

Dust from carpeted and smooth floors. I. Comparative measurements of antigenic and allergenic proteins in dust vacuumed from carpeted and non-carpeted classrooms in Norwegian schools

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Summary

Dust samples from fitted-carpeted and linoleum floors in 12 schools in Norway were collected by vacuum cleaning. The presence of antigens and allergens of alder (*Alnus incana*), birch (*Betula verrucosa*), timothy (*Phleum pratense*), mould (*Cladosporium herbarum*), house dust mite (*Dermatophagoides farinae*), cat and dog dander, codfish, hen egg white and human dander were investigated by crossed immunoelectrophoresis (CIE), crossed radio-immunoelectrophoresis (CRIE) and radio-allergosorbent test (RAST) inhibition. No qualitative differences in allergen contents of dust from both types of floor tested were noted. Similarly, no relationship could be demonstrated between floor-type and allergen concentration under identical experimental conditions. Antigens and allergens of both cat and dog were frequently demonstrated in dust extracts. All extracts included human dander and mould allergens. In addition, most dust samples from both carpeted and smooth floors contained hen egg white and codfish allergens. Furthermore, the study demonstrated that dust from smooth floors and fitted-carpeted was relatively free of mite and pollen from alder, birch and timothy.

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Introduction

Attention has been directed to the disadvantages of carpeted floors as one of the causes of type I allergic symptoms. Carpeted floors have been criticized and are suspected to be a reservoir of dust and allergens [1-5].

No data are available on the quantities of allergenic proteins in dust from carpets as compared to smooth floor coverings. The purpose of the present study was to provide this necessary information for an objective report. In this communication we report a paired collection of dust samples from these two types of floor coverings from schools with different geographical locations, and an estimate of the concentrations of a panel of selected allergens.

Materials and methods

Sample collection

The dust samples were obtained from five schools in

Eastern Norway and seven schools in Western Norway during April to September. The 24 classrooms included in this study were equipped with either fitted-carpeted or smooth floor coverings. The practical procedures of dust collection were performed as follows: in each school, samples from one carpeted and one non-carpeted classroom were collected by daily vacuum cleaning of the whole floor for 20 min at the end of each school day for 5 days. The mouthpiece of each vacuum cleaner was thoroughly cleaned before use and disposable paper bags were replaced. Cross-contamination between samples from smooth and carpeted floors was hence avoided. The same make of vacuum cleaner was used to collect dust samples from carpeted and smooth floor coverings in the respective schools. Samples from carpeted floors were collected twice, at different seasons, and analysed individually. In addition, samples of carpets (dimensions 20 × 30 cm) were taken from investigated classrooms. Dust and carpet samples were sealed in plastic bags and sent to the laboratory where they were stored at +4°C until extracted. A total of 21 dust samples from carpeted

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floors, 12 samples from smooth floors and 12 carpet samples were collected. A questionnaire was completed with pertinent information including cleaning routines, frequency of vacuum cleaning, methods and date for the last carpet cleaning, age of the carpet, room temperature, number of windows that could be opened, room function, topographic location of the school (nearby river, marsh, sea, deciduous wood or meadow) and technical data of the vacuum cleaner. The dust samples from carpeted floors (CF), smooth floor coverings (SF) and carpet segments (C) were enumerated CF001–CF021, SF051–SF062 and C031–C042, respectively.

Extraction

Dust extracts were prepared by suspending 20 g dust in 0.05 M NH_4HCO_3 (pH 8.0) to a total volume of 1000 ml. The suspensions were gently shaken for 24 hr at 4°C and filtered (Schleicher & Schüll Folded Filters, Dassel, FRG). Carpet samples were agitated in 1000 ml 0.05 M NH_4HCO_3 (pH 8.0) for 24 hr at 4°C before filtration. The extracts were lyophilized and stored at –20°C until used.

Allergens

Lyophilized extracts of *Dermatophagoides farinae* and *Cladosporium herbarum* were kindly provided by Dr S. Dale, Nycomed, Oslo, Norway. Lyophilized extracts of timothy pollen, cat and dog dander were supplied by Allergologisk Laboratorium København (ALK), Copenhagen, Denmark. Crude hen egg white extract, partially purified codfish allergen (D III) extract and crude birch and alder pollen extracts were prepared in our laboratory as described previously [6–9].

Rabbit antisera

Rabbit antisera against extracts of the various allergens were produced as described by Harboe and Ingild [10]. Groups of three animals were injected intramuscularly with 0.15 mg of lyophilized extracts of either house dust mite, timothy pollen, cat and dog dander, birch and alder pollen and codfish, respectively. Antigens were emulsified in 50 µl saline and 50 µl Freund's complete adjuvant (FCA). Rabbits immunized with egg white extract [7] received an emulsion of 0.5 ml antigen and 0.5 ml FCA. Booster injections containing the same amount of antigen in Freund's incomplete adjuvant were given every second week for 2 months, then every fourth week. Ten days after the last injection the rabbits were killed under anaesthesia. The three antisera from each group were respectively pooled and stored at –20°C until used.

Rabbit anti-human serum was supplied by Norsk Hoechst, Oslo, Norway.

Human sera

Sera were collected from atopic patients with elevated IgE, clinical history of allergy and RAST class 2–4 to the respective allergen. Fifty patients who had not undergone hyposensitization therapy donated blood samples after informed consent. Sera from a minimum of six patients were pooled and stored at –20°C until used.

Protein concentrations

The protein concentrations of lyophilized extracts (10 mg/ml) were determined by the method of Lowry *et al.* [11], using bovine serum albumin as a standard. Absorbances were registered at 750 nm and standard calibration curves were constructed daily.

RAST inhibition

RAST inhibition tests were performed essentially as described by Yman *et al.* [12] using allergen coated paper discs (Phadebas, Pharmacia AB, Uppsala, Sweden). The 50% inhibition values could not be calculated due to low concentration and great variation in contents of birch, alder and timothy pollen, hen egg white and mite in the extracts. RAST inhibition titration curves for all allergens would require extended sampling periods to obtain sufficient allergens to estimate reliable 50% inhibitions. Therefore, the RAST inhibition was calculated on the basis of identical concentrations of the lyophilized extracts and specific patient sera, and expressed as a percentage using the formula:

$$\frac{(\text{c.p.m. of non-inhibited serum} - \text{c.p.m. of inhibited serum}) \times 100}{\text{c.p.m. of non-inhibited serum}}$$

The presence of the nine different allergens in each dust sample was determined in duplicate using the following procedure: 100 µl of a 20 mg/ml solution of lyophilized dust extract were added to 50 µl of pooled human serum and incubated for 1 hr at room temperature. An allergen disc coated with appropriate allergen was added to the mixture and incubated for 3 hr. The disc was further incubated overnight with 50 µl [^{125}I]anti-IgE (Phadebas RAST isotope, Pharmacia AB, Uppsala, Sweden) and the percentage inhibition calculated by the above formula. For controls (non-inhibited sera) dust extracts were substituted by buffer. The allergen contents of all samples were compared by their percentage inhibition values using identical amounts of freeze-dried extracts. The allergen contents were confirmed by RAST inhibition titration curves when the concentration of allergens in the samples allowed their construction.

Immunoelectrophoresis

Crossed immunoelectrophoresis (CIE) was performed essentially as described previously [13,14]. Twelve millilitres of 1% (w/v) agarose gel (Agarose M, LKB, Sweden) in 0.073 M Tris, 0.024 M barbital, 0.006 M calcium lactate and 0.003 M sodium azide (pH 8.6) were poured onto 8 × 9 cm² glass slides and allowed to set. Aliquots of 250 µg lyophilized dust samples in 5 µl were applied to 2.5 mm diameter wells for the first-dimension electrophoresis using 10 V/cm for 45 min. Gels for the second dimension contained, respectively, 8 µl/cm² antiserum against house dust mite, 10 µl/cm² antiserum against cat dander, 12 µl/cm² antiserum against crude hen egg white, dog dander, codfish (D III) and crude timothy pollen and 24 µl/cm² antiserum against crude birch and alder pollen. Intermediate gels were neutral except for the CIE with rabbit antiserum against cat dander in the antibody gel, where the intermediate gel included 8 µl/cm² of rabbit anti-human serum. This modification was made because the rabbits were immunized with a commercial extract of cat dander which included human serum albumin (HSA), and antibodies against both cat dander and HSA were produced. The intermediate gel containing rabbit anti-human serum was therefore used to trap the HSA in the dust extracts, avoiding the complication of the CIE pattern.

Autoradiography

Dried immunoelectrophoresis plates were incubated overnight at room temperature in 7 ml of 75 mM phosphate buffer, pH 7.2 containing 0.9% sodium chloride, 0.3% sodium azide, 2% Tween 20, 0.1% EDTA and 0.5 ml of the reaginic serum. Gels were washed three times for 10 min with physiological saline, followed by a single wash with 10 ml distilled water and incubation with approximately 2.8 × 10⁵ c.p.m. of [¹²⁵I]-anti-IgE dissolved in 7 ml incubation buffer overnight. Autoradiography was performed with X-ray film (Kodak X-Omat AR). Films were exposed to dried CIE plates at -80°C for 2 days [15].

Results

Allergens detected by RAST inhibition

The results of RAST inhibition of the dust samples are summarized in Table 1.

Dust extracts tested on allergenic activity of alder, birch and timothy pollen showed low RAST inhibition values, indicating minor allergenic activity. However, the samples CF009, CF014 and SF059 gave a RAST inhibition for birch of 60, 69 and 67%, respectively. These

Table 1. Mean protein concentrations in mg per 100 mg lyophilized dust extracts and estimations of allergenic activities of alder, birch, timothy, cat, dog, mite, hen egg white, codfish and mould as expressed by percentage RAST inhibition

Protein	Mean ± s.d. (range)			P values
	Carpeted floors (n = 21)	Smooth floors (n = 12)	Carpet samples (n = 12)	
Protein	9.1 ± 3.1 (4.6–14.9)	6.3 ± 2.3 (4.2–10.4)	4.2 ± 2.3 (0.5–7.7)	P < 0.01
Alder	11 ± 15 (0–22)	9 ± 6 (0–18)	10 ± 6 (0–20)	P = 0.5
Birch	18 ± 18 (0–69)	33 ± 17 (5–67)	25 ± 16 (0–44)	P < 0.01
Timothy	7 ± 6 (0–21)	4 ± 5 (0–12)	8 ± 8 (0–14)	0.05 < P < 0.1
Cat	45 ± 18 (5–72)	54 ± 10 (41–70)	34 ± 17 (1–49)	0.1 < P < 0.2
Dog	39 ± 20 (6–72)	25 ± 12 (6–42)	12 ± 9 (0–23)	P = 0.02
Mite	18 ± 11 (0–49)	25 ± 9 (15–38)	16 ± 10 (4–33)	0.05 < P < 0.1
Egg white	13 ± 12 (0–34)	19 ± 12 (0–41)	5 ± 3 (0–9)	P = 0.2
Codfish	54 ± 24 (4–85)	61 ± 22 (10–80)	12 ± 8 (0–22)	0.2 < P < 0.5
Mould	60 ± 15 (30–84)	47 ± 22 (14–78)	33 ± 6 (25–42)	0.05 < P < 0.1

Percentage RAST inhibition values of dust extracts from carpeted and smooth floors were analysed by Student's *t*-test. *P* values are for comparisons of carpeted and smooth floors.

samples were collected from schools in the vicinity of birch forests.

Cat allergens were detected in all samples. The RAST inhibition values varied from 5 to 72% in samples from carpeted floors, from 41 to 70% in samples from smooth floors, and from 1 to 49% in carpet samples.

Dog allergens were detected in all samples from smooth and carpeted floors. The highest allergenic activity was detected in CF007, and the paired carpet sample C037 showed the highest RAST inhibition value in the carpet series.

Samples CF007, CF021, SF051, SF061 and C041 showed the highest values of RAST inhibition for house dust mite. These samples were collected from schools in Western Norway, where the relative air humidity is high (approximately 80%).

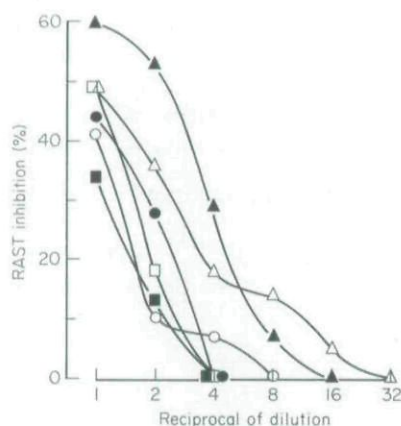


Fig. 1. Inhibition of IgE binding to RAST discs of cat allergens using dust extracts from three different schools: (a) (●) CF005 and (○) SF051; (b) (▲) CF001 and (△) SF057; (c) (■) CF006 and (□) SF052. The initial concentration of freeze-dried dust extract was 2 mg.

Allergenic activity of hen egg white was detected in most samples from both carpeted and smooth floors. The highest value of RAST inhibition was obtained in SF053, the only dust sample collected in a room used for lessons in cooking.

Codfish (D III) allergens were detected in most extracts by RAST inhibition. The inhibition in the carpet sample extracts varied from 0–22%, indicating the presence of minor allergenic material.

All the analysed samples showed allergenic activity of the mould *Cladosporium herbarum*. The lowest allergenic activity was shown in SF059 (RAST inhibition 14%), while the highest value of inhibition was obtained by CF019 (84%).

The detection of allergens was confirmed by RAST inhibition titration curves for the various dust extracts whenever the concentrations of allergens were above the detection limits of the test used. Figure 1 shows examples of RAST inhibition titration curves of dust samples showing allergenic activity of cat dander.

Comparison of allergens in dust from smooth and carpeted floors by RAST inhibition

Statistically significant differences ($P \leq 0.02$, Student's *t*-test) between carpeted and smooth floors were obtained for total protein concentrations, birch pollen and dog dander allergens (Table 1). For total protein and dog dander allergens the highest amounts were found in dust from carpeted floors, whereas the highest amount of birch pollen allergens was in dust from smooth floors.

For all other allergens the amounts in carpeted and smooth floors were not significantly different, although

Table 2. Number of dust extracts from carpeted floors, smooth floors and carpet samples containing antigens and allergens of alder, birch, timothy, cat, dog, mite, hen egg white, codfish and HSA as assessed by CIE and CRIE

	Carpeted floors (n=21)		Smooth floors (n=12)		Carpet samples (n=12)	
	Ag	Al	Ag	Al	Ag	Al
Alder	0	0	0	0	1	0
Birch	1	0	0	0	0	0
Timothy	0	0	0	0	0	0
Cat	5	1	1	1	1	0
Dog	8	11	9	10	0	0
Mite	4	0	0	0	0	0
Egg white	18	5	10	3	1	0
Codfish	0	0	0	0	3	4
HSA	21	n.d.	12	n.d.	8	n.d.

Ag=antigens; Al=allergens.

n.d.=not determined.

P values between 0.05 and 0.1 were calculated for timothy pollen, mite and mould. The type of flooring demonstrating the highest levels of allergen varied from school to school.

The probability of detecting a significant difference, if it actually existed (power of the test) varied and was dependent upon the allergens tested. For four of the allergens (alder, timothy, mite and hen egg white) the probability of detecting a difference, if it existed, exceeded 90%, for cat 76%, mould 51%, and for codfish 41%. On this basis a type II statistical error seems unlikely.

Antigens and allergens detected by CIE and CRIE

Approximately 450 CIE/CRIE runs were performed in this investigation. The CIE and CRIE observations of the various dust samples are summarized in Table 2.

The peaks obtained in CIE were compared to reference patterns produced by ourselves and other laboratories [8,9,16–26]. One to two precipitation lines were accepted as a positive signal for the presence of the antigen in question. No trial was done to establish ideal CIE patterns. Antigens from cat and dog dander were detected in seven and 17 dust samples, respectively, while those from alder and birch pollen were only found in two samples. Furthermore, house dust mite antigens were identified in four samples from carpeted floors. Unexpectedly, hen egg white could be detected in 29 of the 45 dust

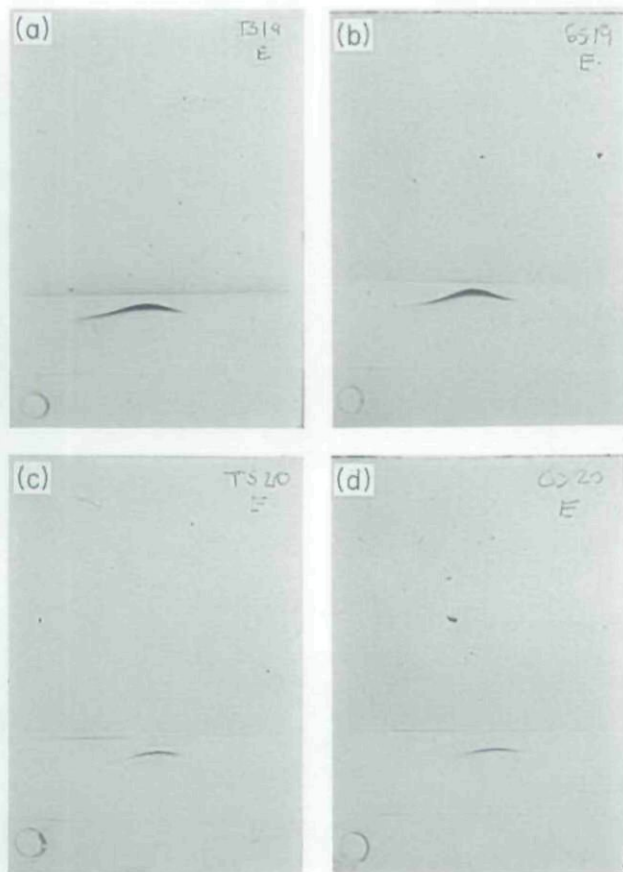


Fig. 2. CIE of 250 μ g lyophilized dust extracts (a) CF014; (b) SF058; (c) CF001 and (d) SF057. The first-dimension electrophoresis was performed towards the anode at 10 V/cm for 45 min, the second-dimension electrophoresis at 2 V/cm for 20 hr using 12 μ l antiserum against crude hen egg white/cm² in the anodic antibody-containing gel.

samples. Figure 2 demonstrates the presence of hen egg white antigen in the dust extracts as shown by the CIE Coomassie brilliant blue-stained precipitation lines. Codfish antigens were identified in three of the 12 carpet samples, but were not available in any of the dust samples collected from smooth and carpeted floors. HSA was identified in all dust samples from carpeted and smooth floors, while eight of the 12 carpet samples included HSA.

Allergens from hen egg white (with ovalbumin as the most dominant major allergen), cat and dog were identified in dust samples from carpeted and smooth floors. Figure 3 illustrates the detection of hen egg white allergens in dust samples. Several allergens could easily be visualized in CRIE. Codfish (D III) allergens were present in four out of 12 carpet samples, while those of alder, birch and timothy pollen could not be detected by CRIE.

Discussion

The concern about the use of carpets, particularly in schools [1,2,27,28] was the impetus for this study. Carpets in schools and offices have been suspected to be of risk for patients with asthma and allergy [1–5]. It is generally assumed that fitted carpets act as a depot for allergens and increase the severity of allergy symptoms. We particularly wanted to investigate whether there were differences in allergen content in dust of different types of floor coverings. Epidemiological data on the number of children who regularly occupied the classrooms who were skin-test positive to the detected allergens or showed symptoms of atopy are not included in this investigation. The prevalence of asthma among schoolchildren in Norway has been covered previously [29–31]. A limited study correlating anamnestic data, SPT and histamine release from sensitized normal basophils to total and specific IgE levels among children from the classes investigated is in progress.

A selection of a relatively broad panel of nine allergens in dust from smooth and carpeted floors was chosen for this first investigation. The allergens selected for analysis were chosen as to their clinical importance and expected presence in the dust collected. The amounts of allergens detected in this investigation will theoretically, as airborne, be capable of eliciting allergic reactions.

In spite of the view that school environment probably represents no significant reservoir for allergens [32], our investigation suggests that children were in contact with cat and dog allergens in most of the investigated classrooms. Patients allergic to animals are often sensitive to floor dust since cat and dog dander are common constituents [33]. It is accepted that cat and dog derived allergens are brought into schools from outside contact with pets. Significant amounts of dog allergens have been found in dust samples from homes in which dogs have never been kept [34].

Most dust samples contained hen egg white and codfish allergens detectable by specific IgE inhibition, indicating that schoolchildren are exposed to these allergens in classrooms. The presence of these allergens is probably due to remains from pupils' lunches eaten in the classrooms, or contamination from classrooms used for cooking. A major source of codfish allergen could be processed cod roe caviar, usually used in lunch sandwiches. Samples SF053 was collected in a classroom used for cooking and obviously included a particularly high content of hen egg white and fish allergens.

Mould inhalant allergens were expected to be present in the dust extracts [35,36]. This was emphasized by the detection of allergens of *Cladosporium herbarum* in all the dust samples.

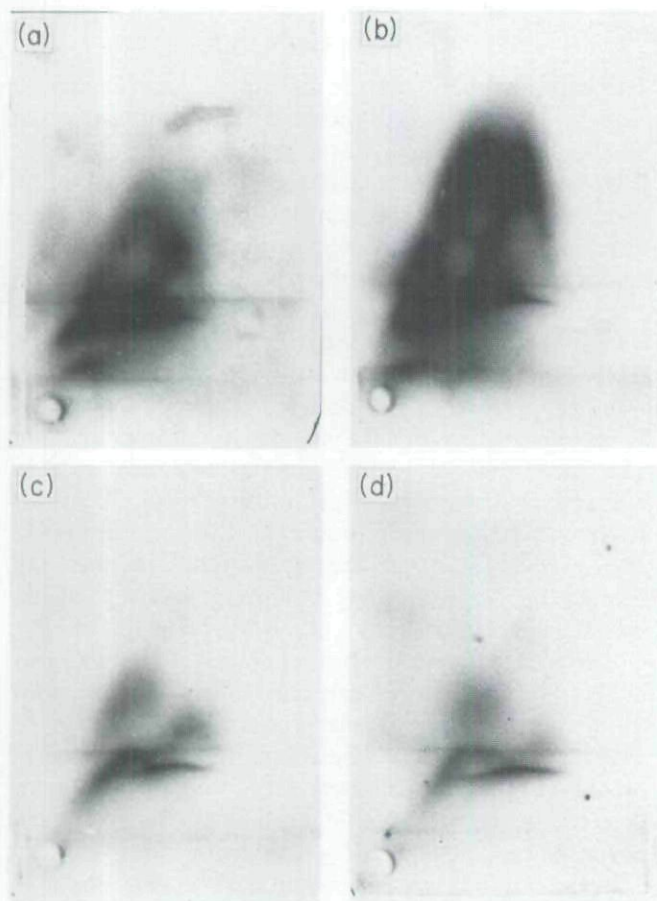


Fig. 3. CIE of the dust extracts shown in Fig. 2 a–d. The CIE plates were incubated with 0.5 ml serum from hen egg white-allergic patients in 7 ml CIE buffer followed by incubation with [^{125} I]-anti-IgE corresponding to 35 000 c.p.m./ml. The CIE plates were then subjected to autoradiography for 2 days. The plates show autoradiographic blacking of allergens not detected by Coomassie brilliant blue staining in CIE, demonstrating the sensitivity differences of the two techniques.

House dust mites represent another potential source of allergens in schools. Four dust samples collected in April from carpeted floors in schools in the western part of Norway contained mite antigens. House dust mites were not present in dust samples from schools in Eastern Norway, where the relative humidity and ambient temperature are lower than on the west coast of the country. Obviously the low mite counts in schools, compared to homes, are due to frequent carpet cleaning and the absence of bedrooms [32].

Floors of the investigated classrooms were relatively free of pollen allergens from birch, alder and timothy. Exceptions were CF009, CF014 and SF059 which included a high content of birch pollen allergens. These samples were collected in schools located in the vicinity of birth tree forests on 16 May, 8 June and 14 June, which are in the birch pollen season. The low contents of pollen allergens indicate that tree pollen allergens are topographically restricted problems. The low timothy allergen concentrations in the samples may be due to the fact that the collecting procedures during springtime were terminated before the peak of the timothy pollen season.

Allergens were detected in extracts of carpet samples,

verifying that proteins are retained in these after vacuum cleaning. The carpet samples investigated represent a minor part of the total floor area, and were used to quantify the allergens retained within the carpet fibres.

We have found no qualitative or quantitative differences in allergen content between dust from carpeted and smooth floors in the investigated schools. The cleaning procedures in the investigated schools were satisfactory: the smooth floors were washed and the carpeted floors vacuumed daily. The frequent vacuum cleaning schedule used was possibly the reason why we could not detect any difference in allergen content between smooth and carpeted floors. Similar studies on dust collected from selected carpets and bare floors reported higher contents of organic macromolecular components from the carpets [1,37]. Contrary to our investigation, the carpet cleaning was infrequent and the protein allergens were neither qualitatively nor quantitatively estimated.

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