

Crete: does farming explain urban and rural differences in atopy?

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Summary

Background Urban-rural differences in the prevalence of atopy and associated allergic diseases have been reported in several non-European countries. Within Europe, where such variations are less striking, a farm childhood has been postulated to be protective.

Objective We aimed to compare the prevalence of atopy in urban and rural children living in Crete and to examine the role of early exposure to a farming environment in explaining any measured differences.

Methods A cross-sectional survey of children attending secondary schools in Iraklion, the capital city, and five villages 100 km to the south. Atopy was determined by the responses to skin prick tests with seven common aeroallergens.

Results Nine-hundred and ninety-seven children participated in the survey. 19.6% of those living in Iraklion, but only 9.6% of those from the villages, had a positive response to one or more skin prick tests. Among urban children there were significant gradients in the prevalence of atopy across several categories of animal contact and consumption of farm (unpasteurized) milk products – before the age of 5 years. These differences, however, were not apparent among the rural children.

Keywords: asthma, atopy, childhood, farming, urban, rural

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Introduction

The prevalence of childhood atopy, asthma and hayfever has risen over the past 40 years in most, if not all European countries. The International Study of Asthma and Allergies in Childhood (ISAAC), in a concerted attempt to understand these increases, used a standardized questionnaire to measure the prevalence of asthma and associated allergies among children aged 6–7 and 13–14 years in 56 countries. Initial results [1] revealed wide variations with up to 15-fold differences in rates of asthmatic symptoms between countries. There was also variation within regions; in

Europe the highest estimates of disease (wheezing > 30%) were observed in the UK with the lowest (< 5%) in southern and eastern areas: including Greece. Differences of this magnitude and occurring over a period of 20–30 years seem likely to be due to environmental factors.

Less is known about within-country variations. The prevalence of childhood atopic diseases is significantly less in Halle and Leipzig as compared to Munich in children from the former East and West Germany, the 'former' perhaps representing an 'older' or less westernised society [2]; a similar pattern has been reported in Estonia and Sweden [3,4]. In several African countries childhood asthma is more prevalent in urban than rural communities, and in towns among more prosperous children [5,6]. In Sweden atopy is 46% more common in children living in the city than the countryside [7]. In contrast, across the UK,

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there was little difference between metropolitan and non-metropolitan areas in the prevalence of asthma symptoms in 12–14 year-old children [8].

Where they exist, urban-rural differences in the overall prevalence of childhood respiratory allergies could be explained by the protective effects of early contact with microbial agents – an extension of the ‘hygiene hypothesis’ [9]. For example, atopy among young soldiers in central and southern Italy was less common in those with evidence of previous hepatitis A infection [10] a putative marker of a ‘dirty childhood’. There is also accumulating evidence, that factors related to farming, whether direct or indirect, decrease the risk of children becoming atopic and developing symptoms of allergic disease [11–13]. While such evidence is, to date, reasonably consistent, it is not clear what it is about farming which is protective; other factors, including perhaps differences in diet, may be important.

To investigate this question, we have compared the prevalence of atopy and associated allergic disease in two Greek communities: children from Iraklion, a busy and rapidly expanding city port and the capital of Crete; and children from the rural, largely agricultural, south of the same island. We hypothesized that rates of atopy would be lower among the rural children, a difference which would be explained by contact with farm animals (or perhaps unpasteurized milk products) in early childhood.

Methods

School children between the ages of 11 and 19 were surveyed in the first two weeks of November 1999. All those within this age group attending a large school (Iraklio V) in Iraklion, and a cluster of five smaller, rural schools (in Asimi, Herakas, Terfilii, Pyrgos and Mesochorio) were invited to take part. The latter, themselves separated by only 15 km, are approximately 100 km south of Iraklion but set in a very different landscape of mountains and wide plains.

Ethical approval was granted by Royal Brompton and Harefield NHS Trust in the UK and The Scientific Committee at the Venzalio Hospital in Crete. All parents gave written informed consent.

The parents of each participating child completed a questionnaire which enquired into the child’s respiratory and allergic symptoms, birth order and sibling numbers, family history of allergic diseases and levels of parental education. Parents were considered ‘allergic’ if they had ever had a diagnosis of asthma, hayfever or eczema. They were also asked whether they ran a farm, full- or part-time, and about their child’s contact with farm animals (goat, chicken, cow, pig, rabbit, horse) and unpasteurized milk products straight from the farm. Core questions on asthma and allergy were taken from the ISAAC protocol; these,

and the questions on relevant exposures, related to both now and the first five years of the child’s life.

Each child was invited to undergo skin prick tests with saline (negative control) histamine (positive control) mixed grass pollen (Mediterranean), house dust mite, cat, *Parietaria*, olive blossom, *Alternaria* and goat allergens, each purchased from ALK (Hungerford, UK) and selected because we felt they might be common aeroallergens in this community. The tests were carried out in accordance with the ISAAC protocol including measures of tester reliability. A positive skin prick test was defined as one with a mean wheal diameter of at least 3 mm. For consistency with studies elsewhere, children were described as atopic if they had at least one positive skin prick test to house dust mite, cat or pollens (mixed grass, *Parietaria* or olive blossom).

Up to 15 mL of venous blood was collected from each child; after separation of serum, by spinning for 10 min at 1000 g, the samples were transported to the laboratory at the Venzalio Hospital and frozen within eight hours. Concentrations of immunoglobulin (Ig)G antibodies to hepatitis A were measured using a commercially available immunoassay kit (Roche Diagnostics, Basel, Switzerland). We used cut-off points recommended by the manufacturer.

The study population consisted of 1392 children and, of these, 997 (72%–64% urban and 85% rural) returned parental questionnaires with consent. Of these 929 (93%) underwent skin prick tests and 847 (85%) gave blood samples for analysis. Urban children (92%) and boys (91%) were less likely to undergo skin prick testing than country children (95%) or girls (95%).

Statistical analysis

Demographic differences and rates of atopy were first examined univariately in an analysis stratified by current geographical location (urban/rural). Differences between the two communities were tested using the chi-squared test for independence, or Fisher’s exact test where appropriate, in the case of categorical variables and the Wilcoxon two-sample for continuous data. Potential determinants of atopic status were then examined among all children and after stratification by location before the age of five, with chi-squared (when appropriate, for trend) statistics calculated to assess statistical significance. Finally, logistic regression analyses were used to study the relationship between atopy and various exposure variables while controlling for specified confounders. The estimated odds-ratios (OR) and 95% confidence intervals associated with exposures are presented. Likelihood ratio tests were performed for each exposure variable to assess whether its inclusion to the core model of confounders improved the fit of the model. All analyses were performed using SAS, EpiInfo6 and Stata software packages.

Table 1. Differences between urban and rural children, according to current place of residence

		Urban	Rural	<i>P</i> -value
<i>n</i>		559	438	–
Male – <i>n</i> (%)		256 (45.8%)	200 (45.7%)	0.966
Age – median (range)		13 (11–19)	13 (11–17)	0.025
Urban (aged < 5 yrs)		509 (91.4%)	30 (6.9%)	< 0.001
Mother’s age – median (range)		24 (14–43)	24 (14–45)	0.541
Father’s age – median (range)		29 (15–61)	32 (18–55)	< 0.001
Parental allergy		151 (27.3%)	112 (26.1%)	0.687
Mother’s years of pre-university schooling – median (range)		12 (3–19)	7 (0–18)	< 0.001
Siblings – <i>n</i> (%)	0/1	350 (62.6%)	142 (32.4%)	< 0.001
	2	153 (27.4%)	145 (33.1%)	
	3 +	56 (10.0%)	151 (34.5%)	
High levels of crowding (> = 2 people/room)	187 (34.6%)	170 (40.5%)	0.063	
Farming parents (aged < 5 yrs)	Full-time – <i>n</i> (%)	18 (3.2%)	254 (58.7%)	< 0.001
	Part-time – <i>n</i> (%)	180 (32.3%)	125 (28.9%)	
Farm animal contact (aged < 5 yrs)	Never	299 (54.1%)	132 (30.6%)	< 0.001
	Sometimes	170 (30.7%)	119 (27.6%)	
	Daily/ > 1 per week	84 (15.2%)	181 (41.9%)	
Farm milk consumption (aged < 5 years)	Never	425 (76.3%)	235 (54.5%)	< 0.001
	Sometimes	99 (17.8%)	119 (27.6%)	
	Daily	77 (17.9%)	33 (5.9%)	
<i>Skin prick test participants</i>				
<i>n</i>		512	418	
Atopy		100 (19.6%)	40 (9.6%)	< 0.001
SPT +: house dust mite		72 (14.1%)	18 (4.3%)	< 0.001
SPT +: cat		16 (3.1%)	2 (0.5%)	0.004
SPT +: grass*		48 (9.4%)	24 (5.7%)	0.038
<i>Parietaria</i>		26 (5.1%)	4 (1.0%)	< 0.001
Olive		29 (5.7%)	12 (2.9%)	0.038
Goat		4 (0.8%)	2 (0.5%)	0.696
<i>Alternaria</i>		1 (0.2%)	1 (0.2%)	1.000

SPT: Skin prick test.

Denominators may vary depending on response rates.

*Mediterranean grass mix, olive blossom or *Parietaria*.

Results

Predictably, the sex distributions of the two communities were similar and, although the difference was statistically significant ($P = 0.025$), there was only a small difference in the age distributions. There were no important differences between maternal ages or rates of parental atopy, but fathers in the urban community were found to be significantly younger. Almost all (91%) of the city children had spent all their lives there; conversely, 93% of rural children had always lived in the country. The parents, particularly the mothers, of city children were more likely to have had a university education whereas half of rural

mothers had less than 8 years of formal education. Rural families tend to be bigger than those living in Iraklion; children from the former were over three times more likely to have three or more siblings. Consequently, rural children more frequently came from crowded homes, although the difference was slight and did not reach statistical significance.

Very few city children had come from families where the main business was farming, although a third of their parents described themselves as part-time farmers. This contrasted strongly with the rural children, over half of whom were from full-time, and a further 29% from part-time, farming families. Up to the age of 5 years half of the urban children

Table 2. Prevalence of atopy (n,%) among all children and separately by residence aged ≤ 5 years

	All children			Urban children (< 5 years)			Rural children (< 5 years)		
	N	Atopic n (%)	P	N	Atopic n (%)	P	N	Atopic n (%)	P
Male	416	78 (18.8)	0.005	229	57 (24.9)	0.009	185	21 (11.4)	0.336
Female	513	62 (12.1)		264	41 (15.5)		245	21 (8.6)	
11–12 yrs	299	50 (16.7)		171	33 (19.3)		126	17 (13.5)	
13–14 yrs	325	41 (12.6)		168	30 (17.9)		156	11 (7.1)	
15+ yrs	305	49 (16.1)		154	35 (22.7)		148	14 (9.5)	
Parental allergy			0.263			0.995			0.094
Yes	243	42 (17.3)		141	28 (19.9)		100	14 (14.0)	
No	672	96 (14.3)		347	69 (19.9)		324	27 (8.3)	
Number of siblings			0.060			0.433			0.985
0/1	460	85 (18.5)		310	69 (22.3)		145	16 (11.0)	
2	273	26 (9.5)		131	16 (12.2)		141	10 (7.1)	
3+	196	29 (14.8)		52	13 (25.0)		144	16 (11.1)	
Crowding			0.913			0.885			0.963
Low	249	40 (16.1)		121	27 (22.3)		128	13 (10.2)	
Medium	309	43 (13.9)		182	30 (16.5)		127	13 (10.2)	
High	334	54 (16.2)		171	38 (22.2)		160	16 (10.0)	
Maternal ed. (yrs)			0.034			0.399			0.652
0–6	252	29 (11.5)		88	15 (17.1)		163	14 (8.6)	
7–9	209	33 (15.8)		95	18 (19.0)		114	15 (13.2)	
10+	400	71 (17.8)		286	60 (21.0)		112	11 (9.8)	
Farming (< 5 year)			0.005			0.888			0.983
No	383	70 (18.3)		334	65 (19.5)		49	5 (10.2)	
Part-time	280	44 (15.7)		142	31 (21.8)		136	13 (9.6)	
Full-time	260	26 (10.0)		15	2 (13.3)		244	24 (9.8)	
Milk products (< 5)			0.005			0.028			0.609
Never	609	105 (17.2)		373	80 (21.5)		234	25 (10.7)	
Sometimes	208	23 (11.1)		86	14 (16.3)		122	9 (7.4)	
Daily	104	9 (8.7)		32	2 (6.3)		71	7 (9.9)	
Farm animals (< 5)			0.006			0.019			0.550
Never	394	76 (19.3)		269	64 (23.8)		125	12 (9.6)	
Sometimes	270	33 (12.2)		152	24 (15.8)		117	9 (7.7)	
Regularly*	254	30 (11.8)		67	9 (13.4)		185	21 (11.4)	
Goat (< 5 year)			0.003			0.026			0.989
Never	529	96 (18.2)		351	78 (22.2)		178	18 (10.1)	
Sometimes	225	27 (12.0)		104	16 (15.4)		120	11 (9.2)	
Regularly*	163	16 (9.8)		33	3 (9.1)		128	13 (10.2)	
Chicken (< 5 years)			0.032			0.018			0.263
Never	505	89 (17.6)		317	73 (23.0)		188	16 (8.5)	
Sometimes	214	26 (12.2)		120	19 (15.8)		92	7 (7.6)	
Regularly*	185	22 (11.9)		47	5 (10.6)		137	17 (12.4)	
Cat (< 5 years)			0.270			0.425			0.964
Never	551	90 (16.3)		311	66 (21.2)		240	24 (10.0)	
Sometimes	176	25 (14.2)		102	18 (17.7)		73	7 (9.6)	
Regularly*	175	23 (13.1)		72	13 (18.1)		101	10 (9.9)	
Dog (< 5 years)			0.091			0.150			0.967
Never	503	85 (16.9)		291	64 (22.0)		212	21 (9.9)	
Sometimes	193	28 (14.5)		109	20 (18.4)		83	8 (9.6)	
Regularly*	216	26 (12.0)		85	13 (15.3)		129	13 (10.1)	

*Daily/> 1 per week.

Table 3. Results of regression analyses for early childhood exposures: adjusted OR (95% CI)

	Variables included in the model				
	Core model: Age, sex, parental allergy, siblings, rural (aged < 5 yrs)	Core + any farm animal contact (aged < 5 yrs)	Core + farm milk (aged < 5 yrs)	Core + farm animal contact (any) + farm milk (aged < 5 yrs)	Core + farm animal contact (any) and/or unpasteurized milk (aged < 5 yrs (any))
Rural (aged < 5 yrs)	0.44 0.29, 0.66	0.48 0.31, 0.74	0.47 0.31, 0.72	0.51 0.33, 0.78	0.50 0.32, 0.78
Farm animal contact		0.67 0.46, 0.99		0.72 0.49, 1.07	
Farm milk			0.65 0.41, 1.00	0.70 0.45, 1.10	
– milk – farm					1.00
– milk + farm					0.56 (0.35, 0.88)
+ milk – farm					0.32 (0.13, 0.78)
+ milk + farm					0.58 (0.34, 0.98)
LRT test for improvement on core model	N/A	0.044	0.047	0.043	0.008

never had contact with farm animals compared with daily contact for nearly half of the country children. This pattern is reflected in the unpasteurized milk consumption of urban and rural children.

Overall, 140 (15.1%) children were atopic. Most of the positive reactions were to house dust mite or Mediterranean mixed grass allergens with very few children having positive tests to cat fur, *Parietaria*, olive blossom, *Alternaria* or goat allergens. Atopy was twice as common (19.6% vs. 9.6%) among urban children. This difference held for all the test allergens, except those where positive reactions were very few. Forty-five children, now living in Iraklion, had spent their first five years in a rural area. Five of them (11.1%) were atopic, a prevalence lower ($P = 0.131$) than that (20.5%) among children who had always lived in the city.

The rates of atopy within each community were analysed in relation to a number of potential determinants; Table 2 summarizes the results of these analyses for all surveyed children and, separately, for those brought up in urban and rural communities.

Boys were more likely to be atopic, a difference found in the unstratified sample and among children who lived in urban communities before the age of 5. Family size and domestic crowding had no clear effect within either community. Amongst all children, those with mothers who had 6 or less years of education were less likely to be atopic, but there was no discernible association between atopy and maternal education in analyses stratified by location early in life. A similar pattern emerged amongst children from a farming background, and particularly where this was the family's full-time occupation; they were less likely to be atopic. These differences were not

retained in the stratified analyses, however. Amongst all children, those who consumed unpasteurized milk products in early life had lower rates of atopy than those who did not. The differences remained among the urban children, but there was no discernible association between atopy and the consumption of unpasteurized milk products amongst rural children. This pattern was reproduced when early contact with farm animals – of any species – was examined. There was no statistically significant relationship between atopy and contact with domestic animals (cat and dog) in the total sample or in the stratified analyses.

A core model was constructed using the following variables: age (continuous), sex, number of siblings (continuous), presence of parental allergy, and location before the age of five. Other exposure variables were then added, singly, with the resultant odds ratios for those with statistical significance displayed in Table 3. Of particular animal species, only the inclusion of any childhood contact with goats made a significant improvement to the core model ($P = 0.048$). We were unable to detect any evidence of an exposure-response relationship across different categories of farm animal or milk exposure (data not shown).

In a further model we included variables for both farm animal and farm milk exposure, resulting in statistically non-significant risk estimates for both (Table 3). Further analysis suggested an interaction between the two exposures; a new variable incorporating both farm animal exposure and milk consumption across four levels was created. The results are displayed in Table 3; the absence of early contact with either farm animals or unpasteurized milk products increased a child's odds of being atopic by 42% compared to children who have been exposed to both.

Even with inclusion of these variables, however, the risk estimate for rural living remained statistically significant and indeed was little changed from the result obtained in the core model.

Of the 847 blood samples tested for hepatitis A-specific IgG, only 19 (2.3%) were positive. This proportion was slightly higher among urban children (3.1% vs. 1.3%) but the difference was not statistically significant ($P = 0.095$). Atopic children (2.3%) were no less likely to have a positive result than non-atopic children (2.3%).

Discussion

As anticipated, we found a marked variation in the prevalence of atopy between children brought up in two communities separated by only 100 km. Although we were able to document differences in family size, farming, animal contact and the consumption of unpasteurized milk products between rural and urban children, none of these satisfactorily explained the variation in atopy. In contrast with a nearby southern European population [10] evidence of previous infection with hepatitis A appears to be irrelevant in this community.

Although we found that a farm childhood was associated with a reduction in atopy among all children, and that the effect was stronger for full-time farming families, the difference disappeared when rural children (20% of whom had no farming background) were examined separately. This is in stark contrast to surveys carried out in Switzerland, Bavaria and Austria [9,11,12]. When farm-associated exposures were examined – contact with animals and consumption of unpasteurized milk products – the same patterns emerged. Among urban children, however, statistically significant trends of decreasing atopy with increasing early contact with farm animals or farm milk were retained. Only when both of these factors were considered in their interaction in a logistic regression analysis did we find a statistically significant and negative relationship. It is possible that our measurements of farming background and associated exposures were insufficiently accurate and that systematic misclassification resulted in the loss of any relationship among rural children. Alternatively, the reported protective effects of central European farms, and in at least one case [12] regular contact with livestock, may not extend to those in Crete. Certainly agricultural practices are very different. Farms in Crete are generally small, often some distance from the family home and in most cases are concerned with the cultivation of olives and the tending of goats. Cows are rare on the island; only 27 farming families had kept a cow.

Although the absolute rates are lower, the differences reported here between near-neighbouring communities are as great as those reported between children brought up in

former East and West Germany and greater than those between urban and rural children in Sweden. In Crete the variation does not seem to be explained by a number of apparently established factors and, presumably represents the effect of some less tangible difference(s) between rural and urban childhoods. These differences may be the same as those that distinguish 'western' from 'non-western' populations. Our findings are consistent with a protective effect of greater 'rurality' with some evidence of a 'dose-response' albeit based on scales of only three categories. Again, these effects may not be universal. Among Finnish students, for example, there was no difference in the cumulative prevalences of allergic rhinitis or asthma between those from urban and non-farming rural backgrounds; the reduction among rural children was confined to those brought up on farms [13]. The elucidation of a specific rural factor or factors may be very difficult. Indeed our experience with this survey, although valuable in identifying a population with sufficient variation in outcome to be useful in future aetiological research, highlights the precariousness of two-point comparisons. These are simple, rapid and cheap to undertake but their results are difficult to interpret with any degree of certainty. It is probable, for example, that a detailed dietary survey would reveal important differences in nutritional intake between the rural and urban communities of Crete; similarly for any study concerned with vehicular pollution. To what extent, however, would these further the argument for causal links between diet or air pollution and the development of atopy or other allergic conditions?

We have established substantial variation in the prevalence of atopy within a European community. We aim, by defining several categories of urban/rural diversity, to further explore those factors which are potentially protective in the development of childhood allergic diseases.

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References

- 1 ISAAC Steering Committee. Worldwide variations in the prevalence of asthma symptoms. the International Study of Asthma and Allergies in Childhood (ISAAC) *Eur Respir J* 1998; 12:315–35.
- 2 Von Mutius E, Fritzsche C, Weiland S, Röhl G, Magnussen H. Prevalence of asthma and allergic disorders among children in united Germany: a descriptive comparison. *BMJ* 1992; 305:1395–9.

- 3 Riikjäär M, Julge K, Vasar M, Bräbäck L, Knutson A, Björkstén B. The prevalence of atopic sensitisation and respiratory symptoms among Estonian school children. *Clin Exp Allergy* 1995; 25:1198–204.
- 4 Bräbäck L, Breborowicz A, Julge K et al. Risk factors for respiratory symptoms and atopic sensitisation in the Baltic area. *Arch Dis Child* 1995; 72:487–93.
- 5 Ng'ang'a LW, Odhiambo JA, Mungai MW et al. Prevalence of exercise induced bronchospasm in Kenyan school children: an urban-rural comparison. *Thorax* 1998; 53:919–26.
- 6 Keeley D, Neill P, Gallivan S. Comparison of the prevalence of reversible airway obstruction in rural and urban Zimbabwean children. *Thorax* 1991; 46:549–3.
- 7 Bräbäck L, Breborowicz A, Dreborg S, Knutsson A, Pieklik H, Björkstén B. Atopic sensitization and respiratory symptoms among Polish and Swedish school children. *Clin Exp Allergy* 1994; 24:826–35.
- 8 Kaur B, Anderson HR, Austin J et al. Prevalence of asthma symptoms, diagnosis and treatment in 12–14 year old children across Great Britain (ISAAC UK). *BMJ* 1998; 316:118–24.
- 9 Braun-Fahrländer CH, Gassner M, Grize L et al. Prevalence of hayfever and allergic sensitisation in farmer's children and their peers living in the same rural community. *Clin Exp Allergy* 1999; 29:28–34.
- 10 Matricardi PM, Rosmini F, Ferrigno F et al. Cross sectional retrospective study of prevalence of atopy among Italian military students with antibodies against hepatitis A virus. *BMJ* 1997; 314:999–1003.
- 11 Von Ehrenstein OS, Von Mutius E, Illi L et al. Reduced risk of hay fever and asthma among children of farmers. *Clin Exp Allergy* 2000; 30:187–93.
- 12 Riedler J, Eder W, Oberfeld G, Schreurer M. Austrian children living on a farm have less hay fever, asthma and allergic sensitisation. *Clin Exp Allergy* 2000; 30:194–200.
- 13 Kilpeläinen A, Terho EO, Helenius H, Koskenvuo M. Farm environment in childhood prevents the development of allergies. *Clin Exp Allergy* 2000; 30:201–8.