general to all intestinal parasites or is specific to individual species, though the greatest individual species effect was seen with hookworm.

After allowance for parasite and allergen exposure effects on the prevalence of asthma, the prevalence of wheeze was still about three times higher in the urban population, and the excess remains unaccounted for. One potentially important factor that we were unable to assess is the use of fossil fuels such as kerosene for domestic heating and cooking, which we have shown[18] is associated with an increased risk of allergy in urban individuals in the original Jimma population survey. Adjustment for kerosene use in this case-control study had no appreciable effect on any of the associations reported. However, the number of individuals in our case-control

sample who used kerosene was very small, especially in the rural area, and the study was therefore underpowered to look at the independent effects of this and other environmental exposures in explaining the urban-rural difference in asthma prevalence.

These findings add to the increasing body of evidence implicating Der p 1 as a risk factor for asthma, previously suggested in more developed countries, and here shown objectively in a less developed country, and also point towards a modification of the relation between allergen skin sensitisation and wheeze in the urban population, as a result of which atopy becomes an adverse health characteristic. If this dissociation is indeed due to the absence or low concentrations of hookworm or other parasite infections, this result clearly has implications for the potential adverse effects of hookworm vaccines, which are under development.[30] If the vaccine does not carry the protective quality, the prevalence of asthma and other allergic diseases is likely to rise substantially as hookworm is eradicated; if it does, the vaccine could perhaps contribute substantially to the eradication of both diseases.

Contributors

Sarah Scrivener, Haile Yemaneberhan, Mehila Zebenigus, Daniel Tilahun, Samuel Girma, and Seid Ali were responsible for data collection in Ethiopia, and Sarah Scrivener for data management, statistical analysis, and preparation of successive drafts of the paper. Paul McElroy did the IgE serology analyses, and Adnan Custovic and Ashley Woodcock the dust allergen measurements. John Britton, Haile Yemaneberhan, Andrea Venn, and David Pritchard designed the study and provided overall supervision of the analysis and preparation of this report.

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