

Bacterial and fungal agents in house dust and wheeze in children: the PARSIFAL study

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Summary

Background Growing up on a farm and an anthroposophic lifestyle are associated with a lower prevalence of allergic diseases in childhood. This might be related to increased inhalatory exposure to microbial agents.

Objective To assess the association between microbial agents in house dust and atopic wheeze in farm children, Steiner school children and reference children.

Methods Levels of bacterial endotoxin, fungal $\beta(1,3)$ -glucans and fungal extracellular polysaccharides (EPS) in mattress and living room floor dust were measured in a population of 270 atopic (= Phadiatop-positive) children with self-reported wheezing, including 168 current atopic wheezers, and 441 non-atopic, non-symptomatic controls. These children were selected from a cross-sectional study in five European countries.

Results In the study population as a whole, average levels of mattress dust endotoxin, EPS and glucans were slightly (1.1–1.2-fold; $P < 0.10$) higher in control children than in atopic wheezers. Atopic wheeze was related to mattress levels of endotoxin, EPS and glucans in farm and farm-reference children. However, when adjusting for group (farm vs. farm-reference children), the associations became non-significant whereas the group effect remained. No associations between atopic wheeze and microbial agents were observed in Steiner and Steiner-reference children. For current atopic wheeze, the farm effect became non-significant after adjustment for microbial agent levels.

Conclusion Not only bacterial endotoxin but also mould components might offer some protection against atopic wheeze in children. However, the protective effect of being raised on a farm was largely unexplained by the mattress microbial agent levels measured in this study.

Keywords asthma, $\beta(1,3)$ -glucans, endotoxin, farm, fungal extracellular polysaccharides

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Introduction

While the prevalence of asthma and allergy has increased worldwide [1], both diseases appear to be less common in children raised on a farm [2–8] and in children from families with an anthroposophic lifestyle [9]. It has been suggested that early childhood exposure to high levels of microbial components leads to a lower risk of asthma and allergy [10]. The activation of innate immunity mechanisms by these agents may inhibit helper T cell type 2 (Th2)-type allergic responses [10].

The cross-sectional PARSIFAL (Prevention of allergy – risk factors for sensitization in children related to farming and anthroposophic lifestyle) study explored factors characteristic of farming and anthroposophic populations that may protect

against the development of atopic diseases in children. We recently reported enhanced levels of house dust-associated bacterial and fungal agents in farm homes, and to a lesser extent, in the homes of Steiner school children of that study [11]. We also reported a lower prevalence of sensitization and rhinoconjunctivitis in farm and Steiner children [12], while the prevalence of wheezing was significantly lower in farm children in some countries, but not in others [12]. This study aimed to describe the overall association between reported wheeze and levels of microbial agents in house dust and to investigate whether the lower prevalence of wheeze in farm children can be explained by microbial agent levels. For this purpose, we selected approximately 100 children with self-reported wheeze and 100 children without symptoms from each country of the PARSIFAL population.

Previous studies regarding microbial agents and atopic diseases in children have mainly focused on bacterial endotoxin [13–16]. One study in farm and farm-reference children in Austria, Germany and Switzerland showed that endotoxin levels in mattress dust were inversely related to the prevalence of hayfever, allergic asthma and atopic sensitization. Non-atopic asthma was not significantly associated with endotoxin [15]. However, it has been suggested that protection against asthma and allergy might also result from exposure to other microbial agents [17], such as mould $\beta(1,3)$ -glucans, which also have known immunomodulatory effects [18–23], and that we found to be increased in farm homes and homes of Steiner children [11]. We therefore examined the relations between wheeze in children and house dust levels of endotoxin, $\beta(1,3)$ -glucans and another marker of fungal exposure [24], extracellular polysaccharides (EPS), from *Aspergillus* and *Penicillium* spp.

Methods

Prevention of allergy – risk factors for sensitization in children related to farming and anthroposophic lifestyle study

A total of 14893 children aged 5–13 years were recruited among farmers (>80% livestock farms), families with an anthroposophic lifestyle and respective reference groups in five European countries: Austria, Germany, the Netherlands, Sweden and Switzerland, as described previously [12]. Children with an anthroposophic lifestyle were recruited through Rudolf Steiner schools. Farm-reference children were selected such that they lived in the neighbourhood of farm children, in rural areas, but did not actually live on a farm. Steiner-reference children lived in the neighbourhood of Steiner children, mainly in urban areas, but did not attend a Rudolf Steiner school. Questionnaires and consent forms were completed by the parents of the participating children. Allergen-specific IgE analysis, including Phadiatop (a mix of common inhalant allergens) and fx5 (a mix of common food allergens), was performed centrally [12].

Selection of cases and controls

Atopic and non-atopic children with wheeze and non-atopic, non-symptomatic controls were selected on the basis of IgE analysis and reported symptoms. Children were defined as

wheezers if their parents reported 'wheeze in the past 12 months', or 'wheeze ever' and 'atopic' if they had a positive Phadiatop test. Control children had a negative Phadiatop test and a negative fx5 test and their parents reported that the child had none of the following symptoms: 'wheeze ever', 'asthma ever', 'doctor-diagnosed asthma, bronchitis or pseudocroup ever', 'sneezing or a runny or blocked nose without a cold, ever', 'rhinitis ever', 'itchy rash for at least 6 months, ever' and 'eczema ever'. For this study, we selected all atopic wheezers (total $N = 270$), a random sample of non-atopic wheezers ($N = 188$) and a random sample of non-atopic controls ($N = 441$), by drawing equal numbers of wheezers (ca. 100) and controls (ca. 100) per country from the population with IgE serology data. The selection was made irrespective of the group (farm, Steiner or reference children) the children belonged to, because we aimed to study whether the protective 'farming' effect could be explained by microbial agent levels; the association between 'group' and atopic wheeze could not have been studied in a selection stratified by group.

Dust collection

House dust from mattresses and living room floors was collected by parents of the participating children. They were sent nylon sampling socks and instructed to vacuum the whole area of the mattress and a square metre of either the carpet, smooth floor or rug in the sitting area for 2 min according to detailed photo-instructions. After sampling, the socks were returned to the local study centre by regular mail, where they were stored at -20°C . All samples were shipped in frozen conditions to one laboratory (IRAS, Utrecht, the Netherlands) for central analysis.

Dust extraction and analysis

Endotoxin, EPS and glucans were extracted sequentially and analysed as described previously [11]. In short, endotoxin was measured with the kinetic chromogenic Limulus Amebocyte Lysate (LAL) test, EPS with a specific sandwich EIA for EPS of *Aspergillus* and *Penicillium* spp. [25] and glucans with an inhibition EIA [26]. Mite (Der p I, Der f I), cat (Fel d I) and dog (Can f I) allergens were measured as described previously [27]. Samples with non-detectable amounts (endotoxin, $n = 23$, EPS, 45, glucans, 36) were given a value of two-thirds of the lowest observed detectable amount per gram of dust or per square metre for the specific component determined.

Statistical analysis

Statistical analyses were performed with SAS statistical software (version 8.2, SAS Institute, Cary, NC, USA). Levels of microbial agents were approximately normally distributed after natural log (ln) transformation. Associations with atopic wheeze were investigated for levels of microbial agents per gram dust only, because levels per square metre were considered less reliable; dust was collected by untrained participants with different types of vacuum cleaners, which might have influenced dust amounts. The relationships between ln-transformed microbial agent levels and atopic wheeze were explored with non-parametric regression by computing smoothed curves, expressing the logit of the rate of atopic wheeze as a continuous function of microbial agent

levels, using the generalized additive models procedure of SAS, and were found to be generally linear. Therefore, logistic regression analyses, in which the microbial agent levels were treated as continuous variables, were performed to calculate odds ratios (ORs) for an interquartile range increase of each component, with adjustment for country, age and sex. In addition, potential confounding by group (farm, farm-reference, Steiner or Steiner-reference), older siblings, parental education level, environmental tobacco smoke, smoking of the mother during pregnancy and allergen levels was evaluated. Models including all groups of children (farm, farm-reference, Steiner and Steiner-reference), as presented in Fig. 2, were not adjusted for group because it would be unclear as to which group should serve as a reference group. However, separate analyses, adjusted for group, were performed for the two branches of the PARSIFAL study: farm/farm-reference and Steiner/Steiner-reference children (Tables 3 and 4). The homogeneity of the ORs across countries was evaluated by including interaction terms in the models (country $\times$ microbial agent levels). In case of heterogeneity ($P < 0.10$), combined effect estimates were calculated using random effects estimation [28].

Results

Figure 1 shows the differences in atopic wheeze prevalence between farm and farm-reference children and Steiner and Steiner-reference children by country, in the population with IgE serology, from which we selected our cases and controls. In the German and Swiss population, farm children had a significantly lower prevalence of atopic wheeze than farm-reference children, whereas the differences were not significant in the other countries. A lower prevalence of atopic wheeze in Steiner children as compared with Steiner-reference children was only observed in the Netherlands.

Characteristics of the selected population

Table 1 shows the numbers and group compositions for the cases and controls selected for this study by country. By design, the total number of controls was nearly equal in each

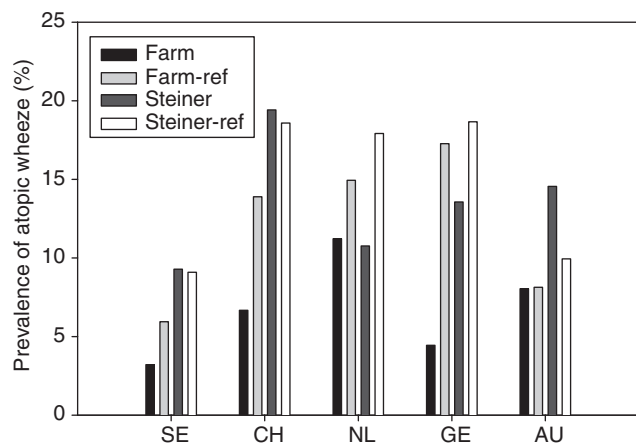


Fig. 1. Prevalence of atopic wheeze by group and by country (SE, Sweden; CH, Switzerland; NL, the Netherlands; GE, Germany; AU, Austria).

country. Although the total number of wheezers was nearly equal as well, the numbers of atopic and non-atopic wheezers were different across countries, as all atopic wheezers were included. In addition, percentages of farm, Steiner and reference children among cases and controls differed across countries due to the differences in the size of the groups in the population with IgE serology (column 1 of Table 1) and group-specific prevalence rates of atopic wheeze (Fig. 1). For example, the German analysis population contained relatively many farm children, because of the large number of farm children in the population with IgE serology. Since the German farm children also showed a very low prevalence of atopic wheeze, controls from Germany largely consisted of farm children (Table 1). Non-atopic wheezers were not included in further analyses because of the low prevalence of 'wheeze in the past 12 months' in this group (20% vs. 63% in the atopic wheeze group).

Table 2 shows the demographic characteristics and microbial agent levels per gram mattress dust of cases and controls. The subject characteristics of this analysis population were essentially similar to the characteristics of atopic wheezers and controls in the total population with IgE serology ($N = 4039$) (not shown). Geometric mean levels of endotoxin, EPS and glucans per gram mattress dust were 1.1–1.2-fold higher in control children than in atopic wheezers. Levels in living room floor dust were also higher in control children than in atopic wheezers, although significantly only for levels per square metre. Further analyses have been restricted to mattress levels. Correlations between levels per gram mattress dust of allergens and microbial agents, between the microbial agents, and between group and microbial agents, were generally low (r for log-transformed data < 0.41). Therefore, their independent effects could be assessed in multivariate analyses.

Associations between microbial agent levels and atopic wheeze

Figure 2 shows the ORs for endotoxin, EPS and glucans in mattress dust and atopic wheeze by country. Heterogeneity was observed for endotoxin ($P = 0.07$); levels were inversely related to atopic wheeze in three countries, particularly in Germany, but for Sweden the association was in the other direction. The ORs for glucans and EPS were more similar across countries and overall ORs were borderline significant. Similar results were obtained when allergens were included in the models (not shown).

Separate analyses were performed for the two branches of the PARSIFAL study: farm children and references and Steiner children and references (Table 3). Protective effects of endotoxin, EPS and glucans in mattress dust were observed for farm and farm-reference children, but when adjusting for group, the effects became non-significant. The effect of group, on the other hand, did not change by including microbial agents in the model. For Steiner and Steiner-reference children, no significant effects of group and microbial agent levels were observed. Results were essentially the same after adjustment for potentially confounding factors, like older siblings, parental education level, environmental tobacco smoke and smoking of the mother during pregnancy.

We performed sensitivity analyses by excluding children who did not report wheeze in the last 12 months (Table 4). In farm

Table 1. Numbers and percentages of atopic wheezers, non-atopic wheezers and controls by group and by country; approximately 100 children with self-reported wheeze and 100 non-atopic, non-symptomatic children were selected per country from the population with IgE serology data

	Source population, <i>N</i>	Analysis population, <i>N</i>	Atopic wheezers, <i>N</i> (%)	Non-atopic wheezers, <i>N</i> (%)	Controls, <i>N</i> (%)
Sweden	833	184	46	49	89
Farm	253	61	7 (11.5)	19 (31.1)	35 (57.4)
Steiner	353	83	27 (32.5)	18 (21.7)	38 (45.8)
Farm-reference	105	19	3 (15.8)	6 (31.6)	10 (52.6)
Steiner-reference	122	21	9 (42.9)	6 (28.6)	6 (28.6)
Switzerland	743	180	83	6	91
Farm	222	47	9 (19.1)	1 (2.1)	37 (78.7)
Steiner	252	64	38 (59.4)	3 (4.7)	23 (35.9)
Farm-reference	150	41	19 (46.3)	1 (2.4)	21 (51.2)
Steiner-reference	119	28	17 (60.7)	1 (3.6)	10 (35.7)
The Netherlands	552	167	35	47	85
Farm	102	26	4 (15.4)	9 (34.6)	13 (50.0)
Steiner	162	59	11 (18.6)	11 (18.6)	37 (62.7)
Farm-reference	181	53	11 (20.8)	18 (34.0)	24 (45.3)
Steiner-reference	107	29	9 (31.0)	9 (31.0)	11 (37.9)
Germany	1154	182	57	39	86
Farm	623	80	12 (15.0)	14 (17.5)	54 (67.5)
Steiner	245	43	18 (41.9)	11 (25.6)	14 (32.6)
Farm-reference	147	43	20 (46.5)	10 (23.3)	13 (30.2)
Steiner-reference	139	16	7 (43.8)	4 (25.0)	5 (31.3)
Austria	757	186	49	47	90
Farm	214	50	10 (20.0)	11 (22.0)	29 (58.0)
Steiner	233	63	21 (33.3)	20 (31.7)	22 (34.9)
Farm-reference	135	29	6 (20.7)	6 (20.7)	17 (58.6)
Steiner-reference	175	44	12 (27.3)	10 (22.7)	22 (50.0)
Total	4039	899	270	188	441

Table 2. Subject characteristics and microbial agent levels in mattress dust

	Atopic wheezers, <i>N</i> = 270	Controls, <i>N</i> = 441	<i>P</i> -value
Age, mean (SD)	9.0 (1.8)	8.8 (1.8)	NS
Male (%)	69.3	46.5	<0.0001
Maternal asthma and/or hayfever (%)	34.5	15.4	<0.0001
Paternal asthma and/or hayfever (%)	32.0	15.3	<0.0001
High parental education (%)	42.3	36.6	NS
Smoking mother during pregnancy (%)	10.9	6.0	0.02
Current environmental smoking (%)	12.0	12.5	NS
Older siblings (%)	65.8	67.6	NS
Microbial agents per gram dust			
Endotoxin (EU/g), GM (GSD)	22 268 (3.06)	26 498 (2.95)	0.05
EPS (EPS U/g), GM (GSD)	58 814 (2.85)	69 139 (3.46)	0.07
β(1,3)-glucans (μg/g), GM (GSD)	2662 (2.12)	2959 (1.94)	0.07
Microbial agents per square metre			
Endotoxin (EU/m ²), GM (GSD)	3396 (4.81)	4570 (4.50)	0.01
EPS (EPS U/m ²), GM (GSD)	8511 (6.34)	12 148 (6.78)	0.02
β(1,3)-glucans (μg/m ²), GM (GSD)	402 (4.60)	519 (3.98)	0.03

NS, not significant: *P* > 0.1; EPS, extracellular polysaccharides.

and farm-reference children, the protective farm effect appeared to become smaller and non-significant when microbial agent levels were included in the model. The farm effect was, however, still largely unexplained. For Steiner and Steiner-reference children, no significant effects of group and microbial agent levels to current atopic wheeze were observed.

Discussion

This study demonstrated that microbial agent levels in house dust were related to atopic wheeze in farm and farm-reference children. After adjustment for living on a farm, however, the effects became non-significant. For current atopic wheeze, the 'farm-effect' became smaller and non-significant after adjust-

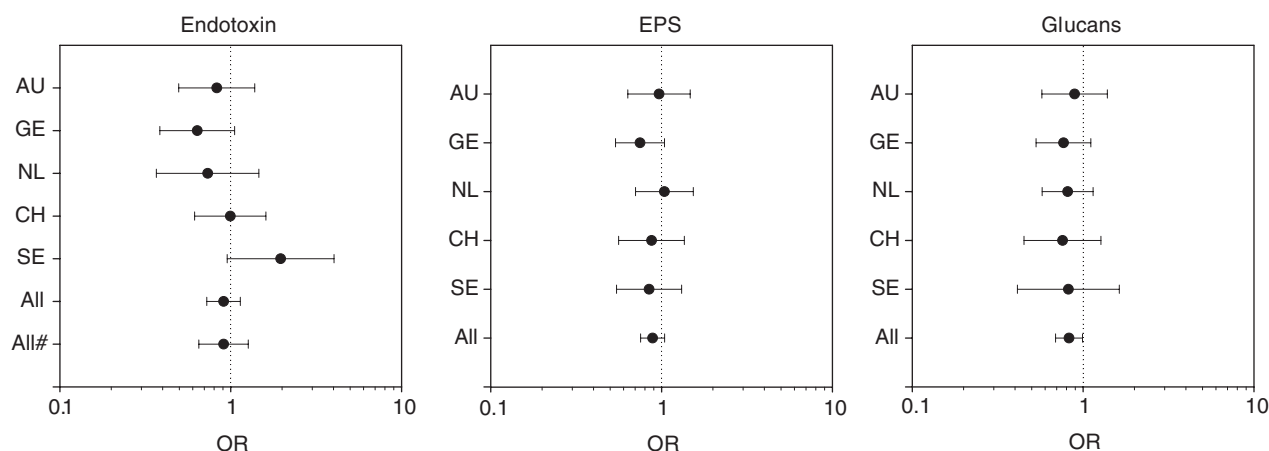


Fig. 2. Odds ratios (ORs) and 95% confidence intervals for atopic wheeze with an interquartile range increase of each microbial agent per gram mattress dust, adjusted for country, age, sex, older siblings, parental education level, environmental tobacco smoke and smoking mother during pregnancy (SE, Sweden; CH, Switzerland; NL, the Netherlands; GE, Germany; AU, Austria). # OR based on meta-analysis using random effects estimation because of heterogeneity.

Table 3. Odds ratios and 95% confidence intervals for atopic wheeze with an interquartile range increase of each component per gram mattress dust*

	aOR†	Mutually adjusted aOR‡
Farm and farm-reference (N§ = 329) children		
Farm vs. farm-reference children	0.34 (0.20–0.56)	0.36 (0.20–0.64)
Endotoxin	0.71 (0.52–0.97)	1.03 (0.69–1.55)
EPS	0.79 (0.63–0.98)	0.95 (0.70–1.30)
Glucans	0.77 (0.58–1.01)	0.83 (0.56–1.22)
Steiner and Steiner-reference children (N§ = 334)		
Steiner vs. Steiner-reference children	0.86 (0.53–1.41)	0.89 (0.52–1.51)
Endotoxin	1.09 (0.78–1.53)	1.22 (0.84–1.77)
EPS	1.02 (0.82–1.28)	0.96 (0.74–1.25)
Glucans	0.90 (0.71–1.12)	0.92 (0.66–1.28)

*A factor 4.0 for endotoxin, 3.0 for EPS and 1.9 for glucans in all models, based on the interquartile ranges in the total population. Results were essentially the same when using group-specific interquartile ranges. †Adjusted for country, age, sex. ‡Adjusted for group, microbial agents and the factors mentioned under †. §Number of children in mutually adjusted model. aOR, adjusted odds ratio; EPS, extracellular polysaccharides.

Table 4. Odds ratios and 95% confidence intervals for *current* atopic wheeze with an interquartile range increase of each component per gram mattress dust*

	aOR†	Mutually adjusted aOR‡
Farm and farm-reference (N§ = 287) children		
Farm vs. farm-reference children	0.45 (0.24–0.86)	0.58 (0.29–1.19)
Endotoxin	0.74 (0.51–1.08)	1.09 (0.66–1.78)
EPS	0.74 (0.57–0.95)	0.77 (0.54–1.10)
Glucans	0.78 (0.57–1.07)	0.91 (0.58–1.43)
Steiner and Steiner-reference children (N§ = 283)		
Steiner vs. Steiner-reference children	0.76 (0.44–1.31)	0.81 (0.45–1.45)
Endotoxin	1.20 (0.82–1.74)	1.38 (0.92–2.09)
EPS	0.94 (0.74–1.19)	0.89 (0.67–1.18)
Glucans	0.89 (0.68–1.16)	0.80 (0.56–1.16)

*A factor 4.0 for endotoxin, 3.0 for EPS and 1.9 for glucans in all models, based on the interquartile ranges in the total population. †Adjusted for country, age, sex. ‡Adjusted for group, microbial agents and the factors mentioned under †. §Number of children in mutually adjusted model. aOR, adjusted odds ratio; EPS, extracellular polysaccharides.

ment for mattress microbial agent levels but the 'farm-effect' was still largely unexplained.

This cross-sectional study is based on house dust analyses in a large number of children with wheeze and controls from five different countries and different subgroups: farm and Steiner children and their respective references. Despite differences across subgroups and across countries, indications of a protective effect of mould components to atopic wheeze in farm and farm-reference children were observed, which have not been reported previously. Whether these effects reflect a down-regulation of the development of atopy and/or the development of wheeze could not be determined on the basis of our data, neither could we determine whether timing of exposure plays a role; it is an area in which further exploration is needed.

Exposure to microbial agents was assessed by analyses of house dust collected by parents of the participating children. Dust amounts might have been influenced by variation in sampling time or sampling area, although parents were instructed to follow standard procedures. In addition, different types of vacuum cleaners may have influenced dust amounts. A previous study showed high correlations between allergen levels of subject- and fieldworker-collected dust samples, but subjects collected lighter dust samples [29]. The validity of the methods of microbial exposure assessment applied in this study, using dust 'socks', collected by participants, will be described in a separate publication. In this study, associations with wheeze were calculated for levels per gram dust only, to factor out variation in dust amounts.

Our results are in line with the previously published ALEX study [15], which showed an OR of 0.89 (0.57–1.39) for the

occurrence of atopic wheeze with an increase in the endotoxin level per gram mattress dust from the lowest to the highest quartile. When restricting the analyses to similar groups of children within the PARSIFAL study, farm and farm-reference children in Germany, Austria and Switzerland, and without adjustment for group (which was not adjusted for in ALEX), we observed an adjusted OR (aOR) of 0.56 (0.35–0.90). For levels per square metre, the aOR was 0.72 (0.49–1.06) vs. 0.62 (0.39–0.99) in ALEX. We showed, however, that after adjustment for group or glucans and EPS, the effect of endotoxin was not significant. Exposures to endotoxin, EPS and glucans were moderately, but significantly, correlated, precluding a firm conclusion on the degree to which specific agents contributed to the observed effect. Moreover, these microbial agents might be markers of a much broader spectrum of microbial agents. However, our results suggest that mould components might also modulate immune responses and thereby protect against allergic diseases, as previously suggested for endotoxin [13–15]. This is in line with a study on the effects of endotoxin and fungal spores on atopy and asthma in adult farmers, in which fungal spores, rather than endotoxin, were inversely related to atopic wheeze [30].

The protective effects of microbial agents were mainly observed in farm and farm-reference children. This might be because the total inhalatory exposure to microbial agents is much higher in these children than in Steiner and Steiner-reference children, as they are not only exposed to microbial agents in house dust, but probably also to microbial agents in stable dust. We previously showed that levels of microbial agents in stable dust were 3–7-fold higher than levels in house dust. We also showed that indoor levels of EPS and glucans were significantly correlated with levels in stable dust [11], while for endotoxin no significant relation could be found. EPS and glucans in mattress dust might be better markers of total inhalatory exposure to several microbial components because of this correlation between levels in stables and levels indoors.

Mattress glucan and EPS levels explained part of the protective 'farming' effect on atopic wheeze, but only when analyses were restricted with wheeze in the last 12 months. The total group of wheezers included children with transient wheeze, which might represent developmental airway abnormalities, resulting in wheeze with viral respiratory infections [31]. The protective effects of microbial agents were more pronounced in the small group of atopic children with severe wheeze (four or more attacks in the last 12 months, $N = 51$, not shown), indicating that the results may have been biased towards the null by including a mixture of wheezing phenotypes. Another explanation for the stronger association between microbial agents and current atopic wheeze as compared to wheeze ever is that house dust analyses and current atopic wheeze refer to the same time window. However, even with these confined definitions of atopic wheeze, the farming effect was not completely explained by microbial agent levels in mattress dust. This is in line with the ALEX study, which demonstrated that independent of endotoxin levels in mattress dust 'exposure to farming during the first year' (exposure to stables and/or consumption of milk directly from the farm) showed a protective effect on atopic wheeze [15]. The associations

between these and other farm-related factors and allergic disease in the PARSIFAL population will be described elsewhere. The effects of microbial agents on current atopic wheeze as described in this paper could, however, not be explained by contact to pets or farm animals, stable visits or drinking milk directly from a farm (not shown).

Our selections of cases and controls was made irrespective of the groups that children originally belonged to, which resulted in an unbalanced group composition across countries. We therefore adjusted our analyses for group and country, and – in case of heterogeneity across countries, like for endotoxin – by calculating an overall OR with meta-analysis techniques (i.e. random effects estimation). The numbers of cases were too low for stratification by group and by country. However, adjustment for group may have resulted in an underestimation of the effects of microbial components on atopic wheeze, because group was shown to be a determinant of mattress microbial agent levels [11]. Moreover, measurement of 'group' is less subject to measurement error when compared with measurement of microbial agent levels, which might explain why group was a stronger determinant of atopic wheeze.

Our results suggest that microbial agent levels, especially mould components, in house dust may offer some protection against atopic wheeze in children. However, the protective effect of growing up on a farm was largely unexplained by the mattress microbial agent levels measured in this study.

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References

- 1 Beasley R, Crane J, Lai CK, Pearce N. Prevalence and etiology of asthma. *J Allergy Clin Immunol* 2000; 105 (Part 2):S466–72.
- 2 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.
- 3 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.
- 4 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.
- 5 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.
- 6 Kilpelainen M, Terho EO, Helenius H, Koskenvuo M. Farm environment in childhood prevents the development of allergies. *Clin Exp Allergy* 2000; 30:201–8.
- 7 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 (Part 1):1829–34.
- 8 Braback L, Hjert A, Rasmussen F. Trends in asthma, allergic rhinitis and eczema among Swedish conscripts from farming and non-farming environments. A nationwide study over three decades. *Clin Exp Allergy* 2004; 34:38–43.
 - 9 Alm JS, Swartz J, Lilja G, Scheynius A, Pershagen G. Atopy in children of families with an anthroposophic lifestyle. *Lancet* 1999; 353:1485–8.
 - 10 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.
 - 11 Schram D, Doekes G, Boeve M et al. Bacterial and fungal components in house dust of farm children, Rudolf Steiner school children and reference children – the PARSIFAL Study. *Allergy* 2005; 60:611–8.
 - 12 Alfvén T, Braun-Fahrlander C, Brunekreef B et al. Allergic diseases and atopic sensitisation in children related to farming and anthroposophic lifestyle – the PARSIFAL study. *Allergy*, in press.
 - 13 Gereda JE, Leung DY, Thatayatikom A et al. Relation between house-dust endotoxin exposure, type 1 T-cell development, and allergen sensitisation in infants at high risk of asthma. *Lancet* 2000; 355:1680–3.
 - 14 Gehring U, Bischof W, Fahlbusch B, Wichmann HE, Heinrich J. House dust endotoxin and allergic sensitization in children. *Am J Respir Crit Care Med* 2002; 166:939–44.
 - 15 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.
 - 16 Park JH, Gold DR, Spiegelman DL, Burge HA, Milton DK. House dust endotoxin and wheeze in the first year of life. *Am J Respir Crit Care Med* 2001; 163:322–8.
 - 17 Douwes J, Pearce N. Invited commentary: is indoor mold exposure a risk factor for asthma? *Am J Epidemiol* 2003; 158:203–6.
 - 18 Zhang K, Petty HR. Influence of polysaccharides on neutrophil function: specific antagonists suggest a model for cooperative saccharide-associated inhibition of immune complex-triggered superoxide production. *J Cell Biochem* 1994; 56:225–35.
 - 19 Suzuki T, Ohno N, Saito K, Yadomae T. Activation of the complement system by (1 → 3)-beta-D-glucans having different degrees of branching and different ultrastructures. *J Pharmacobiodyn* 1992; 15:277–85.
 - 20 Stone B, Clarke A. Chemistry and biology of (1,3)-β-glucans. Victoria: La Trobe University Press, 1992.
 - 21 Sakurai T, Ohno N, Yadomae T. Changes in immune mediators in mouse lung produced by administration of soluble (1 → 3)-beta-D-glucan. *Biol Pharm Bull* 1994; 17:617–22.
 - 22 Goto H, Yuasa K, Rylander R. (1 → 3)-beta-D-glucan in indoor air, its measurement and in vitro activity. *Am J Ind Med* 1994; 25:81–3.
 - 23 Adachi Y, Okazaki M, Ohno N, Yadomae T. Enhancement of cytokine production by macrophages stimulated with (1 → 3)-beta-D-glucan, grifolan (GRN), isolated from *Grifola frondosa*. *Biol Pharm Bull* 1994; 17:1554–60.
 - 24 Chew GL, Douwes J, Doekes G et al. Fungal extracellular polysaccharides, beta (1 → 3)-glucans and culturable fungi in repeated sampling of house dust. *Indoor Air* 2001; 11:171–8.
 - 25 Douwes J, van der Sluis B, Doekes G et al. Fungal extracellular polysaccharides in house dust as a marker for exposure to fungi: relations with culturable fungi, reported home dampness, and respiratory symptoms. *J Allergy Clin Immunol* 1999; 103 (Part 1):494–500.
 - 26 Douwes J, Doekes G, Montijn R, Heederik D, Brunekreef B. Measurement of beta(1 → 3)-glucans in occupational and home environments with an inhibition enzyme immunoassay. *Appl Environ Microbiol* 1996; 62:3176–82.
 - 27 van Strien RT, Koopman LP, Kerkhof M et al. Mite and pet allergen levels in homes of children born to allergic and nonallergic parents: the PIAMA study. *Environ Health Perspect* 2002; 110:A693–8.
 - 28 DerSimonian R, Laird N. Meta-analysis in clinical trials. *Control Clin Trials* 1986; 7:177–88.
 - 29 Arbes SA, Sever M, Vaughan J et al. Feasibility of using subject-collected dust samples in epidemiological and clinical studies of indoor allergens. *Environ Health Perspect* 2005; 113:665–9.
 - 30 Eduard W, Douwes J, Omenaas E, Heederik D. Do farming exposures cause or prevent asthma? Results from a study of adult Norwegian farmers. *Thorax* 2004; 59:381–6.
 - 31 Martinez FD, Wright AL, Taussig LM, Holberg CJ, Halonen M, Morgan WJ. Asthma and wheezing in the first six years of life. The Group Health Medical Associates. *N Engl J Med* 1995; 332:133–8.