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Allergen quantification in surface dust samples from German day care centers

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ABSTRACT

Indoor allergens are among the main causes of allergic rhinitis and asthma. Allergen exposure is not limited to private homes. Mite, cat, and dog allergens were measured in day care centers to determine whether these concentrations detected might exert significant influence on human health. In 20 day care centers across North Rhine–Westphalia, Germany, the surfaces of 171 rooms were vacuumed 4 times a year to collect dust (1340 samples in total). In all samples, domestic mite antigens (DM) and the main allergens of cats (Fel d 1) and dogs (Can f 1) were quantified using enzyme immunoassays. Provisional threshold limits (PTL) for increased risks of sensitization and allergic symptoms were estimated according to published values and conversion factors. The influence of room characteristics on allergen concentrations was analyzed in mixed linear models, also considering values below the limit of detection (LOD). Nearly all samples contained allergens (99% DM, 96% Fel d 1, and 96% Can f 1). The concentrations rarely exceeded levels that were previously found to induce symptoms in home environments, but were frequently higher than estimates for enhanced sensitization risk (13% DM, 43% Fel d 1, and 27% Can f 1). Upholstered furnishings had the highest dust and allergen loads, followed by carpets and smooth floors. Allergen concentrations on different surface types that were sampled in the same room at the same time were significantly correlated and analyzed in separate models. The highest DM concentrations were present in bedrooms and in autumn. Further, DM loads on floors decreased significantly in rooms that were renovated within the last 5 years. If there were no records that furnishings were vacuumed, there were then significantly higher Can f 1 loads. Sweeping floors elevated DM and cat allergen concentrations. In addition to mite allergens, cat and dog allergens were detected in nearly all samples from day care centers. Overall, the present results indicate that allergen concentrations may be reduced by renovation and appropriate cleaning procedures.

Introduction

Children sensitized and exposed to mite, cat and dog allergens have a significantly increased risk of wheezing (Gent et al. 2012; Annesi-Maesano et al. 2013). Individuals who are sensitized to these perennial allergens in the first 3 yr of life exhibit poor lung function, particularly when combined with high exposure to the sensitizing allergen (Illi et al. 2006). In these studies, surface dust samples from floors and furnishings were collected from the homes of the children. However, this is not the only source. Previous studies originating in different countries found mite and pet allergens in vacuumed dust of kindergarten and day care facilities (Annesi-Maesano et al. 2013; Arbes et al. 2005; Cyprowski et al. 2013;

Engelhart et al. 2002; Fernández-Caldas et al. 2001; Instanes et al. 2005; Oldfield et al. 2007; Salo et al. 2009; Tranter et al. 2009; Wickman et al. 1999; Zuraimi et al. 2008).

Children in Germany spend increasing proportions of their time in day care. In 2015, 94.9% of all children between the ages of 3 and 6 years were in regular child care and 43.9% of these spent more than 7 h/d under such care (Statistische Ämter des Bundes und der Länder. 2016). Therefore, allergen exposure outside the home environment needs to be taken into consideration.

In order to measure allergen concentrations in day care centers across Germany, 20 centers were visited 4 times during the different seasons, and vacuumed

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dust from floors and upholstered furnishings was analyzed for mite, cat, and dog allergens. In contrast to previous studies in day care facilities, this was the first study where sampling was performed during all four seasons. In addition, several rooms from each facility, including corridors, group rooms, side rooms, sleeping rooms, and staff rooms were examined. The influence of season, room type, facility and room characteristics, and cleaning procedures was determined on allergen loads, and concentrations on different surfaces were analyzed by multivariate analysis taking into account values that were below the limit of detection (LOD).

Methods

Sampling and Extraction of Surface Dust in Day Care Centers

Sampling took place in 20 day care centers across North Rhine–Westphalia in Germany from 2010 until 2012. Permission for sampling at the facilities was obtained from employers and involved employees. The day care centers were attended by 1–7 groups of 20–25 children (25–160 children per facility) and consisted of 4–11 rooms. The day care centers were visited 4 times, in autumn (3.11–7.12), winter (17.01–18.03), spring (29.03–27.05) and summer (31.05–13.09). Relative air humidity was measured at the time of each visit, and a questionnaire on usage, furniture, and room dimensions and cleaning procedures was completed. Only animals without hair or fur, such as reptiles and fish, were kept in the day care centers. Out of 1077 children (or their parents) who answered the question on pet ownership, 178 (16.5%) had a cat and 140 (13%) a dog at home. Children in all groups had pets at home. However, data on pet ownership were not obtained for all groups throughout the year and therefore are not included in the analysis.

Dust samples were collected from 61 group rooms, 59 adjoining side rooms, 20 staff rooms, 13 bedrooms, and 18 corridors. ALK dust collection cassettes (ALK-Abello, Hørsholm, Denmark) with glass fiber filters (Macherey & Nagel, Düren, Germany) that had been preweighed in a climate-controlled room with constant temperature and air humidity were attached to vacuum cleaners, and areas of 0.8 m² on smooth floors, 0.4 m² on carpets, and 0.2 m²

on upholstered furnishings were vacuumed using rectangular templates with 0.2 m² space. The space within a template was vacuumed for 1 min. Within the next 3 d, samples were frozen (–20°C) for 24 h to destroy mites. After conditioning for 24 h in the climate-controlled room, dust load on the filters was weighed. As a reference, three filters were preweighed and reweighed at each weigh-in. These values were used to calculate mean plus three times the standard deviation to estimate LOD for weighing (0.41 mg/filter). Filters below LOD of dust were excluded from the study because it was assumed that a mistake was made during vacuuming or sample management. Filters with floor dust were extracted in 15 ml phosphate-buffered saline (PBS) with 0.05% Tween 20, and rotated for 1 h at room temperature. All extracts were centrifuged for 15 min at 3000 × g, and supernatants were stored in aliquots at –80°C until testing.

Allergen Quantification With Fluorescence Enzyme Immunoassays (FEIA)

The domestic mite (DM) immunoassay is based on polyclonal antibodies to *Dermatophagoides farinae* allergens and detects antigens from several domestic mite species (Sander et al. 2012). Therefore, concentrations are higher compared to assays used to detect single allergens, such as Der f 1 or Der p 1. According to a previous comparison, significant correlation of DM to Der f 1 values was obtained, but DM values were approximately 144-fold higher than Der f 1 levels obtained using the monoclonal antibody-based immunoassay and multiallergen standard from Indoor Biotechnologies (Sander et al. 2012). To detect the main allergens from cat and dog, monoclonal antibodies and multiallergen standards were purchased from Indoor Biotechnologies Ltd. (Charlottesville, VA). The kit, EL-FD1, was employed to measure Fel d 1. For detection of Can f 1, the monoclonal antibody 6E9 was biotinylated with a 33 molar excess of biotin-ε-aminocaprylic-*N*-hydroxy-succinimide ester (Roche, Mannheim, Germany), and monoclonal antibody 10D4 was utilized as the capture antibody. All assays were performed as amplified fluorescence enzyme immunoassays (FEIA) using streptavidin peroxidase conjugate poly-HRP80-SA (Fitzgerald, Concord, MA) and fluorogenic substrate, Quanta blue (ThermoScientific, Rockford, IL). Sample

concentrations were calculated by interpolation of optical density (OD) values or relative fluorescence units on a four-parameter fitted standard curve using Softmax Pro 5.4.1 (Molecular Devices, Sunnyvale, CA, USA). The LOD for amplified FEIA were 0.05 ng/ml for DM, and 0.01 ng/ml for Fel d 1 and Can f 1. Results obtained for Fel d 1 and Can f 1 in the amplified FEIA were similar those obtained in the Indoor multiplex array (Sander et al. 2013). In order to compare the results obtained with Indoor multiallergen standard to those in the published literature using the Indoor enzyme-linked immunosorbent assay (ELISA) standard, the following conversion factors need to be used: 12.7 for Der f 1, 3.4 for Fel d 1, and 5.9 for Can f 1 a (Filep et al. 2012a). The following cutoff points based on ELISA measurements were employed as provisional threshold allergen levels in vacuumed dust: 10 µg/g for Der f 1 or Can f 1, and 8 µg/g Fel d 1 for symptoms, and 2 µg dust mite allergen or Can f 1 and 1 µg Fel d 1 for sensitization (Salo et al. 2008; 2009; Tranter et al. 2009). Using these thresholds and conversion factors for data obtained in the present study, the cutoff points for symptom- or sensitization-based threshold concentrations are as follows: domestic mite antigens 110 µg/g ($10 \mu\text{g/g} \times 144/12.7$) and 22 µg/g; for Can f 1 1.7 µg/g ($10 \mu\text{g/g}/5.9$) and 0.34 µg/g; and for Fel d 1 2.4 µg/g ($8 \mu\text{g/g}/3.4$) and 0.29 µg/g, respectively.

Statistical Analysis

Data management and descriptive data analysis were performed using SAS, version 9.4 (SAS Institute, Inc., Cary, NC). The potential influences of variables on the concentrations of dust and allergens per vacuumed area stratified by different surfaces were examined using hierarchical models with three levels (level 1: collected sample, level 2: room of collection, level 3: day care center). In order to account for left-censoring in dependent variables, attributed to observations below LOD, normal mixed-effects models with censored responses were applied. A maximum likelihood approach was selected using the expectation maximization algorithm for computation of the estimated parameters in each model. The theory of this modeling approach is based upon findings of Vaida and Liu (2009), and their software solution of a hierarchical model with two levels, written in R (R Core Team. 2015), was enhanced to a model with three levels. The concentrations of dust and allergens

per vacuumed area were log-transformed to meet the assumptions of the normal mixed effects models. The independent (fixed) variables in the models were the number of children in the day care center, maintaining animals at the day care center, location of the day care center (rural, urbanized), renovation in the past 5 years, air humidity (%) on the day of dust collection, type of room (group room, side room, sleeping room, staff room, corridor), floor area of the room (m², log-transformed), height of the room (<2.40 m, ≥2.40m), season, percent carpet covering the floor (0–≤10%, 10–≤50%, >50%), and number of days of vacuuming per week. To estimate the fraction of total variability that is due to the group-level conditional on the fixed effects, intraclass correlation coefficients of the full model were calculated.

In the case of smooth floors, the frequencies of sweeping and mopping per week were additionally included into the models. Missing values in the independent variables were supplemented either by information known for another sample of the same room or by the mode value. In case of missing values with respect to air humidity in a room, the values were predicted by a hierarchical model with two levels (level 1: room of collection, level 2: day care center) based on the measured values in the day care centers. Type of room and a harmonic oscillation of the day of measurement in the year ($\beta_1 \sin(2\pi x/365.25) + \beta_2 \cos(2\pi x/365.25)$) were the independent variables of the model. Air humidity reached a maximum on d 249 and minimum on d 66 of the year. Similar trends in air humidity oscillation over the year were observed when the relative air humidity was measured in school class rooms in North Rhine–Westphalia (Neumann and Buxtrup 2014).

For nonparametric correlations (Spearman) and box plots of dust and allergen loads and concentrations, the software GraphPad Prism 5.04 (GraphPad Software, Inc., La Jolla, CA) was used, and then values below LOD of immunoassays were replaced by 2/3 LOD.

Results

Allergens were detected in nearly all samples that were measured. Only 10 samples (0.75%) were found below the LOD in the domestic mite (DM) assay, whereas 55 samples each (4.1%) were below the LOD in the Fel d 1 and Can f 1 assays. The

dust and allergen levels per square meter in different seasons and on various surfaces are shown in Figure 1. Different seasons appeared to affect especially DM antigen concentrations with the highest amounts (median $0.5 \mu\text{g}/\text{m}^2$) detected in autumn (Figure 1A).

The levels of dust and all allergen loads (ng/m^2) were significantly altered according to surface where they were collected (Figure 1B). These differences and also dispersion of data (ranges and interquartile ranges) were greater for allergens compared to dust concentrations alone. Upholstered furnishings displayed the highest dust and allergen loads, followed by carpets and then smooth floors. The same was true for allergen concentrations per gram dust, although differences between surfaces were less pronounced (data not shown). The geometric means for smooth floors, carpets, and furnishings

were 2.07 , 4.5 , $11.15 \mu\text{g}/\text{g}$ DM, 0.12 , 0.21 , $0.67 \mu\text{g}/\text{g}$ Fel d 1, and 0.1 , 0.15 , $0.33 \mu\text{g}/\text{g}$ Can f 1, respectively.

Significant correlations were observed between dust loads and allergen concentrations of samples that were collected from different surfaces in the same rooms at the same time (Table 1). For all allergens, the highest correlations were between carpets and upholstered furnishings, followed by carpet and smooth floor. The lowest correlations were noted between smooth floor and upholstered furnishings. The correlations were stronger for different allergens than for dust alone, with the highest correlation between DM levels on carpets and furnishings. The highest DM concentrations were measured in bedrooms (median $9.7 \mu\text{g}/\text{g}$), whereas the median cat and dog allergen levels were highest in side rooms: $0.29 \mu\text{g}$ and $0.18 \mu\text{g}/\text{g}$, respectively (Figure 2). All allergen concentrations were lowest in staffrooms

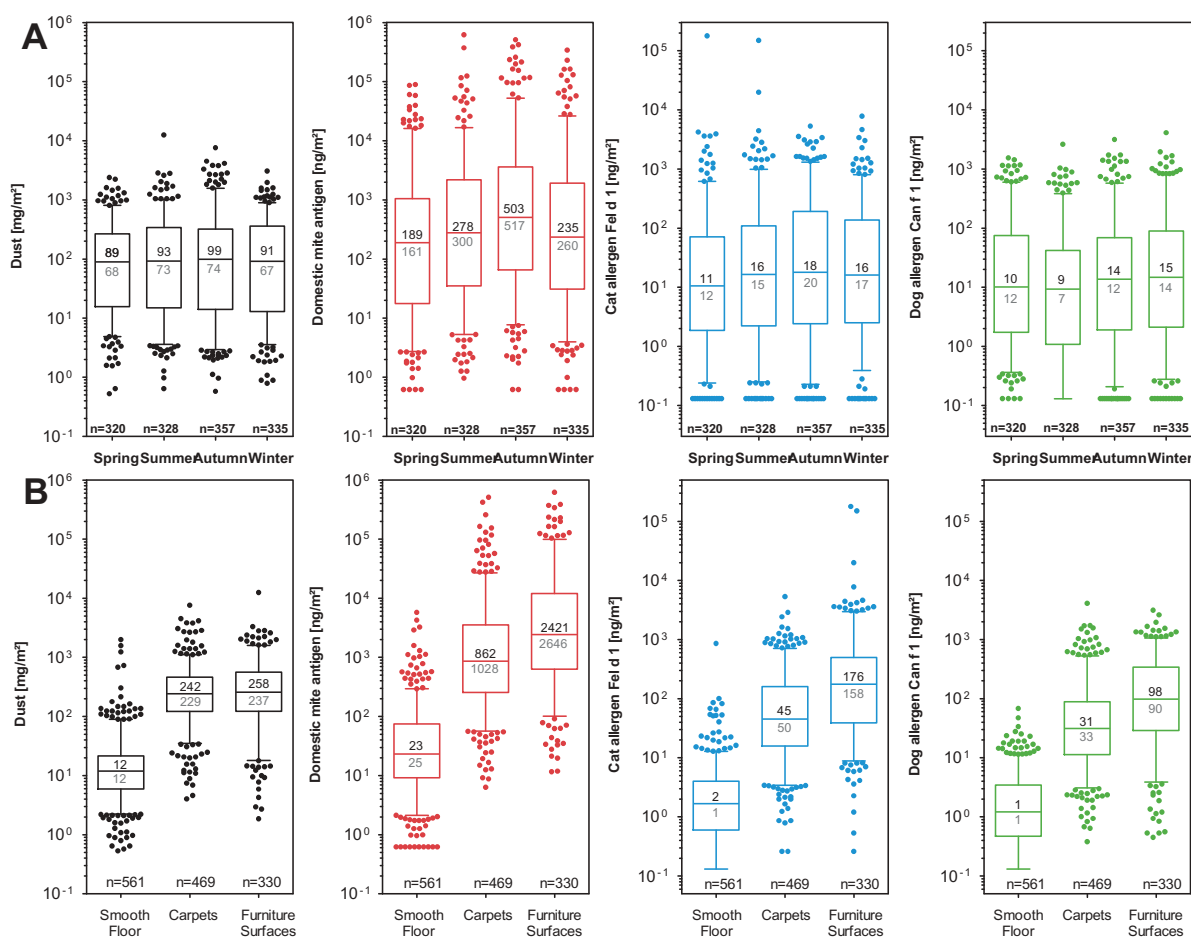


Figure 1. Dust and allergen loads in day care centers depending on (A) the season and (B) on different surfaces. Boxes comprise the 25th and 75th percentiles and the median values above and geometric means below the horizontal line. Vertical bars show the 5th and 95th percentiles, and circles represent values outside of the given percentiles. In addition, the sample numbers are indicated below.

Table 1. Spearman correlation coefficients of dust loads (mg/m²) and allergen concentrations (ng/g) between samples taken in the same rooms at the same time on different surfaces.

	Carpet	Smooth floor
Furnishings Dust	0.44**	0.22*
Domestic mite	0.64**	0.38**
Cat Fel d 1	0.61**	0.29**
Dog Can f 1	0.51**	0.43**
Carpet Dust		0.37**
Domestic mite		0.49**
Cat Fel d 1		0.41**
Dog Can f 1		0.47**

p* = .0003, *p* < .0001.

and corridors. Using provisional threshold allergen concentrations considering different standards by conversion factors for classification of the results (horizontal lines in Figure 2), 4% or 13% of all samples showed elevated DM, 3.2% or 43% elevated Fel d 1, and 2.8% or 27% had increased Can f 1 concentrations that are apparently associated with induction of symptoms or sensitization, respectively.

To analyze the influence of season, facility and room characteristics, and cleaning procedures on allergen loads and concentrations, these parameters were used as fixed variables in multivariate models (Tables 2–4). Each allergen was accounted for in a separate model, either in relation to the vacuumed area (allergen load/m²) or in relation to dust (allergen concentration/dust). Further, different surface types—smooth floors, carpet, and

upholstered furnishings—were analyzed using separate models, and data are presented in Tables 2, 3, and 4, respectively. In addition, the intraclass correlation coefficients (ICC) within day care center or room (random effect variables in the models) were calculated. Tables 2–4 show that in conditions of fixed variables, dust or allergen levels were not markedly correlated within the same day care center. For all models, the within day care center ICC is small (maximal ICC 13.61%, Table 3). Thus, a specific day care center does not seem to be a robust or dominant determinant of allergen concentration. Conversely, DM load (ICC 65.51%) and concentration (ICC 51.67%) that had been collected on the surfaces of upholstered furnishings were predominantly determined by the specific room (Table 4), whereas the dust and allergen loads and levels measured in samples collected on floors in the same rooms were not markedly correlated (see level-two variances and ICC in Tables 2 and 3).

The following fixed variables were analyzed in each multivariate model: the number of children in the day care center, animals in the day care center (only non-fur animals), location, renovation, air humidity, room type, area and height of room, season, floor coverage with carpets, and cleaning frequency (Tables 2–4). To account for multiple comparisons, significance levels were corrected by the number of variables in each

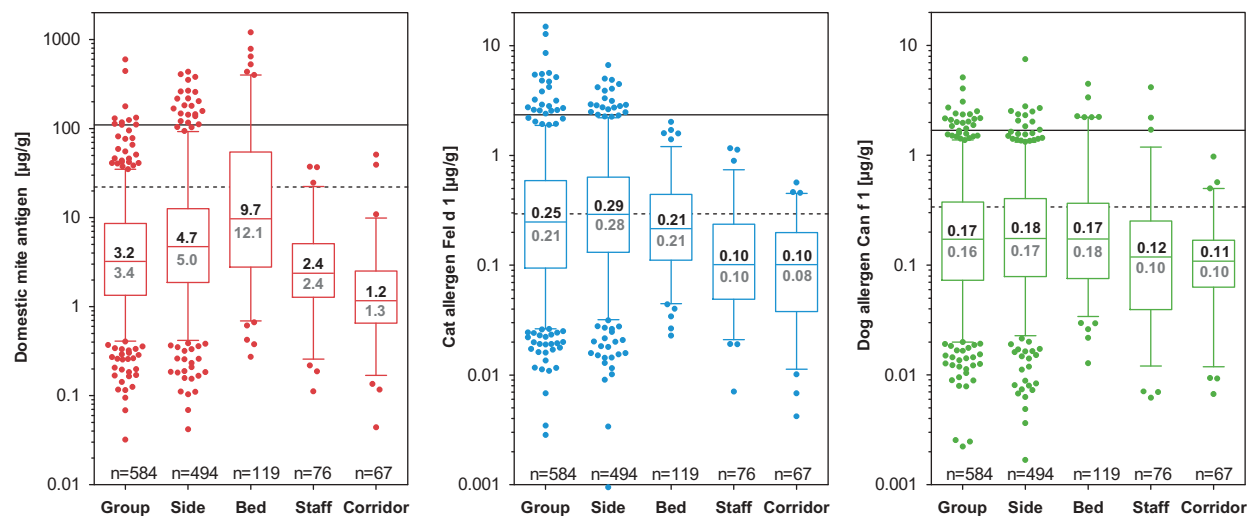


Figure 2. Allergen concentrations per gram dust sampled in different room types in day care centers. Boxes comprise the 25th and 75th percentiles and the median values above and geometric means below the horizontal lines. Vertical bars show the 5th and 95th percentiles, and circles represent values outside of the given percentiles. In addition, the sample numbers are indicated. The broken lines indicate estimated cut-points for elevated allergen concentrations in respect to sensitization rates and the solid lines in respect to symptoms.

**Table 2.** Models of dust and allergens per vacuumed area on smooth floors.

Fixed Effects		Dust / m ²		DM / m ²		DM / dust		Fel d 1 / m ²		Fel d 1 / dust		Can f 1 / m ²		Can f 1 / dust	
Variable	Reference	Estimate	p-value	Estimate	p-value	Estimate	p-value	Estimate	p-value	Estimate	p-value	Estimate	p-value	Estimate	p-value
Number of Children	Intercept	68.90		66.77		1064.79		19.94		315.48		55.90		1005.73	
Animals (non-fur)	(per 1 child increase)	1.00	0.7817	1.00	0.6363	1.00	0.3677	1.01	0.1584	1.01	0.0546	1.00	0.3568	1.00	0.7345
Location	No (n=404)	1.20	0.6372	1.21	0.6490	1.01	0.9810	0.90	0.8100	0.76	0.4913	1.33	0.4014	1.11	0.8120
Renovation	Rural (n=265)	1.16	0.5404	1.33	0.2896	1.15	0.5528	1.01	0.9812	0.87	0.5925	0.91	0.6765	0.76	0.3378
Air humidity	Yes (n=359)	0.67	0.0832	0.43	0.0007	0.64	0.0429	0.48	0.0083	0.72	0.1744	0.82	0.3483	1.27	0.3788
Type of room	(per 1% increase)	0.99	0.1087	0.98	0.0062	0.99	0.98	0.97	<0.001	0.98	0.0005	0.97	<0.001	0.97	<0.001
	Group room (n=223)	0.72	0.0265	1.02	0.9237	1.40	0.0476	0.79	0.2611	1.08	0.6725	0.66	0.0267	0.90	0.5308
	Staff room (n=54)	0.60	0.0105	0.63	0.0876	1.03	0.8896	0.49	0.0085	0.79	0.3470	0.57	0.0223	0.89	0.6035
	bedroom (n=28)	0.34	<0.001	0.85	0.5954	2.42	0.0007	0.44	0.0089	1.22	0.4859	0.40	0.0012	1.08	0.7651
	Corridor (n=63)	1.57	0.0070	1.22	0.3735	0.78	0.2004	1.17	0.5023	0.74	0.1507	1.44	0.0815	0.90	0.5694
Area of the room	(per log m ² increase)	0.81	0.0525	0.85	0.2627	1.04	0.85	0.75	0.0549	0.92	0.5260	0.70	0.0093	0.85	0.2039
Height of room	<2.4m (n=62)	0.88	0.4191	1.09	0.6996	1.23	0.2562	0.75	0.1723	0.85	0.4064	0.72	0.0867	0.83	0.2915
Season	Spring (n=132)	1.03	0.8338	2.31	<0.001	2.28	<0.001	1.57	0.0107	1.54	0.0042	0.82	0.2331	0.81	0.1608
	Summer (n=133)	1.02	0.8952	3.27	<0.001	3.25	<0.001	1.68	0.0022	1.64	0.0005	1.09	0.6009	1.09	0.5348
	Autumn (n=141)	0.87	0.2517	1.34	0.0765	1.54	0.0027	1.26	0.1452	1.44	0.0060	1.00	0.9989	1.12	0.3874
	Winter (n=135)	1.32	0.0172	1.17	0.3122	0.89	0.3961	1.06	0.6984	0.81	0.1562	0.92	0.5640	0.73	0.0147
Carpet (%)	10-50% (n=267)	1.52	0.0396	0.94	0.8086	0.64	0.0556	0.96	0.8710	0.66	0.0915	0.70	0.1605	0.50	0.0021
Sweeping	3-5 times (n=459)	0.88	0.6907	0.35	0.0022	0.40	0.0021	0.53	0.0871	0.59	0.1026	0.95	0.8412	1.06	0.8718
Vacuuming	5 times (n=129)	0.85	0.5147	1.29	0.3484	1.51	0.0877	0.85	0.5856	1.00	0.9936	1.05	0.8315	1.17	0.5807
Mopping	5 times (n=508)	1.01	0.9856	2.01	0.1104	2.00	0.0735	1.10	0.8386	1.11	0.8003	2.43	0.0134	2.39	0.0566
Random Effects		Dust / m²		DM / m²		DM / dust		Fel d 1 / m²		Fel d 1 / dust		Can f 1 / m²		Can f 1 / dust	
Level-three variance (Day care center)		0.02		0.03		0.18		0.04		0.02		<0.01		0.05	
Level-two variance (Room)		<0.01		<0.01		<0.01		<0.01		0.02		<0.01		<0.01	
Level-one variance (Sample)		1.02		1.78		1.32		1.60		1.13		1.51		1.08	
ICC (Day care center)		2.34%		1.43%		1.32%		2.61%		1.82%		0.53%		4.26%	
ICC (Day care center + room)		2.34%		1.46%		1.32%		2.92%		3.24%		0.55%		4.43%	

Note. Influences with *p* values <0.00263 (Bonferroni correction of the significance level of .05 by the number of variables in each model) are printed in boldface. Estimate: This is the transformed coefficient estimate exp (β) of the multilevel model. ICC: Intraclass correlation coefficient of the full model.

Table 3. Models of dust and allergens per vacuumed area on carpets.

Fixed Effects			Dust / m ²		DM / m ²		DM / dust		Fel d 1 / m ²		Fel d 1 / dust		Can f 1 / m ²		Can f 1 / dust	
Variable	Reference	Category	Estimate	p-value	Estimate	p-value	Estimate	p-value	Estimate	p-value	Estimate	p-value	Estimate	p-value	Estimate	p-value
Intercept			85.08		249.41		2490.52		9.29		102.97		28.05		391.41	
Number of Children	(per 1 child increase)		1.00	0.4197	0.99	0.2140	0.99	0.3509	0.99	0.4165	1.00	0.6905	0.99	0.0345	0.99	0.0476
Animals (non-fur)	No (n=348)	Yes (n=124)	2.15	0.0836	3.65	0.1123	1.78	0.3776	3.91	0.0329	1.84	0.1591	3.64	0.0266	1.64	0.2340
Location	Rural (n=235)	Urbanized (n=237)	1.49	0.1695	2.13	0.1573	1.49	0.3499	1.65	0.2347	1.12	0.6955	1.28	0.5157	1.49	0.5608
Renovation	No (n=162)	Yes (n=310)	0.67	0.0826	0.35	0.0131	0.54	0.0740	0.36	0.0022	0.53	0.0049	0.59	0.0789	0.87	0.524
Air humidity	(per 1% increase)		1.00	0.3728	1.01	0.4410	1.01	0.1147	1.00	0.9170	1.00	0.3824	0.99	0.4064	1.00	0.8660
Type of room	Group room (n=234)	Side-room (n=185)	1.27	0.1934	1.72	0.0614	1.45	0.1940	1.61	0.0588	1.28	0.2093	1.39	0.2101	1.05	0.8349
		Staff room (n=22)	0.86	0.6332	0.98	0.9642	1.26	0.6340	0.80	0.6064	0.95	0.8799	0.77	0.5582	0.84	0.6381
		bedroom (n=27)	0.77	0.2861	2.85	0.0077	3.90	0.0004	0.72	0.3346	0.96	0.8628	0.81	0.5551	1.03	0.9085
		Corridor (n=4)	0.64	0.4573	0.24	0.1371	0.44	0.3868	0.27	0.1075	0.42	0.1793	0.33	0.2005	0.62	0.4998
Area of the room	(per log m ² increase)		1.23	0.1433	1.09	0.7151	0.91	0.6730	1.36	0.1155	1.12	0.4589	1.33	0.1627	1.02	0.9030
Height of room	<2.4m (n=58)	>2.4m (n=414)	1.25	0.1685	1.54	0.0922	1.27	0.3473	1.35	0.1792	1.08	0.6565	1.09	0.7097	0.85	0.4092
Season	Spring (n=116)	Summer (n=116)	1.13	0.3866	1.89	0.0027	1.70	0.0001	1.46	0.0419	1.29	0.0303	0.69	0.0593	0.60	0.0001
		Autumn (n=120)	1.52	0.0015	4.30	<0.001	2.85	<0.001	2.70	<0.001	1.77	<0.001	1.53	0.0235	1.00	0.9981
		Winter (n=120)	1.27	0.0517	2.17	<0.001	1.69	<0.001	2.01	<0.001	1.59	<0.001	1.54	0.0120	1.22	0.0790
Carpet (%).	10-50% (n=292)	0-10% (n=77)	1.59	0.0004	2.19	0.0001	1.46	0.0532	1.62	0.0068	1.03	0.8484	1.58	0.0141	1.05	0.7733
		>50% (n=103)	1.16	0.2945	1.47	0.0745	1.18	0.4117	1.12	0.5532	0.97	0.8149	1.15	0.4794	0.98	0.8760
Vacuuming	5 times (n=327)	1 time (n=90)	1.22	0.4999	1.92	0.2310	1.61	0.2695	0.97	0.9476	0.79	0.4085	1.06	0.8753	0.86	0.5671
		3 times (n=55)	1.95	0.1014	1.94	0.3702	1.00	0.9999	3.32	0.0404	1.72	0.1761	3.43	0.0227	1.65	0.2035
Random Effects			Dust / m ²		DM / m ²		DM / dust		Fel d 1 / m ²		Fel d 1 / dust		Can f 1 / m ²		Can f 1 / dust	
Level-three variance (Day care center)			0.02		0.32		0.10		0.11		0.02		0.06		0.01	
Level-two variance (Room)			<0.01		<0.01		0.09		<0.01		<0.01		<0.01		0.02	
Level-one variance (Sample)			0.83		2.02		0.83		1.53		0.62		1.69		0.70	
ICC (Day care center)			2.71%		13.61%		10.11%		6.71%		3.01%		3.65%		1.71%	
ICC (Day care center + room)			2.71%		13.61%		19.15%		6.71%		4.06%		3.65%		3.96%	

Note. Influences with *p* values <0.00278 (Bonferroni correction of the significance level of .05 by the number of variables in each model) are printed in boldface. Estimate: This is the transformed coefficient estimate exp (β) of the multilevel model. ICC: Intraclass correlation coefficient of the full model.

Table 4. Models of dust and allergens per vacuumed area on upholstered furnishings.

Fixed Effects			Dust / m ²		DM / m ²		DM / dust		Fel d 1 / m ²		Fel d 1 / dust		Can f 1 / m ²		Can f 1 / dust	
Variable	Reference	Category	Estimate	p-value	Estimate	p-value	Estimate	p-value	Estimate	p-value	Estimate	p-value	Estimate	p-value	Estimate	p-value
Intercept			303.10		300.98		3065.92		59.03		520.29		491.46		2448.41	
Number of Children	(per 1 child increase)		1.01	0.2115	1.01	0.3088	1.00	0.7917	1.01	0.5516	0.99	0.1364	1.01	0.1906	1.00	0.6430
Animals (non-fur)	No (n=253)	Yes (n=74)	0.83	0.7599	0.85	0.8298	1.17	0.7675	1.96	0.3161	2.69	0.0056	0.41	0.2140	0.50	0.0539
Location	Rural (n=139)	Urbanized (n=188)	0.98	0.9587	1.54	0.3886	1.65	0.1415	1.04	0.9287	1.11	0.6522	0.48	0.1019	0.49	0.0022
Renovation	No (n=107)	Yes (n=220)	1.29	0.4155	0.94	0.8820	0.87	0.6294	0.85	0.6511	0.72	0.0884	2.20	0.0378	1.75	0.0042
Air humidity	(per 1% increase)		0.98	0.0437	1.00	0.7839	1.02	0.0164	0.99	0.2310	1.00	0.8309	0.99	0.2944	1.00	0.6554
Type of room	Group room (n=127)	Side-room (n=136)	0.57	0.1085	1.08	0.9044	1.16	0.7511	0.64	0.3718	0.79	0.4319	0.24	0.0016	0.37	0.0002
		Staff room (n=0)														
		bedroom (n=64)														
		Corridor (n=0)														
Area of the room	(per log m ² increase)		0.89	0.6439	0.91	0.8470	0.76	0.4015	1.26	0.5382	1.11	0.6467	0.60	0.1218	0.61	0.0124
Height of room	<2.4m (n=37)	>2.4m (n=290)	0.92	0.7683	1.50	0.4724	1.78	0.1410	0.98	0.9626	1.16	0.5648	0.73	0.3947	0.78	0.2730
Season	Spring (n=72)	Summer (n=79)	1.57	0.0146	1.51	0.0982	0.97	0.8723	1.41	0.1540	0.92	0.5926	0.77	0.2750	0.50	<0.001
		Autumn (n=96)	0.83	0.2702	1.39	0.1580	1.70	0.0006	1.02	0.9222	1.27	0.0969	0.54	0.0072	0.67	0.0045
		Winter (n=80)	0.76	0.0953	1.13	0.5668	1.48	0.0062	0.97	0.8951	1.28	0.0639	0.76	0.2090	1.00	0.9771
Carpet (%)	10-50% (n=176)	0-10% (n=84)	1.04	0.8405	1.04	0.9104	1.01	0.9831	0.81	0.4936	0.78	0.1807	1.13	0.6595	1.08	0.6536
		>50% (n=67)	1.69	0.0227	2.72	0.0192	1.44	0.2130	2.38	0.0101	1.35	0.1578	1.90	0.0318	1.12	0.5213
Vacuuming	5 times (n=46)	0.25-1 time (n=46)	2.49	0.0837	4.27	0.0256	2.23	0.0698	3.42	0.0347	1.69	0.0821	4.93	0.0114	2.22	0.0118
		3 times (n=25)	1.47	0.6178	1.00	0.9990	0.95	0.9423	4.07	0.1031	3.72	0.0033	1.31	0.7703	0.98	0.9719
		on demand (n=25)	1.39	0.5987	0.57	0.5159	0.46	0.1856	1.85	0.4000	1.44	0.3508	2.07	0.3370	1.59	0.2322
		missing (n=185)	1.48	0.4184	1.90	0.2864	1.77	0.1654	1.65	0.3509	1.43	0.1964	4.50	0.0095	3.38	<0.001
Random Effects			Dust / m ²		DM / m ²		DM / dust		Fel d 1 / m ²		Fel d 1 / dust		Can f 1 / m ²		Can f 1 / dust	
Level-three variance (Day care center)			0.04		<0.01		<0.01		0.02		<0.01		0.06		<0.01	
Level-two variance (Room)			0.06		3.19		0.80		0.59		0.10		0.18		0.02	
Level-one variance (Sample)			0.95		1.68		0.74		1.62		0.66		1.66		0.64	
ICC (Day care center)			3.77%		0.01%		<0.01%		0.68%		<0.01%		3.30%		0.23%	
ICC (Day care center + room)			9.71%		65.51%		51.67%		27.11%		12.89%		12.70%		3.89%	

Note. Influences with *p* values <0.00278 (Bonferroni correction of the significance level of .05 by the number of variables in each model) are printed in boldface. Estimate: This is the transformed coefficient estimate exp (β) of the multilevel model. ICC: Intraclass correlation coefficient of the full model.

model. Significant influences are summarized by the order of confounding variables. Urban locations halved the concentrations of dog allergens measured on upholstered furniture (Table 4). Renovation of rooms in the last 5 yr resulted in reduced dust and allergen loads on both smooth and carpeted floors (Tables 2 and 3). This effect was significant for DM on smooth floors (Table 2) and Fel d 1 on carpets (Table 3). An increase in air humidity significantly decreased cat and dog allergen loads on smooth floors (Table 2), whereas no significant changes were observed on carpets (Table 3) and on upholstered furnishings (Table 4).

The estimates for allergens on all surfaces were predominantly affected by room type and season. Sleeping rooms displayed significantly lower dust and Can f 1 loads on smooth floors (Table 2) and lower dust, Fel d 1, and Can f 1 loads and concentrations on furnishings compared to group rooms (Table 4). In addition, furnishings in side rooms yielded significantly lower Can f 1 loads and levels than furnishings in group rooms. In contrast, DM concentrations were significantly higher on bedroom floors compared to floors of group rooms (2.42-fold higher on smooth floors [Table 2], 3.9-fold higher on carpets [Table 3]). Compared to spring, in autumn on all surfaces significantly higher DM concentrations were measured (Tables 2–4). Smooth floors in rooms with more than 50% carpet coverage were dustier than floors with only 10–50% carpet coverage and, in addition, showed higher dog allergen concentrations (Table 2). Carpets that covered less than 10% of the floor contained significantly higher dust and mite allergen loads than carpets that covered 10–50% of the floor (Table 3). Further, sweeping the floors significantly elevated DM loads and concentrations (Table 2). Less frequent vacuuming of upholstered furnishings raised dust and allergen loads, and significantly higher Can f 1 concentrations were measured on furniture surfaces where there were missing specifications on how often vacuuming was conducted compared to vacuuming five times per week (Table 4).

Discussion

In indoor environments, the allergen content of settled or reservoir dust is typically used to provide estimates of exposure levels (Raulf et al. 2014). Therefore, risk estimates for indoor allergens are based upon

concentrations in vacuumed surface samples, usually in relation to dust in samples taken either from floors with or without carpets, or from furnishings, mostly mattresses (Gelber et al. 1993; Lau et al. 1989; Salo et al. 2008; Wahn et al. 1997). Such published-derived thresholds used to assess elevated allergen concentrations associated with asthma morbidity and allergic sensitization need to be interpreted with caution (Salo et al. 2008), and differences in sampling, extraction procedures, assays, and standards need to be considered (Raulf et al. 2014; Zahradnik and Raulf 2014). Salo et al. (2009) used such provisional threshold limits (PTL) that had been associated with allergic sensitization or asthma symptoms in sensitized individuals in their review on indoor allergens in schools and day care environments. While considering the limitations on transferability of threshold limits obtained by previous investigations in households, allergen levels measured in this study were converted and related to these provisional threshold values for ease of comparison to the literature. Approximately 3% of the presented surface samples in the day care centers showed elevated Fel d 1 or Can f 1 and 4% increased DM concentrations that were associated with an increased asthma frequency. This proportion, however, is smaller than in household samples collected in the United States where approximately 40% of 822 samples displayed elevated Fel d 1, Can f 1, and Der f 1 levels (Salo et al. 2008). Similarly, in comparison to 84 vacuumed floor samples collected from German households (Sander et al. 2012), the concentrations of DM antigens in floor dust of day care centers were approximately 10-fold lower than in households (geometric means on smooth floors: 2.07 vs. 21.7 $\mu\text{g/g}$; geometric means on carpets: 4.5 vs. 40.6, respectively). These results confirm the findings of Engelhart et al. (2002), who reported significantly lower DM allergen levels (Der f 1 and Der p 1) in dust collected from German day care centers than in dust from private homes. However, applying the PTL for an increased risk of sensitization to samples in the present study, 13% were above this limit for DM, 43% of samples were above the threshold for Fel d 1, and 27% of samples above the threshold for Can f 1.

Studies from other countries often demonstrated that there were higher concentrations of dog allergens compared to cat allergens in dust collected from day care centers (Arbes et al. 2005; Cyprowski et al. 2013; Instanes et al. 2005). However, in the present study cat

allergens were higher, which may be due to the greater percent of children with cats (16.5%) than dogs (13%) at home. In addition, reference standards have changed since previous studies. Currently, the principles of “Development of Certified Reference Materials for Allergenic Products and Validation of Methods for their Quantification,” acronym CREATE, are applied to allergen standards, thus complicating comparisons of allergen levels by different conversion factors for Fel d 1 than for Can f 1 levels (Filep et al. 2012a; 2012b). Previously, Can f 1 values were favored by a factor of approximately 1.7 in comparison to Fel d 1 values, when the relation of conversion factors between old and current standards (5.9/3.4) is considered.

In accordance with previous studies (Arbes et al. 2005; Custovic et al. 1996; Engelhart et al. 2002; Tranter et al. 2009), higher allergen loads and levels on upholstered furniture than on carpets, and higher loads on carpets than on smooth floors, were found. One novel finding is that the allergen concentrations on different surfaces that were collected from the same rooms at the same time were significantly correlated. More specifically, correlations between allergen concentrations on carpets and furnishings were stronger than between allergens on carpets and smooth floors. The weakest correlations were observed between smooth floors and furnishings.

Previous studies in day care centers were based on small sample numbers, for example, 57 (Oldfield et al. 2007), 60 (Tranter et al. 2009), 84 (Cyprowski et al. 2013), 113 (Arbes et al. 2005), 123 (Zuraimi et al. 2008), and 143 samples (Engelhart et al. 2002); however, in the present study 1340 surface samples were included into the multivariate analysis with three-level hierarchical models. Further, the longitudinal design of the present study enables an estimation of seasonal effects on allergen concentrations. For all surfaces measured, DM levels were highest in autumn and up to threefold greater than measurements made in spring. The highest air humidity was detected at the beginning of September and the lowest at the beginning of March. Crisafulli et al. (2007) in Australia conducted a study over a 7-yr period including monthly measurements of the mite allergen Der p 1 in children's beds and found two- to threefold seasonal fluctuations in Der p 1 levels that were maximal in late autumn following 2 mo

of peak relative humidity. Seasonal changes that were also at maximum in fall were noted to be more pronounced for Der f 1 than for Der p 1 (van Strien et al. 2002). Further, increased severity in airway responsiveness depending on sensitization to house dust mites was observed in the fall (van der Heide et al. 1997), as was the highest proportion of participants who experienced an exacerbation of asthma (Teach et al. 2015). Thus, seasonal fluctuation of allergen concentrations might be sufficient to influence asthmatic symptoms.

Both DM and pet allergens were significantly affected by different seasons. Higher loads and concentrations were measured on carpets in winter and autumn compared to spring in the case of cat allergens, and summer in the case of dog allergens. Possible explanations may be transfer of allergens on clothes by children playing on the floor (Cyprowski et al. 2013), and a higher allergen load on wool than on cotton clothing (Patchett et al. 1997; Siebers et al. 1996). An elevation in room air humidity significantly reduced cat and dog allergen loads and levels on smooth floors. Perhaps allergen particles stick to clothing at higher air humidity and fewer particles settle on the floor.

DM concentrations in bedroom dust were 2- to 3.9-fold higher than those measured in group room dust, most probably due to shedding of outer skin cells that contain keratin, the primary and most important food source for house dust mites (Calderón et al. 2014). In contrast, pet allergens are postulated to enter day care centers primarily attached to clothing (Berge et al. 1998; Cyprowski et al. 2013; Salo et al. 2009). Consequently, high dust, Fel d 1, and Can f 1 loads were found on smooth floors in group rooms and corridors, on upholstered furnishings in group rooms, and on carpets in side rooms—all places that are heavily used.

Newly renovated rooms and levels of cleanliness influenced dust and allergen loads. Rooms that were renovated in the last 5 yr displayed significantly reduced allergen loads of both DM and Fel d 1 on their floors by more than 50%. Dust and Can f 1 loads on floors of renovated rooms were decreased by 30 or 20–40%, respectively, without reaching a level of significance. If floors were mopped 5 times instead of 3 times per week, DM and Can f 1 loads were diminished by 50 and 60%,

respectively. In addition, vacuuming furnishings 5 times instead of less than once a week lowered allergen loads by 70–80%. In contrast, sweeping the floors 3–5 times a week increased DM and cat allergen concentrations by a factor of 2.5 and 1.7, respectively. Thus, appropriate cleaning procedures help to reduce allergen loads.

A final factor that influenced allergen loads was percent carpet cover. Significantly higher dust and allergen loads were found on carpets covering less than 10% of the floor. Small carpet pieces included door and play mats, which were highly used. Another explanation is that small carpets are more difficult to clean by vacuuming because small area rugs might be sucked up at high suction power.

The strengths of the present study are (1) longitudinal design with measurement during four seasons, (2) high number of samples analyzed using sensitive assay variants with few samples below LOD, (3) application of updated allergen standards for pet allergens, and (4) three-level hierarchical multivariate analysis taking into account left-censoring of data. A weakness of the study is the small number of day care centers (20) included in the study, and missing data on home exposure and health of the children. Further, as in all studies based on surface dust, the validity of reservoir dust as a surrogate marker for airborne exposure has to be questioned. Therefore, as an alternative for exposure assessment indoors, electrostatic dust fall collectors (EDC) were used to quantify the same allergens in the same rooms throughout the year, in addition to the surface data presented in the current study in the 20 day care centers. Allergen assessment with EDC was applied successfully in Dutch homes and schools (Krop et al. 2014), and EDC data on German day care centers and children's homes remain to be published.

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