

Prenatal exposure to a farm environment modifies atopic sensitization at birth

Markus Johannes Ege, MD,^a Ileana Herzum, MD,^b Gisela Büchele, MPH,^c Susanne Krauss-Etschmann, MD,^{a,d} Roger P. Lauener, MD,^e Marjut Roponen, PhD,^f Anne Hyvärinen, PhD,^f Dominique A. Vuitton, MD,^g Josef Riedler, MD,^h Bert Brunekreef, PhD,ⁱ Jean-Charles Dalphin, MD, PhD,^j Charlotte Braun-Fahrländer, MD,^k Juha Pekkanen, MD,^{f,l} Harald Renz, MD,^b Erika von Mutius, MD,^a and the Protection Against Allergy Study in Rural Environments (PASTURE) Study group* Munich, Marberg, and Ulm, Germany, Zurich and Basel, Switzerland, Kuopio, Finland, Besançon, France, Schwarzhach, Austria, and Utrecht, The Netherlands

Background: Previous cross-sectional surveys have suggested that maternal exposure to animal sheds during pregnancy exerted a protective effect on atopic sensitization in children lasting until school age.

Objective: We sought to evaluate the effects of maternal exposure to animal sheds and other farm-related exposures during pregnancy on cord blood IgE levels in a prospective birth cohort.

Methods: Pregnant women living in rural areas in Austria, Finland, France, Germany, and Switzerland were recruited in the third trimester of pregnancy. Information on maternal farm-related exposures, nutrition, and health during pregnancy was obtained by means of interviews. Specific IgE levels for food and common inhalant allergens were assessed in cord blood of 922 children and peripheral blood samples of their mothers.

Results: Different sensitization patterns in cord blood of farm and nonfarm children were observed. In multivariable analysis consumption of boiled, but not unboiled, farm milk during pregnancy was positively associated with specific IgE to cow's milk independently from maternal IgE. In contrast, there was an inverse relationship between maternal exposure to animal sheds and cord blood IgE levels against seasonal allergens (adjusted odds ratio, 0.38; 95% CI, 0.21-0.70). This association was not confounded by maternal IgE levels. Maternal contact with hay enhanced the protective effect of exposure to animal sheds on IgE levels to grass pollen in cord blood.

Conclusions: Maternal exposure during pregnancy influences atopic sensitization patterns in cord blood. The (microbial) context of allergen contact possibly modifies the risk of atopic sensitization. (*J Allergy Clin Immunol* 2008;122:407-12.)

Key words: Prenatal exposure, atopic sensitization, cord blood, farming, microbial components

The potential effects of antenatal origins of diseases manifesting later in life have attracted much attention, and a vast potential for disease prevention has been anticipated.^{1,2} Also, a number of mechanisms have been implicated for the prenatal development of asthma and atopic diseases, and potential strategies to reverse the process are discussed.³⁻⁵ For atopic sensitization, a protective effect by prenatal exposure to a farm environment has been suggested: an inverse association of maternal exposure to animal sheds during pregnancy with atopic sensitization in the offspring at school age has been found in a survey.⁶ The cross-sectional design of that study, the retrospective assessment of the exposure, and the time gap between exposure and outcome evaluation precluded the establishment of a causal relation. Only collection of exposure data before birth and detection of sensitization patterns at birth can with any certainty eliminate recall bias and interfering effects of any exposures between birth and school age. In the meantime, the birth cohort Protection against Allergy Study in Rural Environments (PASTURE) has been set up to assess the role of indoor and

From ^aUniversity Children's Hospital, Munich; ^bthe Department of Clinical Chemistry and Molecular Diagnostics, Philipps University of Marburg; ^cthe Institute of Epidemiology, University of Ulm; ^dHelmholtz Zentrum München German Research Center for Environmental Health, Munich; ^eZurich University Children's Hospital; ^fthe Department of Environmental Health, National Public Health Institute, Kuopio; ^gSERF Research Unit, Université de Franche-Comté, Besançon; ^hChildren's Hospital, Schwarzhach; ⁱthe Institute for Risk Assessment Sciences and Julius Center for Health Sciences and Primary Care, University Medical Center Utrecht; ^jthe Department of Respiratory Disease, University Hospital, Besançon; ^kthe Institute of Social and Preventive Medicine, University of Basel; and ^lthe School of Public Health and Clinical Nutrition, University of Kuopio.

*The Protection Against Allergy Study in Rural Environments (PASTURE) study group: Gertraud Weiß, Ellen Üblagger, Claudia Humer, and Manuela Rußegger (Austria); Raija Juntunen, Reetta Tiitonen, Pekka Tiittanen, Maija-Riitta Hirvonen, Kati Huttunen, Suvi Virtanen, Timo Kauppila, Aino Nevalainen, Sami Remes, Tomi-Pekka Tuomainen, and Anne Karvonen (Finland); Marie-Laure Dalphin, Renaud Piarroux, Gabriel Reboux, Sandrine Roussel, and Bertrand Sudre (France); Susanne Schmid, Sabina Illi, Nicola Korherr, Jon Genuneit, Richard Peter, Serdar Sel, Nicole Blümer, and Petra Pfefferle (Germany); Ulrike Gehring (The Netherlands); Sondhja Bitter, Felix H. Sennhauser, Susanne Loeliger, Johanna Steinle, and Remo Frei (Switzerland).

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Reprint requests: Markus Johannes Ege, MD, Dr. von Haunersche Kinderklinik, Lindwurmstrasse 4, 80337 Munich, Germany. E-mail: markus.ege@med.uni-muenchen.de.

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Abbreviations used

aGMR:	Adjusted geometric mean ratio
ALEX:	Allergy and Endotoxin
aOR:	Adjusted odds ratio
cOR:	Crude odds ratio
EPS:	Extracellular polysaccharide
PARSIFAL:	Prevention of Allergy Risk Factors for Sensitization in Children Related to Farming and Anthroposophic Lifestyle
PASTURE:	Protection Against Allergy Study in Rural Environments

outdoor exposure to various microbial products for the development of childhood asthma and allergies in a prospective design in rural environments across Europe. Furthermore, the project aims to identify the immunologic and genetic mechanisms that determine an individual's response to these environmental influences.⁷

The aim of the present analysis of the PASTURE cohort was to evaluate the effects of maternal farm-related exposures during pregnancy, such as boiled or unboiled farm milk and exposure to hay lofts and animal sheds, on specific fetal IgE production, as determined in cord blood at birth.

METHODS**Study design and population**

The PASTURE study is a prospective birth cohort study involving children from rural areas in 5 European countries: Austria, Finland, France, Germany, and Switzerland.⁷ Pregnant women living in these rural areas were recruited in the third trimester of pregnancy. Women who lived on family-run farms where any kind of livestock was kept were assigned to the farm group. For the reference group, women from the same rural areas but not living on a farm were recruited. Exclusion criteria were living on farms without livestock, maternal age of less than 18 years, twin pregnancy, home births, premature deliveries, genetic disease in the offspring, absent telephone connection, insufficient knowledge of the country's language, intention to move away from the study area, and commuting to a metropolitan area. The last criterion was only applicable in Germany.

Questionnaires

The questionnaires developed within the PASTURE study group used questions on various exposures from the International Study of Allergy and Asthma in Childhood (ISAAC),⁸ the Allergy and Endotoxin (ALEX) study,⁹ and the Prevention of Allergy Risk factors for Sensitization in children Related to Farming and Anthroposophic Lifestyle (PARSIFAL) study.¹⁰ Respiratory health of the parents was assessed by using questions derived from the American Thoracic Society questionnaire.¹¹ Questionnaires were administered in interviews or were self-administered to the mothers at the end of pregnancy and during a home visit when the children were 2 months of age. The questions referred to the general health of the children's families, with a focus on respiratory and atopic diseases and maternal health during pregnancy. In particular, nutrition during pregnancy, as well as intensity and timing of maternal farm-related exposures, were assessed. All mothers were asked how much farm milk they consumed daily during pregnancy and whether the milk was boiled or skimmed. Furthermore, the time they spent weekly or monthly on a farm and particularly in animal sheds or hay lofts was recorded. Mothers living on a farm were asked whether they actively participated in farming activities, such as feeding animals, placing litter, removing dung, milking, cleaning animal sheds, and collecting eggs in hen sheds. Potential confounders, such as smoking during pregnancy, educational level, and number of previous pregnancies, were addressed as well. Parents were also asked whether they grew up on farms and whether they were involved in farm activities during childhood and adolescence.

Specific IgE in serum samples

Specific IgE levels for 7 food and 13 common inhalant allergens were assessed in placental venous cord blood samples (at birth) by using the Allergy Screen test panel for atopy (Mediwiss Analytic, Moers, Germany) in a central laboratory. This method has previously been validated against the *in vitro* IgE CAP system (Pharmacia, Freiburg, Germany) and the skin prick test.¹² Food allergens included hen's egg, cow's milk, peanut, hazelnut, carrot, wheat flour, and soybean; inhalant allergens comprised *Dermatophagoides pteronyssinus*, *Dermatophagoides farinae*, cat, horse, dog, *Alternaria* species, mugwort, plantain, alder, birch pollen, hazel pollen, rye pollen, and a grass pollen mix. In addition, peripheral blood samples of the mothers were taken at birth (in Switzerland and Finland) or at a home visit when the child was 2 months old and were assessed for IgE levels.

Dust samples

When the children were 2 months of age, dust samples were collected by fieldworkers from the mothers' mattresses according to a standardized protocol using nylon dust-sampling socks.¹³ The samples were centrally analyzed at the Institute for Risk Assessment Sciences, Utrecht, The Netherlands. Endotoxin content was quantified with the kinetic chromogenic Limulus Amebocyte Lysate test (Bio Whittaker, Walkersville, Md),¹⁴ and fungal extracellular polysaccharides (EPSs) were quantified with a specific sandwich enzyme immunoassay for EPSs of *Aspergillus* and *Penicillium* species.¹⁵

Statistical analysis

Statistical analysis was performed with SAS 9.1.3 software (SAS Institute, Inc, Cary, NC). Crude odds ratios (cORs), adjusted odds ratios (aORs), and adjusted geometric mean ratios (aGMRs) are presented with 95% CIs. In epidemiologic studies adjustment of *P* values for multiple testing usually is not performed "because it will lead to fewer errors of interpretation when the data under evaluation are not random numbers but actual observations on nature."¹⁶

Specific cord blood IgE levels were dichotomized at the detection limit of 0.2 IU/mL. IgE levels in maternal peripheral blood were dichotomized at the same cutoff point. In addition to specific IgE to the particular allergens, combinations of specific IgE were defined: IgE to food allergens; IgE to perennial allergens (*D pteronyssinus*, *D farinae*, cat, horse, and dog); IgE to seasonal allergens (alder pollen, birch pollen, hazel pollen, rye pollen, grass pollen mix, mugwort, plantain, and *Alternaria* species); and IgE to tree pollen (alder pollen, birch pollen, and hazel pollen).

Prenatal exposures to animal sheds and hay lofts in any trimester of pregnancy were defined for farm and reference children as follows. Continuous variables were created from the respective animal shed and hay loft exposure variables "hours per day," "days per week," and "weeks per month." Values of less than a quarter of an hour per week were regarded as negligible and set to 0. Consumption of boiled or unboiled farm milk was calculated as a continuous variable. Values of less than 10 mL of farm milk per day were regarded as negligible and set to 0.

Because the prevalences of cord blood IgE varied across countries, odds ratios for associations of exposures with IgE outcomes were adjusted for the variable "center" to avoid confounding by study center.

Multivariable models for positive specific IgE levels to seasonal and food allergens, particularly to cow's milk, were established by including farming-related variables that changed the estimate for farming by more than 10%. In addition, the models were adjusted for potential confounders, as shown in the table legends. In addition to these fully adjusted models, parsimonious models were created by means of stepwise logistic regression at a *P* value of less than .15.

Concordance of maternal farm activities was estimated by Kendall's τ partial on center in the subgroup of mothers exposed to animal sheds, and center-adjusted odds ratios for the effect of maternal farm activities on the child's sensitization were calculated in this subgroup.

Prevalences of sensitization to grass pollen were compared among the 3 categories ("no exposure to animal sheds"/"exposure to animal sheds without contact with hay"/"exposure to animal sheds with contact with hay") of a

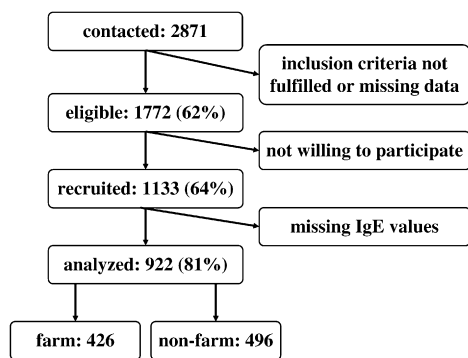


FIG 1. Recruitment of the study population.

combined variable for maternal exposure during pregnancy by using a Cochran-Armitage trend test.

Endotoxin and EPS levels in maternal mattress dust were log-transformed, resulting in normal distribution. For cord blood IgE to seasonal allergens, grass, and tree pollen and their previously detected determinants (ie, maternal exposure to animal sheds and the presence of a dung hill close to the home), aGMRs of endotoxin and EPS levels adjusting for center and farming were calculated.

RESULTS

The study population comprised 1133 newborns, 47% of them born to farm families (Fig 1). Table E1 (available in the article's Online Repository at www.jacionline.org) shows general population characteristics for the 922 (81%) farm and reference children who had complete values for both maternal and cord blood IgE and were subject to this analysis.

The specific IgE levels to the various allergens were consistently less than 3.5 IU/mL, and the 95th percentiles were less than 0.35 IU/mL. At a detection limit of 0.2 IU/mL, however, specific IgE values were measurable in the cord blood of 220 (24%) children, ranging from 12% in Germany to 40% in France. Sensitization patterns at birth varied considerably between farm and reference children (Fig 2). In farm children sensitization to seasonal inhalant allergens (cOR, 0.39 [0.23-0.66]), particularly grass pollen (cOR, 0.16 [0.06-0.42]), was less prevalent. In contrast, sensitization to food allergens (cOR, 1.48 [1.04-2.09]) and to cow's milk (cOR, 1.78 [1.08-2.93]) was more prevalent in farm children. For IgE values to other allergens, including perennial inhalant allergens, no significant differences between these groups were observed. Table E2 (available in the article's Online Repository at www.jacionline.org) shows the country-specific prevalences in analogy to Fig 2.

Next, logistic regression models were established to determine whether the differences in cord blood IgE levels between farm and reference children were attributable to particular pregnancy or farm-related exposures, such as exposure to animal sheds or hay lofts, contact with various farm animals, consumption of boiled or unboiled farm milk, consumption of home-grown products, liquid manuring in the neighborhood, and heating with wood. Exposure to animal sheds during pregnancy changed the center-adjusted estimate of farming on IgE to seasonal allergens most profoundly (from an aOR of 0.38 [95% CI, 0.22-0.66] to an aOR of 0.64 [95% CI, 0.31-1.34], by 68%). The presence of an open dung hill in the surrounding area of the house further changed the estimate (Table I). The distance between dung hill

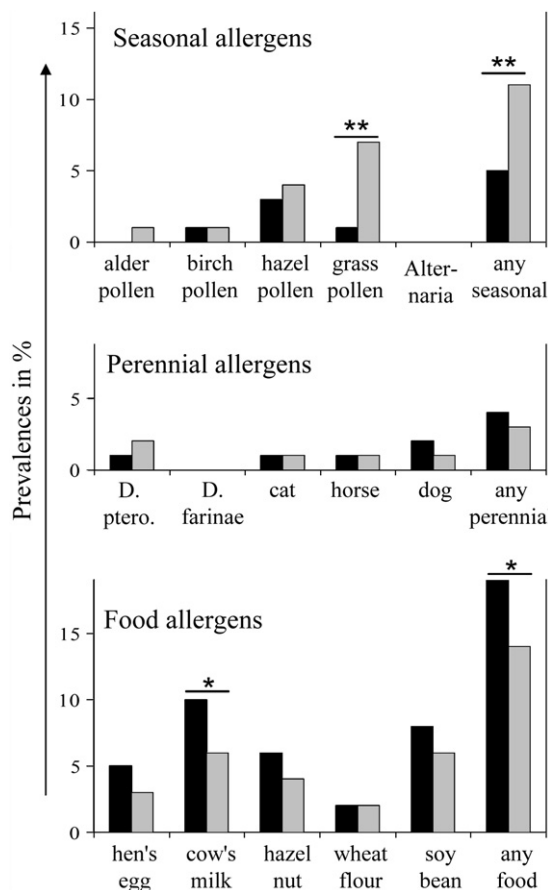


FIG 2. Prevalences of detectable IgE for specific allergens in cord blood of farm and reference children. Farm children are represented by black bars, and reference children are represented by gray bars. Statistically significant differences are indicated as follows: * $P < .05$, ** $P < .001$. *D. pteronissinus*, *D. pteronyssinus*.

and house (<10 meters/10-50 meters/>50 meters) did not play a role (data not shown). Potential confounders did not interfere with these associations (Table I). Further exposure variables, such as exposure to hay lofts during pregnancy or farm milk consumption during pregnancy, were not related to IgE levels against seasonal allergens in multivariable models. Because of lower prevalences for IgE to grass pollen and tree pollen, a separate analysis for these specificities was not feasible in multiple analyses.

In multivariable analysis for IgE to food allergens, consumption of boiled farm milk was positively associated with IgE to food allergens (aOR, 2.29 [95% CI, 1.23-4.29]; Table II) and particularly with IgE to cow's milk (Table II). Another finding was the inverse association of the number of previous pregnancies with IgE to food allergens (aOR, 0.87 [95% CI, 0.76-1.00]; Table II). The associations of maternal and cord blood IgE levels to food allergens and to cow's milk were rather strong (aOR, 9.83 [95% CI, 6.29-15.4] and 28.8 [95% CI, 13.8-59.9], respectively). In contrast, parental histories of atopic diseases were inversely associated with IgE to cow's milk and, to a lesser extent, with IgE to food allergens (Table II). A sensitivity analysis did not reveal confounding by avoidance of milk consumption in atopic mothers (data not shown).

TABLE I. Multivariable models for the outcome: Cord blood IgE to seasonal allergens

Exposures and covariables	Full model* (n = 831)	Parsimonious model (n = 863)
Farm child	1.18 (0.45-3.06), <i>P</i> = .738	
Exposure to animal sheds during pregnancy	0.38 (0.18-0.81), <i>P</i> = .013	0.45 (0.25-0.82), <i>P</i> = .009
Open dung hill in surrounding area	0.49 (0.25-0.96), <i>P</i> = .039	0.51 (0.29-0.90), <i>P</i> = .021
Maternal IgE to seasonal allergens	1.49 (0.82-2.70), <i>P</i> = .189	1.61 (0.94-2.76), <i>P</i> = .083
Paternal history of atopic diseases	1.04 (0.54-2.01), <i>P</i> = .903	
Maternal history of atopic diseases	1.28 (0.67-2.43), <i>P</i> = .460	
No. of previous pregnancies	0.84 (0.65-1.07), <i>P</i> = .159	

Odds ratios are given with 95% CIs and *P* values. An odds ratio of greater than 1 indicates a positive association and an odds ratio of less than 1 indicates a negative association of the respective exposure or covariable with the outcome variable. The exposures and covariables in both models are adjusted mutually and for center.

*The full model is additionally adjusted for sex, mode of birth, season of birth, contact with pets during pregnancy, maternal smoking during pregnancy, maternal and paternal educational level, and maternal and paternal farm exposure during childhood and adolescence.

TABLE II. Multivariable models for the outcomes: Cord blood IgE to cow's milk and to food allergens

Exposures and covariables	IgE to food allergens		IgE to cow's milk	
	Full model* (n = 875)	Parsimonious model (n = 890)	Full model* (n = 875)	Parsimonious model (n = 894)
Farm child	1.25 (0.72-2.19), <i>P</i> = .430		1.08 (0.50-2.33), <i>P</i> = .854	
Boiled farm milk consumption	2.29 (1.23-4.29), <i>P</i> = .009	2.56 (1.41-4.66), <i>P</i> = .002	3.64 (1.35-9.79), <i>P</i> = .010	3.55 (1.35-9.34), <i>P</i> = .010
Maternal IgE to respective allergens	9.83 (6.29-15.4), <i>P</i> < .001	10.1 (6.47-15.9), <i>P</i> < .001	28.8 (13.8-59.9), <i>P</i> < .001	26.6 (13.4-52.8), <i>P</i> < .001
Maternal history of atopic diseases	0.69 (0.44-1.07), <i>P</i> = .095	0.67 (0.43-1.03), <i>P</i> = .069	0.39 (0.18-0.87), <i>P</i> = .021	0.40 (0.19-0.86), <i>P</i> = .019
Paternal history of atopic diseases	0.72 (0.45-1.16), <i>P</i> = .175	0.71 (0.45-1.12), <i>P</i> = .137	0.51 (0.24-1.10), <i>P</i> = .088	0.51 (0.24-1.07), <i>P</i> = .074
No. of previous pregnancies	0.87 (0.76-1.00), <i>P</i> = .052	0.88 (0.78-1.00), <i>P</i> = .051	0.88 (0.69-1.12), <i>P</i> = .297	

Odds ratios are given with 95% CIs and *P* values. An odds ratio of greater than 1 indicates a positive association and an odds ratio of less than 1 indicates a negative association of the respective exposure or covariable with the outcome variable. The exposures and covariables in all models are adjusted mutually and for center.

*The full models are additionally adjusted for sex, mode of birth, season of birth, contact with pets during pregnancy, maternal smoking during pregnancy, maternal and paternal educational level, and maternal and paternal farm exposure during childhood and adolescence.

When replacing the variable "consumption of boiled farm milk" in the model for IgE to cow's milk in Table II with a categorical variable, no dose-response relationship was established, presumably because of low case numbers per category. When additionally assessing consumption of skimmed versus unskimmed farm milk, there was no effect on IgE levels to cow's milk (aOR, 0.93 [95% CI, 0.29-2.94]).

Because of heterogeneity across countries (see Table E2), multilevel analyses for the models presented in Tables I and II were performed. These sensitivity analyses revealed only marginal differences from the center-adjusted models (data not shown).

Because the exposure to animal sheds during pregnancy was related to IgE to seasonal allergens, maternal activities in or around animal sheds were explored in more detail. Some activities were rather correlated, such as handling hay and removing dung, with a τ value of 0.66. Fig E1 (available in the article's Online Repository at www.jacionline.org) shows aORs for the associations of several maternal farm activities with IgE to seasonal allergens in cord blood. The strongest effects were found for activities involving contact with cattle, removing dung, cleaning the henhouse, handling silage, and handling hay (cOR, <0.4). These activities imply exposure to microbes, as well as exposure to grass pollen. When assessing the effects of maternal activities separately for IgE to tree pollen, a significant association was found with removing dung (cOR, 0.23; 95% CI, 0.06-0.87). Conversely, the strongest significant effect on IgE to grass pollen was found for handling hay (cOR, 0.17; 95% CI, 0.03-0.87). Fig 3 shows the effect of additional hay exposure exceeding the effect of animal shed exposure on the frequency of grass pollen sensitization. Prenatal animal shed exposure almost halves

the prevalence of grass pollen sensitization; additionally, contact with hay reduces the prevalence of grass pollen sensitization to 0.6%.

Activities involving contact with cattle, removal of dung, and the presence of an open dung hill nearby suggest exposure to microbial compounds. In mattress dust of mothers exposed to animal sheds, the endotoxin levels were higher (aGMR, 1.35 [95% CI, 1.09-1.68]; see Table E3 in the article's Online Repository at www.jacionline.org). However, the levels were not associated with IgE to seasonal allergens in cord blood (aGMR, 1.03 [95% CI, 0.77-1.36]) or with IgE levels to grass pollen or tree pollen (see Table E3). The aGMRs for EPSs were alike (see Table E3).

Based on the observation that exposure to grass pollen, because it occurs during activities in contact with hay or silage, might be related to the IgE response to grass pollen, one might generally assume an association of prenatal exposure and sensitization to the same allergen. On this note, other pregnancy exposures not necessarily related to farming were investigated. For the exposure to cats, dogs, or horses, no inverse associations with IgE levels to the respective allergens adjusted for maternal IgE levels and center were observed (aORs, 0.95 [95% CI, 0.18-5.09], 1.49 [95% CI, 0.52-4.26], and 1.72 [95% CI, 0.26-11.5], respectively).

DISCUSSION

In the PASTURE birth cohort specific IgE antibodies to various allergens were detected at low levels in cord blood. Intriguingly, a clear distinction of the sensitization patterns of farm and reference children emerged.

Detection of IgE in cord blood

There is an ongoing debate about whether atopic sensitization can occur prenatally.¹⁷⁻¹⁹ In a birth cohort of high-risk children, cord blood T-cell reactivity toward house dust mite was not related to the subsequent development of allergen-specific T_H2 memory cells or production of specific IgE, leading the authors to conclude that priming of T_H2 responses and persistent specific IgE production did not occur prenatally.¹⁸ There is also uncertainty with respect to contamination of cord blood IgE by maternal IgE.²⁰

These are important issues for the interpretation of our findings. Therefore we have addressed these points in detail in a separate article analyzing specific IgE and T-cell responses in cord blood of children enrolled in the PASTURE cohort.²¹ Based on these analyses, there is little evidence to suggest that cord blood IgE is not of fetal origin in the PASTURE population. Furthermore, it is of note that in the present article all observed effects of prenatal exposure on cord blood IgE withstood adjustment for maternal IgE at the same sensitive detection limit of 0.2 IU/mL. If cord blood IgE was mainly of maternal origin, the inclusion of the variable maternal IgE to the respective allergen into the model would have eliminated the offspring's IgE variable. We found the opposite to happen: maternal IgE to seasonal allergens failed statistical significance in the models of Table I. An approach to test fetal origin of IgE in future studies might consist in tracking specific IgE levels over infancy: stability or progressive increase in titers then would argue in favor of an ongoing response in the newborn. Decrease of IgE levels might reflect clearing of maternal IgE or an altered immune response in the infant.

IgE to food allergens

The higher rate of cord blood food sensitization in farm children was rather unexpected. Still, this finding can be attributed to a higher consumption of boiled farm milk in farm mothers (337 mL/d) compared with reference mothers (226 mL/d, $P = .015$). Interestingly, the consumption of unboiled farm milk did not induce higher levels of IgE to cow's milk. This notion might suggest that not only the extent of allergen exposure but also the context in which it occurs plays a role in inducing a fetal immune response. It is conceivable that microbial and other components in raw milk might lose their biologic activity through heat inactivation, resulting in a temporary predominance of the disadvantageous effects of milk allergens. An alternative explanation might be denaturation of specific milk proteins or lipid structures by boiling.²² Independently of farming, specific IgE levels to cow's milk in mother and child were highly correlated, as demonstrated by the high aORs (Table II). This might reflect a higher rate of transmission of the allergen to the fetus, as described for other food allergens, such as ovalbumin.²³ The inverse association of maternal and paternal history of atopic diseases with the child's IgE level to cow's milk (Table II) might indicate that the presence of IgE to cow's milk does not necessarily reflect atopic sensitization or predict later manifestation of cow's milk allergy.

IgE to seasonal allergens

The inverse association of prenatal exposure to animal sheds with atopic sensitization to seasonal allergens agrees with earlier results using atopy at school age and retrospective exposure assessment of the cross-sectional ALEX⁹ and PARSIFAL⁶

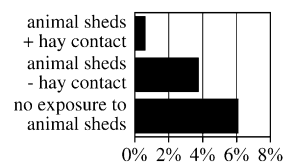


FIG 3. The combined effect of the mother spending any time in animal sheds and handling hay during pregnancy on cord blood-specific IgE levels against grass pollen. Prevalence of sensitization to grass pollen in cord blood is given for the 3 exposure categories. The fourth category (hay contact without exposure to animal sheds) contained only 14 nonsensitized children (<2% of all) and was therefore not included in the figure.

studies. The prospective longitudinal design of the present study provides strong evidence for this notion. Moreover, the present analysis demonstrates that the effect of prenatal exposure to animal sheds is already detectable at birth.

Remarkably, the effect was restricted to sensitization against seasonal allergens, a finding that was retrospectively confirmed in the PARSIFAL population. Sensitization at school age, as determined based on a specific IgE level of 3.5 IU/mL or greater, was related to prenatal exposure to animal sheds for seasonal allergens (aOR, 0.47 [95% CI, 0.25-0.86]), particularly for grass pollen (aOR, 0.40 [95% CI, 0.21-0.77]), whereas for sensitization to food allergens, the odds ratio was close to unity (aOR, 0.89 [95% CI, 0.33-2.40]). Also, in other studies on farm children, grass pollen was found to be the lead allergen for atopic sensitization.^{9,24} In this context the additional effect of maternal contact with hay is important. In spite of cross-correlation of maternal farm activities, handling hay stood out from other activities with respect to IgE levels against grass pollen. In the face of cautious interpretation because of low case numbers, the relation of prenatal exposure and absence of sensitization to the same allergen (ie, grass pollen) is remarkable. This finding might reflect an allergen-specific immune response. In the ALEX study it has been shown that exposure to a farm environment protected against grass-specific but not mite-specific responses at every step along the immunoglobulin class-switching pathway.²⁵

An alternative explanation for the outstanding effect of a farming environment on grass pollen sensitization might be found in allergen-specific tolerance. A susceptible time window for tolerance induction in pregnancy would be one explanation. However, the absence of protective effects by other farm-related allergens (ie, animal dander and milk) argues against this notion. An alternative mechanism suppressing atopic sensitization might consist of the simultaneous presence of the allergen and an immunomodulatory substance in the same environment. One might expect such an immunomodulatory substance to be of microbial origin. As of today, we do not know how strongly low levels of specific IgE in cord blood will predict the incidence of allergic illness later in life. The ongoing follow-up of the PASTURE cohort up to age 6 years will allow such analyses in the future.

Biomarkers of microbial exposure

Levels of biomarkers for gram-negative bacteria (endotoxin) or *Aspergillus* and *Penicillium* species (EPSs) in maternal mattress dust were not related to cord blood IgE levels against grass pollen in the present study. Endotoxin and EPSs are rather general markers; accordingly, more specific detection methods might trace the postulated immunomodulatory substance or substances.²⁶ Still, the presence of specific microbes in hay is

conceivable because active participation in haying was associated with higher gene expression levels of Toll-like receptors independently of other farm-related exposures in school-age children.²⁷ Toll-like receptors are part of the innate immune system's recognition of microbial substances, thereby operating as indirect biomarkers of enhanced exposure to microbial substances.

As a further potential determinant for sensitization to seasonal allergens, the presence of an open dung hill in the surrounding area of the child's home has been identified independently of farming and animal shed exposure. Thus a proper effect on sensitization might be attributable to the microbial milieu emitted by dung hills. Endotoxin and EPS levels in maternal mattress dust did not reflect the presence of a dung hill in the neighborhood (see Table E3), possibly indicating that the effect might not be quantitatively related to a higher microbial burden. Minor but steady microbial exposure might comply with reduced sensitization.

Conclusion

The extent and the context of prenatal exposure to some allergens (seasonal and food allergens) but not to others (perennial allergens) might play a role in the development of sensitization. In the farming environment this might be exemplified by the ubiquitous sources of grass pollen and microbial compounds, ultimately contributing to a consistent protective "farming effect." Subsequent analyses of the PASTURE cohort will evaluate the "farming effect" on immune maturation and disease manifestation.

Clinical implications: Development of atopic sensitization might depend on the time window, extent, and context of allergen contact.

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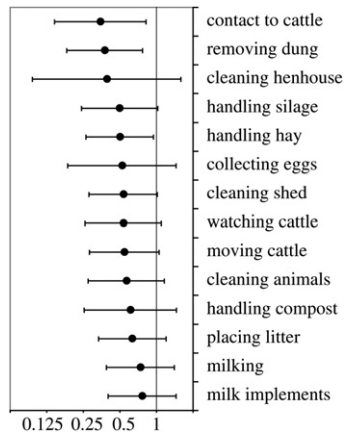


FIG E1. Center-adjusted odds ratios for the effects of maternal farm activities on IgE levels for seasonal allergens in cord blood. The odds ratios are adjusted for study center. Only children whose mothers were exposed to animal sheds during pregnancy are included (n = 464).

TABLE E1. Population characteristics

	Child living on a family-run farm				P value
	Yes (n = 426)		No (n = 496)		
	No.	Percentage	No.	Percentage	
Female sex	209	49	237	49	.842
Primigravida	97	23	183	37	<.001**
Birth in September to December	157	37	157	32	.124
Cesarean section	58	14	81	17	.230
Maternal smoking in pregnancy	38	9	93	19	<.001**
High maternal education	341	80	425	86	.027*
High paternal education	269	65	358	76	<.001**
Mother lived on a farm during childhood	273	64	140	28	<.001**
Father lived on a farm during childhood	388	92	145	30	<.001**
Parental history of atopy†	261	69	374	83	<.001**
Contact with pets during pregnancy	353	83	226	46	<.001**
Unboiled farm milk consumption during pregnancy‡	242	57	44	9	<.001**
Boiled farm milk consumption during pregnancy‡	70	17	20	4	<.001**
Exposure to hay lofts during pregnancy§	288	69	56	12	<.001**
Exposure to animal sheds during pregnancy§	374	90	90	19	<.001**
Dung hill in surrounding area	390	92	146	30	<.001**

Statistically significant differences are indicated as follows:

* $P < .05$.

** $P < .001$.

†Physician-diagnosed asthma or atopic eczema or seasonal rhinoconjunctivitis.

‡At least 10 mL/d versus less than 10 mL/d.

§At least 15 min/wk versus less than 15 min/wk.

TABLE E2. Prevalences in percentage of detectable IgE for specific allergens in cord blood of farm and reference children per country

Allergen	Austria			France			Finland			Germany			Switzerland		
	Farm (n = 105)	Reference (n = 115)	P value	Farm (n = 94)	Reference (n = 109)	P value	Farm (n = 112)	Reference (n = 102)	P value	Farm (n = 112)	Reference (n = 142)	P value	Farm (n = 107)	Reference (n = 135)	P value
Any allergen	19	28	.227	40	41	1.000	24	14	.075	12	12	1.000	28	26	.749
Hen's egg	3	6	.502	1	0	.442	12	3	.029	4	1	.311	3	4	1.000
Cow's milk	7	2	.154	4	1	.322	13	13	1.000	6	3	.284	17	9	.134
Hazelnut	10	9	1.000	6	2	.407	7	1	.065	3	1	.574	2	6	.298
Wheat flour	0	1	1.000	3	1	.584	5	4	1.000	1	2	1.000	2	0	.203
Soybean	6	7	.770	13	10	.623	8	5	.569	3	4	1.000	11	5	.106
Food allergens	17	17	1.000	22	15	.311	22	14	.147	12	8	.440	22	16	.273
<i>D pteronyssinus</i>	0	1	1.000	3	7	.468	0	0		0	1	1.000	1	1	1.000
<i>D farinae</i>	0	0		0	2	.504	0	0		0	0		0	0	
Cat	1	1	1.000	1	2	1.000	1	1	1.000	0	1	1.000	0	0	
Horse	0	0		1	2	1.000	2	0	.498	0	1	1.000	2	0	.203
Dog	1	0	.476	6	1	.171	4	1	.369	1	1	1.000	0	1	1.000
Perennial allergens	1	2	1.000	8	11	.608	6	2	.280	1	2	1.000	3	2	.660
Alder pollen	0	0		1	1	1.000	1	1	1.000	0	1	1.000	0	1	1.000
Birch pollen	2	0	.225	1	2	1.000	1	1	1.000	0	2	.510	0	1	1.000
Hazel pollen	1	2	1.000	15	16	1.000	1	1	1.000	0	1	1.000	0	1	1.000
Grass pollen*	1	13	0.001	0	5	.067	0	0		0	4	.140	4	11	.117
<i>Alternaria</i> species	0	0		0	1	1.000	0	0		0	0		0	1	1.000
Seasonal allergens	3	15	.006	17	22	.433	1	1	1.000	0	7	.022	4	13	.048

**Alopecurus pratensis*, *Phleum pratense*, *Anthoxanthum odoratum*, *Lolium perenne*, *Dactylis glomerata*, *Cynodon dactylon*, *Holcus lanatus*, *Secale cereale*, *Festuca elatior*, *Arrhenatherum elatius*, *Bromus inermis*, *Agropyron repens*, and rye (*Secale cereale*).

TABLE E3. Geometric mean ratios for biomarker levels in mattress dust of mothers for different exposures and IgE levels in cord blood of their children

	Endotoxin	EPSs
Exposure to animal sheds	1.35 (1.09-1.68), $P = .006$	1.36 (1.12-1.65), $P = .002$
Open dung hill in area	1.02 (0.85-1.23), $P = .816$	1.02 (0.86-1.21), $P = .812$
IgE to seasonal allergens	1.03 (0.77-1.36), $P = .853$	1.01 (0.79-1.31), $P = .910$
IgE to grass pollen	0.96 (0.66-1.40), $P = .829$	0.96 (0.69-1.35), $P = .830$
IgE to tree pollen	1.04 (0.69-1.56), $P = .856$	1.01 (0.70-1.46), $P = .964$

The geometric mean ratios are adjusted for center and farming.