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Exposure to microbial agents in house dust and wheezing, atopic dermatitis and atopic sensitization in early childhood: a birth cohort study in rural areas

RUNNING TITLE: Microbial exposure and wheezing, atopic dermatitis and atopy

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ABSTRACT

Background: Early-life exposure to environmental microbial agents may be associated with development of wheezing and allergic diseases.

Objective: To assess the association of microbial exposure in rural homes with the risk of asthma, wheezing, atopic dermatitis and sensitization.

Methods: Birth cohorts of rural children (n=1133), half from farmer families, were followed up from birth to 2 years of age by questionnaires in five European centres. Endotoxin and extracellular polysaccharides (EPS) of *Penicillium* and *Aspergillus spp.* were determined from living room floor and mother's mattress dust samples collected at 2 months of age.

Specific IgE against 19 allergens was measured at 1 year of age. Discrete-time hazard models, generalized estimations equations (GEE) and logistic regression were used for statistical analyses.

Results: The incidence of asthma was inversely associated with the amount of dust (adjusted odds ratio (aOR) 0.73, 95%CI 0.58-0.93) and the loads (units/m²) of EPS (aOR 0.75, 95%CI 0.55-1.04) and endotoxin (aOR 0.79, 95%CI 0.60-1.05) in the mother's mattress. Similar associations were seen with wheezing and with living room floor dust. The microbial markers were highly correlated and their effects could not be clearly separated. The inverse associations were seen especially among non-farmers. The risk of sensitization to inhalant allergens increased with increasing endotoxin exposure from mattress dust. No associations were observed with concentrations (units/g) or with atopic dermatitis.

Conclusion & Clinical Relevance: The amount and microbial content of house dust were inversely associated with asthma and wheezing, but due to high correlation between microbial agents and amount of dust, it was not possible to disentangle their individual effects. New ways to better measure and represent exposure to environmental microbes, including indexes of biodiversity, are needed especially among farmers.

Keywords: Respiratory disease, asthma, atopy, specific IgE, atopic dermatitis, EPS, endotoxin, children

Abbreviations:

EPS: fungal extracellular polysaccharides antigens of *Penicillium* and *Aspergillus* spp.

PASTURE: Protection against Allergy Study in Rural Environments

OR: Odds ratio

aOR: adjusted Odds ratio

95%CI 95% Confidence Interval

sIgE:	Specific immunoglobulin E
kU/L:	kilo units per litre
GEE:	Generalized estimating equations
LAL:	Limulus amebocyte lysate
LPS:	lipopolysaccharide

Introduction

Growing up on a farm may be protective of allergy. Several cross-sectional studies have shown that maternal exposure to cowsheds during pregnancy followed by children's exposure in the early years [1], and also farm milk consumption are associated with a decreased risk of allergic diseases [2]. Mechanisms behind these protective effects are unknown. According to the hygiene hypothesis [3], exposure to high concentrations of microbial agents with pro-inflammatory properties stimulates the maturation of T-helper cell 1 (Th1) -oriented immunity, instead of the atopic Th2-oriented immunity [4]. A recent study suggested that the protective effect of farming maybe due to exposure to a greater variety of fungi and bacteria [5].

The most commonly used indicator of bacterial exposure is endotoxin. Endotoxin commonly stands for lipopolysaccharides (LPS) of the cell wall of gram-negative bacteria. Its biological activity is routinely measured with the Limulus amebocyte lysate (LAL) test. The association between endotoxin in the environment and allergy and asthma in children has been studied extensively [6], however, with inconsistent results. Most studies on wheezing have revealed harmful effects in early age [7-13], whereas the effect in later, e.g. school-age, has turned to protective for wheezing or asthma. Higher levels of endotoxin have been inversely associated with allergic sensitization [4, 14].

The extracellular polysaccharide (EPS) of *Penicillium* and *Aspergillus* is considered a relatively good marker of exposure to indoor moulds, as *Penicillium* and *Aspergillus spp.* belong to the most commonly found viable fungi in indoor air. EPS concentrations have been associated with total concentrations of culturable fungi in house dust [15, 16]. There is also preliminary evidence that fungal exposure as estimated by EPS levels in house dust may protect against atopic sensitization [17, 18], wheezing [19] and/or asthma in children [17, 20, 21]. However, these observations need further confirmation.

In contrast to EPS studies, previous studies with airborne samples have found positive associations between high levels of viable *Penicillium spp.* and respiratory disease, like asthma [22], asthma exacerbations [23], wheeze [24, 25] and lower respiratory infections in urban/suburban environments [26]. Airborne concentrations of culturable *Aspergillus spp.* have been associated with an increased risk of atopy [22]. It has been concluded that moisture damage and mould growth in the building are significantly associated with respiratory symptoms and asthma exacerbations [27, 28] and also with the development of new asthma [28, 29].

The farming environment, which is rich in microbes, offers a good opportunity to evaluate the importance of microbial exposures to maturation of the immune system and children's respiratory health. The purpose of the present study was to evaluate the association between the amount and EPS and endotoxin content of the house dust and the occurrence of asthma, wheezing, atopic dermatitis and atopic sensitization in an international birth cohort study of rural children.

Methods

Study population

The study population consisted of the European PASTURE (Protection against Allergy Study in Rural Environments) birth cohort, which has been followed up prospectively from the third trimester of pregnancy. The PASTURE study has been carried out in rural areas of Austria, Finland, France, Germany and Switzerland [30]. All pregnant women who lived on farms with livestock and an equal numbers of non-farming women who lived in rural areas were invited to join the study: 2871 women contacted, 1772 were eligible for the study and 1133 were recruited to the study [31]. The study was approved by the local ethics committees of all participating study centres, and written informed consent was obtained from all parents.

Dust collection and analysis

Dust samples were collected by fieldworkers when the children were two months old, by vacuuming the living room floor and the mother's mattress, using similar protocol and the same type of vacuum cleaners (Miele 516) in each study centre,. The whole surface of the mother's mattress was vacuumed for 2 minutes after removing duvets or blankets. The living room was defined as the room where the family spends most of their time in the evenings after dinner. The fieldworkers were advised to vacuum either 1 m² of wall-to-wall carpet (for two minutes) or 4 m² of smooth floor (for four minutes)[32]. In the case of a smooth floor with a rug, an additional sample from a 1 m² area of the rug was vacuumed (for two minutes). For these homes, microbial content of rug samples, if available, were selected to the final statistical analyses.

The samples were stored at -20°C until extracted in Utrecht, The Netherlands [32]. The levels of fungal EPS of *Penicillium spp.* and *Aspergillus spp.* were measured with an antigen-specific sandwich enzyme immunoassay [16] and the levels of endotoxin by the kinetic chromogenic Limulus Amebocyte Lysate test (Bio Wittaker, Walkersville, MD) [33]. Levels were expressed as endotoxin units (EU) and EPS units (EPSU) both per square meter of sampled area (load) and per gram of sampled dust (concentration).

The detection limit of the amount of house dust was 20 mg. In the case of the nondetectable amounts of dust (8 from mother's mattress, and 11 from living room floor), a value of 13 mg (two-third of the detection limit) was assigned, and the levels of EPS and endotoxin in these samples were excluded from further data analyses. The detection limit of EPS was 141 EPSU/g. In the case of the 9 samples with nondetectable amounts of EPS, a value of 94 EPSU/g was assigned. All endotoxin levels were detectable. In the case of missing data on the loads of EPS or endotoxin in the rug sample, the given load was replaced from smooth floor samples.

Questionnaire data

The first questionnaire was administered to the mothers during the third trimester of pregnancy, and follow-up questionnaires at 2, 12, 18 and 24 months after delivery. The follow-up questionnaires, except the questionnaire at two months, enquired about the wheezing apart from cold and any wheezing as well as confounders for the time period after the preceding questionnaire. 'Asthma' was defined as parent-reported doctor-diagnosed asthma at least once or doctor-diagnosed asthmatic (or obstructive) bronchitis *more than once* during the follow-up. 'Atopic dermatitis' was defined as parent-reported doctor-diagnosed

dermatitis at least once during the follow-up. Housing characteristics were collected with a questionnaire during the home visit.

Specific IgE determination at the age of one year

Venous blood samples obtained from 1-year-old study children were analyzed for specific immunoglobulin E (sIgE) to 19 common allergens, by using the Allergy Screen Test Panel for Atopy (Mediwiss Analytic, Moers, Germany) [34]. The 13 inhalant allergens tested were two house dust mites (*Dermatophagoides pteronyssinus* and *D. farinae*), 7 pollens (alder, birch, European hazel, grass pollen mixture, rye, mugwort and plantain), and cat, horse and dog dander, and the mould *Alternaria alternata*. The 6 food allergens were hen's egg, cow's milk, peanut, hazelnut, carrot and wheat. Atopic sensitization was defined as sIgE concentration of 0.35 kU/L or greater.

Statistical analysis

For the present statistical analyses, only one living room dust sample for each house was selected in the following order of priority: first wall-to-wall carpet, then rug, and finally smooth floor. The loads and concentrations of EPS and endotoxin and the amounts of dust were not normally distributed and were therefore transformed using natural logarithm (ln-transformed). Correlations were calculated as Spearman's rho correlation coefficients. Mean values were expressed as geometric means with their 95 % confidence intervals (CIs). ANOVA with ln-transformed data was used to analyze associations between levels of microbial exposure and potential determinants. Logistic regression was used to analyze the associations between microbial exposure and health outcomes. The results are presented as odds ratios (ORs) and their 95% confidence intervals (CIs) associated with an overall interquartile range increase in exposure, which corresponds to factors 15.12, 18.95 and 12.31

on the original scale for the amount of dust, EPS and endotoxin loads from living room floor, respectively, and 3.03, 7.72 and 5.36 for the amount of dust, EPS and endotoxin loads in dust from mother's mattress, respectively. The corresponding interquartile ranges increase for concentrations were 3.61 for EPS and 3.74 for endotoxin in dust from living room floor and 3.95 for EPS and 4.12 for endotoxin from mother's mattress.

Generalized estimating equations (GEE) with an exchangeable correlation structure to account for correlation between repeated measures within subjects were used to determine associations between microbial exposure and parent-reported wheezing at ages 12, 18 and 24 months. Discrete-time hazard models were used to evaluate the association between microbial exposure and the onset of asthma and atopic dermatitis.

All models were adjusted for study centre, farming, maternal history of allergic diseases (asthma, atopic dermatitis or hay fever), gender, number of older siblings and smoking during pregnancy. Analyses were in addition carefully tested for potential confounding effects of 20 previously reported confounders i.e. parents' histories of allergic diseases and educations, age of mother, birth weight, mode of the delivery, season and floor type of dust sampling, second hand smoke, dog and cat ownership and staying indoors, moisture damage, mother working in cow shed and hay barn during pregnancy, dung hill, breastfeeding, consumption of farm milk and day care attendance, as well as all the microbial determinants shown in the Supplement Table S1. Although many of these additional variables were significantly associated with the levels of microbial markers, none of them changed any of the tested estimated effects of exposure by more than 10% in any analyses and were therefore not included into any of the final analyses. Effect-modification by farming status and centre,

were tested using variables for interaction variables. The data were analyzed using PASW Statistics 18.0 for Windows. Two-sided *p*-values of less than .05 were considered significant.

Results

Data on house dust, EPS and endotoxin levels and information on sampled area for both living room and the mother's mattress were available for 1001 (88.3%) of the 1133 study children. The highest levels of dust, EPS and endotoxin from the living room floor were found in rug samples (Table 1). Samples from a smooth floor had very low amounts of dust and EPS, even lower than in dust samples from mothers' mattresses. Living room floor samples, which were included in the analyses, were almost solely vacuumed from a rug in Finland and from smooth floor in France. The amount of dust and the levels of EPS and endotoxin from living room floor were quite similar across centres when stratified by floor type (Table 1), except France due to low number of rugs. In every centre, the amount of dust and the loads of EPS and endotoxin were higher in farmers' homes than in non-farmers' homes on the living room floor (Figure 1) as well as in the mother's mattress (data not shown).

Determinants of exposure

The following variables increased significantly the amount of dust and both loads and concentrations of EPS and endotoxin in the living room sample (supporting information Tables S1 and S2): a higher number of siblings, having a cat and/or dog, living on a farm, more working hours of mothers in a stable or hay barn, floor type, older homes and houses with woodstoves. Also, the study country and floor type were in general significant determinants. The effect of a woodstove was independent of the effect of farming. Using a

gas cooker was associated with a lower amount of dust and concentrations of EPS and endotoxin.

Frequency of outcomes

The frequency distribution of the outcomes varied across centres (Table 2). The largest difference was in atopic sensitization (17.9% in Austria vs. 43.4% in France) and atopic dermatitis (7.6% in Switzerland vs. 29.9% in France). The French children also had the highest lifetime prevalence of any wheezing and highest incidence of asthma. At the age of 1 year, 264 (28.1%) of 939 children were sensitized to at least one of the tested allergens, 217 (23.1%) to inhalant and 87 (9.3%) to food allergens. The 19 specific allergens were not equally relevant in all centres. The highest prevalence of sensitization was against dog in Austria and against cat in other centres.

Correlations between markers

The correlations between the amounts of house dust and the loads of EPS ($r = 0.86$) and endotoxin ($r = 0.82$) were high in dust samples from the living room floor. The corresponding correlations between samples from the mother's mattress were slightly lower ($r = 0.71$ for EPS and $r = 0.58$ for endotoxin loads). The correlation between EPS and endotoxin loads in dust from the living room floor was 0.77 and from the mother's mattress 0.60. EPS loads between the two sampled locations correlated only poorly ($r = 0.26$) as was also seen for endotoxin ($r = 0.30$). The correlations between the amount of dust and concentrations of EPS or endotoxin were all much lower ($r < 0.20$) than those between the amount of dust and loads of microbial markers.

Microbial markers, wheezing and asthma

Asthma and wheezing tended to be less common in children who lived in homes with higher amounts of dust and loads of EPS and endotoxin (Table 3). Increased amounts of dust in the mother's mattress decreased the risk of asthma. Endotoxin load in the living room floor sample showed a significantly inverse association with asthma as did EPS load in the living room floor with any wheezing. No significant associations were observed with asthma and wheezing and EPS or endotoxin concentrations (supporting information Table S3).

Microbial markers, serum sIgE and atopic dermatitis

The risk of sensitization to inhalant allergens was increased at the age of 1 year with increasing endotoxin in the mother's mattress (Table 4 for loads and supporting information Table S3 for concentration). Similar associations were found between increasing endotoxin exposure in the mother's mattress and sensitization to any tested allergens (aOR 1.21, 95%CI 0.99-1.48 for endotoxin load and aOR 1.25, 95%CI 1.03-1.52 for endotoxin concentration). The estimates were closer to one when the cut-off level was elevated to 0.7kU/L: sensitization to inhalant allergens (aOR 1.13, 95%CI 0.87-1.45 and aOR 1.14, 95%CI 0.89-1.46, respectively) and sensitization to any tested allergens (aOR 1.16, 95%CI 0.92-1.47 and aOR 1.16, 95%CI 0.92-1.46, respectively). No associations were found with sensitization to food allergens (data not shown) or with atopic dermatitis (Table 4 for loads and supporting information Table S3 for concentration).

Microbial markers analysed together

In an effort to disentangle the roles of amounts of dust, EPS and endotoxin loads, fully adjusted models were run for asthma, where two markers at a time and finally all three markers were entered simultaneously into the same model. Due to the high correlations between the markers, the direction and size of the observed associations for the three markers

changed in general strongly. However, the inverse association between the amount of dust from the mother's mattress and asthma was not substantially confounded by endotoxin and EPS although the association did not reach statistical significance (aOR 0.72, 95% CI 0.50-1.04). Also, the estimate of endotoxin load in dust from living room floor and asthma became more protective after mutual adjustments (aOR 0.55, 95% CI 0.30-1.00). When the association between endotoxin load in the mother's mattress dust and sensitization to inhalant allergens was adjusted for EPS and amount of dust, the association did not change (OR 1.37, 95% CI 1.07-1.77).

Effect modification in farming status

In further stratified analyses, inverse associations between three microbial markers and asthma or wheezing were seen especially among non-farmers' children. Farming status seemed to be an effect modifier particularly in the models using mother's mattress samples, and especially asthma and wheezing apart from cold (Table 5), but not atopic dermatitis or sensitization (data not shown).

Heterogeneity between countries

There was little heterogeneity in the associations between centres (supporting information Table S4). There was significant heterogeneity for endotoxin load from living room floor and wheezing apart from cold. Endotoxin loads tended to be inversely associated with wheezing apart from cold in other countries, but among Finnish children the association was positive. In the German children, microbial markers from living room sample appeared to protect from the risk of atopic dermatitis.

Discussion

In the present study, the amount and microbial content of house dust were inversely associated with asthma and wheezing, but due to high correlation between microbial agents and amount of dust, it was not possible to disentangle their individual effects. The inverse associations were especially seen among non-farmers' children. The associations were clearly weaker when microbial exposures were calculated as concentrations (per gram of dust). The risk of sensitization to inhalant allergens at the age of 1 year increased with increasing concentration of endotoxin in the mother's mattress. No significant associations were found for atopic dermatitis.

High endotoxin load in infancy seemed to decrease the risk of wheezing by 2 years of age. Our results are very similar to the findings of Douwes et al. [17], who observed a decreased risk of wheezing at the age of 4 years with higher amount of dust, endotoxin and EPS. However, the present results are in contrast to most other birth cohort studies with short follow-ups [7, 8, 10, 11], in which endotoxin has been associated with an increased the risk of wheezing, although the association may turn [35]. Other results among children have been inconsistent [14, 20, 36, 37]. In adults endotoxin is a well-known respiratory irritant [38], especially in the workplaces. In contrast, endotoxin exposure has mostly been protective of atopy both among children [39] and among adults in the workplaces [38].

EPS loads tended to be inversely associated with asthma and wheezing, which is in line with earlier studies among older children [17, 19, 21]. To date, knowledge on the health effects of EPS in early age is limited. In contrast to studies on EPS, two studies with airborne samples have found associations between high levels of viable *Penicillium spp.* and increased risk of wheeze [22, 24, 25] in 1-year-old children with high risk of asthma. A potential explanation for these opposite findings might be the difference in the measure of exposure used. EPS is extracellular polysaccharide of the fungal species *Penicillium* and *Aspergillus* and is measured from house dust. In contrast, viable fungi were measured from airborne samples. Cultivation also only measures viable, biologically active fungi, in contrast to EPS, which measures cumulative exposure to both viable and non-viable fungi.

Another explanation for the present results on endotoxin and EPS might be that most of the earlier birth-cohort studies have been done in urban/suburban areas, whereas the present study was done in rural areas with very different sources of microbes, such as stables and dung hills. In addition, most of the houses in the present study were detached houses and half of them were farm homes, in contrast to other studies, where most homes have probably been apartments. Measurements of endotoxin and EPS are only crude markers of the total microbial exposure and [5, 40, 41] in different environments may reflect quite different microbial exposures. The microbial diversity has been shown to be much higher in farming homes than in non-farming homes and there are also significant differences in prevalence of dominant microbes [5]. It can also be hypothesized that microbial exposure coming from different sources, e.g. farm environment or moisture damage, may have different effects on health.

The above may also partly explain our finding that inverse associations with respiratory outcomes were stronger among non-farmers than farmers. Another possible explanation is that farmers' children may be exposed to high levels of microbes outside the home, e.g. in the stables. Endotoxin and EPS levels in stable dust are 5- to 7 fold higher than in dust from living room floor in farm houses [32]. Thus, there may be greater misclassification of exposure among farm children than among non-farm children.

High endotoxin exposure in mattress dust increased the risk of sensitization to inhalant allergens, as was also suggested in another birth cohort study [10]. No association was found in two other birth cohort studies among children of similar age as in our study [11, 42]. On the contrary, cross-sectional studies have mostly found an association between high endotoxin exposure and lower prevalence of atopy in school-aged children [39] as was also observed in a birth cohort study in 5-year-old children [43]. Early sensitization in most children is transient and part of the normal maturation of the immune system [44]. Most children with low levels of specific IgE (0.35-1.0 kU/L) at the age of 2 years lose their sensitization until the age of 5 years, whereas stronger sensitization with higher levels of specific IgE (above 3.5 kU/L) is more persistent [44]. In our study, only 2% of the study children had such stronger sensitization, and so, most of the sensitization at age 1 is likely to be transient. Therefore, also the association between endotoxin and sensitization is likely to depend on age.

We did not find any associations between EPS or endotoxin and atopic dermatitis during the first two years of life. Birth cohort studies among children around 1 year have found inverse associations between endotoxin and dermatitis or atopic dermatitis [7, 12, 45]. A birth cohort study found inverse association only in children with the genotype CC of the CD14 gene

[43]. No association between EPS and atopic eczema has been observed in a cross-sectional study [46]. Thus, the evidence from earlier studies and from the present study suggests that the role of microbial exposure in the development of atopic dermatitis is weaker in early childhood than in case of asthma or atopic sensitization or that the association may have been masked by genetic variation.

The strongest and most significant associations were observed with diagnosed asthma, whereas wheezing without cold and especially any wheezing showed few associations. This is likely to be due to the higher specificity of asthma definitions, as compared to parent-reported wheezing, but may also indicate severity of the disease. The follow-up of the children in the present study continued only for the first 2 years of life, which is too short a time to draw definite conclusions concerning asthma in children.

The main strengths of the present study are the prospective birth cohort design, over 88% participation rate and dust collections by field workers using a standardized protocol in 5 European centres. The lack of assessments for other microbial products than fungal EPS and endotoxin and the short follow-up are the main limitations of the study. We also did not do microbial measurements from children's mattresses, as other studies have shown that the mattresses of infants are often new and the levels of microbes low [47]. Although mother's mattress dust is only an indirect indicator of child's exposure, at least for the children who do not sleep with their mothers, it reflects the exposure at home and also in the stables, as there is a fair correlation between the time that the mother and her baby spend in the stables ($r=.45$ in our study).

In conclusion, the amount and microbial content of house dust were inversely associated with asthma and wheezing, but due to high correlation between microbial agents and amount of dust, it was not possible to disentangle their individual effects. New ways to better measure

and represent exposure to environmental microbes, including indexes of biodiversity, are needed especially among farmers.

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A conflict of interest

E. von Mutius has received funding from Airsonett AB for conducting a clinical trial. Also, she has been serving as a paid consultant for GlaxoSmithKline, Novartis, and Protectimmun. The rest of the authors have declared that they have no conflict of interest.

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Legends to figure

Figure 1. Amount of dust and the loads of EPS and endotoxin in dust from living room floors among non-farmers and farmers in Austria (A), Switzerland (C), France (F), Germany (G) and Finland (S). EPSU= extracellular polysaccharides units, EU=endotoxin units. Extreme values have been removed from the figures. Note that the differences between centres are mainly explained by differences in the dust sample type (smooth floor, rug or wall-to-wall carpet).

Table 1. Geometric means (GM) and geometric standard deviations (GSD) of amount of dust, the loads (units/m²) of EPS and endotoxin from living room and mother's mattress.

	A1			Austria			Finland			France			Germany			Switzerland											
Living room floor dust (m ²)	N	GM	95%CI	N	GM	95%CI	N	GM	95%CI	N	GM	95%CI	N	GM	95%CI	N	GM	95%CI									
Wall-to-wall carpet																											
Amount of dust (mg/m ²)	105	645	539	772	14	1,115	947	1,487	0	3	189	29	1,250	29	593	426	822	59	629	495	798						
EPS (EPUs/m ²)	104	54,345	41,245	71,805	14	74,037	35,718	153,447	0	3	14,350	1,163	174,132	26	50,561	27,804	91,945	59	55,921	40,000	79,178						
Endotoxin (EU/m ²)	103	17,007	12,472	23,192	14	27,713	13,017	59,900	0	3	3,833	254	60,792	26	18,921	11,264	31,782	58	15,468	10,052	23,863						
Rug																											
Amount of dust (mg/m ²)	501	894	825	968	105	977	829	1,151	208	1,074	966	1,193	23	255	151	388	101	827	694	1,000	66	795	646	955			
EPS (EPUs/m ²)	498	58,462	52,135	65,595	105	88,184	76,697	122,488	204	53,059	45,514	61,956	23	13,436	6,947	29,719	100	42,826	47,946	51,861	66	52,649	38,381	70,564			
Endotoxin (EU/m ²)	496	22,494	19,764	25,801	105	31,749	23,725	42,488	202	19,880	16,374	24,136	23	9,279	5,452	15,792	100	27,727	20,308	37,857	66	19,918	13,872	25,529			
Smooth floor																											
Amount of dust (mg/m ²)	434	57	51	64	89	64	50	82	8	89	40	197	140	54	45	85	96	80	61	106	97	40	32	49			
EPS (EPUs/m ²)	433	3,477	2,944	4,106	89	4,111	2,878	5,872	8	3,304	1,051	10,394	143	3,360	2,489	4,534	97	5,996	4,178	6,806	97	1,828	1,360	2,459			
Endotoxin (EU/m ²)	435	2,582	2,225	2,997	89	2,064	1,509	2,824	10	2,755	1,221	6,217	143	3,028	2,427	3,777	97	3,805	2,640	5,483	96	1,883	1,213	2,333			
Mother's mattress dust (m²)																											
Amount of dust (mg/m ²)	1,052	218	204	230	213	280	588	245	321	211	188	174	203	172	119	87	101	142	239	259	233	269	217	252	19	219	290
EPS (EPUs/m ²)	1,042	19,955	12,726	15,301	212	21,229	17,231	26,165	209	7,092	5,969	8,425	170	11,789	9,273	14,972	237	17,357	14,438	20,085	215	15,985	13,077	18,492			
Endotoxin (EU/m ²)	1,051	1,837	1,505	1,782	212	2,391	2,013	2,839	211	1,070	901	1,271	172	1,122	891	1,414	239	2,362	1,877	2,797	217	1,549	1,295	1,854			

EU/m² = extracellular polysaccharides units

EU/m² = endotoxin units

EPUs= extracellular polysaccharides units

EU=endotoxin units

Table 2. The lifetime prevalence of any wheezing and wheezing apart from cold and the cumulative incidence of asthma and atopic dermatitis during the first two years of life and the prevalence of atopic sensitization ($\geq 0.35\text{ kU/l}$) at the age of 1 year.

	All		Austria		Finland		France		Germany		Switzerland	
	n/N	%	n/N	%	n/N	%	n/N	%	n/N	%	n/N	%
Wheezing¹												
Any	408/1 042	(39.2)	82/209	(39.2)	62/204	(30.4)	88/174	(50.0)	86/231	(38.5)	87/224	(38.8)
Apart from cold	130/1 042	(12.1)	16/209	(7.7)	38/204	(18.6)	29/174	(15.5)	21/231	(9.1)	26/224	(11.6)
Asthma²	95/1 025	(9.4)	8/209	(3.8)	11/202	(5.4)	30/166	(18.1)	29/228	(12.7)	8/220	(3.8)
Atopic dermatitis³	170/1 040	(16.3)	31/209	(14.8)	41/204	(20.1)	52/174	(29.9)	30/230	(13.0)	17/223	(7.6)
Atopic sensitization												
Inhalant allergens	217/939	(23.1)	29/197	(14.7)	41/193	(21.2)	62/173	(35.8)	42/199	(21.1)	43/177	(24.3)
Food allergens	87/939	(9.3)	9/197	(4.6)	18/193	(9.3)	24/173	(13.9)	16/199	(8.0)	20/177	(11.3)
Any	264/939	(28.1)	35/197	(17.8)	49/193	(25.4)	75/173	(43.4)	51/199	(25.6)	54/177	(30.5)

¹ Parent-reported wheezing symptom in any time points between 2-24 months

² Parent-reported doctor-diagnosed asthma at least once and/or asthmatic bronchitis more than once until the age of 2 years

³ Parent-reported doctor-diagnosed atopic dermatitis until the age of 2 years

Table 3. Associations between microbial exposure loads (units/m²) and asthma or wheezing during the first two years of life.

	Asthma		Wheezing apart from cold		Any wheezing	
	N ¹	Adjusted OR (95%CI)	N ¹	Adjusted OR (95%CI)	N ¹	Adjusted OR (95%CI)
Living room floor						
Amount of dust (mg/m ²)	989	0.79 (0.54-1.17)	1006	0.68 (0.46-1.00)	1006	0.82 (0.67-1.00)
EPS (EPSU/m ²)	984	0.91 (0.64-1.13)	1001	0.74 (0.54-1.03)	1001	0.82 (0.68-0.99)
Endotoxin (EU/m ²)	984	0.71 (0.51-0.99)	1001	0.84 ² (0.62-1.15)	1001	0.85 (0.72-1.00)
Mother's mattress						
Amount of dust (mg/m ²)	1003	0.73 (0.58-0.93)	1020	0.87 (0.68-1.11)	1020	0.93 (0.82-1.06)
EPS (EPSU/m ²)	993	0.75 (0.55-1.04)	1010	0.85 (0.62-1.18)	1010	0.94 (0.78-1.12)
Endotoxin (EU/m ²)	1002	0.79 (0.60-1.05)	1019	0.88 ² (0.65-1.12)	1019	0.89 (0.77-1.04)

N = number of subjects included in the analysis. ¹Subjects contributed up to three repeated observations to this longitudinal analysis

Models are adjusted for study center, farming status, gender, maternal history of allergic diseases (hay fever, atopic dermatitis and/or asthma), smoking during pregnancy and the number of siblings. Odds ratios are calculated for an interquartile change. EPSU= extracellular polysaccharides units, EU=endotoxin units

² Test for heterogeneity between centers $p < 0.2$

Table 4. Associations between microbial exposure loads (units/m³) and atopic dermatitis or sensitization to inhalant allergen.

	Atopic dermatitis		Sensitization to inhalant allergen [‡]	
	N [‡]	Adjusted OR (95%CI)	N	Adjusted OR (95%CI)
Living room floor				
Amount of dust (mg/m ³)	979	0.92 [‡] (0.67-1.27)	892	0.89 (0.66-1.21)
EPS (EPSU/m ³)	975	0.87 [‡] (0.66-1.14)	887	0.91 (0.71-1.18)
Endotoxin (EU/m ³)	974	0.93 [‡] (0.72-1.21)	887	1.01 (0.79-1.29)
Mother's mattress				
Amount of dust (mg/m ³)	993	0.92 (0.76-1.12)	900	0.98 (0.81-1.19)
EPS (EPSU/m ³)	983	0.97 (0.75-1.25)	892	0.98 (0.76-1.24)
Endotoxin (EU/m ³)	992	0.96 (0.77-1.20)	899	1.25 (1.01-1.55)

N = number of subjects included in the analysis, [‡]Subjects contributed up to three repeated observations to this longitudinal analysis

[‡] Sensitization to at least one inhalant allergen (≥ 0.35 kU/L) at the age of 1 year

Models are adjusted for study centre, farming status, gender, maternal history of allergic diseases (hay fever, atopic dermatitis and/or asthma), smoking during pregnancy and the number of siblings.

Odds ratios are calculated for an interquartile change

EPSU= extracellular polysaccharides units, EU=endotoxin units

[‡] Test for heterogeneity between centers $p < 0.2$

Table 5. Associations between microbial exposure loads (units/m³) and asthma, wheezing apart from cold and atopic dermatitis stratified by farming status.

	Asthma			Wheezing apart from cold			Atopic dermatitis		
	non-farmer	farmer	inter	non-farmer	farmer	inter	non-farmer	farmer	inter
	Adjusted OR (95%CI)	Adjusted OR (95%CI)	p - value	Adjusted OR (95%CI)	Adjusted OR (95%CI)	p - value	Adjusted OR (95%CI)	Adjusted OR (95%CI)	p - value
Living room floor									
Amount of dust (mg/m ³)	0.79 (0.46-1.36)	0.93 (0.46-1.90)	0.86	0.45 (0.25-0.79)	1.07 (0.63-1.81)	0.07	0.88 (0.58-1.35)	1.03 (0.62-1.71)	0.88
EPS (EPSU/m ³)	1.82 (0.63-5.66)	0.77 (0.46-1.29)	0.46	0.65 (0.41-1.04)	0.82 (0.51-1.32)	0.47	0.95 (0.66-1.34)	0.77 (0.50-1.20)	0.58
Endotoxin (EU/m ³)	0.69 (0.42-1.10)	0.72 (0.45-1.17)	0.97	0.65 (0.40-1.05)	1.04 (0.70-1.53)	0.17	0.87 (0.60-1.26)	1.01 (0.69-1.48)	0.57
Mother's mattress									
Amount of dust (mg/m ³)	0.58 (0.42-0.80)	0.95 (0.65-1.37)	0.06	0.68 (0.48-0.96)	1.15 (0.84-1.57)	0.03	0.79 (0.61-1.03)	1.12 (0.83-1.52)	0.09
EPS (EPSU/m ³)	0.55 (0.36-0.85)	1.06 (0.67-1.69)	0.04	0.66 (0.43-1.00)	1.09 (0.69-1.71)	0.08	0.82 (0.59-1.15)	1.19 (0.81-1.75)	0.20
Endotoxin (EU/m ³)	0.68 (0.47-0.99)	0.95 (0.62-1.46)	0.22	0.71 (0.53-0.95)	1.06 (0.74-1.54)	0.05	0.86 (0.65-1.15)	1.09 (0.78-1.53)	0.31

Models are adjusted for study center, gender, maternal history of allergic diseases (hay fever, atopic dermatitis and/or asthma), smoking during pregnancy and the number of siblings

Odds ratios are calculated for an interquartile change, EPSU= extracellular polysaccharides units, EU=endotoxin units

Interaction p-values are for interaction term for farming status

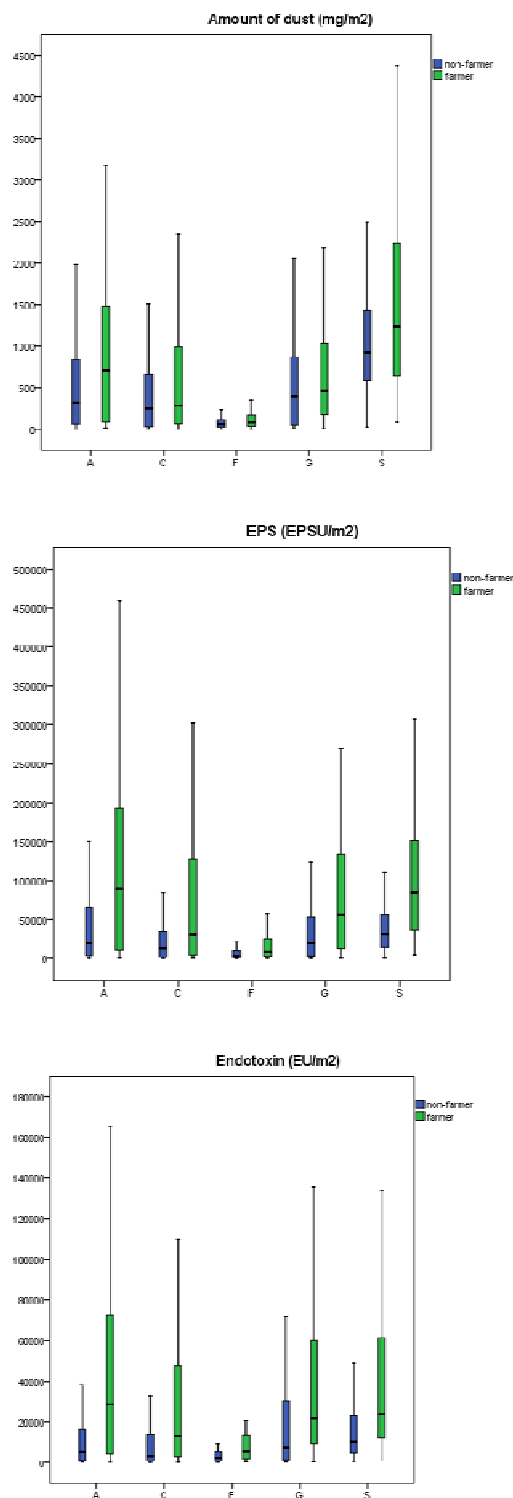


Figure 1. Amount of dust and the loads of EPS and endotoxin in dust from living room floors among non-farmers and farmers in Austria (A), Switzerland (C), France (F), Germany (G) and Finland (S). EPSU= extracellular polysaccharides units, EU=endotoxin units. Extreme values have been removed from the figures. Note that the differences between centres are mainly explained by differences in the dust sample type (smooth floor, rug or wall-to-wall carpet).