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Asthma and allergies: is the farming environment (still) protective in Poland? The GABRIEL Advanced Studies

S. J. MacNeill[1], B. Sozanska[2], H. Danielewicz[2], A. Debinska[2], A. Kosmeda[2], A. Boznanski[2], S. Illi[3], M. Depner[3], C. Strunz-Lehner[3], M. Waser[4,5], G. Büchele[6], E. Horak[7], J. Genuneit[6], D. Heederik[8], C. Braun-Fahrländer[4,5], E. von Mutius[3], P. Cullinan[1] & the GABRIELA study group*

[1]Department of Occupational and Environmental Medicine, National Heart and Lung Istitute, Imperial College London, London, UK; [2]Department of Paediatrics, Allergology and Cardiology, Wrocław Medical University, Wrocław, Poland; [3]Asthma and Allergy Research Group, University Children's Hospital, Munich, Germany; [4]Swiss Tropical and Public Health Institute, Basel, Switzerland; [5]University of Basel, Basel, Switzerland; [6]Institute of Epidemiology and Medical Biometry, University of Ulm, Ulm, Germany; [7]Department of Paediatrics and Adolescents, Division of Cardiology and Pulmonology, Innsbruck Medical University, Innsbruck, Austria; [8]Division of Environmental Epidemiology, Institute for Risk Assessment Sciences, Utrecht University, Utrecht, The Netherlands

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Correspondence
Stephanie J. MacNeill, PhD, Department of Occupational and Environmental Medicine, National Heart and Lung Institute, Imperial College London, 1b Manresa Road, London SW3 6LR, UK.
Tel.: +44-20-7351-8397
Fax: +44-20-7351-8336
E-mail: s.macneill@imperial.ac.uk

*See Appendix A for the GABRIELA study group.

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Abstract

Background: Evidence exists that a farming environment in childhood may provide protection against atopic respiratory disease. In the GABRIEL project based in Poland and Alpine regions of Germany, Austria and Switzerland, we aimed to assess whether a farming environment in childhood is protective against allergic diseases in Poland and whether specific exposures explain any protective effect.
Methods: In rural Poland, 23 331 families of schoolchildren completed a questionnaire enquiring into farming practices and allergic diseases (Phase I). A subsample (n = 2586) participated in Phase II involving a more detailed questionnaire on specific farm exposures with objective measures of atopy.
Results: Farming differed between Poland and the Alpine centres; in the latter, cattle farming was prevalent, whereas in Poland 18% of village farms kept ≥ 1 cow and 34% kept ≥ 1 pig. Polish children in villages had lower prevalences of asthma and hay fever than children from towns, and in the Phase II population, farm children had a reduced risk of atopy measured by IgE (aOR = 0.72, 95% CI 0.57, 0.91) and skin prick test (aOR = 0.65, 95% CI 0.50, 0.86). Early-life contact with grain was inversely related to the risk of atopy measured by IgE (aOR = 0.66, 95% CI 0.47, 0.92) and appeared to explain part of the farming effect.
Conclusion: While farming in Poland differed from that in the Alpine areas as did the exposure–response associations, we found in communities engaged in small-scale, mixed farming, there was a protective farming effect against objective measures of atopy potentially related to contact with grain or associated farm activities.

Recent findings suggest that the rise in prevalence of childhood asthma has relented in western Europe, but persists in eastern regions where it has traditionally been low (1). These countries appear to be experiencing an 'allergic transition' parallel to and intertwined with their socio-political transformation, as 'westernization' may include factors directly influencing the incidence of asthma and related allergies (2).

Farm living appears to provide some protection against asthma (3–10), wheeze (4, 6, 8, 11–14), hay fever (8, 9, 14, 15) and atopy (3, 6, 8, 9, 14–23) in children. Specifically, contact with animals and their stables (4, 5, 8–10, 14, 20, 21, 23–25), cultivation farming (4, 5) and consumption of farm milk (5, 8, 13, 14, 20, 26) have been shown to be protective. Most of this evidence came from rural communities in western Europe practicing particular forms of dairy-based farming. Surveys elsewhere (5, 11, 17) are less consistent, suggesting that protection may depend on the type of farming. The GABRIEL Advanced Surveys were developed to explore this in greater detail, taking advantage of variations in farming practices in Europe.

Methods

Study design

Phase I

The GABRIEL Advanced Surveys took place in five rural regions: two in Germany and one each in Switzerland, Austria and Poland (lower Silesia) (27). Here, we report findings from the Polish centre; the remaining areas are hereafter referred to as 'Alpine centres' and are presented elsewhere (28). In Poland, Phase I comprised, in 2006, a questionnaire distributed to parents of all children attending targeted schools in years 1–6 in villages and, distinct from Alpine centres, small towns. Polish schools were in communities of <15 000 inhabitants (27), and participating children lived in these or nearby similarly small communities. The questionnaire enquired into current farming practices, respiratory and allergic diseases and family characteristics. Due to anticipated cultural differences, questions regarding farm characteristics differed slightly between Polish and Alpine centres and these regions were studied separately.

Children were grouped into strata reflecting Phase I farming experiences. In Poland, these were 'farm' children, 'exposed nonfarm' children and 'unexposed nonfarm' children (27). These mutually exclusive groups were used for stratified sampling for Phase II; they were not used in analysis (analytic categories are below).

Phase II

Phase II included a comprehensive questionnaire enquiring into the timing and frequency of the child's specific farm-related exposures. Children were also invited to participate in blood sampling, genetic analyses, skin prick testing and environmental dust sampling.

Approval was received from the ethics committee of the Medical University of Wrocław and from school authorities. Parents provided written informed consent.

Definitions

Farming

Based on previous findings in Poland showing town–village differences in the prevalence of atopy (22), farm exposures were classified according to where children lived and Phase I farming characteristics. 'Farm' children live in villages where either parent farms. 'Village nonfarm' children live in villages but neither parent farms. 'Town nonfarm' children live in towns. Both nonfarm categories were combined to compare farm and nonfarm children.

Phase I outcomes

International Study of Asthma and Allergies in Childhood (ISAAC) core questions were used to measure current symptoms of wheeze and allergic rhinitis (29). 'Childhood asthma' was defined as current symptoms of wheeze, asthma inhaler use (ever), at least one doctor's diagnosis of asthma or diagnosis of bronchitis with wheeze more than once. 'Hay fever' was defined as a doctor diagnosis of hay fever or current nasal symptoms and itchy, watery eyes without flu.

Phase II outcomes

Blood samples were collected from consenting children, and specific serum immunoglobulin E (IgE) antibodies measured to: *D. pteronyssinus*, cat dander, grass mix and common silver birch (27). Atopy was defined by specific IgE $\geq$ 0.7 kU/l against *D. pteronyssinus*, cat or birch or a positive reaction (0.35 kU/l) to the grass mix.

In Poland, skin prick testing (SPT) was performed using extracts from *D. pteronyssinus*, *D. farinae*, mixed grasses, birch and cat epithelia with histamine and saline controls (ALK-Abelló, Hungerford, UK). Atopy was defined as a mean wheal size 3 mm greater than the mean negative control for any of cat, *D. pteronyssinus*, grass or birch.

Statistical method (Data S1)

For Phase I, data are presented as frequencies and proportions. The chi-square test assessed differences between farm and nonfarm children. For Phase II, data are presented as weighted proportions and univariate comparisons were performed using Pearson's chi-square test with the Rao and Scott correction (30).

Adjusted logistic regression models assessed the effects of farm characteristics (Phase I) on outcomes among village children. Models for atopy were weighted for Phase II sampling. For each outcome, a separate adjusted model was fitted for each farming exposure. All exposures significantly associated with the outcome were added to a final model.

To assess the effects of early-life farm exposures (Phase II) on atopy, weighted logistic regression models adjusting for farming and potential confounders were fitted for each exposure. To detect the exposures potentially explaining the overall farm effect, we constructed weighted stepwise logistic regression models.

Regression model results are presented as adjusted odds ratios (aOR) with 95% confidence intervals. Analyses were performed in STATA v11 (StataCorp, College Station, TX, USA).

Results

Phase I population

In Phase I, 46 488 questionnaires were distributed and 23331 (50%) returned. Participants were aged 9.2 years (SD = 1.8); 49% were boys (Table 1).

Seventy-one percent of children lived in villages; 28% were classified as 'farm children', 43% as 'village nonfarm children' and 29% as 'town nonfarm children'. Farm and nonfarm children were comparable in sex and age, but farm children had larger families, less often had a family history of hay fever and eczema, and their parents were less likely to smoke or have had tertiary education.

While current wheeze and diagnoses of 'bronchitis with wheeze' and 'recurrent bronchitis' were common, a diagnosis

Table 1 Phase I population – demographic and farming characteristics and outcomes. *N* (%)

	Village Farm (*n* = 6089)	Non-farm		*P*	
		Village (*n* = 9289)	Town (*n* = 6348)	*	†
Demographic characteristics					
Sex					
Male	3012 (49.5)	4590 (49.5)	3055 (48.2)	0.45	0.08
Age; mean (SD)	9.2 (1.8)	9.2 (1.8)	9.1 (1.8)	<0.01	0.01
Number of siblings ≥2	3268 (54.7)	4131 (45.4)	2216 (35.5)	<0.01	<0.01
Family history					
Asthma	531 (9.1)	796 (9.0)	658 (10.9)	0.16	<0.01
Hay fever	946 (16.4)	1401 (16.0)	1219 (20.3)	0.02	<0.01
Eczema	640 (11.1)	974 (11.1)	830 (13.8)	0.03	<0.01
Maternal smoking (ever)					
Never	2690 (49.9)	3545 (42.6)	2663 (45.8)	<0.01	0.81
Ex	945 (17.5)	1442 (17.3)	1021 (17.6)		
Current	1753 (32.5)	3337 (40.1)	2130 (36.6)		
Paternal smoking (ever)					
Never	1756 (31.4)	2244 (26.6)	1647 (28.6)	<0.01	0.01
Ex	1076 (19.3)	1551 (18.4)	1178 (20.5)		
Current	2754 (49.3)	4649 (55.1)	2927 (50.9)		
Highest level of parental education					
Post-secondary	754 (12.5)	1621 (17.6)	1834 (29.0)	<0.01	<0.01
Illness and symptoms					
Current wheeze	1050 (18.0)	1602 (17.9)	1236 (20.2)	0.15	<0.01
Doctor diagnosis (ever)					
Asthma	354 (6.1)	616 (6.9)	521 (8.5)	<0.01	<0.01
Bronchitis with wheeze	1751 (30.5)	2750 (31.2)	2158 (35.6)	<0.01	<0.01
Recurrent bronchitis	990 (17.5)	1586 (18.4)	1281 (21.6)	<0.01	<0.01
Inhaler (ever)	1320 (22.2)	2049 (22.7)	1851 (29.8)	<0.01	<0.01
Childhood asthma‡	2001 (33.0)	3059 (33.1)	2515 (39.8)	<0.01	<0.01
Hay fever§	1358 (23.1)	2091 (23.2)	1696 (27.5)	0.01	<0.01
Doctor diagnosis of atopic dermatitis/eczema (ever)	709 (12.0)	1171 (13.0)	1067 (17.2)	<0.01	<0.01

*Chi-square test comparing farm and nonfarm children.

†Chi-square test comparing village and town children.

‡Defined as either current symptoms of wheeze, asthma inhaler use (ever), a doctor's diagnosis of asthma at least once or doctor's diagnosis of bronchitis with wheeze more than once.

§Defined as either a doctor's diagnosis of hay fever or current (in the last 12 months) nasal symptoms and itchy, watery eyes without flu.

of asthma was rarer (7% overall). At least 20% of children had ever used an asthma inhaler. Combining these variables, we estimated a prevalence of 'childhood asthma' of 35% overall. While most outcomes were less common among farm children, differences were most apparent between village and town children. Defining asthma on diagnoses – doctor diagnosis of asthma or >1 diagnosis of bronchitis with wheeze – yielded a lower prevalence (19%) with similar trends with farming (farm: 18%; village nonfarm: 19%; town: 22%; $P < 0.01$ comparing town with village and farm with nonfarm).

Village farms in Poland were different from those in Alpine centres (Fig. 1). Alpine farms were more likely to have cows, whereas in Poland just 18% of village farms kept cows and 34% pigs. The regions also differed in animal feeds used: grass and corn silage, grain shred and hay pellets were common in the Alpine centres, but relatively uncommon in Poland.

Among village children, mutually adjusted models showed that children living on farms with cows had a reduced odds of childhood asthma and those on farms growing fruit or using grain shred had increased odds (Table 2). Similar findings were found for hay fever although only grain shred reached statistical significance. When defining asthma on doctor diagnoses, only fodder beet cultivation was statistically significant [aOR = 0.73 (0.57, 0.94)].

For atopy measured by IgE in the Phase II population (Table 2), only commercial grain cultivation was significantly and inversely associated after adjusting for cattle and pig farming, cultivation of feed grain and use of grain shred. For atopy measured by SPT, none of pig farming, commercial grain cultivation or use of hay pellets were significantly associated.

Phase II population

6908 (30%) of Phase I participants attended schools selected for Phase II and consented for further assessments. Of these, 3951 (57%) were selected by exposure strata and 2541 returned a Phase II questionnaire, 2436 had specific IgE measured, and 2493 had a valid SPT. 2586 (65% of 3951 selected) completed

Table 2 Specific farm characteristics (Phase I) among village children and their associations with asthma and allergic outcomes in the Phase I and II populations

	Mutually adjusted model including potential confounders		
	aOR	95% CI	*P*
Phase I population			
Childhood asthma*			
Cattle farming	0.76	0.65–0.89	<0.01
Fruit cultivation	1.55	1.14–2.11	0.01
Grain shred as animal feed	1.33	1.14–1.55	<0.01
Hay fever*			
Cattle farming	0.84	0.69–1.02	0.08
Cultivation of corn feed	0.76	0.53–1.08	0.12
Grain shred as animal feed	1.34	1.13–1.58	<0.01
Phase II population†			
Atopic sensitization (IgE)‡			
Commercial grain cultivation	0.64	0.44–0.93	0.02
Cattle farming	0.87	0.47–1.59	0.64
Pig farming	0.74	0.46–1.19	0.21
Cultivation of feed grain	0.87	0.52–1.46	0.60
Grain shred as animal feed	0.77	0.46–1.29	0.33
Atopic sensitization (skin prick test)‡			
Pig farming	0.67	0.40–1.12	0.13
Commercial grain cultivation	0.69	0.45–1.06	0.09
Hay pellets as animal feed	0.63	0.29–1.36	0.24

*Potential confounders included in models: age at Phase I, having two or more siblings, maternal and paternal smoking and parental education.

†Models are weighted to the Phase I population eligible for Phase II.

‡Potential confounders included in models: having two or more siblings, maternal smoking, level of parental education and whether the child's mother was raised on a farm.

at least one of these. In weighted analyses of children selected for Phase II, participants (n = 2586) were less likely to be boys, have a mother who ever smoked, were more likely to have parents achieving post-secondary education and have a family history of hay fever and eczema than nonparticipants (n = 1365). Participants were more likely to be from farming households (data not shown). When examining the maternal history of farming among participants, 65% of farm children's mothers were raised on farms compared with 41% of village nonfarm and 33% of town children.

The prevalence of atopy was significantly lower in farm than in nonfarm children after adjusting for age, number of siblings, parental education and whether or not the mother was raised on a farm [positive IgE: aOR = 0.72 (0.57, 0.91); positive SPT: aOR = 0.65 (0.50, 0.86)].

Farm exposures were usually established by age 3 years (data not shown); thus, subsequent exposures relate to the period from pregnancy to age three and are classified as 'any' or 'none' (Table 3). While many had contact with small animals – whether farm or nonfarm children – this was most common among farm children. Exposure to cows, pigs and their sheds was common among farming households as was contact with straw, hay, grain, corn, grass and pellet feed.

We also explored the number of animals kept on farms that the children visited. Among farm children exposed in the last 12 months, the number of cows and pigs on these farms was small (cows: none = 73%, 1–10 = 24%, 11–50 = 2%, >50 = 1%; pigs: none = 55%, 1–10 = 34%, 11–50 = 9%, >50 = 2%) compared with the number of chickens (none = 29%, 1–10 = 27%, 11–50 = 35%, >50 = 9%).

Association between allergic disease and sensitization

Given the high prevalence of respiratory outcomes, we studied their association with atopy. Only 22% of those with

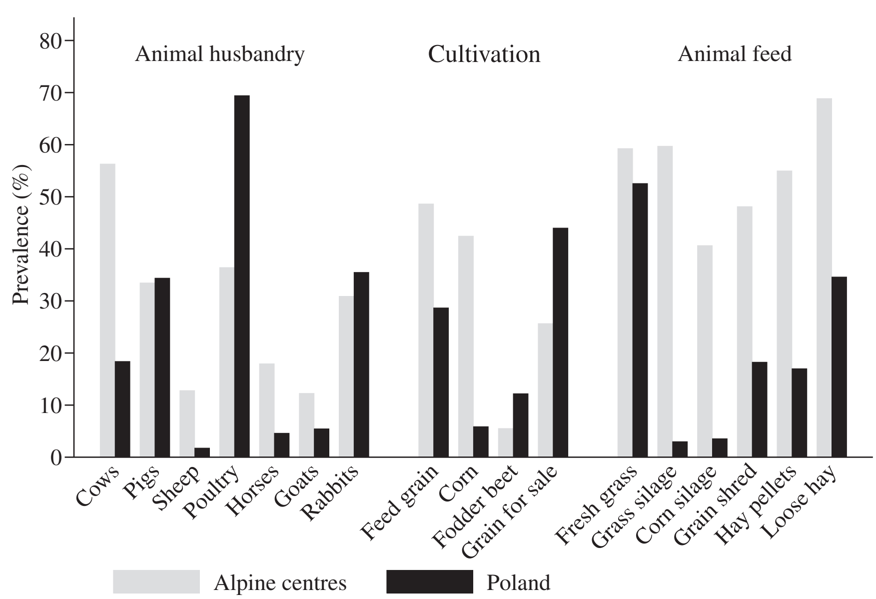

Figure 1 Prevalence of current farming practices among farm children in Poland and Alpine GABRIELA centres

Table 3 Phase II population – exposures and outcomes. Weighted prevalence*

	Village Farm	Nonfarm		
		Village	Town	
	(n = 1145)	(n = 759)	(n = 536)	P†
Any farm exposures (pregnancy to age 3 years)				
Animals				
Cat	57.0%	32.3%	18.4%	<0.01
Dog	60.3%	36.3%	22.0%	<0.01
Cow	31.5%	9.3%	5.3%	<0.01
Pig	28.4%	7.6%	3.9%	<0.01
Poultry	52.9%	21.4%	13.7%	<0.01
Animal sheds				
Cattle	25.4%	6.2%	2.2%	<0.01
Pig	24.2%	5.1%	2.5%	<0.01
Poultry	44.9%	14.5%	9.8%	<0.01
Cultivation/forage				
Straw	27.5%	5.8%	3.0%	<0.01
Hay‡	30.5%	8.7%	5.4%	<0.01
Grain‡	42.4%	11.3%	6.2%	<0.01
Corn‡	17.3%	7.2%	3.7%	<0.01
Grass	30.2%	9.5%	6.1%	<0.01
Pellet feed	18.2%	3.0%	2.6%	<0.01
Storage rooms				
Barn	31.6%	8.6%	5.0%	<0.01
Fodder storage room	29.1%	7.7%	4.6%	<0.01
Consumption of any farm milk	56.2%	40.4%	23.4%	<0.01
Atopy				
Atopic sensitization (IgE)	17.2%	21.9%	25.2%	<0.01
Atopic sensitization (skin prick test)	10.3%	13.2%	21.1%	<0.01

*Weighted to the Phase I population defined as eligible for Phase II.
†Pearson chi-square test with Rao and Scott correction comparing farming and nonfarming groups.
‡Combination of several variable items: hay (contact with forage hay or present while parents were harvesting or handling hay), grain (contact with forage grain or present while parents were harvesting, threshing or kibbling grain) and corn (contact with forage corn or present while parents were harvesting or kibbling corn).

childhood asthma were sensitized to indoor allergens (cat or dust) and 17% to outdoor allergens (grass mix or birch pollens). Similarly, when asthma was defined on doctor diagnoses (diagnosis of asthma or >1 diagnosis of bronchitis with wheeze): only 25% of those were sensitized to indoor allergens and 19% to outdoor allergens. Hay fever also seemed discordant with sensitization, whereby only 21% of those with hay fever were sensitized to outdoor allergens. The following analyses are therefore focussed on the more objective outcome of atopy.

Impact of specific childhood farm exposures on atopy

In adjusted models, several exposures were associated with a reduced risk of atopy (Table 4). For IgE sensitization, these included contact with dogs, poultry, horses, straw, hay and grain. For sensitization as measured by SPT, these and exposure to cats, cows, pigs, cattle stables, pig sheds and grass were associated with a reduced risk. Most children with early-life farm contact were exposed to multiple factors. Highly correlated factors ($\tau_b \geq 0.7$) were dogs and cats, hay and straw, and grain and straw.

Consumption of farm milk was inversely associated with atopy, but did not reach statistical significance when adjusted for farming and potential confounding variables (Table 4). When stratified by childhood contact with any of cows, pigs, horses or sheep, farm milk consumption was not significantly associated with sensitization in either subgroup. There was no evidence of interaction (atopy by SPT: $P = 0.23$; atopy by IgE: $P = 0.53$).

To identify exposures contributing most to the impact of farming, we constructed stepwise models (Table 5). For atopy measured by IgE, grain exposure resulted in the greatest change in the farming aOR, and after inclusion in the model, no other exposures contributed in a sufficiently significant manner. For atopy measured by SPT, poultry exposure resulted in the greatest change. By including these exposures, the farming aOR was reduced and no longer statistically significant, but remained 'protective'.

To assess whether the diversity of farm-related exposures is protective, separate 'exposure–response' analyses were performed. Increasing diversity was associated with a reduced odds of atopy as measured by SPT, reaching statistical significance when comparing ≥5 with none [none: aOR = 1.00; 1 exposure: aOR = 0.97 (0.63, 1.52); 2–4 exposures: aOR = 0.70 (0.45, 1.09); ≥5 exposures: aOR = 0.61 (0.38, 0.99)]. Similar trends were found for sensitization by IgE, but did not reach statistical significance (data not shown).

Associations in the village farm environment

In the Phase II population, poultry and grain exposure contributed towards explaining the inverse association between farming and atopy. Using stepwise models in village farm children alone, early-life exposure to hay was significantly and inversely associated with atopy measured by IgE [aOR = 0.53 (0.34, 0.83)]. As in the full Phase II population (Table 5), early-life exposure to poultry was inversely associated with atopy measured by SPT [aOR = 0.57 (0.34, 0.96)]. In the same model, exposure to hay was also inversely associated with atopy [aOR = 0.31 (0.14, 0.68)]. After subsequently fitting an interaction term between these two exposures, which was statistically significant ($P = 0.027$), the model was refitted using a combined exposure variable. When both exposures were present, there was a significant reduction in the risk of atopy compared with when neither was present [no exposure aOR = 1; poultry only: aOR = 0.69 (0.42, 1.14); hay only: aOR = 0.83 (0.31, 2.21); poultry and hay: aOR = 0.11 (0.04, 0.30)].

Discussion

In a large survey of Polish children, rural life was seemingly protective against childhood asthma and hay fever with little

Table 4 Farm exposures (pregnancy to age 3 years) associated with outcomes in the Phase II population*

	Atopic sensitization – IgE			Atopic sensitization – skin prick test		
	aOR†	95% CI	*P*	aOR†	95% CI	*P*
Animals						
Cat	0.85	0.65–1.11	0.24	0.71	0.52–0.97	0.03
Dog	0.71	0.55–0.91	0.01	0.68	0.50–0.91	0.01
Cow	0.81	0.57–1.15	0.23	0.54	0.34–0.84	0.01
Pig	0.74	0.52–1.06	0.10	0.53	0.32–0.86	0.01
Poultry	0.74	0.55–0.98	0.04	0.54	0.38–0.77	<0.01
Horse	0.39	0.17–0.89	0.03	0.23	0.07–0.78	0.02
Animal shed						
Cow	0.73	0.50–1.06	0.10	0.57	0.35–0.93	0.02
Pig	0.72	0.49–1.07	0.10	0.56	0.33–0.97	0.04
Poultry	0.87	0.65–1.17	0.37	0.86	0.60–1.24	0.43
Animal feed						
Straw	0.66	0.45–0.95	0.03	0.59	0.36–0.95	0.03
Hay‡	0.68	0.47–0.98	0.04	0.41	0.24–0.68	<0.01
Grain‡	0.66	0.47–0.92	0.02	0.56	0.37–0.85	0.01
Grass	0.80	0.57–1.10	0.17	0.54	0.35–0.82	<0.01
Pellet feed	0.84	0.54–1.31	0.44	0.70	0.41–1.20	0.19
Fodder beet	0.52	0.27–1.02	0.06	0.58	0.27–1.25	0.17
Storage room						
Barn	0.90	0.65–1.25	0.54	0.73	0.48–1.13	0.16
Fodder	0.77	0.53–1.11	0.16	0.92	0.59–1.45	0.73
Farm milk	0.88	0.69–1.12	0.30	0.82	0.62–1.09	0.18

*Analyses weighted to the Phase I population eligible for Phase II.

†Analyses adjusted for farming and potential confounders (age at Phase I, number of siblings, parental education and whether the mother grew up on a farm).

‡Combination of several variable items: hay (contact with forage hay or presence while parents were harvesting or handling hay), grain (contact with forage grain or presence while parents were harvesting, threshing or grinding grain).

Table 5 Farm exposures (pregnancy to age 3 years) that best explain the overall effect of 'farming' on outcomes as identified in weighted multivariate stepwise regression models* in the Phase II population

	Model including farming and potential confounders†			Model including farming exposures, farming and potential confounders†		
	aOR‡	95% CI	*P*	aOR	95% CI	*P*
Atopic sensitization – IgE						
Contact with grain				0.66	0.47–0.92	0.02
Farming	0.72	0.56–0.93	0.01	0.81	0.62–1.06	0.12
Atopic sensitization – skin prick test						
Contact with poultry				0.54	0.38–0.77	<0.01
Farming	0.66	0.50–0.87	<0.01	0.76	0.57–1.03	0.08

*Weighted logistic regression models with stepwise variable selection based on statistical significance and largest change in estimate for farming after adjusting for potential confounding variables. All significant ($P < 0.05$) exposure variables (pregnancy to age 3 years) from farm and confounder-adjusted analyses that induced a change in the farming estimate of $\geq 10\%$ were considered in the selection process. Models are weighted to the Phase I population eligible for Phase II.

†Potential confounders included in models: age at Phase I, number of siblings, parental education and whether the mother grew up on a farm.

‡To ensure comparability between models, the models that exclude the specific childhood farming exposures are based on the same sample as the models including these exposures.

difference in village children who did and did not live on a farm. Detailed examinations, in a subpopulation, suggested that a farm childhood afforded additional protection against atopy, possibly reflecting contact with poultry or grain used for animal feed, or other related factors. The protective effect of farming against atopy was observed using IgE and SPT although the effect was more pronounced for the latter. While cautioning against over-interpreting the singular importance of particular factors where there is likely to be much confounding, the findings are broadly in keeping with those from other GABRIEL centres (28) and elsewhere (4). Specifically, the seemingly protective effect of contact with poultry has been reported previously (5, 9, 14).

In other respects, these findings differ from those in the Alpine centres (28). First, the 'farm' effects in the latter were stronger than in Poland. Notably, cattle breeding, the commonest type of Alpine farming, showed inverse associations with childhood asthma and hay fever. In the Polish village population, this appears counterbalanced by exposures conferring risks – fruit cultivation and use of grain shred as animal feed – potentially evening out a 'farm effect'. Furthermore, respiratory outcomes in Poland were only loosely related to atopy potentially reflecting different phenotypes. Also, despite using the same definitions as the Alpine centres (28), appropriately translated, the prevalence of childhood asthma and hay fever was unusually high in Poland. The high rates of inhaler use compared with asthma diagnoses here suggest that a definition of asthma incorporating this may result in some misclassification.

Importantly, these rural communities practise very different types of farming. Over half of Alpine farms kept cows, the remainder keeping other large animals. In Poland, over half of village farms kept only small animals or none, and only a fifth owned cows. Differences in the use of animal feeds may reflect these differences in animal husbandry. Also, farms in Poland were small – the number of cows and pigs on farms recently visited by village farm children was low (≤ 10) compared with the number of chickens. Additionally, only half of Polish farm children fitted in the three farming typologies identified in the Alpine centres (28), suggesting a more mixed farming. If a protective farm effect is dependent on (large) animal exposure (4, 5, 8, 10, 20, 21, 24, 25, 28) or closely related factors, then it is likely to be less prominent in Poland.

Another striking difference between Polish and Alpine centres is the apparent lack of a significant protective effect of farm milk on atopy. In the Alpine centres (28) – and other studies in Europe (8) and the UK (13) – consumption of farm milk was inversely and significantly associated with sensitization. While protective effects against sensitization were found in Poland (Table 4), these did not reach statistical significance and were of smaller magnitude [Poland: aOR = 0.88 (0.69, 1.12); Alpine: aOR = 0.73 (0.64, 0.84)]. There was no evidence of heterogeneity of effect ($P = 0.8$), however, suggesting that the result in Poland may reflect its smaller sample size.

Despite these differences, we detected an approximately 35% reduction in the odds of atopy that could be attributed to grain-related exposures in this Polish community. This corroborates the findings in the Alpine centres where contact with material from grain (i.e. straw) was protective (28).

The protective effect of farm upbringing was, however, less pronounced than the contrast between villages and towns. This may stem partly from exposure misclassification as 'nonfarm' village children had exposure to animals. Our earlier work in the same region, however, suggests that this is not new (22). Failure to discern with confidence the precise nature of a 'protective factor' is unsurprising, but more important is not any current protective effects but their future prospects. Since joining the European Union in 2004, Poland's agriculture has changed radically with a significant reduction in not only the total number of farms but in particular the number of small, family-run farms in villages (31, 32). Additionally, the number and range of animals kept by small-scale farmers have declined and cultivation practices changed importantly. It seems probable that these rapid changes in Polish village life will diminish or abolish those related exposures which were earlier protective against childhood allergy.

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Author contributions

DH, CBF, EvM, PC and members of the GABRIELA study group conceived the study design. BS, HD, AD, AK and AB conducted the Polish data collection. JG managed the databases for all GABRIEL centres. SJM performed the statistical analyses with contributions from SI and MD. SJM and PC wrote the manuscript. All authors contributed to the interpretation of the results and editing of the manuscript and have approved the final manuscript.

Conflicts of interest

BS, HD, AD, AK, AB and DH received research support from the European Commission. MD received research support from the European Commission/European Research Council. EvM consulted for Novartis, GlaxoSmithKline, ALK-Abelló and Protectimmune; received a speaker's fee from InfectoPharm; received research support from Airsonnett AB; and served as a member of the Expert Panel for the UK Research Excellence Framework. All remaining authors declare that they have no relevant conflicts of interest.

Supporting Information

Additional Supporting Information may be found in the online version of this article:

Data S1. Statistical methods.

References

1. Pearce N, Aït-Khaled N, Beasley R, Mallol J, Keil U, Mitchell E et al. Worldwide trends in the prevalence of asthma symptoms: phase III of the International Study of Asthma and Allergies in Childhood (ISAAC). *Thorax* 2007;**62**:758–766.
2. Strachan DP. Hay fever, hygiene, and household size. *BMJ* 1989;**299**:1259–1260. Epub 1989/11/18.

3. Alfvén T, Braun-Fahrländer C, Brunekreef B, von Mutius E, Riedler J, Scheynius A et al. Allergic diseases and atopic sensitization in children related to farming and anthroposophic lifestyle – the PARSIFAL study. *Allergy* 2006;**61**:414–421.
4. Douwes J, Cheng S, Travier N, Cohet C, Niesink A, McKenzie J et al. Farm exposure in utero may protect against asthma, hay fever and eczema. *Eur Respir J* 2008;**32**:603–611.
5. Ege MJ, Frei R, Bieli C, Schram-Bijkerk D, Waser M, Benz MR et al. Not all farming environments protect against the development of asthma and wheeze in children. *J Allergy Clin Immunol* 2007;**119**:1140–1147.
6. Ernst P, Cormier Y. Relative Scarcity of Asthma and atopy among rural adolescents raised on a farm. *Am J Respir Crit Care Med* 2000;**161**:1563–1566.
7. Midodzi WK, Rowe BH, Majaesic CM, Senthilselvan A. Reduced risk of physician-diagnosed asthma among children dwelling in a farming environment. *Respirology* 2007;**12**:692–699.
8. Riedler J, Braun-Fahrländer C, Eder W, Schreuer M, Waser M, Maisch S et al. Exposure to farming in early life and development of asthma and allergy: a cross-sectional survey. *The Lancet* 2001;**358**:1129–1133.
9. Riedler J, Eder W, Oberfeld G, Schreuer M. Austrian children living on a farm have less hay fever, asthma and allergic sensitization. *Clin Exp Allergy* 2000;**30**:194–200.
10. von Ehrenstein O, von Mutius E, Illi S, Baumann L, Böhm O, von Kries R. Reduced risk of hay fever and asthma among children of farmers. *Clin Exp Allergy* 2000;**30**:187–193.
11. Chrischilles E, Ahrens R, Kuehl A, Kelly K, Thorne P, Burmeister L et al. Asthma prevalence and morbidity among rural Iowa schoolchildren. *J Allergy Clin Immunol* 2004;**113**:66–71.
12. Klintberg B, Berglund N, Lilja G, Wickman M, van Hage-Hamsten M. Fewer allergic respiratory disorders among farmers' children in a closed birth cohort from Sweden. *Eur Respir J* 2001;**17**:1151–1157.
13. Perkin MR, Strachan DP. Which aspects of the farming lifestyle explain the inverse association with childhood allergy? *J Allergy Clin Immunol* 2006;**117**:1374–1381.
14. Wickens K, Lane JM, Fitzharris P, Siebers R, Riley G, Douwes J et al. Farm residence and exposures and the risk of allergic diseases in New Zealand children. *Allergy* 2002;**57**:1171–1179.
15. Braun-Fahrländer C, Riedler J, Herz U, Eder W, Waser M, Grize L et al. Environmental exposure to endotoxin and its relation to asthma in school-age children. *N Engl J Med* 2002;**347**:869–877.
16. Braun-Fahrländer C, Gassner M, Grize L, Neu U, Sennhauser F, Varonier H et al. Prevalence of hay fever and allergic sensitization in farmer's children and their peers living in the same rural community. *Clin Exp Allergy* 1999;**29**:28–34.
17. Downs SH, Marks GB, Mitakakis TZ, Lëuppi JD, Car NG, Peat JK. Having lived on a farm and protection against allergic diseases in Australia. *Clin Exp Allergy* 2001;**31**:570–575.
18. von Hertzen L, Mäkelä MJ, Petäys T, Jousilahti P, Kosunen TU, Laatikainen T et al. Growing disparities in atopy between the Finns and the Russians: a comparison of 2 generations. *J Allergy Clin Immunol* 2006;**117**:151–157.
19. Horak F, Studnicka M, Gartner C, Veiter A, Tauber E, Urbanek R et al. Parental farming protects children against atopy: longitudinal evidence involving skin prick tests. *Clin Exp Allergy* 2002;**32**:1155–1159.
20. Radon K, Windstetter D, Eckart J, Dressel H, Leitritz L, Reichert J et al. Farming exposure in childhood, exposure to markers of infections and the development of atopy in rural subjects. *Clin Exp Allergy* 2004;**34**:1178–1183.
21. Remes ST, Iivanainen K, Koskela H, Pekkanen J. Which factors explain the lower prevalence of atopy amongst farmers' children? *Clin Exp Allergy* 2003;**33**:427–434.
22. Sozanska B, MacNeill SJ, Kajderowicz-Kowalik M, Danielewicz H, Wheatley M, Newman Taylor AJ et al. Atopy and asthma in rural Poland: a paradigm for the emergence of childhood respiratory allergies in Europe. *Allergy* 2007;**62**:394–400.
23. Viinanen A, Munhbayarlah S, Zevgee T, Narantsetseg L, Naidansuren T, Koskenvuo M et al. The protective effect of rural living against atopy in Mongolia. *Allergy* 2007;**62**:272–280.
24. Kramer MS, Matush L, Bogdanovich N, Dahhou M, Platt RW, Mazer B. The low prevalence of allergic disease in Eastern Europe. *Clin Exp Allergy* 2009;**39**:708–716.
25. Remes ST, Pekkanen J, Soininen L, Kajosaari M, Husman T, Koivikko A. Does heredity modify the association between farming and allergy in children? *Acta Paediatr* 2002;**91**:1163–1169.
26. Loss G, Apprich S, Waser M, Kneifel W, Genuneit J, Büchele G et al. The protective effect of farm milk consumption on childhood asthma and atopy: the GABRIELA study. *J Allergy Clin Immunol* 2011;**128**: 766–773.
27. Genuneit J, Büchele G, Waser M, Kovacs K, Debinska A, Boznanski A et al. The GABRIEL Advanced Surveys: study design, participation and evaluation of bias. *Paediatr Perinat Epidemiol* 2011;**25**: 436–447.
28. Illi S, Depner M, Genuneit J, Horak E, Loss G, Strunz-Lehner C et al. Protection from childhood asthma and allergy in Alpine farm environments—the GABRIEL Advanced Studies. *J Allergy Clin Immunol* 2012;**129**:1470–1477.
29. Asher M, Keil U, Anderson H, Beasley R, Crane J, Martinez F et al. International Study of Asthma and Allergies in Childhood (ISAAC): rationale and methods. *Eur Respir J* 1995;**8**:483–491.
30. Rao JNK, Scott AJ. On chi-squared tests for multiway contingency-tables with cell proportions estimated from survey data. *Ann Stat* 1984;**12**:46–60.
31. Central Statistical Office. Information about preliminary results of National Agriculture Census 2010 (in Polish). 2011 [cited 2011 August 24]; Available from: http://www.stat.gov.pl/cps/rde/xbcr/gus/PUBL_psr_Info_wstepne_wyniki_psr_2010_300611.pdf. Last accessed 28 March 2013.
32. Central Statistical Office. Structure of agricultural holdings 2007 (in Polish). 2008 [cited 2011 August 24]; Available from: http://www.stat.gov.pl/gus/5840_1771_PLK_HTML.htm. Last accessed 28 March 2013.

Appendix A

The members of the GABRIELA study group are in alphabetical order: Silvia Apprich PhD[G], Andrzej Boznanski MD, PhD[J], Charlotte Braun-Fahrländer MD[D,E], Gisela Büchele PhD[C], William Cookson MD, DPhil[A], Paul Cullinan MD[A], Hanna Danielewicz MD[J], Anna Dębińska[J], Martin Depner PhD[B], Markus Ege MD[B], Urs Frey MD, PhD[Q], Oliver Fuchs MD[K], Jon Genuneit MD[C], Dick Heederik PhD[F], Elisabeth Horak MD[L], Anne Hyvärinen PhD[H], Sabina Illi PhD[B], Michael Kabesch MD[M], Katalin Kovacs[L], Aleksandra Kosmęda PhD[J], Wolfgang Kneifel PhD[G], Philipp Latzin MD, PhD[K], Roger Lauener MD[O], Georg Loss MSc[D,E], Stephanie MacNeill PhD[A], Bernhard Morass MD[L], Anne-Cécile Normand PhD[P], Ilka Noss PhD[F], Renaud Piarroux MD, PhD[P], Helena Rintala PhD[H], Mascha K Rochat MD[B], Nikolaos Sitaridis[C], Barbara Sozanska MD[J], David Strachan MD[N], Christine Strunz-Lehner MPH[B], Bertrand Sudre MD, PhD[I], Erika von Mutius MD, MSc[B], Marco Waser PhD[D,E], Juliane Weber MD[B] and Inge Wouters PhD[F.]

A. Imperial College London, National Heart and Lung Institute, South Kensington Campus, London SW7 2AZ, United Kingdom.

B. LMU Munich, University Children's Hospital, Lindwurmstrasse 4, D 80337, Munich, Germany.

C. Ulm University, Institute of Epidemiology and Medical Biometry, Helmholtzstraße 22, D–89081 Ulm, Germany.

D. Swiss Tropical and Public Health Institute, Socinstr. 57, PO Box 4002, Basel, Switzerland.

E. University of Basel, Petersplatz 1, 4003 Basel, Switzerland.

F. Utrecht University, Institute for Risk Assessment Sciences (IRAS), Division of Environmental Epidemiology, PO Box 80178, 3508TD, Utrecht, The Netherlands.

G. BOKU Vienna, University of Natural Resources and Life Sciences, Department of Food Science and Technology, Muthgasse 18, A-1190 Vienna, Austria.

H. THL Kuopio, National Institute for Health and Welfare, PL 95 70701 Kuopio, Finland.

I. Université de Franche-Comté, UMR 6249 Chrono-Environnement, Département de Parasitologie/Mycologie, UFR SMP, 19 rue A. Paré, 25000 Besançon, France.

J. Wrocław Medical University, 1st Department of Paediatrics, Allergology and Cardiology, ul. J.M. Hoene-Wronskiego 13C, 53-376 Wrocław, Poland.

K. Division of Pulmonology, Department of Paediatrics, Bern University Hospital, Bern, Switzerland.

L. Department of Pediatrics and Adolescents, Division of Cardiology and Pulmonology, Innsbruck Medical University, Anichstr. 35, A-6020, Innsbruck, Austria.

M. Children's University Hospital Regensburg (KUNO), Camus St. Hedwig, Steinmetzstrasse 1–3, 93049 Regensburg, Germany.

N. St George's, University of London, Cranmer Terrace, London SW17 0RE, United Kingdom.

O. High Mountain Hospital Davos, Herman-Burchard-Str. 1, CH-7265 Davos-Wolfgang, Switzerland.

P. Department of Parasitology and Mycology, Hôpital de la Timone, Assistance Publique-Hôpitaux de Marseille, 13385 Marseille, France.

Q. University Children's Hospital (UKBB), Spitalstrasse 33, 4056 Basel, Switzerland.