Original article

Not all farming environments protect against the development of asthma and wheeze in children

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Background: In recent years, studies have shown a protective effect of being raised in a farm environment on the development of hay fever and atopic sensitization. Inconsistent data on the relation of farming to asthma and wheeze have raised some doubt about a true protective effect.

Objective: We sought to study the differential effects of farm-associated exposures on specific asthma-related health outcomes.

Methods: The cross-sectional Prevention of Allergy Risk Factors for Sensitization in Children Related to Farming and Anthroposophic Lifestyle study included 8263 school-age children from rural areas in 5 European countries. Information on farm-related exposures and health outcomes was obtained by using questionnaires. In subsamples allergen-specific IgE and RNA expression of *CD14* and *Toll-like receptor* genes were measured, and dust from children's mattresses was evaluated for microbial components.

Results: Inverse relations with a diagnosis of asthma were found for pig keeping (odds ratio [OR], 0.57; 95% CI, 0.38-0.86), farm milk consumption (OR, 0.77; 95% CI, 0.60-0.99), frequent stay in animal sheds (OR, 0.71; 95% CI, 0.54-0.95), child's involvement in haying (OR, 0.56; 95% CI, 0.38-0.81), and use of silage (OR, 0.55; 95% CI, 0.31-0.98; for nonatopic asthma) and in Germany for agriculture (OR, 0.34; 95% CI, 0.22-0.53). Protective factors were related with higher expression levels of genes of the innate immunity. Potential risk factors for asthma and wheeze were also identified in the farm milieu. Levels of endotoxin and extracellular polysaccharides were related to the health outcomes independently of the farm exposures.

Conclusions: The protective effect of being raised in a farm environment was ascribed to distinct exposures.

Clinical implications: The development of atopic sensitization and atopic and nonatopic asthma is most likely determined by different environmental factors, possibly reflecting distinct pathomechanisms. (J Allergy Clin Immunol ■■■■;■■■:■■■-■■■.)

Key words: Asthma, wheeze, atopic sensitization, farming, microbial components

Numerous studies have shown a protective effect of being raised in a farm environment on the development of hay fever and atopic sensitization among children.[1-11] The effects on asthma, however, are inconsistent. Whereas in some studies being raised on a farm was inversely associated with asthma or wheeze ever,[1,2,6,7,9,12,13] a substantial number of studies did not find any significant association,[4,5,10,11,14-16] and in some regions a tendency toward a positive association of farming with asthma was revealed.[2,17] These controversial results have raised some

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Abbreviations used
EPS: Extracellular polysaccharide
OR: Odds ratio
PARSIFAL: Prevention of Allergy Risk Factors for Sensitization in Children Related to Farming and Anthroposophic Lifestyle
TLR: Toll-like receptor

doubt about a true farm effect on asthma. However, a more differentiated look at distinct farm-related exposures and specific asthma phenotypes might be useful.

Moreover, little is known about potential specific determinants of asthma and atopy in the farm environment. As candidate protective factors, consumption of unpasteurized farm milk[7,18] and exposure to animal sheds have been identified. Dust from animal sheds is rich in endotoxin[19] and muramic acid,[20] both bacterial components that can activate innate immunity, thereby potentially affecting the development of asthma and allergic diseases.

Farming practices and hence microbial exposures vary between farms. The global analysis of the multicenter Prevention of Allergy Risk Factors for Sensitization in Children Related to Farming and Anthroposophic Lifestyle (PARSIFAL) study revealed a weak effect of being raised on a farm on asthma, with substantial heterogeneity across study regions.[21] The present analysis aimed at disentangling the differential effects of various farm-associated exposures on specific asthma-related health outcomes, thereby explaining the heterogeneous findings. Furthermore, the contribution of several microbial components to the identified effects was studied.

METHODS

Population and study areas

The PARSIFAL study was a cross-sectional survey on children of farmers, children attending Rudolf Steiner schools, and their respective reference groups. The study was carried out as described previously.[21] In the farming branch of the study, children aged 5 to 13 years from rural areas of Austria, Germany, The Netherlands, Sweden, and Switzerland were invited to participate. Of the 11,969 invited children, 8402 (70%) returned the questionnaires. A total of 139 children were excluded because of missing values for sex and age or because they did not, on review, meet the age criteria of 5 to 13 years. Of the included 8263 children, 88% had complete values for all farm exposure variables. The study was approved by the national ethical boards of the 5 study centers, and informed consent was obtained from the children's parents or guardians for questionnaires and blood samples.

Questionnaire

The questionnaire included questions on sociodemographic background, family history of asthma and atopy, exposure to farm environment, animals, pets, housing, nutrition, and the child's health. Questions on health outcome and farm exposure were derived from the internationally validated International Study of Asthma and Allergies in Childhood II[22] questionnaire and the Allergy and Endotoxin study.[7] Children with reported physician-diagnosed asthma once or obstructive bronchitis more than once in their lifetime were defined as having *asthma ever*. Wheezing during the past 12 months was considered *current wheeze*. A child who lived on a farm and whose family ran the farm was coded as being a *farm child* (n = 2823), whereas all other children were termed *reference children* (n = 5440). Questions concerning farm life and farm exposure were asked to both farm and reference children, covering regular stays in animal sheds or barns; regular participation in farm activities, such as harvesting of hay; consumption of farm milk; regular contact with farm animals; and maternal involvement in farm work during pregnancy. Farmers were asked whether they performed livestock farming, agriculture (cultivation of grain), or combinations of these. Data on farm characteristics, such as animal species kept on the farm (cattle, pigs, poultry, horses, sheep, goats, hares, and rabbits) or animal feed (pressed hay, loose hay, pellet feed, and silage), were also collected. The children's farm activities at present were dichotomized into rarely or regularly (at least once a week).

Measurement of allergen-specific serum IgE

Blood analysis was performed in a subsample of 2086 children, which did not differ substantially from the whole sample with respect to farm exposures. Allergen-specific IgE for common inhalant (Phadiatop) and food allergens (fx5; Pharmacia CAP System; Phadia AB, Uppsala, Sweden) was measured in the serum. Definition of atopic sensitization and distinction between atopic and nonatopic asthma or wheeze was based on IgE values of 0.35 kU/L or greater for inhalant or food allergens.

Expression of *CD14* and *Toll-like receptor*

Gene expression of *CD14* and *Toll-like receptor* (*TLR*) was assessed in 268 Swiss children, among them 56% farm children. Children with available RNA samples (95.3% of children who provided blood samples) did not differ significantly from the total Swiss PARSIFAL population with respect to farm exposures and health outcomes (data not shown). Detection methods are described in the Online Repository at www.jacionline.org.

Detection of microbial components

Levels of endotoxin, $\beta(1\rightarrow3)$-glucan, and fungal extracellular polysaccharides (EPSs) were measured in mattress dust samples of 440 children. Sampling and detection methods are described elsewhere[23] (see also this article's Supplementary Methods text in the Online Repository at www.jacionline.org).

Statistical analysis

Statistical analysis was performed with SAS 9.1.3 (The SAS Institute, Cary, NC). Odds ratios (ORs) from bivariate analysis or logistic regression models are given with 95% CIs. Frequencies of farm characteristics were calculated as percentages of all farms (Table I), and children's activities on farms were calculated as percentages of all children (Table II).

Parsimonious models (Fig 1) were established for every outcome separately by means of backward elimination with a significance level of .157, corresponding to Akaike's information criterion, to identify the individual effects of single farm characteristics and the children's activities. The backward elimination started with the following set of variables: family history of asthma or atopy, respectively; sex, being a farm child, and study center; agriculture; keeping pigs, poultry, horses, sheep, goats, hares, or rabbits; feeding pressed hay, loose hay, silage, or pellet feed at the farm; and predominant farm milk consumption by the child, regular stays in animal sheds, regular stays in barns, regular contact with farm animals, and helping with haying. Models for atopic sensitization additionally included

TABLE I. Characteristics of farms in the PARSIFAL study

Farm characteristics	Percentage of farm children (n = 2813)
Farm types	
Livestock farming	82%
Agriculture	40%
Animals kept on farm	
Cattle	70%
Poultry	46%
Pigs	33%
Hares and rabbits	33%
Horses	21%
Sheep	16%
Goats	15%
Fodder used on farm	
Pellet feed	64%
Loose hay	57%
Silage	53%
Pressed hay	42%

TABLE II. Children's farm-related activities in the PARSIFAL study

Children's activities	Farm children (n = 2813)	Reference children (n = 5440)
Regular* farm milk consumption	68%	18%
Regular stays in animal sheds	75%	13%
Regular stays in barns	61%	8%
Regular involvement in haying	51%	3%
Regular contact with farm animals	93%	34%
Regular contact with cattle	77%	16%
Regular contact with pigs	38%	8%
Regular contact with sheep	21%	8%
Regular contact with poultry	47%	16%
Regular contact with horses	29%	17%
Regular contact with goats	20%	7%
Regular contact with hares/rabbits	39%	29%

*"Regular" is defined as at least once a week.

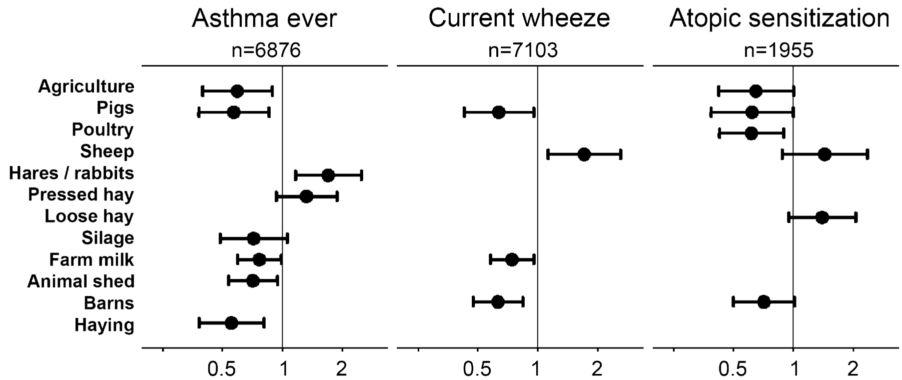

FIG 1. Farm-related specific determinants of asthma, wheeze, and atopic sensitization. Mutually adjusted ORs with 95% CIs additionally adjusted for sex, study center, group, and family history of asthma are shown. Atopic sensitization was adjusted for sex, study center, family history of atopy, and additionally for maternal exposure to animal sheds during pregnancy. The models were established by using backward elimination based on Akaike's information criterion.

maternal exposure to animal sheds during pregnancy.[24] In some study regions, being a farm child, livestock farming, and cattle keeping were collinear, and therefore the latter 2 variables were not included in backward elimination.

The specific determinants for the atopic and nonatopic phenotypes of asthma and wheeze were explored by using the same procedure starting from the same set of variables (Fig 2). In a sensitivity analysis further variables (age, parental educational level, daycare attendance, duration of breast-feeding, contact with pets, reported childhood infections, worm infestation, and use of antibiotics or antipyretics) were explored but did not confound the associations.

All exposures that contributed significantly to the models in Fig 1 were used to calculate propensity scores for being a farm child (ie, the probability of being a farm child dependent on the farming exposures). The resulting propensity scores were then used as scalar variables to adjust for being a farm child in the analyses investigating the effects of microbial components on asthma, wheeze, and atopy.[25] Values for exposure levels of microbial contaminants were obtained by means of division of measured concentrations by weight of collected mattress dust followed by log transformation, resulting in normal distribution. Data on microbial components were only available for a subsample selected for wheezing and atopy status,[23] and therefore stratified weighted logistic regression was performed.[26] Each observation was weighted by the inverse probability of being selected to the subsample of dust analysis.

In a final step heterogeneity across study regions of the effect of farming on asthma was assessed, looking at farms with and without agriculture separately. ORs for farming were calculated stratified for study regions and compared by using Breslow-Day tests. In multiple regression models interaction terms for farming and study regions were explored.

RESULTS

Characteristics of farms and children's activities on farms

Information on farms is provided in Table I. The questions regarding farm characteristics were not mutually exclusive, and therefore percentages do not sum up to 100%.

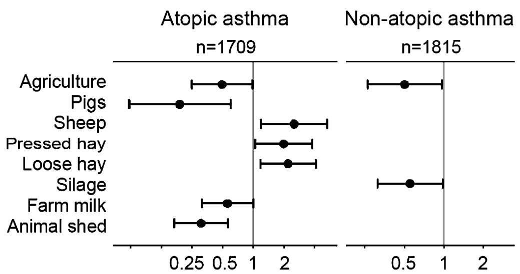

FIG 2. Farm-related specific determinants for atopic and nonatopic asthma. Mutually adjusted ORs with 95% CIs additionally adjusted for number of older siblings, sex, study center and family history of asthma, where appropriate, are shown. The models were established by using backward elimination based on Akaike's information criterion in the subsample of children selected for measurement of allergen-specific IgE levels.

Because the PARSIFAL study was deliberately performed in regions of dairy farming, the predominant form of farming was livestock, in particular cattle. Yet substantial numbers of other animals were kept on the farms as well. Of the livestock farms, 37% also performed agriculture, most likely to provide fodder for their animals. Only 24% of agricultural farms did not also raise livestock. Because farm characteristics might reflect a child's exposure only to a limited extent, children's activities on a farm were assessed also for farm and farm reference children (Table II). A third of the reference children in these rural areas were exposed to farm animals on a regular basis (ie, at least once a week). The prevalences of the lifetime diagnosis of asthma and of current wheeze in farm children were 6.3% and 5.0%, respectively, and in reference children they were 9.1% and 7.7%, respectively.

Effects of farm characteristics and children's activities on asthma, wheeze, and atopic sensitization

Fig 1 shows the mutually adjusted models for asthma, wheeze, and atopic sensitization resulting from backward elimination. Variables with nonsignificant associations with the health outcomes in these models are not listed (ie, horses kept on the farm, goats kept on the farm; pellet feed or other animal feed; and children's regular contact with specific farm animal species [cattle, pigs, sheep, poultry, horses, goats, and hares/rabbits]). The lifetime diagnosis of asthma was inversely associated with agriculture (OR, 0.60; 95% CI, 0.40-0.89), pig farming (OR, 0.57; 95% CI, 0.38-0.86), farm milk consumption (OR, 0.77; 95% CI, 0.60-0.99), frequent stay in animal sheds (OR, 0.71; 95% CI, 0.54-0.95), and child's involvement in haying (OR, 0.56; 95% CI, 0.38-0.81), and there was borderline significance with using silage as feed (OR, 0.72; 95% CI, 0.49-1.06). Keeping of hares and rabbits (OR, 1.70; 95% CI, 1.16-2.50) and the use of pressed hay (OR, 1.32; 95% CI, 0.93-1.88) were positively associated. Current wheeze was inversely related to pig farming (OR, 0.64; 95% CI, 0.43-0.95), farm milk consumption (OR, 0.74; 95% CI, 0.58-0.96), and frequent stays in barns (OR, 0.63; 95% CI, 0.47-0.84); the presence of sheep, however, was positively associated with wheezing (OR, 1.72; 95% CI, 1.13-2.61). Frequent stays in barn were also inversely associated with atopic sensitization, as well as agriculture and keeping pigs and poultry on the farm. The use of loose hay as fodder was positively associated with atopic sensitization. When adjusting for the child's activities and farm characteristics, the variable of being a farm child was not any longer inversely related with asthma, wheeze, or atopy; in other words, the variables included in the final models explained the effect of farming on asthma, wheeze, and atopy completely (data not shown).

Effects of farm characteristics and children's activities on the expression of *CD14* and *TLR* genes

Table III gives the geometric means ratios for the expression of *CD14* and *TLR* genes, depending on the various farm characteristics and children's activities that were related to asthma or wheeze from Fig 1. Interestingly, the different exposures exhibited specific effects on different genes. Because of a high correlation ($r > 0.8$) of the variables regular stays in animal sheds, regular stays in barns, and maternal exposure to stables during pregnancy with being a farm child in the Swiss subsample, independent effects of these variables could not be studied.

Atopic and nonatopic phenotypes of asthma and wheeze

Besides common determinants for asthma and atopic sensitization, some specific determinants for either asthma or atopic sensitization were also detected. This observation prompted a differential evaluation of the asthma and wheeze phenotypes with respect to a child's atopy status. As shown in Fig 2, only agriculture exhibited an inverse relation with both phenotypes of asthma. Further effects on atopic asthma were exerted by pig farming (OR, 0.19; 95% CI, 0.06-0.60), regular consumption of farm milk (OR, 0.56; 95% CI, 95% CI, 0.31-1.01), and stays in animal sheds (OR, 0.31; 95% CI, 0.17-0.57). Sheep farming and feeding loose or pressed hay were positively associated with the atopic phenotype. Interestingly, the use of silage was protective for nonatopic asthma (OR, 0.55; 95% CI, 0.31-0.98). For wheeze, almost the same determinants were found (data not shown): pig farming, silage, and barn stays were inversely related to atopic wheeze, and sheep farming and feeding loose hay were positively related; barn stays tended to be protective for nonatopic wheeze.

Protective and risk-associated farm characteristics with respect to asthma and wheeze

The 742 of 2813 farm children who were growing up in a strongly protective farm environment (ie, exposed to 4 or 5 ''asthma-protective'' factors) had an asthma prevalence of less than 3%. On the other hand, 210 farm children were exposed to asthma risk factors (keeping hares or rabbits and feeding pressed hay), and only 1 or no protective factors. This group of children had an asthma prevalence

TABLE III. Geometric means ratios for expression of CD14 and TLR dependent on children's farm exposures and farm characteristics

Exposure variables	*CD14*	*TLR1*	*TLR2*	*TLR3*	*TLR4*	*TLR5*	*TLR6*	*TLR7*	*TLR8.1**	*TLR8.2**	*TLR9*	*TLR10*
Being a farm child	1.67, $P < .001$	1.38, $P = .006$	1.52, $P < .001$		1.20, $P = .046$			1.26, $P = .048$	1.29, $P = .034$	1.34, $P = .032$		
Haying†				1.72, $P = .067$				1.53, $P = .021$				1.83, $P = .011$
Farm milk consumption†								1.38, $P = .040$		1.42, $P = .060$		
Keeping pigs†						1.41, $P = .054$						
Feeding silage†							1.35, $P = .027$			1.39, $P = .065$	1.32, $P = .079$	
Keeping sheep†												
Keeping hares†			1.27, $P = .072$									
Feeding pressed hay†						0.53, $P = .001$						

Only geometric means ratios with P values of less than .1 are shown. The geometric means ratios are adjusted for age, sex, and for being a farm child (†).
*Isoform 1 (TLR8.1) encodes the longer isoform, which has an extended N-terminus compared with isoform 2 (TLR8.2).

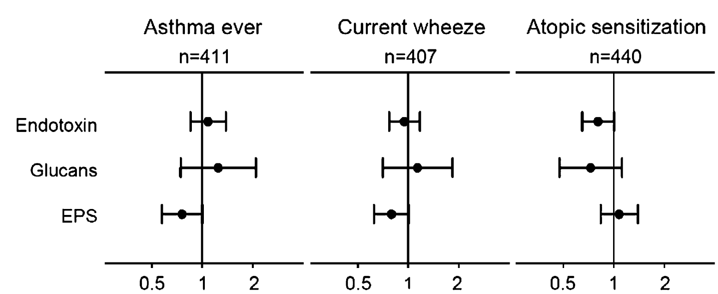

FIG 3. Effects of microbial compounds measured in mattress dust on asthma, wheeze, and atopic sensitization. Mutually adjusted ORs with 95% CIs additionally adjusted for all variables contributing to the respective models in Fig 1 are shown.

of 17%. Children living on farms where sheep but no pigs or cows were kept had prevalences of wheeze and asthma up to 4 times higher than their peers living on farms where pigs or cows but no sheep were kept (12% vs 3% and 14% vs 3% for wheeze and asthma, respectively). Farm children with risk factors for atopy (sheep and loose hay) but no protective factors had a prevalence of 14% for atopic sensitization, whereas among the 109 farm children with no risk-associated factors but at least 3 protective factors, only 2 (1.8%) children were sensitized.

Effects of microbial substances on asthma and related health outcomes

Fig 3 shows the mutually adjusted effects of endotoxin, glucans, and EPS levels in mattress dust on asthma, wheeze, and atopic sensitization. Endotoxin was inversely related to atopic sensitization ($P = .0531$). Glucans were not significantly associated with any of the 3 outcomes but tended to be protective for atopic sensitization. EPSs were inversely related to asthma ($P = .0483$) and with borderline significance to wheeze ($P = .0581$). Further distinction of atopic and nonatopic phenotypes did not reveal more information, most likely because of limited sample sizes. The protective effect of being a farm child on current wheeze was explained by the levels of exposure to endotoxin, glucans, and EPSs (adjusted OR, 0.89), whereas for asthma (adjusted OR, 0.55) and atopic sensitization (adjusted OR, 0.38), the protective effect of being a farm child was not explained by the levels of these exposures. Although children from farms with pigs but without sheep had endotoxin levels (geometric mean, 41,438 EU/g; 29,551-58,107 EU/g) in mattress dust almost twice as high as those seen in children from sheep farms without pigs (26,909 EU/g; 17,192–42,118 EU/g), this difference is unlikely to reflect the underlying mechanisms of the effects of pig and sheep keeping on asthma and sensitization because the variables of keeping sheep and keeping pigs did not change the OR for endotoxin (data not shown).

Heterogeneity across countries

The crude asthma OR for being a farm child versus that seen in reference children varied substantially across countries.[21] A low OR was found in Germany (OR, 0.45; 95% CI, 0.32-0.64), whereas in the other 4 countries the OR was close to unity (OR, 0.92; 95% CI, 0.72-1.17). The effect was not heterogeneous among these 4 countries ($P = .85$), but it was heterogeneous between them and Germany ($P = .013$). Thus the 2 subsamples (children

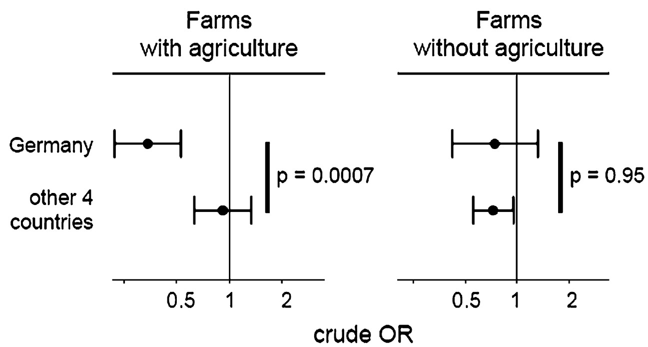

FIG 4. Effect of being a farm child on asthma in agricultural and nonagricultural farms. Crude ORs are given with 95% CIs for asthma diagnosis dependent on being a farm child separately for the German subsample and the subsample of the 4 other countries. *P* values refer to Breslow-Day tests for homogeneity of effects.

from countries without a farm effect and the German subsample) were analyzed for differences in farm characteristics. When comparing children from farms without agriculture with reference children, the effect of farming on asthma did not vary between the 2 subsamples (Fig 4). However, when comparing children from farms with agriculture with reference children, the effect was strong in Germany (OR, 0.35; 95% CI, 0.23-0.55) but marginal in the other 4 countries (OR, 0.93; 95% CI, 0.65-1.33). Heterogeneity of effects between the 2 subsamples was formally proved by using a highly significant Breslow-Day test ($P = .0007$) and a significant interaction term in multiple regression analysis ($P = .0015$).

DISCUSSION

The present study revealed distinct protective exposures for asthma and wheeze (agriculture, pig farming, silage, haying, farm milk, animal sheds, and barns). Most of these exposures were related to the atopic phenotypes, but silage exerted a protective effect only on the nonatopic phenotype. Furthermore, potential risk factors for asthma and wheeze have been identified in the farm milieu (sheep and hare keeping and using hay as feed). For atopic sensitization, agriculture, pig, and poultry farming and barns were identified as protective factors. Collectively, the identified factors explained the protective effect of being a farm child on asthma. Endotoxin and EPS levels were related to asthma, wheeze and atopic sensitization independently of the farm characteristics and the child's activities.

Potential determinants of asthma, wheeze, and atopic sensitization

The effects of frequent stays in animal sheds and farm milk consumption have been described previously.[7,24] A detailed analysis of the effects of farm milk and other dairy products is the subject of a separate article.[18]

The large sample size of the PARSIFAL study provided the opportunity to differentiate between several animal species usually kept on farms. In this context the identification of pig keeping, in our study populations mostly in combination with cattle keeping, as a potential protective factor for all 3 health outcomes is an interesting finding. An inverse relation of pig keeping with atopic sensitization has also been detected in two studies performed in Guinea-Bissau[27] and New Zealand.[16] In contrast, in an Iowa population pigs were found to be positively related to asthma.[17] In our analysis the protective effects of pigs on asthma and atopy were consistent across all countries (data not shown). The discrepancy between the American study and the other studies might be explained by different farm sizes and hence children's exposure. At least in our study population, more than 80% of pig farmers kept less than 10 pigs, and exclusive pig farming was very uncommon in the study population (n = 34). Four (12%) children from exclusive pig farms had a physician's diagnosis of asthma, whereas only 35 (4%) of 884 children from dairy farms with additional pig keeping had asthma ($P = .0514$). An exploratory analysis of the number of pigs kept on a farm did not reveal any dose-response effect. An explanation for the effects of pig keeping might be found in specific microbial exposures present in farms where pigs are kept in addition to cattle.

In contrast to pig keeping, sheep farming increased the risk for atopic asthma and wheeze. This might be a plausible finding because for sheep breeders, a higher risk of respiratory syndromes has been described, possibly caused by disinfectants, such as formaldehyde or copper sulfate.[28] Whether this explanation can be applicable to children remains to be elucidated. Nevertheless, sheep keeping might obscure a protective farm effect on asthma in populations where sheep breeding is common.[16]

Harvesting, storage, and feeding of hay in various forms is another key feature of farms. Active participation in haying, however, might be avoided by children with asthma or allergies, implying potential reverse causation. In the PARSIFAL study only 62 families (<1% of all) reported that the child avoided haying because of manifest disease or for prevention, and the effect of haying was still present when the analysis was restricted to children without sensitization to grass pollen (data not shown). One could imagine that small dust particles inhaled during haying might act as carriers of protective agents, such as microbial antigens or immunomodulatory substances (eg, mycotoxins). This notion is supported by the gene expression studies showing that active participation in haying was associated with higher gene expression of several *TLR* genes (Table III).

In turn, a positive association of pressed hay with asthma and of loose hay with atopic asthma and atopic sensitization was found. Yet this does not necessarily contradict the potential protective effect of active participation in haying because the microbial colonization of fresh hay differs substantially from that of stored hay.[29,30] Barns do not only comprise hay lofts but are also storage places for other farm products, such as crop, grain, and straw. Therefore it is conceivable that the protective effect of barns is present despite a disadvantageous effect of pressed or loose hay.

Silage is of special interest because it was only clearly protective for the nonatopic phenotypes of asthma and wheeze. Silage contains vast amounts of lactobacilli but also other microbes with potential immunomodulatory effects, such as aspergilli[31] and listeriae.[32] Small but continuously inhaled or ingested doses of microbial contaminants, such as mycophenolic acid from aspergilli, might suppress T-cell activity.[31] *Listeria monocytogenes*[32] is known for its ability to stimulate the innate immune system and to convert T_H2-dominated immune responses into T_H1-dominated responses.[33]

The exact molecular mechanisms of the farm-related exposures and their beneficial and, in some cases, also probably harmful effects on asthma remain, however, unknown. The observation that these farm-related exposures are associated with the differential expression of the *CD14* and *TLR* genes (Table III) supports the idea of distinct effects on a molecular level. Various exposures might differentially affect the innate immune system in qualitative and quantitative terms.

A striking finding of the present analysis was the identification of agriculture as having the power to explain the heterogeneous effects of farming on asthma between Germany and the other study regions. Because the effect of agriculture on asthma was only present in Germany, comparing the practices and products of agriculture of the German areas with those of the other PARSIFAL study regions might be helpful. Unfortunately, no supplemental data on agriculture were available in the data set. However, because most of the agricultural farms (>80%) of the German subsample concomitantly raised livestock (cattle/pigs), cultivation of feed grain is the most probable form of agriculture. Culture of fodder beets, oilseeds, potatoes, or cereals for human nutrition is likely to play a minor role in these areas. Contamination of feeding grain with immunomodulatory mycotoxins is a well-known phenomenon,[34-37] and one might speculate that feeding grain could contain beneficial immunomodulatory substances. In particular, pigs are fed with bruised grain, which can be contaminated with deoxynivalenol, a trichothecene mycotoxin that enhances proinflammatory gene expression in macrophages after costimulation by TLR ligands, such as endotoxin.[38]

Independent effects of endotoxin and glucans

Mattress dust samples of selected farm and reference children were examined to back up the questionnaire data with objective biologic parameters. A previous analysis of the same data set focused on atopic wheeze ever as an outcome because of the stratified sampling design.[39] After adjustment of farming, no significant associations of the outcomes with the biocontaminants under investigation were seen. The present analysis used weighted stratified logistic regression to overcome problems of the sampling design, thereby rendering exploration of further outcomes, such as lifetime diagnosis of asthma, current wheeze, and atopic sensitization, possible.

The protective effect of endotoxin on atopic sensitization is in accordance with that seen in previous studies.[19,40] $\beta(1\rightarrow3)$-glucans are cell-wall constituents of most fungi, but are also found in some bacteria and many plants.[41] The inverse association of glucans with atopic sensitization did not reach statistical significance, potentially because of limited sample size, but the estimate was stronger than for endotoxin. EPSs of *Aspergillus* and *Penicillium* species are more specific markers of indoor fungal exposure.[41] The inverse association of EPSs with asthma is a novel finding. Of all 3 microbial substances, EPSs were most closely related to being a farm child (data not shown).

The associations of the 3 biocontaminants with the asthma-related health outcomes were independent of the farm characteristics and the children's activities. Hence they might represent exposures not adequately covered by questionnaire data. On the other hand, the exposures identified through questionnaires, such as agriculture or pig farming, were not traced back to the microbial compounds under investigation, ultimately prompting exploration of further microbial substances (eg, those found in agriculture or animal feed).

Conclusion

The asthma-protective effect of being raised on a farm in the PARSIFAL study can be attributed to pig farming, feeding silage, child's involvement in haying, farm milk consumption, and regular stay in animal sheds and barns. In Germany performing agriculture contributed importantly to the asthma-protective effect. The microbial compounds investigated thus far do not explain the inverse relation of asthma prevalence with farm characteristics and children's activities but exert independent effects. The identification of highly protective farms might direct future research to investigate microbial contamination of distinct farms with their individual animal species and feeds.

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