Farm environment during pregnancy and childhood and polysensitization at the age of 31 – Prospective birth cohort study in Finland

Running title: Farm environment and sensitization

Karvonen AM[1*], Lampi J[1*], Keski-Nisula L[2,3], Auvinen J[4,5], Toppila-Salmi S[6,7], Järvelin MR[4,8,9,10], Pekkanen J[1,11]

[1]Environmental Health Unit, Department of Health Security, Finnish Institute for Health and Welfare, Kuopio, Finland

[2]Department of Obstetrics and Gynecology, Kuopio University Hospital, Kuopio, Finland

[3]Department of Health Sciences, Clinical Medicine, University of Eastern Finland, Kuopio, Finland

[4]Center for Life Course Health Research, Faculty of Medicine, University of Oulu, Finland

[5]Oulunkaari Health Center, Ii, Finland

[6]Haartman Institute, University of Helsinki, Helsinki, Finland

[7]Skin and Allergy Hospital, Helsinki University Hospital and University of Helsinki, Helsinki, Finland.

[8]Department of Epidemiology and Biostatistics, MRC–PHE Centre for Environment & Health, School of Public Health, Imperial College London, UK

[9]Unit of Primary Care, Oulu University Hospital, Finland

[10]Biocenter Oulu, University of Oulu, Finland

[11]Department of Public Health, University of Helsinki, Helsinki, Finland

*These authors contributed equally to this work.

Correspondence author:

Anne M. Karvonen

Finnish Institute for Health and Welfare (THL). Department of Health Security, Environmental Health Unit. P.O.Box 95, FIN-70701 Kuopio, Finland

doi: 10.18176/jiaci.0455

Abstract

Background: Farm environment, especially contact with farm animals in early childhood may prevent from allergic sensitization during adulthood. However, prospective associations between exposure to farm environment and polysensitizationhave not been studied. Polysensitization is a risk factor for asthma and asthma morbidity.
Objective: To investigate whether farming environment in the early childhood, especially animal exposure, is associated with sensitization to specific allergens and polysensitization at the age of 31.

Methods: In a prospective birth cohort study, 5509 subjects born in northern Finland in 1966 were skin prick testedat the age of 31against birch, timothy, cat and house dust mite. Prenatal exposure to the farming environment was documented at birth, whereas information on childhood exposure to pets was only collected retrospectively at the age of 31. Logistic regression was used in the statistical analyses.

Results: Being born to a family with farm animalswas associated with the reducedrisk of sensitization against birch, timothy or cat(adjusted odds ratio,aOR=0.55[95% confidence interval 0.43-0.70];aOR=0.62[0.48-0.79];aOR=0.60[0.47-0.75]) and polysensitization at the age of 31 (aOR=0.62[0.48-0.80]). Sensitization against birch, timothy and cat as well as polysensitization was dose-dependently and inversely associated with number of animal species present during childhood. No association was found with sensitization against house dust mite.
Conclusions: Growing up on a farm and contact with the higher numbers of animal species in childhood is associated with less sensitization against birch, timothy and cat allergensand polysensitization in adulthood, but not with sensitization against house dust mite.

Key words: Atopy, Sensitization, Farm environment, Adults, Polysensitization, Monosensitization

doi: 10.18176/jiaci.0455

Resumen

Antecedentes:
El ambiente de granja, especialmente el contacto con animales de granja en la primera infancia, puede prevenir la sensibilización alérgica durante la edad adulta. Sin embargo, no se han estudiado las posibles asociaciones entre la exposición al entorno agrícola y la polisensibilización. La polisensibilización es un factor de riesgo para el asma y su morbilidad.

Objetivo:
Investigar si el entorno agrícola en la primera infancia, especialmente la exposición a animales, está asociado con la sensibilización a alérgenos específicos y la polisensibilización a la edad de 31 años.

Métodos:
En un estudio prospectivo de cohorte de nacimiento, 5509 sujetos nacidos en el norte de Finlandia en 1966 se sometieron a pruebas cutáneas a la edad de 31 años con abedul, hierba timotea, gato y ácaros del polvo doméstico. La exposición prenatal al ambiente agrícola se documentó al nacer, mientras que la información sobre la exposición infantil a las mascotas solo se recopiló retrospectivamente a la edad de 31 años. Se utilizó La regresión logística en los análisis estadísticos.

Resultados:
Nacer en una familia con animales de granja se asoció con un menor riesgo de sensibilización frente a abedul, hierba timotea o gato (odds ratio ajustado, aOR = 0.55 [intervalo de confianza del 95% 0.43-0.70]; aOR = 0.62 [0.48-0.79] ;aOR = 0.60 [0.47-0.75]) y polisensibilización a la edad de 31 años (aOR = 0.62 [0.48-0.80]). La sensibilización frente a abedul, hierba timotea y gato, así como la polisensibilización, se asociaron de forma dependiente e inversa a la dosis con el número de especies animales presentes durante la infancia. No se encontró asociación con la sensibilización frente a los ácaros del polvo doméstico.

Conclusiones:
Crecer en una granja y el contacto con un mayor número de especies animales en la infancia se asocia con una menor sensibilización frente al abedul, la hierba timotea, alérgenos de gato y polisensibilización en la edad adulta, pero no con sensibilización frente a los ácaros del polvo doméstico.

Palabras clave: Atopia, Sensibilización, Ambiente agrícola, Adultos, Polisensibilización, Monosensibilización.

doi: 10.18176/jiaci.0455

Introduction

Farming environment, especially exposure to farm animals and consumption of raw cow's milk in early childhood, has been associated with lower prevalence of asthma and atopy in children growing up on from farms [1].The farming environments are rich in microbes; microbial diversity and quantityare higher on farm houses than in more urban environments[1,2]. The microbial exposuremay stimulate the immune system, affect the development of immune system, allergic sensitization and prevent from clinical manifestation of allergic diseases[1].The protective effect of farming environment in *utero* and during early childhood may extend to adulthood[3-5]as we have also previously shown in this prospective birth cohort study[6,7].

The protective effect of farm environment during infancy against allergic sensitization hasnot been consistently associated with all allergens. In the previous studies with retrospective information on exposure and the number of participants varying mainly between 290 and 1700 growing up on a farm was inversely associated with sensitization to birch, timothy and/or cat in adulthood[4,8,9,10].The resultswith sensitization to house dust mite has been contradictory[4,8,9,10].

Polysensitization has been recognized as a risk factor for morbidity among asthmatic children[11], and has been associated with risk of asthma in adults[12].It has also been proposed that mono- and polysensitization are two distinct phenotypes in multimorbidity of allergic diseases in children [13].A European cross-sectional study among 858 children aged 6-12 years found an inverse association with farming and polysensitization in

doi: 10.18176/jiaci.0455

children[14]. A study from Greek revealed that urban children were more often polysensitized than children from rural areas, but no difference was found in farming [15]. No such association was found when 101 swine adult breedersand 82 non-breeders were studied in a cross-sectional study in Italy[16].

Our aim was to investigate the prospective associations of farming environment, especially exposure to farm animals in *utero* and early childhood with specific sensitization to birch, timothy, cat and house dust mite and polysensitization at the age of 31. This is the first prospectivestudy investigating the effect of farm environment during infancy and polysensitization in adulthood.

doi: 10.18176/jiaci.0455

Methods

The original cohort consisted of 12 058 live born subjects, whose expected time of birth was between 1st January and 31st December in 1966, in the two northernmost provinces of Finland, Oulu and Lapland. The study population originally covered 96% of the children born in that region in1966. In 1997, 8463 survivors still living in Northern Finland or in the capital city area received a postal questionnaire and invitation to clinical examinations.A detailed description of the number of participants and flow charts of the 31-year follow-up study are shown on the study website [17]. The University of Oulu Ethics Committee approved the study and the participants gave written informed consent.

Assessment at age 31 years

During the 31-year follow-up, participants filled in the self-administrated questionnaires which enquired on health and lifestyle factors, socio-demographic factors, as well as allergic conditions[6]. They also underwent a clinical examination including skin prick test.

The participants were skin prick tested (SPT) using three of the most common allergens (cat, birch and timothy) in Finland, and the house dust mite (*Dermatophagoides pteronyssinus*). Histamine dihydrochloride (10 mg/mL) and diluent of the allergen extracts were used as positive and negative controls, respectively. Skin reactions to each allergen tested were recorded after 15 min as the average of the maximum wheal diameter and the diameter perpendicular to the maximum. Subjects with a wheal reaction ≥ 3 mm to specific allergen tested were considered sensitized. Subjects with a positive

doi: 10.18176/jiaci.0455

reaction to the negative control (n=35) or negative reaction to histamine (<3 mm) were excluded (n=23).Subjects who were sensitized only against 1 allergen of tested 4 allergens were defined as monosensitized.Polysensitization was defined as sensitization to more than 1 of the 4 tested allergens.

Assessment of antenatal and childhood factors

Farm related determinantsand possible confounding factors were collected from women during pregnancy and/or immediately after delivery. The farm related determinants included, whether parental professional was farming; family had farm animals [species (cows, pigs, sheep, poultry and mink) and their counts]; mother worked with farm animals during pregnancy (not at all, worked with an assistance, or done all by herself); andthe place ofresidence(town, village or outlying district). Also, maternal (education, age,BMI, smoking during pregnancy from the second month, age of menarche, andparity) and child's (gestational age at birth, birth weight and height) related determinants, and the residential density (the number of people in household divided by the number of rooms in the household) were inquired as possible confounding factors. Information on keeping cats and dogsin childhood (before the age of 7 years), and parental history of allergic diseaseswas collected retrospectively at the age of 31 years. Exposures to cats or dogs were used not only as exposure factors by themselves, but also as a combined exposure to animals (farm animals, cats and dogs) during early childhood.

doi: 10.18176/jiaci.0455

Statistical analyses

We included in to the final analyses those 5509 subjects who had a positive reaction to histamine, a negative reaction to control and sufficient data about atopic sensitization available. Among these subjects, the maximum prevalence of missing data for disease outcomes was 1.5 %, for farm characteristics collected during pregnancy 1.3 %, and for data on pet ownership collected at the 31-year follow-up 4.5 %. The highest prevalence of missing information for the confounders was 12.8 % (paternal allergy). Logistic regression models were used for statistical analyses. Same selection of the confounders was used in the multivariate models as has been used in our previous analyses described earlier[6]. In the multivariate models, missing data for each confounder were classified as its own category. In the models, the reference category was always non-sensitized participants expect the models where only sensitized participants were analyzed (the polysensitized participants were compared to monosensitized group, Table 3). There were no major differences between original cohort and subjects included in the present analysis with respect to general characteristics (see supplemental material, Table E1).All analyses were conducted with IBM SPSS Statistics 22.

doi: 10.18176/jiaci.0455

Results

Description of the study population stratified by professional farming during infancy is shown in the supplemental material (Table E2).Twenty-three percent of the studied 31-year old adults had been born to families in which parents were farmers, and 27 % of the mothers were working with farm animals during pregnancy by themselves or with assistance (Table 1). The prevalence of sensitization against cat, birch and timothy allergens was 16% (in each allergen); 7% against house dust mite; 15% for monosensitization (only one positive SPT) and 15% for polysensitization(at least two positive SPT) in the whole study population.

Being born to a family in which parents were farmers, having farm animals in infancy or mother worked with farm animals during pregnancywereassociated with decreased risk of sensitization to cat, birch and timothy at the age of 31 years (Table 1). The number of cows in infancy and number of different animal species in childhood were dose-dependently and inversely associated with decreased sensitization againstto cat, birch and timothy. Farm-related factors during infancy were not associated with house dust mite sensitization at the age of 31 years (Table 1).

Being born to a family where parents were farmers, or the family had farm animals in infancy or mother worked with farm animals during pregnancy wereinversely associated with the risk of polysensitization (Table 2). The risk of polysensitization was dose-dependently reduced with the number of cows and the number of different animal

doi: 10.18176/jiaci.0455

species. Similar estimates, although weaker and often non-significant, were observed with monosensitization (sensitized against only one of the tested allergen) (Table 2). The associations between professional farming or farm animals and monosensitization or polysensitization were similar among women and men (interaction terms' *p-values >0.2*) (data not shown).

For sensitized participants only, the risk of being polysensitized compared to those who were only monosensitized,was decreased among the participants born in families with farm animals and was also dose-dependently associated with the higher number of cows in infancy (Table 3).

Growing up on a family with cats or dogs (before the age of 7 years)was associated with a decreased risk of sensitization to cat, birch and timothy (Table1) and polysensitization (Table2). Having cats was inversely associated with monosensitization (Table 2). No such protective associations were found with sensitization against house dust mite (Table 1). Among sensitized participants only, having dog(s) in childhood decreased the risk of polysensitization compared tomonosensitized participants (Table 3).

doi: 10.18176/jiaci.0455

Discussion

This prospective birth cohort study shows that growing up on a farm, especiallywith farm animals, protects from allergic sensitization in adulthood but not against all allergens. Protective effect of farming was observed with sensitization to birch, timothy,cat, and polysensitization, but not with house dust mite. These associations were independent of potential confounders.

This is the first prospective birth cohort study reporting associations between growing up on a farm with farm animals and lesspolysensitization during adulthood. Similar results have been found in a cross-sectional study among children: children from farms were more often monosensitized than polysensitized[14].Furthermore, polysensitization to tested allergens was also dose-dependently and inversely associated with number of animal species during childhood. These findings suggest that quality and/or quantity of microbial exposure during infancy may protect also from polysensitization. This isa clinically important finding, since polysensitization seems to be more strongly associated with the risk of asthma than monosensitization[12,18], and has also been recognized as a risk factor for increased morbidity among asthmatic children [11]. Although polysensitization seems to be more a stronger risk factor for asthma or asthma-related morbidity than monosensitization, the preventable risk factors of polysensitization have been rarely studied.

Our study shows that farm-related factors during infancy were consistently associated with decreased risk of sensitization to birch, timothy and cat. There was also a dose-

doi: 10.18176/jiaci.0455

dependent and inverse association between number of animal species present during early childhood and sensitization to these three allergens in adulthood. These results are in line with previous studies among adults[4,5,8,9,19]. Furthermore, protective effect of farming environment with sensitization to cat and timothy in our study had similar effectsthan in a recent meta-analysis from 29 cross-sectional studies mostly with farming exposure before 1 year of age and follow-up until adulthoodwith 40 % reduce in atopy prevalence [20].

In this present study, farm environment during infancy did not protect against house dust mite sensitization during adulthood. This finding is in line with some of the previous adult studies [4,5], but is in contrast with a Danish study, which retrospectively assessed childhood farming exposure and found less sensitization against house dust mite in adulthood [10] and with a cross-sectional study among European children [14]. In the present study, there was even a weak positive association with house dust mite sensitization among subjects born to farming families, as has been found in a previous Finnish study among adults [8]. Risk of house dust mite sensitization seems to be associated with exposure to house dust mite allergen [21,23],which has not been observed for exposure to and risk of sensitization to cat allergen[22,23]. Many environmental factors are associated with house dust mite allergen levels[21] and there are large geographical and sosio-economic differences in house dust mite allergen and sensitization levels in Europe. In Nordic countries with low temperatures and low indoor relative humidity during winter,house dust mite allergen levels[24] and sensitization prevalence are relatively low[25].Furthermore, in non-farming and urban homes house dust mite allergen levels are often lower compared to farming[26] and rural homes[27].

doi: 10.18176/jiaci.0455

House dust mite sensitization rates in our study population was quite similar compared to other Finnish study[28] and also in other Nordic countries[25],as approximately 7 % of the subjects were sensitized to house dust mite at the age of 31.

House dust mite sensitization could also be explained by cross-reactivity with other allergens. House dust mite allergens are known to cross-react with other allergens such as shellfish, shrimp or other invertebrates[21] and also with storage mite *Lepidoglyphys destructor* which is common in farming environment[29]. We cannot rule out that cross-reactivity could modify therate of house dust mite sensitization in our study as we only have skin prick test data on four allergens, i.e. birch, timothy, cat and house dust mite.

The hypothesized biological mechanisms behind the protective effect of farming environment during infancy against allergic sensitization and allergic diseases are complex[1]. In short, the protective effect of farm environment, especially contact with farm animals or farm milk consumption, is hypothesized to be attributable to differences in the quantity, quality and/or diversity of microbial exposure[1,2]. Exposure to environmental microbes may affect the development of the immune system[30-32] and thereby protect from development of allergic sensitization and prevent from allergic diseases[2,30]. Furthermore, the development of allergic sensitization may be a combination of environmental and genetic factors during infancy since the protective effect seem to be mediated by gene-environment interactions[33,34].

doi: 10.18176/jiaci.0455

The strength of the study is the prospective design with 31years of follow-up with good participation rate. The farming related factors were assessed before or at birth, but there may be some recall bias in the assessment of pet exposure. There are few weaknesses in our study. Firstly, as our study lacked detailed information on exposure to farming environment during follow-up, assessments of the effect of exposure to farming in different time-points could not be performed. Secondly, there is the possibility that some parents of the children avoided the farm environment since they experienced allergic or respiratory diseases, which is similar to the healthy worker effect. This potential bias can partially be controlled for by adjustment for parental allergies.

In conclusion, growing up on a farm and contact with the higher numbers of animal species in childhood is associated with less sensitization against birch, timothy and cat allergens and polysensitization in adulthood, but not with sensitization against house dust mite.

doi: 10.18176/jiaci.0455

Conflicts of interest

The authors have no conflicts of interest.

Funding

Financial support was received from the Academy of Finland, Ministry of Health and Social Affair, the University of Oulu; from the University Hospital of Oulu; and from EU H2020-PHC-2014 (grant number 633595): DynaHEALTH - Understanding the dynamic determinants of glucose homeostasis and social capability to promote Healthy and active aging. AM Karvonen acknowledges Academy of Finland (grant 287675), JuhoVainio's Foundation, Päivikki and SakariSohlberg Foundation and Foundation for Pediatric Research.

Acknowledgements

We thank Professor Paula Rantakallio† for launch of NFBC1966 and initial data collection.

doi: 10.18176/jiaci.0455

References

1. von Mutius E, Vercelli D. Farm living: effects on childhood asthma and allergy. Nat Rev Immunol. 2010;10:861-8.

2. Ege MJ, Mayer M, Normand AC, Genuneit J, Cookson WO, Braun-Fahrländer C, et al. Exposure to environmental microorganisms and childhood asthma. N Engl J Med 2011;364:701-9.

3. House JS, Wyss AB, Hoppin JA, Richards M, Long S, Umbach DM, et al. Early-life farm exposures and adult asthma and atopy in the Agricultural Lung Health Study. J Allergy Clin Immunol. 2017;140:249,256. e14.

4. Leynaert B, Neukirch C, Jarvis D, Chinn S, Burney P, Neukirch F, et al. Does living on a farm during childhood protect against asthma, allergic rhinitis, and atopy in adulthood? Am J RespirCrit Care Med. 2001;164:1829-34.

5. Portengen L, Sigsgaard T, Omland O, Hjort C, Heederik D, Doekes G. Low prevalence of atopy in young Danish farmers and farming students born and raised on a farm. Clin Exp Allergy 2002;32:247-53.

6. Lampi J, Canoy D, Jarvis D, Hartikainen AL, Keski-Nisula L, Järvelin MR, et al. Farming environment and prevalence of atopy at age 31: prospective birth cohort study in Finland. Clin Exp Allergy. 2011;41:987-93.

7. Lampi J, Koskela H, Hartikainen AL, Ramasamy A, Couto Alves A, Järvelin MR, et al. Farm environment during infancy and lung function at the age of 31: a prospective birth cohort study in Finland. BMJ Open. 2015;5:e007350,2014-007350.

8. Kilpelainen M, Terho EO, Helenius H, Koskenvuo M. Childhood farm environment and asthma and sensitization in young adulthood. Allergy. 2002;57:1130-5.

9. Rennie DC, Lawson JA, Karunanayake CP, Pahwa P, Chen Y, Chu L, et al. Farm Exposure and Atopy in Men and Women: The Saskatchewan Rural Health Study. J Agromedicine. 2015;20:302-9.

10. Elholm G, Linneberg A, Husemoen LL, Omland O, Gronager PM, Sigsgaard T, et al. The Danish urban-rural gradient of allergic sensitization and disease in adults. Clin Exp Allergy. 2016;46:103-11.

11. Gabet S, Just J, Couderc R, Bousquet J, Seta N, Momas I. Early polysensitization is associated with allergic multimorbidity in PARIS birth cohort infants. Pediatr Allergy Immunol. 2016;27:831-7.

12. Toppila-Salmi S, Huhtala H, Karjalainen J, Renkonen R, Mäkelä MJ, Wang DY, et al. Sensitization pattern affects the asthma risk in Finnish adult population. Allergy. 2015;70:1112-20.

doi: 10.18176/jiaci.0455

13. Anto JM, Bousquet J, Akdis M, Auffray C, Keil T, Momas I, et al. Mechanisms of the Development of Allergy (MeDALL): Introducing novel concepts in allergy phenotypes. J Allergy Clin Immunol. 2017;139:388-99.

14. Fuchs O, Genuneit J, Latzin P, Buchele G, Horak E, Loss G, et al. Farming environments and childhood atopy, wheeze, lung function, and exhaled nitric oxide. J Allergy Clin Immunol.2012;130:382,8.e6.

15. Priftis KN, Anthracopoulos MB, Nikolaou-Papanagiotou A, Matziou V, Paliatsos AG, Tzavelas G, et al. Increased sensitization in urban vs. rural environment--rural protection or an urban living effect?Pediatr Allergy Immunol. 2007;18:209-16.

16. Galli L, Facchetti S, Raffetti E, Donato F, D'Anna M. Respiratory diseases and allergic sensitization in swine breeders: a population-based cross-sectional study. Ann Allergy Asthma Immunol. 2015;115:402-7.

17. Northern Finland Birth Cohort 1966, 31-year follow-up study. http://www.oulu.fi/nfbc/node/18097 and http://www.oulu.fi/nfbc/node/30344 (June 27th2019).

18. Bousquet J, Anto JM, Wickman M, Keil T, Valenta R, Haahtela T, et al. Are allergic multimorbidities and IgE polysensitization associated with the persistence or re-occurrence of foetal type 2 signalling? The MeDALL hypothesis. Allergy. 2015;70:1062-78.

19. Koskela HO, Happonen KK, Remes ST, Pekkanen J. Effect of farming environment on sensitisation to allergens continues after childhood. Occup Environ Med. 2005;62:607-11.

20. Campbell BE, Lodge CJ, Lowe AJ, Burgess JA, Matheson MC, Dharmage SC. Exposure to 'farming' and objective markers of atopy: a systematic review and meta-analysis. Clin ExpAllergy.2015;45:744-57.

21. Calderon MA, Linneberg A, Kleine-Tebbe J, De Blay F, Hernandez Fernandez de Rojas D, Virchow JC, et al. Respiratory allergy caused by house dust mites: What do we really know? J Allergy Clin Immunol. 2015;136:38-48.

22. Dharmage SC, Lodge CL, Matheson MC, Campbell B, Lowe AJ. Exposure to cats: update on risks for sensitization and allergic diseases. Curr Allergy Asthma Rep. 2012;12:413-23.

23. Carlsten C, Dimich-Ward H, Becker AB, Ferguson A, Chan HW, DyBuncio A, et al. Indoor allergen exposure, sensitization, and development of asthma in a high-risk birth cohort. Pediatr Allergy Immunol. 2010;21:e740-6.

24. Zock JP, Heinrich J, Jarvis D, Verlato G, Norback D, Plana E, et al. Distribution and determinants of house dust mite allergens in Europe: the European Community Respiratory Health Survey II. J Allergy Clin Immunol. 2006;118:682-90.

doi: 10.18176/jiaci.0455

25. Bousquet PJ, Chinn S, Janson C, Kogevinas M, Burney P, Jarvis D, et al. Geographical variation in the prevalence of positive skin tests to environmental aeroallergens in the European Community Respiratory Health Survey I. Allergy. 2007;62:301-9.

26. Radon K, Schottky A, Garz S, Koops F, Szadkowski D, Radon K, et al. Distribution of dust-mite allergens (Lep d 2, Der p 1, Der f 1, Der 2) in pig-farming environments and sensitization of the respective farmers. Allergy. 2000;55:219-25.

27. Wardzynska A, Majkowska-Wojciechowska B, Pelka J, Korzon L, Kaczala M, Jarzebska M, et al. Association of house dust allergen concentrations with residential conditions in city and in rural houses. World Allergy Organ J. 2012;5:22-7.

28. Remes ST, Koskela HO, Iivanainen K, Pekkanen J. Allergen-specific sensitization in asthma and allergic diseases in children: the study on farmers' and non-farmers' children. Clin Exp Allergy. 2005;35:160-6.

29. Olsson S, van Hage-Hamsten M. Allergens from house dust and storage mites: similarities and differences, with emphasis on the storage mite Lepidoglyphus destructor. Clin Exp Allergy. 2000;30:912-9.

30. 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;347:869-77.

31. Roponen M, Hyvärinen A, Hirvonen MR, Keski-Nisula L, Pekkanen J. Change in IFN-gamma-producing capacity in early life and exposure to environmental microbes. J Allergy Clin Immunol. 2005;116:1048-52.

32. Kääriö H, Huttunen K, Karvonen AM, Schaub B, von Mutius E, Pekkanen J, et al. Exposure to a farm environment is associated with T helper 1 and regulatory cytokines at age 4.5 years. Clin Exp Allergy. 2016;46:71-7.

33. Smit LA, Siroux V, Bouzigon E, Oryszczyn MP, Lathrop M, Demenais F, et al. CD14 and toll-like receptor gene polymorphisms, country living, and asthma in adults. Am J RespirCrit Care Med. 2009;179:363-8.

34. Leynaert B, Guilloud-Bataille M, Soussan D, Benessiano J, Guenegou A, Pin I, et al. Association between farm exposure and atopy, according to the CD14 C-159T polymorphism. J Allergy Clin Immunol. 2006;118:658-65.

doi: 10.18176/jiaci.0455

Table 1. Adjusted associations between exposure to farming environment and animals *in utero* or childhood and sensitization to common allergens and house dust mite at the age of 31 years.

	Study population		Sensitization against cat					Sensitization against birch					Sensitization against timothy					Sensitization against house dust mite				
	N	%	n	%	aOR	95%CI		n	%	aOR	95%CI		n	%	aOR	95%CI		n	%	aOR	95%CI	
Professional farming																						
No	4 247	77	767	18.1	1			795	18.7	1			780	18.4	1			278	6.6	1		
Yes	1 262	23	118	9.4	0.60	0.47	0.75	105	8.3	0.55	0.43	0.70	104	8.2	0.62	0.48	0.79	93	7.4	1.13	0.84	1.52
Farm animals																						
No	3 811	70	724	19.0	1			745	19.6	1			737	19.4	1			258	6.8	1		
Yes	1 653	30	151	9.1	0.52	0.41	0.65	149	9.0	0.56	0.45	0.71	139	8.4	0.60	0.47	0.76	109	6.6	0.91	0.67	1.22
Number of cows																						
0	3 851	71	729	19.0	1			751	19.5	1			743	19.3	1			263	6.8	1		
1 - 4	860	16	85	9.9	0.59	0.45	0.77	85	9.9	0.65	0.49	0.85	77	9.0	0.68	0.51	0.91	49	5.7	0.74	0.52	1.08
≥ 5	746	14	60	8.0	0.44	0.32	0.60	58	7.8	0.46	0.34	0.63	55	7.4	0.49	0.36	0.68	54	7.2	0.98	0.69	1.40
Maternal work with farm animals during pregnancy																						
No farm animals	3 881	70	724	19.0	1			745	19.6	1			737	19.4	1			258	6.8	1		
No maternal work with farm animals	164	3	20	12.2	0.66	0.40	1.08	25	15.2	0.87	0.55	1.37	24	14.6	1.02	0.64	1.63	11	6.7	0.99	0.51	1.90
Maternal work with farm animals with assistance	917	17	82	8.9	0.49	0.37	0.65	78	8.5	0.51	0.38	0.67	68	7.4	0.49	0.36	0.66	65	7.1	0.96	0.68	1.34
Maternal work with farm animals	543	10	49	9.0	0.55	0.39	0.78	45	8.3	0.56	0.39	0.79	46	8.5	0.67	0.47	0.96	31	5.7	0.74	0.47	1.15
Cats¤																						
No	2 683	51	540	20.2	1			544	20.3	1			552	20.6	1			177	6.6	1		
Yes	2 576	49	305	11.8	0.63	0.54	0.75	322	12.5	0.73	0.62	0.86	293	11.4	0.64	0.54	0.76	180	7.0	1.04	0.82	1.31
Dogs¤																						
No	2 411	45	478	19.9	1			497	20.6	1			494	20.5	1			170	7.1	1		
Yes	2 909	55	373	12.8	0.69	0.59	0.82	372	12.8	0.70	0.60	0.82	358	12.3	0.70	0.60	0.83	188	6.5	0.82	0.65	1.04
Number of animal species¤#																						
0	1 428	28	331	23.2	1			339	23.8	1			345	24.2	1			98	6.9	1		
1	1 500	30	268	17.9	0.76	0.63	0.92	281	18.7	0.81	0.67	0.97	270	18.0	0.75	0.62	0.91	98	6.5	0.91	0.68	1.23
2	1 017	20	120	11.8	0.48	0.37	0.62	126	12.4	0.56	0.44	0.72	120	11.8	0.53	0.41	0.68	80	7.9	1.01	0.72	1.42
3	645	13	56	8.7	0.35	0.25	0.50	56	8.7	0.42	0.30	0.59	53	8.2	0.42	0.29	0.60	40	6.2	0.76	0.48	1.19
≥ 4	472	9	38	8.1	0.34	0.23	0.51	36	7.6	0.38	0.26	0.57	26	5.5	0.28	0.18	0.45	30	6.4	0.80	0.49	1.30

N number of observations; % percentage of observations in the given class; n the number of cases in the given class; aOR Adjusted odds ratios; Models are adjusted for sex, maternal age, maternal education, smoking during pregnancy, maternal BMI, place of residence, residential density, current education, current BMI, paternal asthma, paternal allergy, maternal asthma, maternal allergy, gestational age, mother's age of menarche, parity and birth height; 95%CI 95 percentage confidence intervals; ¤ Before the age of 7 y; # Includes cows, pigs, sheep, poultry, minks, cats and dogs; Subjects with a weal reaction ≥ 3mm in skin prick test were considered to be sensitized and the reference group is non-sensitized subjects.

Table 2. Adjusted associations between exposure to farming environment and animals *in utero* or childhood and mono- or polysensitization at the age of 31 years.

	Monosensitization						Polysensitization					
	N	n	%	aOR	95%CI		N	n	%	aOR	95% CI	
Professional farming												
No	3 489	667	19	1			3 557	735	21	1		
Yes	1 152	149	13	0.78	0.62	0.98	1 113	110	10	0.62	0.48	0.80
Farm animals												
No	3 088	600	19	1			3 190	702	22	1		
Yes	1 514	205	14	0.83	0.67	1.04	1 446	137	10	0.53	0.42	0.68
Number of cows												
0	3 122	607	19	1			3 223	708	22	1		
1 - 4	785	108	14	0.86	0.66	1.13	750	73	10	0.57	0.42	0.76
≥ 5	689	90	13	0.77	0.58	1.01	656	57	9	0.46	0.34	0.64
Maternal work with farm animals during pregnancy												
No farm animals	3 088	600	19	1			3 190	702	22	1		
No maternal work with farm animals	145	31	21	1.28	0.83	1.97	133	19	14	0.76	0.45	1.26
Maternal work with farm animals with assistance	843	115	14	0.79	0.61	1.02	802	74	9	0.49	0.37	0.66
Maternal work with farm animals	497	55	11	0.69	0.49	0.96	486	44	9	0.55	0.38	0.79
Cats¤												
No	2 160	458	21	1			2 212	510	23	1		
Yes	2 268	322	14	0.71	0.60	0.84	2 246	300	13	0.66	0.56	0.78
Dogs¤												
No	1 929	387	20	1			2 010	468	23	1		
Yes	2 558	406	16	0.85	0.72	1.01	2 494	342	14	0.66	0.55	0.78
Number of animal species¤#												
0	1 091	228	21	1			1 191	328	28	1		
1	1 245	272	22	1.09	0.89	1.34	1 220	247	20	0.73	0.60	0.89
2	897	136	15	0.74	0.57	0.95	879	118	13	0.50	0.39	0.64
3	587	65	11	0.54	0.38	0.76	578	56	10	0.39	0.27	0.55
≥ 4	440	51	12	0.59	0.41	0.86	421	32	8	0.32	0.21	0.48

N number of observations; % percentage of observations in the given class; n the number of cases in the given class; aOR Adjusted odds ratios; Models are adjusted for sex, maternal age, maternal education, smoking during pregnancy, maternal BMI, place of residence, residential density, current education, current BMI, paternal asthma, paternal allergy, maternal asthma, maternal allergy, gestational age, mother's age of menarche, parity and birth height; 95%CI 95 percentage confidence intervals; ¤ Before the age of 7 y; # Includes cows, pigs, sheep, poultry, minks, cats and dogs; Subjects with a weal reaction ≥ 3mm in skin prick test were considered to be sensitized and the reference group is non-sensitized subjects; Monosensitization= sensitization against only1 out of 4 tested allergens; Polysensitization= sensitization against at least 2 out of 4 tested allergens.

Table 3. Among sensitized participants, adjusted associations between farming environment and the risk of being polysensitized, and not only monosensitized, at the age of 31 years.

	Polysensitization					
	N	n	%	aOR	95% CI	
Professional farming						
No	1 402	735	52.4	1		
Yes	259	110	42.5	0.78	0.57	1.08
Farm animals						
No	1 302	702	53.9	1		
Yes	342	137	40.1	0.62	0.46	0.85
Number of cows						
0	1 315	708	53.8	1		
1 - 4	181	73	40.3	0.65	0.45	0.95
≥ 5	147	57	38.8	0.57	0.38	0.86
Maternal work with farm animals during pregnancy						
No farm animals	1 302	702	53.9	1		
No maternal work with farm animals	50	19	38.0	0.62	0.33	1.16
Maternal work with farm animals with assistance	189	74	39.2	0.60	0.41	0.86
Maternal work with farm animals	99	44	44.4	0.76	0.47	1.21
Cats¤						
No	968	510	52.7	1		
Yes	622	300	48.2	0.93	0.74	1.15
Dogs¤						
No	855	468	54.7	1		
Yes	748	342	45.7	0.73	0.59	0.91
Number of animal species¤#						
0	556	328	59.0	1		
1	519	247	47.6	0.64	0.50	0.83
2	254	118	46.5	0.63	0.45	0.89
3	121	56	46.3	0.66	0.42	1.05
≥ 4	83	32	38.6	0.51	0.30	0.87

N number of observations; n the number of cases in the given class; % percentage of observations in the given class; aOR Adjusted odds ratios; Models are adjusted for sex, maternal age, maternal education, smoking during pregnancy, maternal BMI, place of residence, residential density, current education, current BMI, paternal asthma, paternal allergy, maternal asthma, maternal allergy, gestational age, mother's age of menarche, parity and birth height; 95%CI 95 percentage confidence intervals; ¤ Before the age of 7 y; # includes cows, pigs, sheep, poultry, minks, cats and dogs; subjects with a weal reaction ≥ 3mm in skin prick test were considered to be sensitized; Monosensitization, which is a reference group, is defined as sensitization against only 1 out of 4 tested allergens; Polysensitization is defined as sensitization against at least 2 out of 4 tested allergens.