

CONCENTRATIONS OF DUST, ALLERGENS AND ENDOTOXIN IN STABLES, LIVING ROOMS AND MATTRESSES FROM CATTLE FARMERS IN SOUTHERN BAVARIA

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Abstract: Agricultural work is considered to be a major risk factor for occupational diseases. In particular, allergic reactions to cow dander cause numerous cases of airway disorders. We measured the concentration of allergens (e.g. Bos d2, Der p1) and endotoxin in the stables, living-rooms and mattresses of 46 farmers with a diagnosis of occupational asthma or allergic rhinitis caused by cow dander allergen. The concentration of cow dander allergen was highest in stables (median 20,400 µg/g) but also noticeable in dust samples from living-rooms (median 155 µg/g) and mattresses (median 195 µg/g). The sensitization threshold (20–50 µg/g) was exceeded in most cases. Thus, allergen transport from the stables to bed must be prevented by optimizing the hygiene of farmers and family members.

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INTRODUCTION

Agricultural work is considered to be a major risk factor for occupational airway diseases [8, 29]. Iversen and co-workers found Danish farmers to have bronchial asthma with a prevalence of 8% [16]. The European Community Respiratory Health Survey Study Group calculated an odds ratio of 2.62 for farmers after assessing more than 15,000 people in 12 industrialized countries [17]. For farmers exposed to livestock, the pathogenic role of gases, dusts and aeroallergens from mammals, poultry, insects and mites has been well characterized [22]. In southern Germany, cattle husbandry occurs predominantly in stables and most recognized occupational airway diseases are allergies induced by cattle allergens. A German study found the sensitization threshold for the major cow allergen Bos d2 to be 25 µg/g

for atopic subjects and 50 µg/g for non-atopic subjects [14]. Thus, primary prevention, which prevents sensitization by reducing allergen exposure, is very important. Two other studies examined Bos d2 and the use of dust respirators during stable work [23, 33]. However, they measured only the effects of dust respirators on acute symptoms and lung function changes during work.

The role of endotoxin in the farming environment is unclear. Endotoxin might act as a co-allergen facilitating sensitization to other allergens [24] or it may increase the severity of allergic disease [21]. However, there is also evidence that high levels of exposure to endotoxin during childhood might reduce atopy and prevent the development of asthma [4, 5].

The aim of this study was to measure concentrations of dust, endotoxin, dust mites, cat and cow dander allergens in the stables, living-rooms and mattresses of farmers

allergic to cow dander. Our intention was to examine allergen transfer between working and living areas in order to provide suggestions for evidence-based preventive measures.

MATERIALS AND METHODS

Study group. The study included 46 Bavarian cattle farmers with a diagnosis of occupational asthma or allergic rhinitis caused by cow dander allergen. In all cases, this diagnosis was based on a history of dyspnoea and/or rhinitis during work with cattle, positive skin prick testing and a positive bronchial challenge test with extract of cow allergen. Of the farmers, 23 were male, aged between 20–75 years (mean 49.5 years) and 23 were female, aged between 38–80 years (mean 51.1 years). Among the 46 subjects, were none members of the same family or farm. At the time of dust sampling, all farmers either still held livestock or had given up cattle husbandry for at least two years. At the time of the study, the farmers were divided into three groups according to their exposure:

Group 0 (n=10): had no contact with the cow shed at all. These farmers had given up cattle husbandry for at least two years, had no contact with cattle and their family members also had no professional contact with cattle.

Group 1 (n=13): had indirect exposure through family members. These farmers no longer worked with cattle anymore, but lived together with family members regularly working with cows.

Group 2 (n=23): had regular contact with cattle and worked in the cow shed.

Measurements. In total, 302 airborne and settled dust samples were taken in the sheds and dwellings (Tab. 1).

Sampling in cow sheds. In all sheds of group 2, airborne dust and endotoxin concentrations were measured during actual work by personal and stationary sampling (Tab. 1). Airborne concentrations of inhalable and respirable fractions were sampled.

Sampling in farmers' dwellings. Dust samples were taken from each patient's living room and mattress. The procedure has been described in detail elsewhere [36]. Briefly, a 2 m² area of carpeted floor in the living-room was vacuumed for 4 minutes, and mattresses for 2 min/m². Dust was sampled with an ALK (Denmark) sampling device. The samples were stored at room temperature until analysis.

Airborne concentration of shed dust. Inhalable dust and respirable dust concentrations were measured gravimetrically according to approved German guidelines [3]. Dust was sampled on fibre glass filters (37 mm, Macherey-Nagel). The suction pumps were operated at a rate of 3.5 L/min for total dust sampling, and a rate of 2.0 L/min for fine dust sampling. Personal sampling was

Table 1. Number of samples taken from sheds, living rooms and mattresses.

Exposure Group	0	1	2	all
Farmers	10	13	23	46
Airborne dust (inhalable and respirable)				
Stationary sampling				
Dust	0	18	46	64
Endotoxin	0	18	46	64
Personal sampling				
Dust	0	0	46	46
Settled dust				
Shed	0	13	23	36
Living room samples	10	13	23	46
Mattress samples	10	13	23	46

performed at upper shoulder height, with the pump hanging from the waist belt. The same type of pumps were used for stationary sampling, but were placed in the middle of the shed.

Concentration of endotoxin in airborne and settled dust. For endotoxin measurements, stationary samples for inhalable and respirable dust fractions were collected. All filters were stored at 5–10°C and transferred within one week to the laboratory of the Institute for Occupational and Environmental Medicine, University of Munich. Endotoxin concentration was measured according to the European Guideline EN 14031. The Endotoxin content of all dust samples was determined by a kinetic Limulus assay previously described [36]. As this test measures the activities of different types of endotoxin, the results are expressed in Endotoxin Units (EU). Our assay had a potency of 10 EU/ng against *Escherichia coli* 05B0.

Allergen concentration in settled dust (Bos, Der). The allergens Bos d2, Der p1, Der p2, Der f1 and Fel d1 were quantified in the Allergy Laboratory of the University of Paderborn. After extraction, the major cow allergen Bos d2 was measured with Rocket immunoelectrophoresis using an anti-Bos d2 antibody [13]. House dust mite allergens and cat allergen were measured with a sandwich ELISA according to previously published procedures [19].

Statistical analysis. Statistical calculations were performed with the software packages Winstat and SPSS for Windows. Since most data was not normally distributed, we used minimum and maximum values, the median and percentiles. Correlations were calculated using Spearman rank correlation. As numerous parameters were determined for a relatively small group of subjects, multivariate regression models were not used.

RESULTS

Ambient air sampling in sheds

Airborne Dust. As expected, personal sampling showed higher concentrations than area sampling (Tab. 2). This was true for the inhalable fraction (median: 1,780 versus 244 $\mu\text{g}/\text{m}^3$) and for the respirable fraction (median: 124 versus 11 $\mu\text{g}/\text{m}^3$). We calculated a factor of 7.3 for the inhalable dust and a factor of 11.3 for the respirable dust fraction. These big differences are also valid for 90th percentiles and maximum values. These two fractions did not correlate significantly for personal or for area sampling.

Airborne Endotoxin. Endotoxin levels in inhalable dust and respirable dust fractions from sheds are shown in Table 3. The inhalable fraction ranged from 4–561 EU/m^3 with 90% of the samples below 137 EU/m^3 . The respirable fraction had much lower values, but correlated significantly with the inhalable fraction ($r_{\text{spear}} = 0.68$; $p < 0.001$). There was no association between airborne endotoxin and dust concentrations for either fraction.

Settled dust sampling. The results of endotoxin and allergen determinations in settled dust from 36 cow sheds are presented in Table 4. Endotoxin concentrations ranged from 22–832 EU/mg dust, with a median of 202 EU/mg . Evaluation of cow dander allergen revealed high concentrations of Bos d2 in settled shed dust. The concentration ranged from 0.68–55.4 mg/g dust, with a median of 20.4 mg/g . The concentration of cat allergen Fel d1 was relatively low (median: 0.13 $\mu\text{g}/\text{g}$) with 90% of all values below 1.1 $\mu\text{g}/\text{g}$. As expected, the concentration of house dust mite allergens was also low in all sheds. Der p1 concentration ranged from 0.001–9.26 $\mu\text{g}/\text{g}$ dust, with a median of 0.007 $\mu\text{g}/\text{g}$. The median concentrations of Der p2 and Der f1 were 0.005 $\mu\text{g}/\text{g}$ and 0.010 $\mu\text{g}/\text{g}$ dust respectively.

Indoor sampling – Living room and mattress

Endotoxin. The concentration of endotoxin in living room dust samples ranged from 23–417 EU/mg dust, with a median of 98 EU/mg (Tab. 5). There was no significant difference in endotoxin levels between the three exposure groups (data not shown). The living room endotoxin levels of farmers who were still directly (group 2) or indirectly (group 1) exposed to cattle correlated weakly with the endotoxin concentration in respirable shed dust ($r_{\text{spear}} = 0.44$; $p = 0.02$). The correlation with inhalable dust concentrations was of borderline significance ($r_{\text{spear}} = 0.35$; $p = 0.05$). There was no significant association between endotoxin in settled shed dust and endotoxin levels in living room dust. Endotoxin concentrations in mattress dust (Tab. 6) were about half as high as living room levels, and they correlated weakly ($r_{\text{spear}} = 0.27$; $p = 0.03$). Endotoxin concentrations in mattress dust did not

Table 2. Inhalable and respirable dust concentrations in shed air.

Air samples	Dust concentrations ($\mu\text{g}/\text{m}^3$)			
	inhalable	respirable	inhalable	respirable
	person related sampling		stationary sampling	
No. of cases	23	23	31	31
Minimum	247	0	9	0
Median	1,780	124	244	11
90. Percentile	7,029	416	596	116
Maximum	58,224	1,000	2,433	138

Table 3. Airborne endotoxin concentrations in sheds.

Stationary air samples	Airborne endotoxin concentrations (EU/m^3)	
	inhalable	respirable
No. of cases	32	32
Minimum	4	0
Median	36	2
90. Percentile	137	8
Maximum	561	18

Table 4. Allergen and endotoxin concentrations in settled shed dust.

Settled dust	Bos d2	Fel d1	Der p1	Der p2	Der f1	Endotoxin
	$\mu\text{g}/\text{g}$	$\mu\text{g}/\text{g}$	$\mu\text{g}/\text{g}$	$\mu\text{g}/\text{g}$	$\mu\text{g}/\text{g}$	EU/mg
No. of cases	36	36	36	36	36	36
Minimum	680	0.01	0.001	0.001	0.000	22
Median	20,400	0.13	0.007	0.005	0.010	202
90. Percentile	44,000	1.1	0.773	0.068	0.135	453
Maximum	55,400	2.08	9.262	0.531	0.470	832

Table 5. Allergen and endotoxin concentrations in living room dust samples.

Living room dust	Fel d1	Der p1	Der p2	Der f1	Endotoxin
	$\mu\text{g}/\text{g}$	$\mu\text{g}/\text{g}$	$\mu\text{g}/\text{g}$	$\mu\text{g}/\text{g}$	EU/mg
No. of cases	46	46	46	46	46
Minimum	0.01	0.003	0.003	0.003	23
Median	2.58	0.496	0.152	0.106	98
90. Percentile	337	18.333	1.910	1.136	183
Maximum	1,375	43.531	3.984	5.163	417

correlate significantly with endotoxin concentration in sheds, and were not significantly different between the exposure groups.

Major cat allergen Fel d1. In living room dust (Tab. 5), Fel d1 concentration ranged from 0.01–1375 $\mu\text{g}/\text{g}$ dust (median: 2.58 $\mu\text{g}/\text{g}$). Mattress dust samples (Tab. 6) had a median concentration of 1.89 $\mu\text{g}/\text{g}$ (range: 0.07–2096 $\mu\text{g}/\text{g}$ dust). Neither living room nor mattress dust

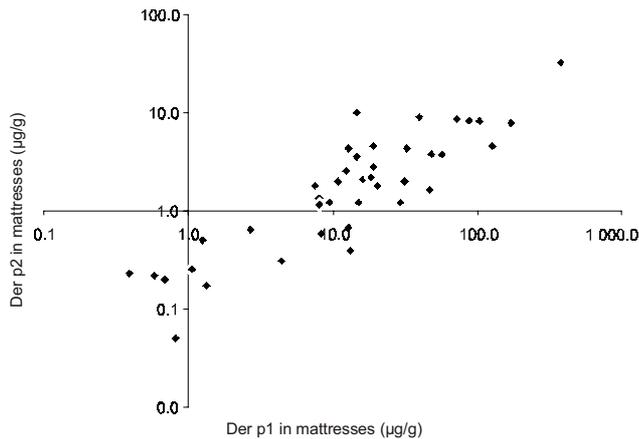


Figure 1. Correlation of dust mite allergens Der p1 and Der p2.

concentrations of Fel d1 correlated with shed dust concentration. However, Fel d1 dust concentrations in the living room and in mattresses were strongly correlated ($r_{\text{spear}} = 0.64$; $p < 0.001$). The small number of cases did not allow the influence of pet presence on the Fel d1 levels in mattresses to be evaluated. Although the highest level of cat allergen was found in the mattress of a cat owner, high concentrations of Fel d1 were also detected in mattresses of farmers who denied the presence of a cat in the home. There was no difference in concentration levels of Fel d1 between the exposure groups for either living room or mattress samples.

Dust mite allergens. Living room and mattress concentrations of dust mite allergens Der p1, Der p2 and Der f1 are shown in Tables 5 and 6. In general, the concentrations of Der p1 were higher than the other two

Table 6. Allergen and endotoxin concentrations in mattress dust samples.

Mattress dust	Fel d1 µg/g	Der p1 µg/g	Der p2 µg/g	Der f1 µg/g	Endotoxin EU/mg
No. of cases	46	46	46	46	46
Minimum	0.07	0.011	0.003	0.004	4
Median	1.89	12.951	1.479	0.259	39
90. Percentile	47	94.174	8.512	22.407	107
Maximum	2,096	377.060	32.682	98.462	262

Table 7. Bos d2 concentrations in mattress and living room dust.

µg/g dust	Bos d2 – living room				Bos d2 - mattresses			
	0	1	2	all	0	1	2	all
Exposure group	0	1	2	all	0	1	2	all
n	10	13	23	46	10	13	23	46
Min	3	34	46	3	4	15	31	4
Median	13	148	316	155	12	195	265	194
90. Percentile	41	1,913	2,478	1,037	347	381	765	547
Maximum	43	2,929	4,209	4,209	381	403	1,268	1,268

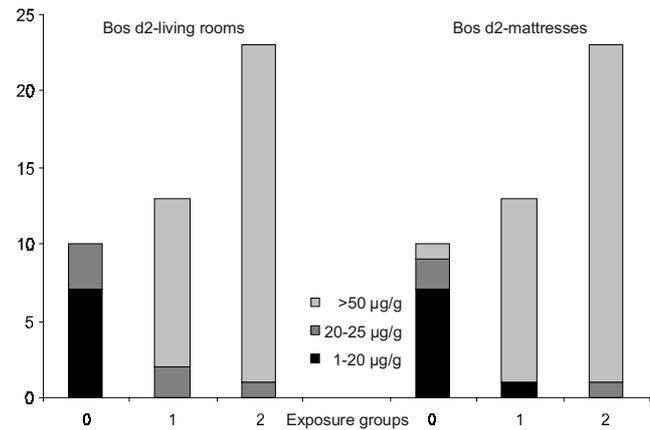


Figure 2. Number of cases exceeding the sensitization threshold for Bos d2.

dust mite allergens. The median values of living room dust samples were 0.50 µg/g for Der p1, 0.15 µg/g for Der p2 and 0.11 µg/g for Der f1, respectively. As expected, mattress dust samples had much higher concentrations (median values: 12.95, 1.48, 0.26 µg/g, respectively). The range of concentrations was very large, but there were no significant differences between the exposure groups. Concentrations of Der p1 and Der p2 correlated significantly in living room samples ($r_{\text{spear}} = 0.86$; $p < 0.001$), and mattress (Fig. 1) samples ($r_{\text{spear}} = 0.89$; $p < 0.001$).

Major cow allergen Bos d2. The concentrations of cow allergen Bos d2 in living room and mattress dust samples for each exposure group are presented in Table 7. Concentrations in living room dust differed significantly between the three exposure groups. The median value for those who had no more contact with cows (group 0) was 13 µg/g, whereas group 1 and group 2 reached 148 and 316 µg/g, respectively. These differences were also of the same magnitude for 90th percentiles and maximum values. Only one sample of group 0 had a higher concentration than the lowest concentration for groups 1 and 2. Transport of Bos d2 from the shed to the living room was supported by the correlation of Bos d2 in living room dust with airborne dust concentrations in the shed ($r_{\text{spear}} = 0.46$; $p = 0.018$). There was only a weak association between Bos d2 concentrations in settled shed dust and living room samples ($r_{\text{spear}} = 0.24$; $p = 0.09$).

The picture for concentrations in mattress dust samples was very similar. Farmers who had no contact with cow shed had a Bos d2 concentration in mattress dust ranging from 4–381 µg/g (9 of 10 values below 30 µg/g!) with a median of 12 µg/g. Mattress dust concentration of Bos d2 from farmers with indirect contact with cows through family members ranged from 15–403 µg/g, with a median of 195 µg/g. Mattress dust concentration of Bos d2 from those with regular contact to cattle ranged from 31–1,268 µg/g, with a median of 265 µg/g. The differences between exposure groups were statistically significant ($p < 0.001$). The strong correlation between mattress and living room dust concentrations ($r_{\text{spear}} = 0.68$; $p < 0.001$) supports that

cow dander allergens are transported from the living room area to bed.

The most important findings concerning the potential risk for allergic reactions to cow dander are summarized in Figure 2. These data show clearly that only one of 10 farmers in group 0 had Bos d2 concentrations in mattress dust exceeding the sensitization threshold of 50 $\mu\text{g/g}$, but nearly all (34 of 36) in groups 1 and 2 did. For living room data, the findings had a very similar pattern.

DISCUSSION

Airborne dust. Inhalable dust concentrations measured in our cow sheds compare very well with those from a Finnish study [35]. Virtanen *et al.* reported a range of 0.2–7.4 mg/m^3 with a median of 2.4 mg/m^3 . Data from a study in Norway [11] also showed similar dust concentrations (GM: $0.31 \pm 4.2 \text{ mg/m}^3$). Concentrations in pig confinements are usually much higher [28]. It is not surprising that personal sampling showed much higher dust concentrations than area monitoring, and this has been found in many occupational studies. Compared to the German TLV for dust (4 mg/m^3), our data give no reason for concern about dust exposure. This finding should be valid for most other cow sheds of this type in Southern Germany.

Endotoxin. The airborne inhalable endotoxin concentrations are well within the expected range. However, since these data are from area samplers, they are probably lower than they would be by personal sampling. Nevertheless, the proposed endotoxin TLV of 50 EU/m^3 [9] seems to cause no airway symptoms by endotoxin for most of the cattle farmers in our investigation. Our finding that there is no significant correlation between dust and endotoxin concentrations leads to the conclusion that only part of the dust has an endotoxin load. The big concentration differences between inhalable and respirable fractions show clearly that endotoxin is bound to larger particles in the stables. The endotoxin concentrations in settled stable dust compare well with previous results [36], which showed a geometric mean of 258 EU/mg in settled dust from 300 sheds. The concentrations in the mattresses (GM: 81.8 EU/mg) and living room dust (GM: 37.8 EU/mg) of farmers' children are also similar to our findings. Thus, shed dust is definitely a relevant source of endotoxin. Endotoxin must be transported from the shed to the living room. Looking at our data of living room dust (median: 98 EU/mg) and mattress dust (median 39 EU/mg), there seems to be a clear gradient between sheds and homes. The correlation of endotoxin concentrations in airborne shed dust with those in living rooms supports the assumption of endotoxin transportation. The role of natural ventilation for carrying dust from shed to dwellings cannot be excluded but should be of minor importance because we found an association with farmers' shed activities only.

Dust mite allergens Der p1, Der p2, Der f1. The very low dust mite concentrations in settled shed dust confirm that these organisms need human dander to grow. Many dust samples from pig confinements [30] also have concentrations below 0.01 $\mu\text{g/g}$. Parvaneh [25] reported a median level of < 0.055 $\mu\text{g/g}$ Der p1 in living room and 0.094 $\mu\text{g/g}$ in mattress samples, which is much lower than our data. In Northern Germany, Radon *et al.* [30] found a median of 53.4 $\mu\text{g/g}$ (range 13.6–190 $\mu\text{g/g}$), which agrees well with our findings. The concentrations of Der p1 from the mattresses of farmers' children in Bavaria, Austria and Switzerland [5] with a GM of 7.09 (5th/95th 0.13/104) also agree with our findings. Compared to the suggested sensitization threshold of 2 $\mu\text{g/g}$ (12), 72% of all mattress samples had concentrations above this level. Furthermore, 59% of all mattress samples had concentrations higher than the sensitization threshold of 9 $\mu\text{g/g}$ dust for non-atopic persons. Thus, many cases are potentially at risk for sensitization and allergic reactions. Since the house dust mites Der p1 and Der p2 correlate very well, it seems sufficient to measure only one species in future investigations. We propose the measurement of Der p1, since its concentration is 10-fold higher than that of Der p2. The much lower concentrations of Der f1 compared to Der p1 in dust samples from living rooms and mattresses confirms our previous findings [5].

Major cat allergen Fel d1. Our findings compare well with data collected in other countries. Parvaneh *et al.* [26] reported a wide range of Fel d1 concentrations in Swedish farming households (0.1–200 $\mu\text{g/g}$), with a median of 4.0 $\mu\text{g/g}$ with a cat and of 3.2 $\mu\text{g/g}$ without a cat in the household. Custovic *et al.* [7] measured concentrations between 0.1–34 $\mu\text{g/g}$ in living room dust samples from households without a cat, and levels between 3–3,000 $\mu\text{g/g}$ when a cat was present. In mattresses, the concentrations ranged between 0.1–2.3 $\mu\text{g/g}$ and between 0.1–3,400 $\mu\text{g/g}$ respectively. A correlation of Fel d2 concentrations in living room and mattress dust samples ($r = 0.48$) was also reported by others [20]. Chapman *et al.* [6] reported a sensitization threshold level of 8 $\mu\text{g/g}$. Compared to this value, 16 (35%) living room and 8 (17.5%) mattress dust samples were higher. This confirms the opinion that many farmers do not allow cats in their beds.

Major cow allergen Bos d2. The high concentrations of major cow allergen Bos d2 measured in settled shed dust is probably explained by the long-term stability of Bos d2 [10]. Although comparable data is not available, Virtanen *et al.* [35] measured airborne concentrations of bovine epithelial allergen in Finnish cow sheds. They found concentrations of $460 \pm 300 \text{ ng/m}^3$, which is equivalent to about 200 $\mu\text{g/g}$ in airborne dust. Such high concentrations can be dispersed during work and thus may lead to airway reactions in sensitized subjects. Furthermore, this seems to be a potent reservoir for allergen transfer into farmers' houses. Our data support

such transport very clearly. A high correlation between bovine allergen concentrations in shed and home dust samples is not expected because stable levels are 10,000-fold higher.

Hinze *et al.* [13] measured Bos d2 in dust samples from farms. They found a range of 0–91 µg/g Bos d2 (median: 0 µg/g) in living room dust samples if farmers had no more contact with sheds. If shed contacts still existed the concentrations of Bos d2 in living room dust samples are much higher (median 29 µg/g, range 10–520 µg/g). We also observed this trend but our values were even higher (see Tab. 7).

Hinze *et al.* [15] calculated sensitization threshold levels for Bos d2 in house dust, which produced specific IgE, to be 20 µg/g for atopic subjects and 50 µg/g dust for non-atopic persons. In our study, nearly all samples from farmers, where shed work was carried out by themselves (exposure group 2) or family members (exposure group 1), exceeded these levels (Tab. 8). Only those farmers without further cattle exposure (group 0) were below this threshold. One exception was a farmer who occasionally helped in another cow shed. All 46 farmers investigated suffered from allergic diseases (asthma or rhinitis) caused by sensitization to Bos d2. Controlling allergen transfer from the cow shed into the living area is an important preventative strategy. This study shows that terminating work in the cow shed reduces Bos d2 levels in the living room and mattress of asthmatic farmers. This reduction is even more significant if family members also discontinue exposure to cow sheds. This shows that a highly significant association exists between level of exposure and level of allergen concentration in living room and mattress. Considering that all cases with continued exposure through shed work (group 2) or with indirect exposure through family members (group 1) had living room concentrations exceeding the threshold level of 20 µg/g, it becomes evident that the use of respirator devices during work cannot sufficiently inhibit the development or persistence of a cow hair allergy.

These examples clearly show the importance of reducing the daily amount of inhaled allergen by reducing the indoor allergen concentration. However, these findings show that the effectiveness of preventive measures depends highly on the individual response to exposure. Although Bos d2 allergen concentration in mattresses was lower than in living room samples, all mattress samples of farmers still working in cow sheds and of those only exposed through family members, exceeded the threshold value for sensitization.

The importance of also measuring allergens in living rooms to optimize disease management (rhinitis, asthma) has been previously shown [13, 31]. Several studies demonstrated that strict allergen avoidance can prevent sensitization during childhood [2] and the sensitization to house dust mites in school age children [1]. In sensitized asthmatic children, airway resistance improved following a stay in an allergen free environment [27]. It has also been shown that the cumulative duration of exposure

predicts the level of sensitization [18] and the impairment on pulmonary function testing [34].

Spitzauer *et al.* [32] describes spouses of deer hunters sensitized to deer dander without having direct contact to deer. The same publication demonstrates a cross reactivity of deer and cow allergens. Thus, it seems plausible that family members could also be indirectly sensitized to cow allergens. For the cat allergen Fel d1 it was shown that significant amounts of allergen are transferred by clothes and can thus lead to exposure [12]. In this context, it is likely that indirect exposure is due to allergen adherent to clothes and hair.

Our study clearly shows that all persons living in a farmer's household must be included in preventative measures.

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