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Cow Hair Allergen (Bos d 2) Content in House Dust: Correlation with Sensitization in Farmers with Cow Hair Asthma

Key Words

Bos d 2
Cow hair asthma
House dust
Measures of avoidance

Abstract

Farmers (N = 45) suffering from occupational cow hair asthma were visited at home to evaluate the concentration of cow hair major allergen Bos d 2 in the house dust and to correlate these results with measures of avoidance, degree of sensitization, clinical symptoms, and lung function. Bos d 2 was determined by rocket immunoelectrophoresis. In dust of tiles and linoleum Bos d 2 was difficult to detect, whereas dust samples of carpets often contained high concentrations of the allergen (50–520 µg/g fine dust). Bos d 2 levels were significantly higher when barn and living quarters were in the same building. Concentrations of cow hair-specific IgE were correlated with concentrations of Bos d 2 in house dust samples. A concentration of 20–29 µg Bos d 2 per gram of house dust could be established as threshold value for relevant IgE sensitization. Avoiding the barn is not a sufficient avoidance measure for cow hair asthmatics if the partner continues cattle farming. Cessation of cattle farming and avoiding the former barn results in a marked reduction in Bos d 2 concentration in living quarters, a decreased degree of sensitization, and a reduced symptom score. Farmers with cow hair asthma should avoid cattle and thoroughly clean all carpets in the living quarters to avoid continuous cow allergen exposure.

Introduction

Occupational bronchial asthma due to cow hair and dander is not well researched, although there are reports on the influence of farming on respiratory symptoms and pulmonary function [1–3]. In Germany, this occupational disease is compensated for by the relevant insurance company if the affected farmers cease cattle farming.

One aim of this study was to investigate the effect of different measures of avoidance by cow hair asthmatics on clinical symptoms, sensitization, and serum IgE. Despite giving up cattle farming, many patients still suffer from a

distinct bronchial hyperreactivity, as in marked bronchial asthma. Therefore, the environmental influences of cow hair allergens in the house dust of these subjects were examined with respect to the degree of sensitization. Additionally, this investigation aimed to establish a threshold concentration of Bos d 2 in house dust leading to relevant IgE sensitization.

A dose-response relationship has been proposed between exposure to indoor allergens and risk of sensitization, and development of respiratory symptoms [4–6]. It has been demonstrated that certain doses of Fel d 1 in the ambient air of a room induce a significant drop in FEV₁ in pa-

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tients with cat asthma and hay fever [7]. Indoor Fel d 1 is known to cause asthma and rhinitis [8], therefore measures were established to reduce the indoor concentration of Fel d 1 [9]. A low amount of allergens in house dust and in the ambient air is expected to decrease sensitization rates and result in improved bronchial hyperreactivity [4].

Allergen sensitization is considered a high risk for development of bronchial asthma [10–13]. Therefore, threshold values were proposed for several indoor allergens that lead to relevant IgE sensitization [5, 6, 10, 14, 15] (table 1). In this context a good correlation was shown between exposure to high levels of indoor major allergens of the cockroach (Bla g 1, Bla g 2) and clinical IgE sensitization [5]. The cockroach has been identified as an important cause of perennial rhinitis and asthma [16]. In the living quarters of some patients with cockroach sensitization Bla g 1 and Bla g 2 could be demonstrated although there was no visible cockroach infestation [15]. This shows the importance of indoor allergen measurements as they were performed in the present study for the major cow hair allergen Bos d 2.

Materials and Methods

Patients

The study includes 45 patients (28 male, 17 female) with a mean age of 57 (range 23–72) years referred to our clinic because of cow hair asthma. The diagnosis was based on the following parameters: positive case history, intracutaneous and bronchial provocation test with cow hair extract, and RAST to demonstrate specific IgE antibodies against cow epithelia. All patients had been farmers, 22 were atopic subjects with a history of infantile atopic eczema, hay fever, or milk crust, whereas 23 had no atopic diathesis.

House Dust Samples

All 45 patients were visited at home. House dust samples were collected using the 'Indoor Allergen System' (ALK-Scherax, Hamburg, Germany) connected to a vacuum cleaner. For each sample a new filter was used. Following the manufacturer's instructions, a carpet area of approximately 1 m² was vacuumed for 5 min. In rooms without carpets, areas covered with dust were selected and vacuumed. In four homes no dust was traceable and no samples were taken.

In each home dust samples were collected from the corridor, living room and bedroom (one sample from each area). In total 131 dust samples were taken from 43 corridors, 45 living rooms, and 43 bedrooms; 86 samples were collected from carpets (17 corridors, 35 living rooms, 34 bedrooms), 28 from tiles (23 corridors, 3 living rooms, 2 bedrooms), and 17 samples from new carpets acquired after removal of cattle.

Determination of Cow Hair Allergen Bos d 2 by Means of Rocket Immuno-electrophoresis (RIE)

The collected house dust samples were tested for Bos d 2. Dust extracts were prepared using an extraction ratio of 1:10 (w/v) by dissolving the sample in 0.125 M NH₄HCO₃ buffer containing 0.1%

Table 1. Proposed threshold values for indoor allergens leading to IgE sensitization

Source	Allergen	Threshold	Reference
Cockroach	Bla g 1	2 U/g dust	5
	Bla g 2	2 U/g dust	15
Cat	Fel d 1	8 µg/g dust	5, 10
Housedust mite (<i>Dermatophagoides pteronyssinus</i>)	group 1 allergens	10 µg/g dust	5
	Der p 1	2 µg/g dust	6
		9 µg/g dust	14
Dog	Can f 1	10 µg/g dust	10

NaN₃ overnight at +5°C under constant rotation. The suspension was then centrifuged for 10 min at 3,000 g. Dust extracts were stored at –20°C until use.

The protein content of purified Bos d 2 was determined according to the method of Lowry et al. [17] with bovine serum albumin as a reference protein. The amount of allergen Bos d 2 in the dust Samples was determined by RIE [18] using rabbit anti-Bos d 2 (0.29 µl/cm²) and Bos d extract and purified Bos d 2 as a reference. The content was measured in micrograms of Bos d 2 per gram of dust.

Clinical Symptoms and Measures of Avoidance

To determine serum IgE and specific IgE (CAP-RAST, Pharmacia, Sweden) against cow epithelia, samples of venous blood were taken. Clinical symptoms (rhinitis, conjunctivitis, expectoration, coughing, dyspnea, and urticaria) and measures of avoidance were collected. The investigated patients were divided into three groups: group A (N = 14) – patients having abandoned cattle farming; group B (N = 12) – patients avoiding the barn; group C (N = 19) – patients without any measures of avoidance.

Statistical Analysis

Comparisons were made using the Mann-Whitney U rank sum test, Student's t, and the χ^2 test. A Gerhardt plot [19] was computed of the data obtained from each sampling location to establish a Bos d 2 concentration which is capable of discriminating between high and low sensitization in CAP-RAST class with a sufficient sensitivity and specificity. The most suitable discriminator was fixed by calculating the Youden value (sensitivity + specificity – 100).

Results

Concentrations of Bos d 2 were found in house dust samples of 34 patients, 4 of whom had already removed their cattle. Bos d 2 was not found in samples of 11 patients, 10 of whom had ceased cattle farming.

Carpets in corridors, living rooms, and bedrooms had significantly more Bos d 2 (up to 520 µg/g dust) than tiles,

linoleum and new carpets acquired after giving up cattle farming (table 2).

In carpets of patients who had removed the cattle the amount of Bos d 2 was significantly lower than in rooms of patients avoiding the cow barn or in rooms without any measures of avoidance (table 3). Comparison of dust samples of the latter two groups showed no significant difference in Bos d 2 concentrations.

The amount of Bos d 2 detected also depends on the architectural setup (table 4): when the barn and living quarters were separate, the amount of Bos d 2 in carpets and tiles was significantly lower than in samples from buildings where the barn and living quarters were together.

Five patients were excluded from further investigation because they had given up cattle farming immediately prior to the study and their homes were provided with new carpets.

Low IgE sensitization (CAP-RAST <0.7 kU/l; CAP-RAST class 0–1) was found in 13 patients while the other 27 showed relevant IgE sensitization (CAP-RAST >0.7 kU/l; CAP-RAST class >1). The house dust of patients with low sensitization contained significantly lower amounts of Bos d 2 than that of cow hair asthmatics with high sensitization (fig. 1).

The house dust Bos d 2 concentrations that separated patients with low (CAP-RAST <0.7 kU/l; CAP-RAST class 0–1) and high (CAP-RAST >0.7 kU/l; CAP-RAST-class >1) sensitization with the maximum Youden value were 24 µg/g dust for corridors, 20 µg/g dust for living rooms, and 29 µg/g dust for bedrooms. The maximum Youden value of the Bos d 2 discriminator for living rooms was 68.7 with a sensitivity of 77.8% and a specificity of 84.6% (fig. 2, table 5).

In all subgroups of the study population (groups A, B, and C) the mean value of serum IgE was increased. No statistically significant difference in serum IgE was found between the three subgroups. High sensitization to cow epithelia in RAST was more common in patients taking no measures of avoidance than in the others (table 6).

Allergic rhinitis, conjunctivitis, and urticaria were found more often in group C than in the other subgroups. Expectoration, difficult breathing, dyspnea on exertion and dyspnea during exposure to cold or humid air were noted more often in groups B and C than in group A, whereas coughing at night and dyspnea without exertion were more common in group B than in the other subgroups. Coughing in the daytime and dyspnea during exposure to dust or mist occurred approximately equally in all groups. The commonest symptoms were difficult breathing, dyspnea on exertion and dyspnea during exposure to dust or mist (table 7). The calculated symptom score (sum of symptoms) was significantly

Table 2. Concentrations of Bos d 2 (µg/g dust) in carpets, on tiles/linoleum, and in new carpets acquired after giving up cattle farming of patients with cow hair asthma

	Carpets		Tiles-linoleum		New carpets	
	N	mean ± SD	N	mean ± SD	N	mean ± SD
Corridor	17	79.1±95.7	23	34.0±42.0	3	0±0.0
Livingroom	35	85.0±119.1	3	3.3±5.8	7	3.4±9.1
Bedroom	34	85.6±95.5	2	0±0.0	7	2.4±6.4

		Corridor	Livingroom	Bedroom
Carpets vs. tiles		p<0.05	p<0.01	p<0.05
New carpet vs. carpets		p<0.05	p<0.01	p<0.001
New carpet vs. tiles		p<0.05	p = NS	p = NS

Table 3. Influence of different measures of avoidance on Bos d 2 quantity (µg/g dust) in carpets of house dust samples of patients with cow hair asthma

	Group A (N=14)		Group B (N=12)		Group C (N=19)	
	N	mean ± SD	N	mean ± SD	N	mean ± SD
Corridor	13	6.8±14.5	11	66.1±89.3	19	68.9±73.6
Livingroom	14	9.1±24.1	12	98.0±151.4	19	89.7±107.1
Bedroom	13	4.3±12.4	12	70.3±74.5	18	112.7±108.3

		Corridor	Livingroom	Bedroom
Group A vs. group B		p<0.001	p<0.001	p<0.001
Group A vs. group C		p<0.0001	p<0.0001	p<0.0001
Group B vs. group C		p = NS	p = NS	p = NS

Group A = Patients having abandoned cattle farming; group B = patients avoiding the barn; group C = patients without any measures of avoidance.

Table 4. Comparison of Bos d 2 quantity (µg/g dust) in house dust of cattle raising cow hair asthmatics related to architectural setup

	Barn and living quarters			
	separated		in the same building	
	N	mean ± SD	N	mean ± SD
Corridor*	17	40.6±42.5	13	103.6±99.7
Livingroom	17	82.4±115.7	13	112.1±139.6
Bedroom*	16	56.3±38.7	13	150.4±122.8

*p<0.05.

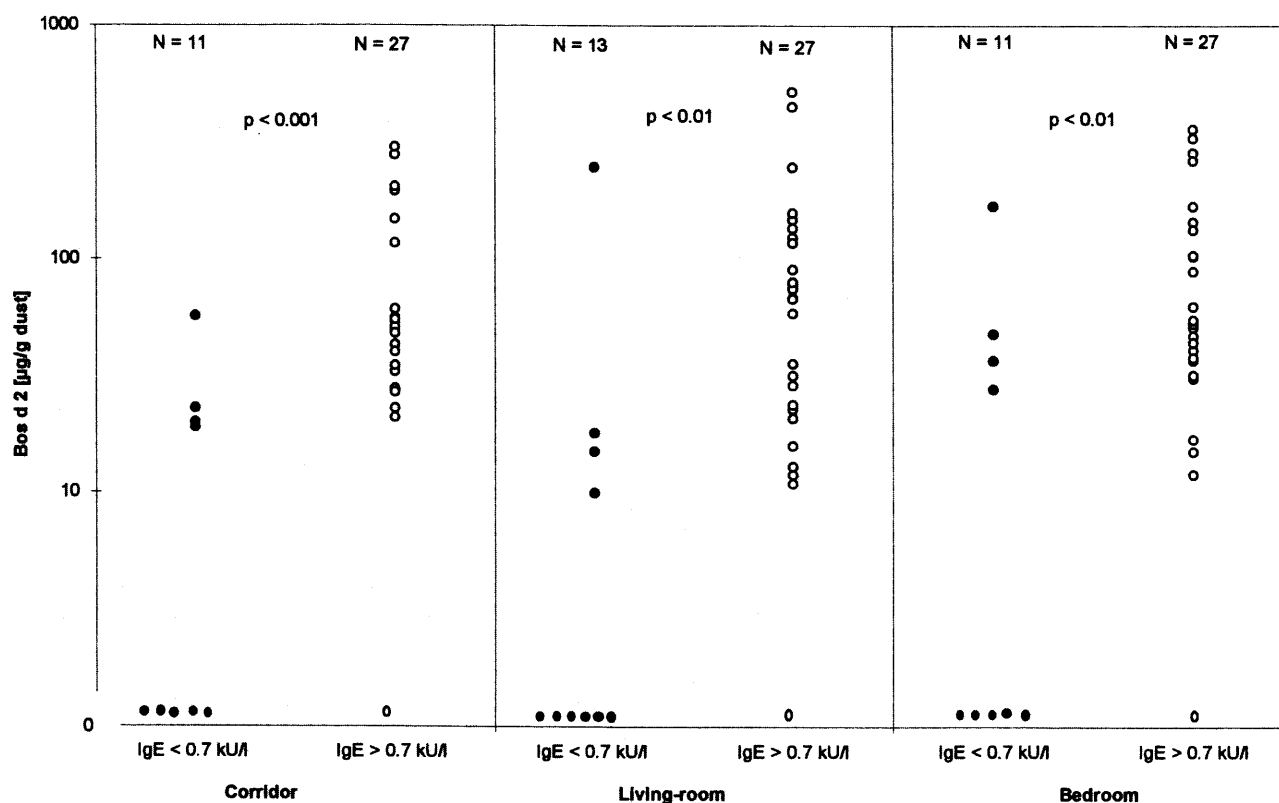


Fig. 1. Bos d 2 concentrations in house dust and degree of sensitization in CAP-RAST.

Table 5. Calculated house dust Bos d 2 threshold values for relevant IgE sensitization (CAP-RAST > 0.7 kU/l) towards cow epithelia

	Thres- hold Bos d 2	Maximum Youden value	Sensi- tivity %	Speci- ficity %	Correct classification	
					N	%
Corridor						
µg/g dust	24	68.7	77.8	90.9	31	81.6
Livingroom						
µg/g dust	20	62.4	77.8	84.6	32	80
Bedroom						
µg/g dust	29	45.1	81.5	63.6	29	76.3

Sensitivity = Percentage of patients with high sensitization and high house dust Bos d 2 concentrations (> calculated threshold allergen concentration); specificity = percentage of patients with low sensitization and lower house dust Bos d 2 concentrations (> calculated threshold allergen concentration); Maximum Youden value = maximum value of the equation (sensitivity + specificity - 100).

lower in patients without cows than in the others. Avoiding the cow barn with continuation of cattle farming by the partner (group B) did not lead to a reduced symptom score.

Discussion

Isolation and characterization of allergens in extracts from cow hair and dander have been studied [20–22]. These investigations demonstrated 23 allergens and characterized 4 of them (Ag 1, 3, 6, and 14) as major allergens [23]. Ag 1 (molecular weight 24 kD) and Ag 3 (20 kD) seem to be the most important concerning sensitization to cow hair and dander [21]. In the allergen nomenclature of 1988 [24] these allergens are designated as Bos d(omesticus) I and Bos d II, and later as Bos d 1 and Bos d 2 [25]. High amounts of these major allergens were found in cow hair and dander. They could also be identified in bovine whey, saliva, urine, amniotic fluid, beef, and leather [26].

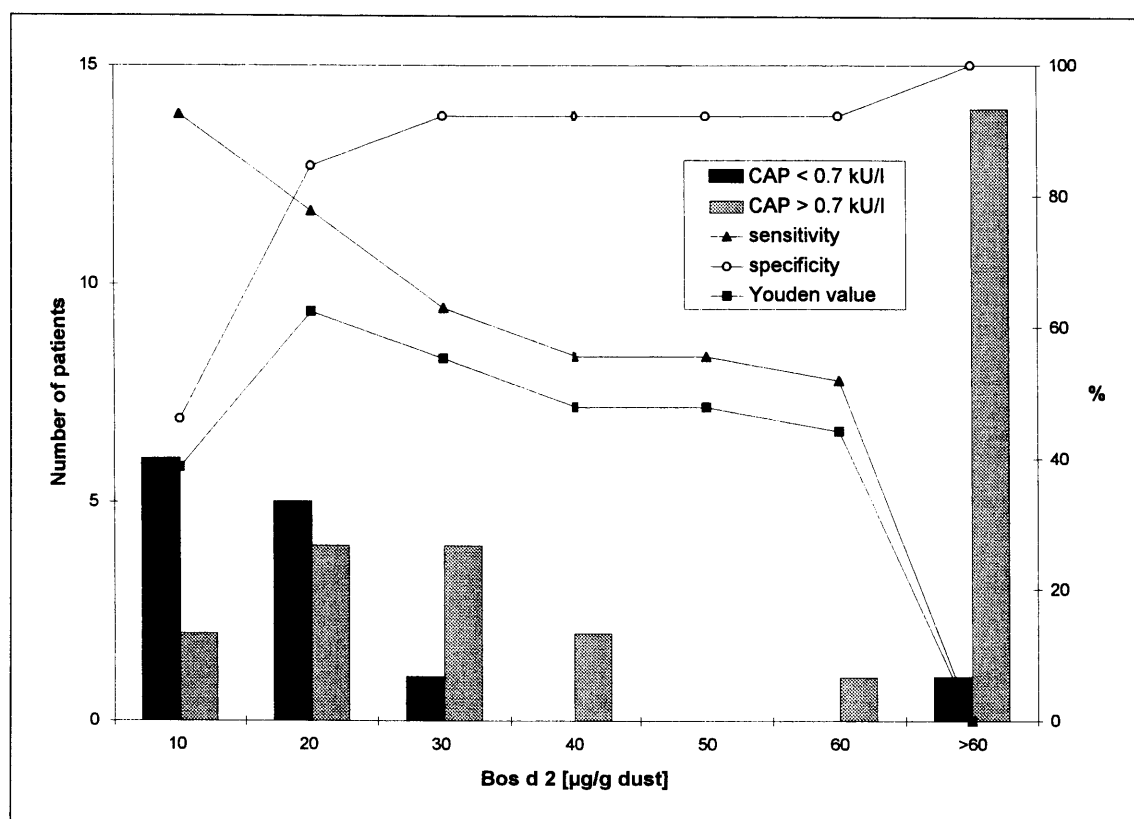


Fig. 2. Gerhardt plot for Bos d 2 concentrations in the house dust of living rooms. The discriminatory Bos d 2 concentrations are represented on the abscissa. The bars show the patients with CAP-RAST <0.7 kU/l and CAP-RAST >0.7 kU/l. The numbers of patients are shown on the left ordinate. Diagnostic sensitivity, specificity, and Youden value are indicated on the right ordinate.

We are not aware of any publication on the quantification of cow hair allergens in house dust samples. Only levels of airborne bovine epithelial antigen in barns have been determined by the ELISA inhibition method [27, 28]. In the present study the major allergen Bos d 2 of cow hair and dander in house dust samples was quantified by means of RIE. Bos d 2 concentrations on tiles and linoleum were significantly lower than in carpets, indicating that tiles prevent high local concentrations of allergen.

Furthermore, a separation of barn and living quarters leads to a marked reduction in Bos d 2 in house dust. Concerning the measures of avoidance, it was remarkable that only the cessation of cattle farming is accompanied by a significant reduction in Bos d 2 levels in carpets, and a reduced sensitization towards cow epithelia in RAST. Avoiding the barn with continuation of cattle farming by the partner is not a suitable method to reduce allergen concentrations in house dust. The degree of sensitization seems to depend on the amount of Bos d 2 in house dust. Allergen concentra-

Table 6. Degree of sensitization of cow hair asthmatics related to different measures of avoidance

	Group A (N = 9)	Group B (N = 12)	Group C (N = 19)
CAP-RAST <0.7 kU/l; CAP-RAST class 0–1	6	5	2
CAP-RAST >0.7 kU/l; CAP-RAST class >1	3	7	17

$$\chi^2 = 9.43; p < 0.01.$$

Group A = Patients having abandoned cattle farming; group B = patients avoiding the barn; group C = patients without any measures of avoidance.

Table 7. Clinical symptoms of cow hair asthmatics related to different measures of avoidance

	Group A (N = 14)		Group B (N = 12)		Group C (N = 19)	
	N	%	N	%	N	%
Allergic rhinitis	2	14	3	25	9	47
Allergic conjunctivitis	2	14	2	17	7	37
Expectoration	3	21	8	67	9	47
Coughing at daytime	4	43	6	50	8	42
Coughing at night	1	7	7	58	4	21
Difficult breathing	9	64	10	83	16	84
Dyspnea without exertion	2	14	5	42	2	11
Dyspnea on exertion	10	71	12	100	16	84
Urticaria	0	0	2	17	9	47
Flu-like prodromi	0	0	1	8	1	5
Dyspnea during exposure to cold or humid air	8	51	10	83	13	68
Dyspnea during exposure to dust or mist	8	86	12	100	16	84
Mean number of symptoms						
Group A	4.0					
Group B	6.5					
Group C	5.7					
Comparison of number of symptoms						
A vs. B	p<0.05					
A vs. C	p<0.05					
B vs. C	NS					

tions of 20–29 µg/g dust can be considered as a threshold value for IgE sensitization, suggesting that long-term exposure to cow hair allergens in house dust is as important to the degree of sensitization as the shorter time of exposure in the cow barn.

A relation between high cow hair allergen sensitization and the occurrence of respiratory symptoms has been re-

ported, the prevalence of allergic rhinitis and asthma in sensitized subjects being 27% [29–32].

In the present study, symptoms of allergic rhinitis and conjunctivitis occurred mainly in patients taking no measures of avoidance, whereas symptoms of nonspecific hyper-reactivity (such as dyspnea during exposure to cold air or dust) were common in all patients. The latter may be a consequence of long-term unsheltered exposure to cow hair allergens.

Patients without cows showed a significantly lower symptom score than others, and their house dust samples had lower Bos d 2 levels. Therefore, it can be concluded that giving up cattle farming is the only suitable measure of avoidance. High sensitization in CAP-RAST occurred more often in patients still farming cattle than in those without cows. This suggests that avoiding the barn alone is not accompanied by a reduced level of cow hair-specific IgE, if cattle raising is continued by the partner.

The results of this study underline the importance of quantitative measurements of allergens at the place of work and in house dust samples. Farmers usually enter living quarters in their working clothes (e.g. in barns), resulting in the transfer of allergens, besides those that are transferred airborne.

Both atopic and nonatopic subjects suffer from cow hair asthma, therefore cattle-raising farmers should take the following measures: (1) Change all clothing before entering the living quarters. (2) Barn and living quarters should be separated. (3) Install tiles or linoleum instead of carpet.

Farmers suffering from cow hair asthma should (if possible) cease cattle farming; thorough cleaning of the entire house may serve to avoid a continuous cow allergen exposure for months or years. The success of these measures can be assessed by means of RIE.

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