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Development of a Sandwich ELISA to Measure Exposure to Occupational Cow Hair Allergens

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Key Words
Agricultural environment · Cattle farmers · Cow hair allergens · Occupational allergen exposure · Sandwich ELISA

Abstract
Background: Cow hair and dander are important inducers of occupational allergies in cattle-exposed farmers. To estimate allergen exposure in farming environments, a sensitive enzyme immunoassay was developed to measure cow hair allergens. ***Methods:*** A sandwich ELISA was developed using polyclonal rabbit antibodies against a mixture of hair extracts from different cattle breeds. To assess the specificity of the assay, extracts from other mammalian epithelia, mites, molds and grains were tested. To validate the new assay, cow hair allergens were measured in passive airborne dust samples from the stables and homes of farmers. Dust was collected with electrostatic dust fall collectors (EDCs). ***Results:*** The sandwich ELISA was found to be very sensitive (detection limit: 0.1 ng/ml) and highly reproducible, demonstrating intra- and interassay coefficients of variation of 4 and 10%, respectively. The assay showed no reactivity with mites, molds and grains, but some cross-reactivity with other mammalian epithelia, with the strongest reaction with goat. Using EDCs for dust sampling, high concentrations of bovine allergens were measured in cow stables (4,760–559,400 $\mu g/m^2$). In addition, bovine allergens were detected in all areas of cattle farmer dwellings. A large variation was found between individual samples (0.3–900 $\mu g/m^2$) and significantly higher values were discovered in changing rooms. ***Conclusion:*** The ELISA developed for the detection of cow hair proteins is a useful tool for allergen quantification in occupational and home environments. Based on its low detection limit, this test is sensitive enough to detect allergens in passive airborne dust.

Introduction

Bovine allergens are important inducers of occupational allergic airway diseases in agricultural workers. The European Farmers' Project Study Group has determined that the prevalence of work-related respiratory symptoms was 21.8% among cattle farmers [1]. The Ger-

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man Cattle Allergy Study has indicated that 9.1% of the 5,627 case reports of occupational airway diseases among farmers were on cattle-allergic patients [2], with a range from 4.1 to 18.7% across different regions of Germany.

The main sources of bovine allergens are cow hair and dander, but allergens are also found in urine, saliva, milk and beef [3]. Early investigations of bovine dander extracts have identified 17 different antigenic components. Three of these, having molecular weights (MWs) of 24, 22 and 20 kDa, have been characterized as major allergens [3–5]. Subsequent studies have shown that the 20-kDa protein, designated as Bos d 2, is the most important allergen in cow antigen extracts [6–9]. As for the major allergens of other mammals, including dogs, mice, rats and horses, Bos d 2 belongs to the lipocalin family of proteins [10, 11]. Lipocalins share common biological functions that are predominantly related to the transport of pheromones. Measurements of Bos d 2 in dust samples from the homes of farmers have indicated that high indoor Bos d 2 levels are strongly correlated with close contact to cattle and with the occurrence of IgE sensitization [12, 13]. The threshold values have been calculated as 1–20 μg Bos d 2/g dust for the sensitization of atopic subjects and 25–50 μg/g for non-atopic subjects [14]. Thus, monitoring and reduction of allergen exposure is a very important preventive measure to decrease the risk of sensitization. Although significant relationships have been demonstrated between the allergen content in settled dust and various health effects, the sampling of airborne dust should be considered more representative as a measure of inhaled allergens. In addition, lipocalin allergens remain airborne for extended periods of time and are easily respirable [15]. Until now, measurements of airborne concentrations of bovine epithelial allergens have been carried out in cow sheds [16, 17]. No data exist for airborne samples from the living areas of farmers.

The sampling of airborne dust on filters using stationary or person-carried pumps may be regarded as the gold standard in the occupational setting [18]. This method, however, requires expensive equipment and trained staff and has a high risk for technical difficulties. Recently, a new sampling strategy for airborne dust has been developed by Noss et al. [19, 20]. They have designed a new electrostatic dust fall collector (EDC) that enables passive sampling of airborne particles that are sedimenting on dust-binding cloths. This low-cost technique is simple to handle and suitable for large-scale exposure studies.

The aim of the present study was to assess bovine allergen exposure in working and living areas of cattle farmers by sampling passive airborne dust using EDCs. For this purpose, we developed a sensitive sandwich ELISA based on polyclonal antibodies (pAbs) against a mixture of hair extracts from different cattle breeds representative of the German dairy and beef farming industries.

Materials and Methods

In-House Cow Hair Extracts

Extracts were made from 74 hair samples of female (n = 60) and male animals (n = 14) of 15 different cattle breeds used for dairy and beef production in Germany and Austria (Simmental, n = 28; Holstein, n = 14; German Brown, n = 11; Pinzgau, n = 4; Tyrolese Grey, n = 3; Charolais, n = 2; Limousin, n = 2; German Red Pied, n = 1; Angeln, n = 1; Angus, n = 1; Montbéliard, n = 1; Belgian White Blue, n = 1; Murboden, n = 1; Murnau-Werdenfels, n = 1, and Simmental crossbreeds, n = 3). The extractions were performed by rotation of 10 g of hair in 140 ml of distilled water for 1 h at room temperature. After centrifugation at 3,000 *g* for 15 min, the supernatant was lyophilized and stored at –80°C. For further analysis, the freeze-dried extracts were dissolved in phosphate-buffered saline (PBS) and centrifuged again at 30,000 *g* for 15 min. Every extract was tested for its protein content using the Bradford assay (Bio-Rad Protein Assay, Munich, Germany) and for the protein pattern using sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE). In addition, all extracts were tested for mite and grain contamination using *Dermatophagoides farinae*, *Lepidoglyphus destructor* and rye flour sandwich ELISA, as described previously [21, 22].

For the preparation of the immunizing antigen, equal protein amounts (2.5 mg) of extracts from the hair of female and male Simmental, female and male Holstein, female and male German Brown, female Pinzgau, female Montbéliard, male Belgian White Blue, female Simmental × Holstein and female Simmental × German Red Pied cattle were pooled.

Commercial Cow Hair Extracts

Commercial cow hair and cow dander substances were obtained from the manufacturer Allergon (Ångelholm, Sweden). The raw material was homogenized in PBS at 3,000 rpm for 10 min and centrifuged at 30,000 *g* for 15 min. The supernatant was stored at –80°C until analysis. The protein content was determined by the Bradford assay.

SDS-PAGE and IgE Immunoblotting

SDS-PAGE was performed under reducing conditions at 200 V using 10% NuPAGE Bis-Tris Gel (Invitrogen, Carlsbad, Calif., USA) and 2-(N-morpholino)ethanesulfonic acid (MES) running buffer (1 M MES, 1 M Tris, 69 mM SDS, 2.5 mM EDTA). Protein test mixtures 4 and 5 (Serva, Heidelberg, Germany) were used as MW markers. The separated proteins were silver stained according to the method of Blum et al. [23]. Unstained proteins were transferred onto polyvinylidine difluoride (PVDF) membrane (0.45-μm pore size; Millipore, Bedford, Mass., USA) for 1 h at 1 mA/cm^2 in a semidry blot apparatus with the transfer buffer (50 mM Tris, 50 mM boric acid, 10% methanol). After transfer, the MW marker lane was cut off and stained with Coomassie brilliant

blue. The remaining PVDF membrane was blocked with blocking buffer [1% bovine serum albumin (BSA), 1% polyvinylpyrrolidone 40 in Tris-buffered saline (TBS), pH 7.4] for 2 h, then cut into strips and incubated overnight with 12 sera from cattle-sensitized farmers. All sera were diluted with 2% BSA in TBS to the cow dander-specific IgE value of approximately 2 kU/l (e4; ImmunoCAP system; Phadia, Uppsala, Sweden). After washing with TBS-0.1% Tween 20, the strips were incubated for 2 h with 1:1,000-diluted alkaline phosphatase-conjugated anti-human IgE (Sigma-Aldrich, Steinheim, Germany), and the bound antibodies were detected with the BCIP/NBT substrate system (Sigma-FAST tablets). As a negative control, one strip was incubated with 2% BSA-TBS instead of human serum.

Production, Purification and Biotinylation of pAbs

Two female New Zealand White rabbits were immunized subcutaneously with 0.5 mg of cow hair mixture extract that was emulsified in TiterMax Gold (TiterMax USA, Norcross, Ga., USA). Two boosts with the same allergen concentration in TiterMax Gold were carried out at 4-week intervals. The sera collected 4 weeks after the last injection were pooled and stored at –80°C. For antibody purification, the IgG fraction of the antiserum was isolated by affinity chromatography with the HiTrap protein G column (GE Healthcare, Uppsala, Sweden) following the manufacturer's instructions. A portion of purified anti-cow hair pAb was biotinylated by mixing with a 30-fold molar excess of biotinoyl-ε-aminocaproic acid-N-hydroxysuccinimide ester (Roche, Mannheim, Germany) dissolved in dimethylsulfoxide for 4 h at room temperature.

Cow Hair Allergen Sandwich ELISA

MaxiSorp microtiter plates (Nunc, Roskilde, Denmark) were coated overnight with 100 μl/well of anti-cow hair pAb in 0.1 M carbonate buffer (1 μg/ml; pH 9.6) at 4°C. After blocking with 200 μl/well of 1% gelatin in PBS-0.05% Tween 20 (PBST) for 2 h, the plates were incubated for 1 h at 22°C with standards, assay controls and samples diluted in PBST. A standard curve was obtained using eight serial one-half dilutions of the cow dander extract (Allergon), with concentrations ranging from 10 to 0.08 ng/ml. Cowshed settled dust extract was used as a positive control and wheat flour extract as a negative control. Every sample was tested using three serial dilutions. The bound proteins were incubated with 100 μl/well of biotinylated anti-cow hair pAb (0.1 μg/ml) for 1 h at 22°C, followed by 100 μl/well of streptavidin-peroxidase conjugate (1/20,000 in PBST, 1 h, 22°C; poly-HRP80-SA; Fitzgerald, Concord, Mass., USA) and finally 100 μl/well of ABTS substrate [2,2′-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid)diammonium salt, 10 mg/tablet; Sigma] in 50 mM phosphate-citrate buffer (pH 4.2) with 0.015% hydrogen peroxide. The reaction was stopped with 100 μl/well of 0.32% sodium fluoride, and the absorbance was read at 414 nm. Sample concentrations were calculated by interpolation of optical density (OD) values on a four-parameter fitted standard curve using Softmax Pro 4.7.1 (Molecular Devices, Sunnyvale, Calif., USA). The lower limit of detection was the concentration corresponding to the minimal value of the four-parameter curve fit function plus the 6-fold standard deviation of the zero standard (OD_{414} = 'parameter A' + 0.15). The upper limit of detection was the concentration corresponding to OD_{414} = 3.0.

Specificity Analysis and Validation of the Assay

An analysis of the assay specificity was performed using extracts from molds *(Alternaria alternata, Aspergillus fumigatus, A. niger, Cladosporium herbarum, Penicillium chrysogenum, Trichoderma viride* and *Stachybotris chartarum)*, mites *(Acarus siro, Lepidoglyphus destructor, Tyrophagus putrescentiae, Glycyphagus domesticus, Dermatophagoides farinae* and *D. pteronyssinus)*, grains (wheat, rye, barley, oat and soy) and mammalian epithelia (goat, sheep, swine, horse, cat, dog, rabbit, mouse, rat and human). The mold and mite raw materials were obtained from Allergon, and the cereal grains were bought from a health food shop. The mammalian epithelia were obtained as skin prick test solutions from the following manufacturers: HAL Allergie, Düsseldorf, Germany (goat and sheep); ALK-Abelló, Madrid, Spain (horse, dog, cat, rabbit and rat), and Lofarma, Milan, Italy (swine and mouse). Human hair extract was prepared in-house according to the extraction procedure used for cow hair.

For validation of the assay, settled dust samples from the cow sheds, living rooms and mattresses of cattle farmers were used (n = 29). The samples had previously been tested for the concentration of the major cow allergen Bos d 2 [12]. The Bos d 2 content was measured with Rocket immunoelectrophoresis using an anti-Bos d 2 rabbit antibody and purified Bos d 2 as a reference [13].

Passive Airborne Dust Sampling

Passive airborne dust samples were collected with the EDCs developed by Noss et al. [19, 20]. The sampler consisted of four electrostatic cloths (area: 0.032 m^2 each) that were mounted in a plastic folder (40 × 30 cm). The EDCs were left for 14 days in a horizontal position (0.8–2 m above the floor) to collect settling airborne dust on cloths exposed to air. Dust sampling was performed in different areas of the cattle farms. EDCs were placed in cow stables (n = 37) and in different rooms of the farmer dwellings, including changing, living and dining rooms, bedroom, kitchen and home office (n = 128). As a control for the cow sheds, dust samples were also taken in pig (n = 4), horse (n = 4) and sheep stables (n = 2), and chicken coops (n = 4). As a control for the farmer dwellings, dust sampling was carried out in the urban homes of persons without any cattle exposure (n = 32).

Extraction of Dust Samples

After sampling, the EDCs were frozen overnight to eliminate any mite growth on the cloths. From every EDC, only one cloth was used for the measurement of cow hair allergens. Each cloth was weighed before and after sampling to assess the dust load. All cloths were conditioned for 48 h prior to weighing. A weighing error of up to 3 mg was determined by repeated measurements of reference cloths. Each cloth was extracted by rotation in 20 ml PBST for 1 h at room temperature. After extraction, the cloth was removed, the extract was centrifuged at 3,000 *g* for 15 min and the supernatant was stored in aliquots at –80°C until analysis.

Statistical Methods

Column statistics and correlation analyses (Pearson's and Spearman's rank tests) were performed with the GraphPad Prism software (GraphPad Software, San Diego, Calif., USA). Significance calculations were performed with the statistical software SAS, version 9.2 (SAS Institute, Cary, N.C., USA). A mixed linear model was fitted with the log-transformed cow hair allergen values as the outcome, the room as a fixed effect and the farm as a

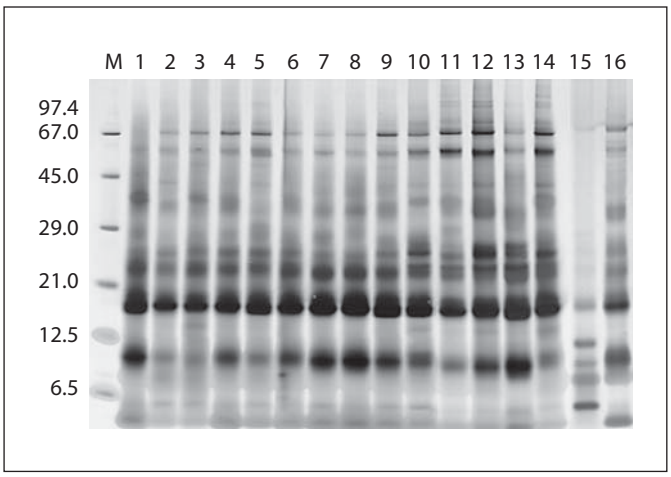

Fig. 1. Silver-stained SDS-PAGE of in-house and commercial cow hair extracts. The lanes were loaded with 1 μg of protein. Lanes 1–4 = male Simmental; lanes 5, 6 = female Holstein; lanes 7, 8 = female Simmental; lanes 9, 10 = female German Brown; lane 11 = male German Brown; lanes 12, 13 = female Pinzgau; lane 14 = male Pinzgau; lane 15 = cow hair from Allergon; lane 16 = cow dander from Allergon; M = MW marker.

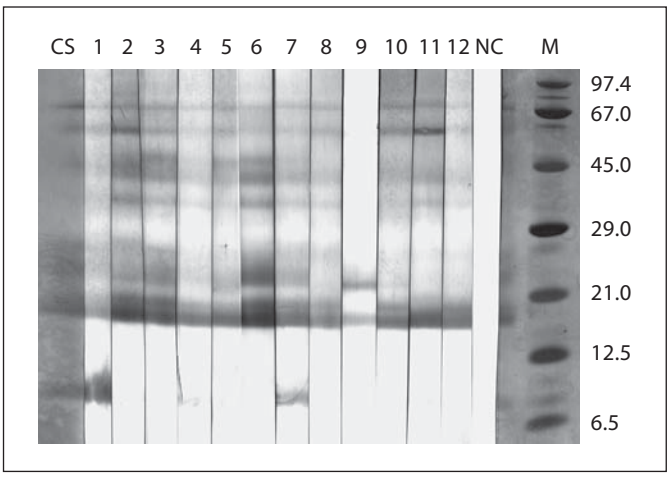

Fig. 2. IgE immunoblot of mixed cow hair extracts developed with the sera of cow dander-sensitized farmers (lanes 1–12); CS = Coomassie stain; NC = negative control; M = MW marker.

random effect. The covariance structure used was variance components. The estimation method applied was the restricted maximum likelihood method, and the number of degrees of freedom was determined using the Satterthwaite approximation. The tests were conducted as two-sided calculations with a global significance level of 5%. The p values presented herein have been adjusted according to the Bonferroni method.

Results

Characterization of Cow Hair Extracts for Immunization

The protein content of the 74 cow hair samples varied from 0.3 to 9.6 mg/g hair without significant differences between males and females or between different breeds. Some of the samples were marginally contaminated by mite or grain proteins, with *L. destructor* accounting for up to 0.01% of the protein content, *D. farinae* for up to 0.09% and rye for up to 0.5% (data not shown). SDS-PAGE analysis of the cow hair extracts showed nearly identical protein patterns with a dominant band at 20 kDa (fig. 1). There were some differences in the bands between 45 and 25 kDa, as well as in the intensities of single bands. These variations appeared both within the same cattle breed, as well as between different breeds and genders. All in-house extracts showed stronger bands compared to the commercial extracts. Although the commercial cow dander extract was very similar to the in-house cow extracts, the commercial cow hair extract lacked some of the high-molecular-weight bands seen in the in-house extracts. For immunization, several extracts were selected using the following criteria: (1) the lowest content of rye and mites to avoid antibody production against these impurities and (2) different breeds and genders to represent all differences in the protein pattern. The prepared cow hair mixture was tested for the presence of allergens using an IgE immunoblot with 12 sera from farmers sensitized to cow dander (fig. 2). All sera showed very similar immunoreactions, reflecting almost the entire protein pattern of the cow hair mixture, with the exception of a band at ~9 kDa, which was recognized in only 3 cases. All the patients had the strongest reaction to the 20-kDa protein, the major allergen Bos d 2.

Cow Hair Allergen Sandwich ELISA

A sandwich ELISA was developed to quantify cow hair allergens using affinity-purified pAbs against an extract prepared from hair of different cattle breeds. A commercial cow dander extract was used as a standard. The working range of the assay defined by the lower and upper limits of detection was between 0.1 and 8.0 ng/ml. The reproducibility was assessed by analyzing the positive control performed in duplicate in 20 independent assays. The mean intra-assay coefficient of variation (CV) was

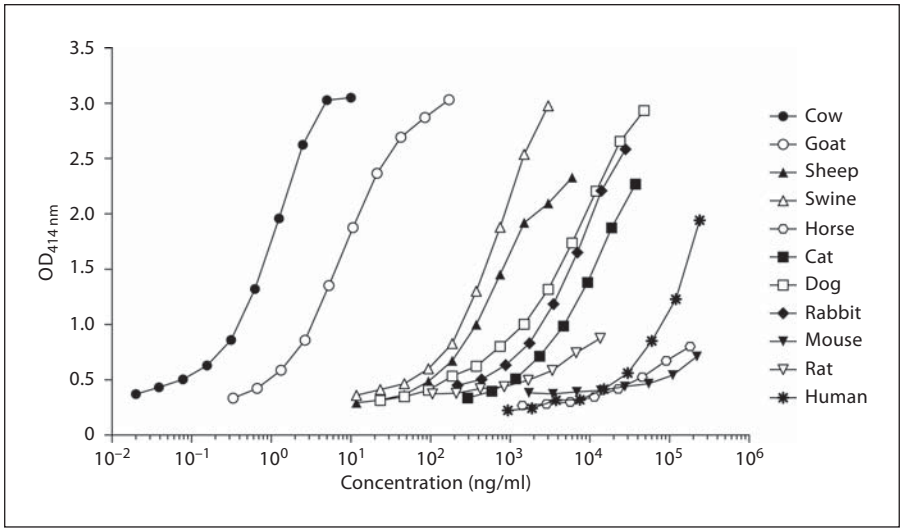

Fig. 3. Specificity of the cow hair allergen sandwich ELISA based on tests using various mammalian epithelial extracts.

4%, and the interassay CV was 10%. The sandwich ELISA showed no reactivity with mold and mite extracts tested at protein concentrations of up to 100 μg/ml, as well as no reactivity with grain extracts of up to 500 μg/ml (data not shown). The ELISA showed cross-reactivity with almost all tested mammalian epithelial extracts with differing intensities (fig. 3). The strongest reaction was observed with goat extract (10-fold less reactivity than cow), followed by swine and sheep (500-fold less). Mouse, rat and horse extracts showed only weak reactivity at the highest concentrations tested. Human hair extract reacted only at very high protein concentrations, demonstrating 200,000-fold less activity compared with the cow standard. To validate the assay, settled dust samples with known concentrations of Bos d 2 were analyzed. There was a very good correlation ($r_{Pearson} = 0.875$, $p < 0.0001$; $r_{Spearman} = 0.974$, $p < 0.0001$) between the cow hair allergen concentrations and the Bos d 2 values (fig. 4).

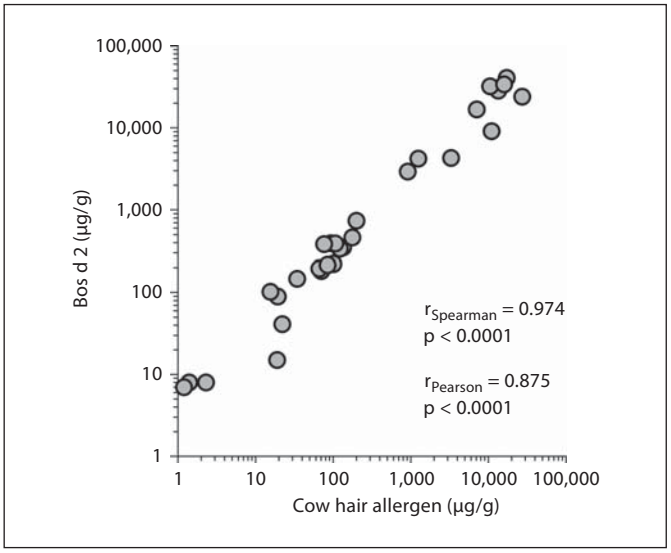

Fig. 4. Correlation between cow hair allergen concentrations (measured by sandwich ELISA) and Bos d 2 content (measured by Rocket immunoelectrophoresis) in 29 settled dust samples from cow stables.

Cow Hair Allergen Levels in Dust Samples

The dust levels collected on the EDC cloths from the stables were relatively high (up to 22 g/m^2), whereas those from both rural and urban homes were very low. Of the latter, 80% of the samples were within the range of the weighing error (2–3 mg).

The concentrations of dust and cow hair allergens on the EDC cloths from the stables are given in table 1. The allergen levels in the cow stables differed about 100-fold, ranging from 4,760 to 559,400 μg/m^2, with a median of 51,700 μg/m^2. There was a strong correlation between the cow hair allergen levels and the dust loads ($r_{Pearson} = 0.78$, $p < 0.0001$). A median allergen concentration of 14.1 mg/g dust was calculated for the cow stable dust (ranging from 0.68 to 59.9 mg/g). In most of the other animal stables (e.g. horse stables), only trace amounts of allergens were detectable (up to 5.6 μg/m^2), although the dust loads were similar to those in the cow stables. The goat stables, however, had slightly increased allergen levels (median: 315.7 μg/m^2).

Table 1. Dust and cow hair allergen concentrations on EDCs from stables

Area	n	Dust (g/m^2)			Cow hair allergen ($\mu g/m^{2)}$		
		median	minimum	maximum	median	minimum	maximum
Cow stables	37	3.4	0.4	21.3	51,700	4,760	559,400
Other stables	20	1.8	0.1	22.0	2.8	0.08[1]	701.4
Goat stables	6	2.4	0.4	3.5	315.7	91.0	701.4
Sheep stables	2	0.5	0.3	0.6	3.9	2.5	5.4
Pig stables	4	9.7	1.8	22.0	1.5	0.1	3.5
Horse stables	4	3.1	0.4	4.3	2.0	0.2	6.5
Poultry stables	4	0.1	0.1	1.1	0.4	0.08[1]	1.5

[1] Not detectable, value expressed as 2/3 of lower detection limit.

The allergen concentrations in individual dust samples from cattle farmer dwellings varied widely, with a nearly 3,000-fold difference between the lowest and highest values (range: 0.3–900.1 $\mu g/m^2$; median: 22.6 $\mu g/m^2$). The results sorted by room type are presented in table 2. The highest cow hair allergen levels were found in changing rooms (median: 104.5 $\mu g/m^2$) and home offices (median: 63.1 $\mu g/m^2$). The allergen concentrations found in the changing rooms were significantly different from those found in all other rooms, except for the home offices (table 2). There was also a significant difference between offices and living rooms ($p = 0.0044$).

Of the 32 control EDC samples from urban homes, 23 gave positive results in the cow hair allergen assay, with values reaching 2.7 $\mu g/m^2$ and with a median of 0.2 $\mu g/m^2$. These concentrations were very low in comparison with farmer homes, where the median level was 100-fold higher.

Table 2. Cow hair allergen concentrations on EDCs from farmer dwellings

Room classification	n	Cow hair allergen, $\mu g/m^2$			p value[1]
		median	minimum	maximum	
Changing room	30	104.5	1.7	594.3	
Home office	8	63.1	12.2	844.4	NS
Living room	28	4.3	0.3	900.1	<0.0001
Dining room	9	7.5	1.8	498.5	0.0032
Bedroom	29	11.8	1.3	194.1	0.0003
Kitchen	24	19.4	1.2	812.2	0.0193

[1] In comparison to changing rooms. NS = Not significant.

Discussion

Bovine hair and dander are the most important inducers of occupational allergic diseases in cattle farmers. To assess the sensitization risk, measurements of allergen concentrations at the workplaces and in the living environments of exposed subjects are essential. In the present study, we developed a highly sensitive sandwich ELISA to quantify bovine allergens in passive airborne dust samples from cattle farms. This assay is based on pAbs against a mixture of all proteins extracted from the hair samples of several cattle breeds.

To determine if there were any breed- or gender-specific differences, 74 hair samples were assessed from female and/or male animals of 15 different cattle breeds that are relevant to dairy and beef farming in Germany. SDS-PAGE analysis revealed that all hair extracts showed nearly identical protein patterns and that each had a dominant Bos d 2 band. These results are in accordance with previous studies that have shown only minor inter-individual differences in protein patterns [3, 24]. In contrast, considerable inter- and within-breed variations were seen in horse and dog allergen extracts [25, 26], but no breed-specific allergens were found. Although considerable variability with up to 30-fold differences could be seen in the protein content from the 74 cow hair samples, this variability was not found to be associated with a certain breed or gender. Heutelbeck et al. [24] have also reported 30-fold differences in the Bos d 2 content among the hair samples from different breeds.

Several different methods have been described for the measurement of cow allergens, including: ELISA inhibition with rabbit antiserum against bovine dander antigens [27], Rocket immunoelectrophoresis with rabbit antiserum against the major allergen Bos d 2 [13] and sandwich ELISA with anti-Bos d 2 monoclonal antibodies [17]. None of these tests are commercially available, and only the sandwich ELISA technique offers the high sensitivity that is required to detect the low allergen levels present in airborne dust samples. The cow hair immunoassay described herein has a detection limit of 0.1 ng/ml, intra-assay CV of 4% and interassay CV of 10%. Compared with the sandwich ELISA based on anti-Bos d 2 monoclonal antibodies, the assay presented here was found to be 10-fold more sensitive and showed similar intra- and intertest variations. In addition, the present assay was shown to correlate strongly with a Bos d 2-related Rocket immunoelectrophoresis.

In the cross-reactivity study, no reactions were found with the mite, mold and grain extracts. Their recognition would have led to false-positive results, especially in stable dust containing high concentrations of these substances. On the other hand, cross-reactivity was observed with almost all tested mammalian epithelial extracts. Immunoelectrophoretic analyses of animal hair and dander extracts have already demonstrated partial identities between cow and goat, sheep, swine, horse, dog, cat and guinea pig antigens [4]. Significant cross-reactivity with deer and goat epithelia has been observed by immunoblot inhibition studies with sera from patients [28, 29]. Similarly, the strongest cross-reaction in the present assay was obtained with the goat extract. The cross-reactivity between different animal allergen extracts may be caused by albumins and milk proteins, which have often been reported to be highly cross-reactive due to their similarities in sequence, structure and biological function [30–32]. Whether lipocalins, the most important respiratory sensitizers, represent cross-reactive allergens is still not clear. The sequence identities between lipocalins are often <20%, but they share a common tertiary structure [11]. A previous IgE ELISA inhibition study [33] with five recombinant animal lipocalins (Can f 1, Can f 2, Mus m 1, Equ c 1 and Bos d 2) and human tear lipocalin has revealed some IgE cross-reactivity between several of the lipocalins. The major cow allergen Bos d 2, however, could not inhibit IgE binding to any of the other lipocalins tested.

The cow hair allergen levels in passive airborne dust collected with EDC differed about 100-fold between cow stables. These results are in accordance with previous findings that have also shown a wide range of allergen concentrations among cow stables [16, 17]. Moreover, individual stables seem to have steady allergen levels in their ambient air. In some cow stables, bovine allergen concentrations tend to be low, whereas in others the levels are consistently high [34]. These variations can probably be explained by factors associated with cow stable characteristics, such as size, heating, ventilation and construction details of the building. The calculated cow allergen concentrations per dust amount (range: 0.68–59.9 mg/g, median: 14.1 mg/g) were very similar to the Bos d 2 levels measured in settled shed dust by Berger et al. [12], where their reported Bos d 2 concentrations were between 0.68 and 55.4 mg/g dust, with a median of 20.4 mg/g. Due to cross-reactivity, the dust samples from the stables of other animals were also positive in the cow hair allergen assay. The allergen concentrations in pig, sheep, horse and chicken stables were negligible, but those in the goat stables were considerably higher, confirming the results of the cross-reactivity study presented here.

As a control for the assessment of bovine allergen exposure in the home environment of farmers, dust sampling was performed in urban dwellings. Although none of the household members had any contact with cattle or cattle farms, the majority of urban EDC samples were positive in the assay, though at very low concentrations (median: 0.2 $\mu g/m^2$). The dispersal of cow allergens from rural to urban environments through the ambient air is quite implausible. Measurements of horse allergen dispersion have shown that allergen levels decline rapidly with increasing distance from the stables and are not detectable 500 m distant from the source area [35, 36]. One possible explanation for the positive results could be the cross-reactivity to human and pet hair or dander. The samples collected from the houses of pet owners, however, did not show increased values over those of non-pet owners. Another possible reason for the positive values could be the detection of bovine allergens derived from foods, e.g. milk and beef, or from leather materials. The presence of allergens in such products has been demonstrated by Prahl [3]. In concordance with this observation, milk gave a positive reaction in the present assay (data not shown).

In cattle farmer dwellings, cow hair allergens were detected with a wide variation between individual samples (0.3–900 $\mu g/m^2$). The median of 22.6 $\mu g/m^2$ was 100-fold higher in comparison with urban homes. These high allergen levels may be due to allergen transfer from the workplace through the clothes and hair of farmers. Human hair and clothing have been reported as a main car-

rier for mouse and cat allergens [37–39]. The data presented here support this route of exposure based on the high bovine allergen burden measured in changing rooms. The high level of allergen contamination found in home offices, living rooms or kitchens suggests that some farmers enter their homes in their work clothes. This allergen transport should be avoided, as high indoor Bos d 2 levels have been shown to correlate with the degree of sensitization [13]. Moreover, transportation of other occupational allergens, such as rodent allergens, has been reported to lead to an enhanced risk of allergy development for the family members of laboratory animal workers [40]. These examples clearly demonstrate the relevance of controlling and reducing indoor allergen concentrations as an effective prevention strategy. A comparison of cow hair allergen concentrations in the homes of cattle farmers with the results of previous studies [12, 13] is difficult due to the different dust sampling methods (settled vs. passive airborne dust) and different units of measurement (μg/g vs. μg/m^2). Expression in microgram per gram dust for the results presented here was not possible because the dust load of 80% of EDC cloths from homes could not be estimated with sufficient accuracy. Despite the low dust levels, allergens were detected in all rural EDC samples. In addition, the use of EDCs allowed differences in allergen concentrations to be assessed between different areas in the home environment of farmers. Thus, this passive dust sampling method is quite applicable for allergen exposure measurements. Due to its low cost and ease of use, this method is a desirable alternative compared to the active collection of airborne dust using pumps.

In conclusion, the ELISA for the detection of cow hair proteins is a useful tool for the quantification and monitoring of allergen levels in occupational and home environments. Based on its demonstrated low detection limit, this test is sensitive enough to measure cow allergens in airborne dust samples and to detect allergen contaminants in the living areas of cattle farmers.

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