

Determinants of endotoxin levels in living environments of farmers' children and their peers from rural areas

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Summary

Background Lower frequencies of asthma and hayfever have been observed in children with contact to livestock. At school age, the amount of endotoxin measured in the dust of children's mattresses is inversely related to the occurrence of atopic asthma, hayfever and atopic sensitization both in children from farming and non-farming households.

Objective The aim of the present study was to investigate which home and lifestyle characteristics of farm and non-farm families contribute to endotoxin levels measured in different indoor home environments.

Methods In the framework of the Allergy and Endotoxin (ALEX) Study, endotoxin was measured in dust samples from the living room floor and the child's mattress of 319 farmers' families and 493 non-farming families, and in settled dust from stables. Endotoxin content of all dust samples was determined by a kinetic Limulus assay (*Limulus-Amebocyte-Lysate* test). Information about the child's activities on farms, home characteristics and cleaning behaviours was obtained from parental questionnaires.

Results Endotoxin levels in stables did not predict the amount of endotoxin measured in floors or mattresses. However, a dose-dependent association between the child's activity on the farm and indoor home endotoxin levels was observed, both in farm and non-farm children. In non-farm children pet keeping and the frequency of floor cleaning were additionally associated with endotoxin levels, whereas in farm children parental farm activities, study area, time since last cleaning, the mattress type as well as younger age of the children contributed to increased microbial exposure.

Conclusion These results demonstrate that regular contact to farm animals increases indoor home endotoxin concentrations, both in farm and non-farm children, and might thus explain the protective effect of contact to livestock on atopic outcomes. To assess children's individual exposure to a microbial environment, measures of mattress dust exposure are needed as stable endotoxin concentrations were not associated with indoor home levels.

Keywords allergy, children, exposure assessment, farm animals and pets, farming, house and stable dust, indoor endotoxin, living room floor, mattress

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Introduction

Lower frequencies of asthma, hayfever and atopic sensitization have been observed in children growing up on farms in Europe, Canada and Australia [1–7]. In comparison to children from non-farming families, farmers' children are exposed to higher concentrations of endotoxin both in stables and in dust from kitchen floors and mattresses [8]. We have recently shown that the timing of exposure to a farming environment is crucial for its protective effect on the

development of asthma and allergy [9]. In addition, we could demonstrate that at school age the amount of endotoxin measured in the dust of children's mattresses is inversely related to the occurrence of atopic asthma, hayfever and atopic sensitization [10] both in children from farming and non-farming households. Thus, in addition to farm abode, other factors must be important in determining the levels of endotoxin in rural farm and non-farm households. However, little is known about these factors. Endotoxin is a stable glycolipid component of the outer membrane of Gram-negative bacteria that can be collected and assayed in settled dust from mattresses or floors [11–13]. From occupational studies, it is known that high levels of endotoxin can be measured in swine confinement and poultry houses [14–16], but it has not yet been investigated whether endotoxin levels

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measured in stables determine the indoor home environment of farmers' children. In urban environments, elevated levels of endotoxin have been reported from homes where cats and dogs were kept [17–20]. In US metropolitan homes, regular use of central air conditioning decreased the levels of indoor endotoxin concentrations, but other home characteristics for living conditions such as home dampness, number of inhabitants, cleaning frequency or presence of smokers did not influence endotoxin levels in house dust [18].

The aim of the present study was to investigate which home and lifestyle characteristics of farm and non-farm families might determine the endotoxin levels measured in the dust of the children's mattresses and in the living room floor dust, and to determine whether the same factors were relevant for both groups. For the farmers' subgroup, we evaluated whether the endotoxin levels measured in stables were directly associated with the respective levels in the dust of children's mattresses or the living room. In addition, we investigated which farm and stable characteristics determine the endotoxin levels in the stable. The analyses are based on the questionnaire data and endotoxin measurements of 812 farm and non-farm families which are part of the collaborative cross-sectional study *Allergy and Endotoxin (ALEX)* [9, 10].

Materials and methods

Study population

The ALEX Study was carried out in rural areas of Austria, Germany and Switzerland as previously described [9]. With respect to population density and farming characteristics, these regions were shown to be comparable concerning usually small, traditional farms, mainly run by members of the family and only occasionally by farmworkers. Ninety-eight percent of the farms have farm animals. The predominant farming type in these areas is traditional dairy farming with a mean number of 35 cows and calves per farm, and only small numbers of pigs, chicken, horses, sheep and goats.

Participating parents (2618 of 3504 potential participants; 74.7%) were asked to consent to blood sampling in their children and to collection of dust samples in different home environments. For these additional investigations, 1406 (53.7%) families accepted. Of these, all the children from farming families, all non-farming children with regular contact to a farming environment through neighbours and a random sample of children not being raised on a farm and not exposed to a farm environment were invited to participate ($n = 901$). The final analysis was restricted to 812 children with complete data and to children with German (471 of 511; 92.2%), Austrian (221 of 253; 87.4%) and Swiss nationality (120 of 137; 87.6%), to avoid potential confounding by ethnicity. They consisted of 319 farmers' children and 493 children from non-farming families. No relevant participation bias was found [9].

Questionnaire

Demographic factors and home characteristics such as age, sex, number of siblings, house location, heating system,

parents education, pets and indoor smoking, as well as information about the child's activities on farms (contact to stable animals, stay in stables and barns, helping with harvesting) and stable characteristics (type and amount of farm animals, feeding material, manure system) were assessed by parental questionnaire. 'Farmers' children' were defined as children whose parents answered 'yes' to the question 'Does your child live on a farm?'. For farm children, the time they assisted their parents with stable work was assessed. For non-farm children, a 'stable activity score' was defined combining regular contact to stable animals (No = 0/Yes = 1) and the frequency of staying in the stable (never = 0/rarely or several times per week = 1/at least daily = 2). The score ranges from 0 (no contact to stable animals and never in a stable) to 3. In addition, information on the date of dust sampling, the age and type (foam rubber, inner spring, or latex) of the mattress, the number of mattress covers, time since last cleaning the mattress, the type of floor, days since last cleaning the living room floor, and the presence of animals in stables during dust sampling were recorded in a sampling protocol during the home visit for dust collection.

Dust collection

The dust collection was performed according to a standardized protocol. Seven fieldworkers sampled in three rural areas: three in Germany, two in Austria and two in Switzerland. Dust samples were taken using new vacuum cleaners of 1200 W (Miele Bodenstaubsauger S624, Miele AG Spreitenbach, Switzerland), calibrated with a flow of 200 L/min with a clean filter, operating power of about 800 W. ALK filters (ALK Allergologisk Laboratorium, Copenhagen, Denmark) were used for dust collection according to the International Study of Asthma and Allergic Disease in Childhood (ISAAC) phase II manual (ISAAC, 1998). To ensure comparability of the method of dust collection at the different study sites, all fieldworkers were centrally trained. Each fieldworker visited approximately an equal number of farmers' and non-farm families, and all samples were collected between November 1999 and February 2000.

The child's mattress was chosen by asking the parents 'Which mattress did your child sleep on most in the last four weeks?'. After removing all sheets apart from mite protecting covers or plastic sheets, the mattress was vacuumed for 2 min/m². In the living room, an area of 2 m² carpeted floor was chosen if there was any kind of carpet in this room. Otherwise, the fieldworker chose an area of 4 m² of smooth floor (without displacing the furniture). The living room floor was vacuumed for 4 min. The pre-weighed ALK-filter holders (± 0.01 g) were re-weighed after dust collection with only particles bigger than 2 mm being removed by tweezers. For the stable dust sample, the stable was chosen, in which the child spent most time. Settled dust from dry surfaces in stables at a height between 0.5 and 1.5 m above ground level was collected with a spatula. Fine dust was separated from hay and straw with a household sieve.

All samples were stored at room temperature and transferred within 1 week after collection to the laboratory of the Institute for Occupational and Environmental Medicine, University of Munich, for endotoxin analyses.

Measurements of endotoxin levels

Endotoxin content of all dust samples was determined by a kinetic *Limulus* assay (kinetic-QCL, BioWhittaker Inc., Walkersville, MD, USA). For extraction, 100 mg from each sample (for stable dusts two replicates) was extracted by rapid shaking with 7-mL endotoxin-free water for 1.5 h. Thereafter, the suspension was diluted 1:100 for samples from mattresses and floors, and 1:1000 for samples from stables. Directly after dilution, an aliquot of 100 µL was added to a microtitre plate (96 well, Falcon) and assayed with *Limulus-Amebocyte-Lysate* (LAL). To get information about possible enhancement or inhibition reactions of the LAL assay, replicate of each sample was spiked with an endotoxin standard. A standard calibration curve (0.05–0.5–5–50 EU/mL), a laboratory blank and an internal laboratory standard were performed on each plate. As recommended by the manufacturer, optical density at 405 nm was measured by an automatic reader (PowerWave™, MWG Biotech Inc., Mendelhall Oaks Parkway, NC, USA). If spike recovery was below 45%, the suspension was diluted higher and the analysis was repeated. The intra-assay variability (EU/mg dust) was less than 10%, whereas the inter-assay variability was lower than 20%. Resulting endotoxin levels were expressed as EU/mg dust (endotoxin concentration) and relative to the size of the sampling area and weight of the sampled dust, as EU/m² (endotoxin load). In case of dusts from stables, the mean of the two replicates was calculated. All measured endotoxin levels were above the limit of detection.

Repeated dust sampling from the same locations at 31 stables after at least 3 days yielded a Pearson correlation coefficient of 0.73, $P < 0.001$ for log endotoxin concentrations. The corresponding mean coefficient of variation was 15.2%. To assess the variation in indoor home endotoxin concentrations over time, repeated dust sampling was obtained 2–4 months after initial sampling from 12 living room floors and seven mattresses. The Pearson correlation coefficients for log endotoxin concentrations in the living room floor dust were 0.66, $P: 0.018$, and 0.78, $P: 0.040$ for the mattress dust. The corresponding coefficients of variation were 20.1% and 12.9%, respectively.

Statistical analyses

Endotoxin levels were best described by a lognormal distribution and therefore log transformed. Geometric means, geometric standard deviation, as well as *t*-tests on log scale data were calculated to describe differences in the endotoxin concentration (EU/mg dust), of mattress and living room floor dust of farm and non-farm families. For the subgroup of farm children, Pearson correlation coefficients were calculated to evaluate whether the log endotoxin concentrations measured in settled dust of stables were correlated with the respective levels in the living room floor and mattress dust. Likewise, for farm and non-farm families the correlation between log endotoxin levels in mattress dust and living room floor dust was determined.

Univariate associations between stable dust endotoxin concentrations ln(EU/mg) and farm or stable characteristics such as the number and type of farm animals kept in the respective stable, were calculated. All variables with a

univariate association ($P \leq 0.25$) were subsequently tested in multivariate regression models.

Univariate associations between endotoxin concentrations ln(EU/mg) in living room and mattress dust samples and potential predictive factors of indoor endotoxin levels were calculated separately for the subgroups of farmers' and non-farm children. We included factors related to the farm environment, and other factors that have been shown to influence the indoor endotoxin levels, such as pets [17–19, 21], house location, heating system, smoking [18] and the frequency of vacuum cleaning [21]. Available information on mattress types and floor covering [21] were also tested. To control for potential confounding all multivariate models were additionally adjusted for sex, age, amount of sampled dust, sampling area, fieldworker and the date of dust sampling. The association of a given predictor variable with endotoxin was expressed as the ratio of covariate-adjusted geometric means relating to a unit increase of the predictor variable (means ratio). All analyses were carried out using Intercooled Stata 8.0 (Stata Corporation, College Station, TX, USA).

Results

The study population consisted of 319 farmers' children with a mean age of 9.42 years (SD 1.63), and 493 children from non-farming families, mean age 9.49 (SD 1.60). The characteristics of the study population and the geometric mean endotoxin concentrations in the measured compartments for farmers' and non-farm children are given in Table 1. No statistically significant correlation existed between stable endotoxin concentrations and the respective living room floor or mattress dust concentrations (Fig. 1a). However, there was a clear positive correlation between the endotoxin levels of living room floor dust and the respective mattress dust levels in both subgroups of children, and for both the endotoxin concentration (Fig. 1b) and the endotoxin load (Fig. 1c).

Crude and adjusted means ratios of the variables associated with endotoxin levels in settled stable dust are presented in Table 2. Full- and part-time farmers did not differ with respect to endotoxin levels in the stables, but increasing number of cattle kept in the stable increased the endotoxin level. However, there was no clear dose-response relationship. Stable animals had mostly been inside the stable for at least 1 month before dust sampling (93.1%). Sixty-five (23.3%) of the farms kept horses in addition to cattle. Keeping horses was independently associated with increased endotoxin levels. Feeding the cattle with hay was associated with increased endotoxin levels, compared to feeding with silage and hay which is a less traditional form of feeding. Using both solid and liquid muck in combination increased stable endotoxin levels. Stables in the Austrian and Swiss study areas yielded endotoxin levels higher than those in Germany. Other variables such as the number of people running the farm, the presence of additional pigs, goats or sheep in the stable did not independently increase the stable endotoxin levels. The adjusted model explained 21.0% of the variation in settled stable dust endotoxin concentration.

Table 1. Study population characteristics, stratified by farmers' children and non-farm children

		Farmers' children N = 319, n (%)	Non-farm children N = 493, n (%)	P-value*
Regular contact to stable animals	Any	299 (93.7)	188 (38.1)	< 0.001
Regular stay in stable	Up to several times weekly	153 (48.0)	306 (62.1)	< 0.001
	At least daily	153 (48.0)	30 (6.1)	
Regular stay in barn	Any	301 (94.4)	292 (59.2)	< 0.001
Helping with harvesting	Any	300 (94.3)	199 (40.4)	< 0.001
Pets regularly indoors	Only cats	77 (24.1)	153 (31.0)	0.082
	Cats and dogs	36 (11.3)	44 (8.9)	
Pets regularly on child's bed	Only cats	52 (16.3)	127 (25.8)	0.003
	Cats and dogs	4 (1.3)	11 (2.2)	
Sex	Girls	147 (46.1)	247 (50.1)	0.263
Number of older siblings, Ref: 0	1–2	160 (50.1)	262 (53.1)	< 0.001
	> 2	56 (17.6)	18 (3.7)	
Fathers education, Ref: None, Low	Medium	132 (41.4)	196 (39.8)	< 0.001
	High	40 (12.5)	114 (23.1)	
Indoor smoking	Any	46 (14.4)	116 (23.5)	0.001
House location, Ref: Inside village	Outside village	188 (58.8)	45 (9.1)	< 0.001
Heating system, Ref: Oil, gas or electricity	Wood, coal	196 (61.4)	82 (16.6)	< 0.001
Age of the mattress (mean year $\pm$ SD)		8.7 $\pm$ 8.0	7.1 $\pm$ 6.8	0.002
Type of mattress, Ref: Foam rubber	Inner spring	159 (49.8)	277 (56.2)	0.071
	Latex	40 (12.5)	77 (15.6)	
Type of floor, Ref: Smooth floor	Carpets	277 (86.8)	451 (91.5)	0.034
Days since last cleaning the living room floor (mean $\pm$ SD)		4.0 $\pm$ 3.3	4.2 $\pm$ 3.0	0.553
Stable dust endotoxin concentration†		258 (1.86)		
Living room floor dust endotoxin concentration†		81.8 (1.81)	44.9 (2.00)	< 0.001
Mattress dust endotoxin concentration†		37.8 (1.77)	22.8 (1.90)	< 0.001

Reference if no other statement: Never.

*P-value for farmers' children vs. non-farm children. †Geometric mean (EU/mg dust) (geometric standard deviation).

Table 3 shows the crude and adjusted means ratios of the variables associated with endotoxin levels in living room floor dust, both for farming and non-farming households. In farming households, the amount of farming activities of the parents (full-time vs. part-time) and the frequency of the child's assistance in the stable were positively associated with endotoxin concentrations, whereas the stable endotoxin concentration *per se* was not related to living room endotoxin levels. For non-farm children, contact to the farm environment was expressed as a stable activity score. A higher stable activity score was positively associated with endotoxin concentrations. Adjustment for all other variables somewhat reduced these effects.

Cats indoors, especially in combination with dogs, increased the endotoxin levels in floor dust in both farming and non-farming households. Father's education was inversely associated with endotoxin concentrations in farming households only. Austrian farm households showed the highest house dust endotoxin levels. Finally, although indoor smoking has been reported to contain biologically active endotoxin [22], we did not find an independent effect on endotoxin levels in the living room floor dust, nor could we detect an association with any of the other variables additionally included in the multivariate regression models such as the number of people running the farm, number of older siblings, the heating system, or the type of floor (smooth or carpeted). The adjusted models explained 34.0% and 20.1% of the variation in living room dust endotoxin concentration in farming and non-farming households, respectively.

Table 4 presents the crude and adjusted means ratios of the variables associated with endotoxin levels in mattress dust, stratified by farm and non-farm children. In non-farm children, the stable activity score increased mattress endotoxin concentrations in a dose-dependent way. Other farming activities such as helping with harvesting, or staying in barns were highly correlated with stable activities, and did not have an independent effect on mattress dust endotoxin levels. Allowing pets to stay on the child's bed also increased the measured endotoxin levels significantly.

In farm children, two or less people running the farm (mostly the parents only) was associated with increased endotoxin levels in the child's mattress, as was the amount of the child's own activity in the stable when assisting the parents. Again, no association was observed with stable endotoxin levels. Few farm children allowed their pets to stay on the bed. Cats and dogs on the bed increased endotoxin levels although not statistically significantly. Mattress endotoxin levels decreased with increasing age of the farm children, and were non-significant in non-farm children. Living on a farm in Austria or Switzerland was associated with increased endotoxin levels compared to the German study area. Inner spring mattresses in farming households were associated with higher endotoxin levels. Time since last cleaning only affected endotoxin levels in mattresses of farm children. The other potential predictors of mattress endotoxin concentration included in the regression models did not show independent additional effects, neither in the farm, nor in the non-farm subgroup. The adjusted models explained 31.7%

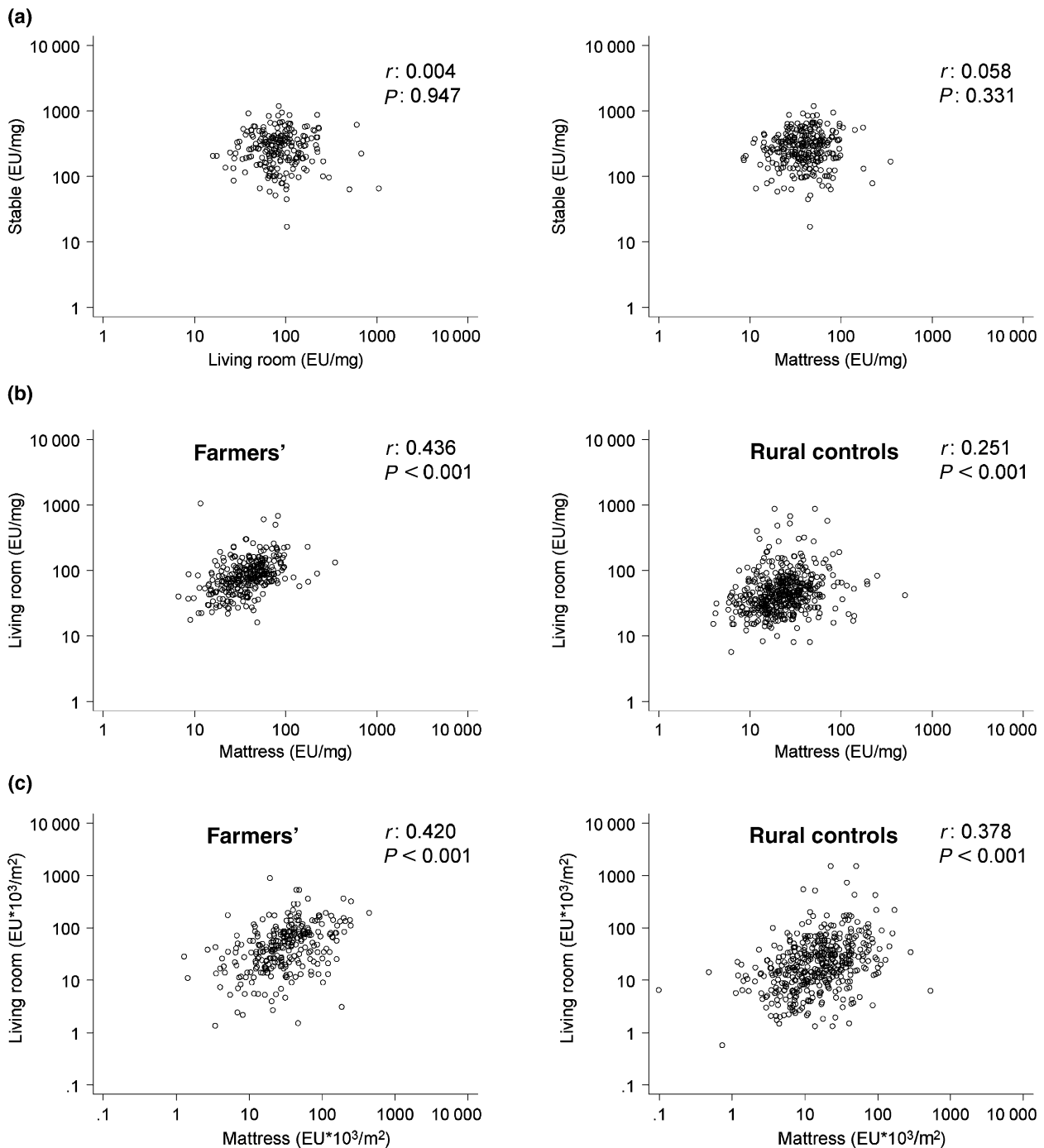


Fig. 1. Correlations of endotoxin levels in different home environments. (a) Stable vs. living room floor and mattress (endotoxin concentration). (b, c) Living room floor vs. mattress stratified by farmers and rural controls ((b) endotoxin concentration, (c) endotoxin load).

and 29.6% of the mattress dust endotoxin variation for farm and non-farm children, respectively.

Discussion

The present analyses show that endotoxin concentrations measured in stables are not associated with the amount of endotoxin measured in dust samples from floors or from

mattresses of farm children. However, a dose-dependent association between the child's activity on the farm and indoor home endotoxin levels was observed. This dose-dependent association was not limited to farmers' children but also seen in non-farm children with contact to the farming environment. Thus, to assess children's individual exposure to their microbial environment, measurements of mattress dust exposure is needed, as information about the number of stable animals or stable endotoxin levels does not predict

Table 2. Crude and adjusted† means ratios‡ (MRs) and 95% confidence intervals (CIs) of endotoxin concentrations in *settled stable dust*

Predictor variables		<i>n</i>	Crude MR (CI)	Adjusted* MR (CI)
Farming as parental occupation	Part-time	105	1	1
	Full-time	180	1.07 (0.92–1.24)	0.96 (0.79–1.17)
Keeping cattle	None	30	1	1
	1–25	95	1.29 (0.91–1.83)	1.51 (0.95–2.40)
	26–50	87	1.51 (1.06–2.15)	1.72 (1.04–2.84)*
	> 50	73	1.15 (0.80–1.64)	1.63 (0.96–2.77)
Keeping horses	No	214	1	1
	Yes	65	1.31 (1.10–1.55)	1.44 (1.20–1.72)**
Feeding material	Silage and hay	218	1	1
	Only hay	66	1.27 (1.07–1.50)	1.26 (1.03–1.53)*
Mucking system	Only solid muck	122	1	1
	Only liquid muck	46	0.87 (0.70–1.07)	1.09 (0.82–1.44)
	Both solid and liquid	109	1.11 (0.95–1.31)	1.23 (1.01–1.51)*
Study area	Germany	128	1	1
	Austria	98	1.31 (1.12–1.54)	1.45 (1.16–1.82)**
	Switzerland	59	1.43 (1.18–1.72)	1.56 (1.20–2.04)**

P*-value < 0.05, *P*-value < 0.01 (*P*-values are only given for the adjusted model). †All MRs were adjusted for part-time/full-time farming, number of people running the farm, stable animals: number of cattle, presence of horses, sheep, goats, pigs, animals in stable during dust sampling, feeding material, mucking system, study area, date of dust sampling. ‡Back-transformed regression coefficient: e^{β} .

Table 3. Crude and adjusted means ratios (MRs) and 95% confidence intervals (CIs) of endotoxin levels in *living room floor dust*

Predictor variables		Farming households			Non-farming households		
		<i>n</i>	Crude MR (CI)	Adjusted† MR (CI)	<i>n</i>	Crude MR (CI)	Adjusted‡ MR (CI)
Farming as parental occupation	Part-time	132	1	1			
	Full-time	186	1.41 (1.24–1.61)	1.29 (1.08–1.53)**			
Stable endotoxin level (EU/mg)	Per unit change	285	1.00 (0.90–1.12)	0.95 (0.84–1.07)			
Current assistance in stable	Never	46	1				
	At least once per week	199	1.30 (1.08–1.56)	1.15 (0.92–1.43)			
	At least daily	68	1.50 (1.21–1.86)	1.44 (1.11–1.87)**			
Stable activity score	0				137	1	1
	1				163	1.20 (1.02–1.40)	1.15 (0.97–1.37)
	2				162	1.31 (1.12–1.53)	1.24 (1.04–1.48)*
	3				22	1.49 (1.09–2.03)	1.28 (0.91–1.80)
Pets regularly indoors	No indoor pets	205	1		293	1	1
	Only cats	77	1.23 (1.06–1.44)	1.17 (0.98–1.39)	151	1.39 (1.21–1.58)	1.35 (1.16–1.58)**
	Cats and dogs	36	1.33 (1.08–1.64)	1.46 (1.08–1.97)*	44	1.63 (1.32–2.02)	1.40 (1.09–1.80)**
Father's education	None or low	139	1		169	1	1
	Medium	132	0.97 (0.84–1.12)	0.81 (0.66–1.00)*	192	0.87 (0.75–1.00)	0.89 (0.75–1.06)
	High	39	0.86 (0.69–1.06)	0.64 (0.48–0.86)**	114	0.95 (0.80–1.12)	0.90 (0.75–1.08)
Study area	Germany	155	1		314	1	1
	Austria	104	1.13 (0.98–1.31)	1.77 (1.17–2.68)**	113	0.93 (0.81–1.08)	0.91 (0.65–1.26)
	Switzerland	59	1.45 (1.22–1.73)	1.25 (0.73–2.15)	61	0.73 (0.60–0.88)	0.81 (0.60–1.10)
Last floor cleaning	< 4 days	182	1		254	1	1
	4–5 days	73	0.90 (0.76–1.06)	0.87 (0.73–1.04)	137	1.08 (0.94–1.25)	1.20 (1.02–1.40)*
	> 5 days	58	0.97 (0.81–1.15)	1.10 (0.87–1.38)	87	1.21 (1.02–1.44)	1.28 (1.06–1.54)**

P*-value < 0.05, *P*-value < 0.01 (*P*-values are only given for the adjusted models). †MRs were adjusted for all the variables in the table, and additionally for number of people running the farm, age, sex, older siblings, house location, heating system, fieldworker, date of dust sampling, indoor smoking, amount of sampled dust, type of floor. ‡MRs were adjusted for all the variables in the table, and additionally for age, sex, older siblings, house location, heating system, fieldworker, date of dust sampling, indoor smoking, amount of sampled dust, type of floor.

indoor home exposure. Parental farming activities, assessed as full- or part-time farming, independently contributed to mattress endotoxin levels of farm children. Other farming activities, such as helping with harvesting or staying in barns, were highly correlated with stable activities and had no independent effect on house dust endotoxin levels. Since endotoxin (lipopolysaccharides) was shown to be very sticky

to different fatty acid binding proteins [23], it is likely that children carry dust loaded with bacteria and endotoxin on clothes and shoes from the stables into the indoor environment, similar to the role of clothing as a means of distributing cat allergen into cat-free environments [24]. In addition, it is conceivable that the amount of endotoxin in the mattress also depends on the child's personal cleanliness. In farm children,

Table 4. Crude and adjusted† means ratios (MRs) and 95% confidence intervals (CIs) of endotoxin levels in *mattress dust*

Predictor variables		Farmers' children			Non-farm children		
		<i>n</i>	Crude MR (CI)	Adjusted MR (CI)	<i>n</i>	Crude MR (CI)	Adjusted MR (CI)
Farming as parental occupation	Part-time	133	1	1			
	Full-time	185	1.21 (1.06–1.37)	1.08 (0.90–1.29)			
People running the farm	>2	145	1	1			
	1 or 2	155	1.15 (1.01–1.30)	1.18 (1.00–1.40)*			
Stable endotoxin level (EU/mg)	Per unit change	284	1.05 (0.95–1.17)	0.96 (0.84–1.09)			
Current assistance in stable	Never	46	1	1			
	At least once per week	198	1.16 (0.96–1.39)	1.11 (0.87–1.41)			
	At least daily	69	1.38 (1.12–1.71)	1.33 (1.00–1.76)*			
Stable activity score	0				140	1	1
	1				164	1.11 (0.96–1.28)	1.08 (0.91–1.27)
	2				162	1.19 (1.03–1.37)	1.23 (1.05–1.44)**
	3				22	1.38 (1.03–1.83)	1.35 (1.00–1.83)*
Pets regularly on child's bed	No pets on child's bed	261	1	1	354	1	1
	Only cats	52	1.15 (0.97–1.37)	1.07 (0.87–1.32)	127	1.14 (1.00–1.30)	1.20 (1.04–1.38)*
	Cats and dogs	4	1.60 (0.91–2.81)	1.81 (0.94–3.50)	11	1.49 (1.01–2.18)	1.68 (1.14–2.46)**
Child's age (years)	≤8	103	1	1	158	1	1
	9	64	0.89 (0.74–1.06)	0.85 (0.68–1.05)	93	1.03 (0.87–1.21)	1.02 (0.86–1.23)
	10–11	116	0.82 (0.71–0.95)	0.74 (0.61–0.89)**	187	0.89 (0.78–1.02)	0.91 (0.79–1.06)
	>11	35	0.67 (0.54–0.83)	0.65 (0.51–0.84)**	51	0.83 (0.68–1.02)	0.82 (0.66–1.03)
Study area	Germany	154	1	1	316	1	1
	Austria	105	1.24 (1.08–1.42)	1.31 (1.00–1.70)*	115	1.03 (0.90–1.18)	1.22 (0.96–1.57)
	Switzerland	59	1.54 (1.30–1.82)	1.42 (1.04–1.94)*	61	0.98 (0.82–1.17)	1.08 (0.80–1.45)
Type of mattress	Foam rubber	98	1	1	121	1	1
	Inner spring	158	0.97 (0.84–1.12)	1.22 (1.01–1.48)*	277	1.01 (0.88–1.16)	1.05 (0.89–1.24)
	Latex	40	0.91 (0.74–1.13)	1.11 (0.82–1.50)	76	0.98 (0.82–1.17)	1.09 (0.87–1.36)
Last cleaning the mattress	≤20 weeks	173	1	1	273	1	1
	>20 weeks	132	0.98 (0.86–1.11)	1.19 (1.01–1.40)*	216	0.99 (0.88–1.11)	1.00 (0.88–1.13)

P*-value < 0.05, *P*-value < 0.01 (*P*-values are only given for the adjusted models). †MRs were adjusted for all the variables in the table, and additionally for parents education, sex, older siblings, heating system, fieldworker, date of dust sampling, indoor smoking, amount of sampled dust, age of mattress, additional covers.

continuously living in an environment rich in microbial products, endotoxin concentrations in mattress dust decreased with increasing age of the children indicating that cleanliness or other personal habits may change with age and, therefore, differentially influence mattress endotoxin levels. Alternatively, it may indicate that older farm children spend less time on the farm due to school activities.

It might be argued that the lack of an association between stable endotoxin concentrations and indoor home endotoxin levels was due to a poor assessment of stable endotoxin levels. However, in this study, endotoxin concentrations of dust samples taken in a subset of stables on two separate occasions were highly correlated and thus seem to be reliable. In addition, a series of farm characteristics predicted stable endotoxin levels as expected. But it has to be kept in mind that the ALEX Study focuses on children's exposure to endotoxin and, therefore, only a limited number of variables that might be associated with stable endotoxin levels were available for analysis. The endotoxin concentrations increased with the number of cattle, but only up to the highest quartile. This might be due to the fact that in these colline to subalpine areas, farms with larger numbers of cattle usually own modern stables with better ventilation systems that are known to reduce airborne endotoxin concentrations in swine stables [25]. Similarly, the positive association between hay

feeding and endotoxin levels compared to feeding of silage and hay might be interpreted as indicating more traditional farming. Likewise, running the farm by two or less people compared to more than two people, which was associated with higher endotoxin levels, may be a surrogate measure of a traditional, less advanced family business. Horse keeping was also associated with increased endotoxin levels. Farms that kept horses did not differ from non-horse keeping farms with respect to study area, part- or full-time farming activity, or number of cattle kept, but they were more likely to accommodate additional farm animals such as pigs, sheep or goats. Hence, either horse keeping truly increases endotoxin levels or it might be a surrogate for a range of other animals contributing to endotoxin levels in stables. Other farm characteristics such as the distance of the stable from the farm house or whether the farm house and the stable were built under the same roof were not available. None of the farms in our study was a pure pig or poultry farm business, thus, endotoxin was essentially measured in cattle stables. This might also explain why the variability of the endotoxin measurements was rather limited.

Stable endotoxin levels and indoor home endotoxin concentrations were significantly higher in Austrian and Swiss farm homes compared to German ones. The reasons for these differences remain unclear, with current European studies

comparing air contaminants in occupational farm environments, focused on greenhouse workers and on pig and poultry farms [26]. However, Swiss and Austrian farms might have been smaller and more traditional, as the number of cattle in German stables were higher than in Austria and Switzerland (mean of 43 (95% CI: 38–48), 27 (22–31), 29 (25–32), respectively).

As previously reported in the literature we found that pet keeping indoors increased the indoor endotoxin levels in floor dust [17, 18, 20]. The association with pets and rodents in the home with higher house dust endotoxin levels suggest that animal species whose gastrointestinal tracts are typically colonized with Gram-negative bacteria contribute substantially to indoor endotoxin levels. Pets staying on the child's bed contributed even more to mattress endotoxin levels. Although farmers' families were less likely to have pets on a bed, the association between endotoxin levels and pets on beds was seen in farm and non-farm families. While the number of dogs on beds was small, it still had a strong impact on mattress endotoxin concentration indicating that dogs specifically increase endotoxin concentration. Several factors such as time since last mattress cleaning, mattress age and type, and the number of mattress covers, were not associated with endotoxin concentration in non-farm children, but farm children who slept on old inner spring mattresses and whose mattresses had not been cleaned for several months were exposed to slightly higher endotoxin levels.

The role of carpeting and cleanliness as determinants of indoor endotoxin is controversial. In a small study of US homes, Gereda et al. found no association between cleanliness and carpeting and house dust endotoxin concentrations [18]. Park et al., however, reported mattress type, type of rug on floor and operating humidifiers to be significant determinants of airborne endotoxin concentrations in homes in the Boston area [19]. Although we found a series of significant determinants of indoor endotoxin levels in these rural populations, only about one-third of the variability of indoor endotoxin levels could be explained by the multivariate models suggesting that many other, yet unknown sources, contribute to house dust endotoxin levels.

In conclusion, the present analyses demonstrate that regular contact to livestock, which has previously been shown to be inversely related to the occurrence of allergic diseases [3, 4] is indeed associated with increased mattress endotoxin concentrations, both in farm and non-farm children. In non-farm children, pet keeping and floor cleaning additionally increased endotoxin levels, whereas in farm children, parental farm activities, the study area, younger age and mattress cleaning, also contributed to increased microbial exposure. However, it must be kept in mind that other bacterial components such as CpG-motifs, or cell-wall components from atypical mycobacteria or Gram-positive bacteria which are known to affect immune responses in ways similar to that of endotoxin were not measured. Endotoxin may thus be a surrogate marker of a much broader spectrum of microbial compounds than Gram-negative bacteria alone. By adjusting for the total amount of dust in our analyses, we aimed to taking into account additional microbial exposure, and were thus determining specific predictors of endotoxin exposure. It therefore seems premature to translate current knowledge on the 'protective' effect of microbial exposure on allergic outcomes into immediate preventive action or therapy.

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References

- Braun-Fahrlander C, Gassner M, Grize L et al. Prevalence of hay fever and allergic sensitization in farmers' children and their peers living in the same rural community. SCARPOL team. Swiss Study on Childhood Allergy and Respiratory Symptoms with Respect to Air Pollution. *Clin Exp Allergy* 1999; 29:28–34.
- Kilpelainen M, Terho EO, Helenius H, Koskenvuo M. Farm environment in childhood prevents the development of allergies. *Clin Exp Allergy* 2000; 30:201–8.
- Riedler J, Eder W, Oberfeld G, Schreuer M. Austrian children living on a farm have less hay fever, asthma and allergic sensitization. *Clin Exp Allergy* 2000; 30:194–200.
- Von Ehrenstein OS, Von Mutius E, Illi S, Baumann L, Bohm O, von Kries R. Reduced risk of hay fever and asthma among children of farmers. *Clin Exp Allergy* 2000; 30:187–93.
- Leynaert B, Neukirch C, Jarvis D, Chinn S, Burney P, Neukirch F. Does living on a farm during childhood protect against asthma, allergic rhinitis, and atopy in adulthood? *Am J Respir Crit Care Med* 2001; 164 (Pt 1):1829–34.
- Ernst P, Cormier Y. Relative scarcity of asthma and atopy among rural adolescents raised on a farm. *Am J Respir Crit Care Med* 2000; 161:1563–6.
- Downs SH, Marks GB, Mitakakis TZ, Leuppi JD, Car NG, Peat JK. Having lived on a farm and protection against allergic diseases in Australia. *Clin Exp Allergy* 2001; 31:570–5.
- Von Mutius E, Braun-Fahrlander C, Schierl R et al. Exposure to endotoxin or other bacterial components might protect against the development of atopy. *Clin Exp Allergy* 2000; 30:1230–4.
- Riedler J, Braun-Fahrlander C, Eder W et al. Exposure to farming in early life and development of asthma and allergy: a cross-sectional survey. *Lancet* 2001; 358:1129–33.
- Braun-Fahrlander C, Riedler J, Herz U et al. Environmental exposure to endotoxin and its relation to asthma in school-age children. *N Engl J Med* 2002; 347:869–77.
- Milton DK, Johnson DK, Park JH. Environmental endotoxin measurement: interference and sources of variation in the Limulus assay of house dust. *Am Ind Hyg Assoc J* 1997; 58:861–7.
- Rizzo MC, Naspitz CK, Fernandez-Caldas E, Lockey RF, Mimica I, Sole D. Endotoxin exposure and symptoms in asthmatic children. *Pediatr Allergy Immunol* 1997; 8:121–6.
- Douwes J, Zuidhof A, Doekes G et al. (1→3)-beta-D-glucan and endotoxin in house dust and peak flow variability in children. *Am J Respir Crit Care Med* 2000; 162 (Pt 1):1348–54.
- Vogelzang PF, van der Gulden JW, Folgering H et al. Endotoxin exposure as a major determinant of lung function decline in pig farmers. *Am J Respir Crit Care Med* 1998; 157:15–8.
- Nowak D. Health effects of airborne pollutants, particularly in swine confinement stalls, from the viewpoint of occupational medicine. *Dtsch Tierarztl Wochenschr* 1998; 105:225–34.

- 16 Radon K, Danuser B, Iversen M, Monso E, Weber C, Hartung J. Air contaminants in different European farming environments. *Ann Agric Environ Med* 2002; 9:41–8.
- 17 Heinrich J, Gehring U, Douwes J et al. Pets and vermin are associated with high endotoxin levels in house dust. *Clin Exp Allergy* 2001; 31:1839–45.
- 18 Gereda JE, Klinnert MD, Price MR, Leung DY, Liu AH. Metropolitan home living conditions associated with indoor endotoxin levels. *J Allergy Clin Immunol* 2001; 107:790–6.
- 19 Park JH, Spiegelman DL, Gold DR, Burge HA, Milton DK. Predictors of airborne endotoxin in the home. *Environ Health Perspect* 2001; 109:859–64.
- 20 Park J, Gold D, Spiegelman D, Burge H, Milton D. House dust endotoxin and wheeze in the first year of life. *Am J Respir Crit Care Med* 2001; 163:322–8.
- 21 Gehring U, Douwes J, Doekes G et al. Beta(1–>3)-glucan in house dust of German homes: housing characteristics, occupant behavior, and relations with endotoxins, allergens, and molds. *Environ Health Perspect* 2001; 109:139–44.
- 22 Hasday JD, Bascom R, Costa JJ, Fitzgerald T, Dubin W. Bacterial endotoxin is an active component of cigarette smoke. *Chest* 1999; 115:829–35.
- 23 van Rozendaal BA, van de Lest CH, van Eijk M et al. Aerosolized endotoxin is immediately bound by pulmonary surfactant protein D *in vivo*. *Biochim Biophys Acta* 1999; 1454:261–9.
- 24 De Lucca SD, O'Meara TJ, Tovey ER. Exposure to mite and cat allergens on a range of clothing items at home and the transfer of cat allergen in the workplace. *J Allergy Clin Immunol* 2000; 106:874–9.
- 25 Attwood P, Brouwer R, Ruigewaard P et al. A study of the relationship between airborne contaminants and environmental factors in Dutch swine confinement buildings. *Am Ind Hyg Assoc J* 1987; 48:745–51.
- 26 Radon K, Danuser B, Iversen M et al. Air contaminants in different European farming environments. *Ann Agric Environ Med* 2002; 9:41–8.