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Respiratory symptoms in relation to indoor exposure to mite and cat allergens and endotoxins

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ABSTRACT: The authors investigated the relationship between respiratory symptoms in adults and exposure to mite and cat allergens, the role of endotoxins in house dust, the effects of mixtures of several allergens, and interactions between allergen exposure and allergic sensitization.

Within a nested case-control study, 405 subjects aged 25–50 yrs from two German cities answered a standardized questionnaire. Allergen-specific immunoglobulin-E was measured. Dust samples were taken from the subjects' homes to determine exposure to mite (*Dermatophagoides pteronyssinus* antigen 1 *Der p* 1) and (*D. farinae* antigen 1 *Der f* 1) and cat (cat antigen d1 *Fel d* 1) allergen and endotoxin content in settled house dust.

Exposure to *Der f* 1 and *Der p* 1 plus *Der f* 1 >10 $\mu g \cdot g^{-1}$ of mattress dust, respectively, increased the risk of wheeze and breathlessness (odds ratios (OR): 4.04, 95% confidence interval (CI): 1.53–10.64, OR: 2.78, 95% CI: 1.06–7.28). *Fel d* 1 >8 $\mu g \cdot g^{-1}$ was positively associated with cough at night (OR: 2.74, 95% CI: 1.22–6.17), noteworthy also in the nonsensitized subjects. Subjects exposed to elevated concentrations of more than one allergen had an up to seven-fold increase in the risk of respiratory symptoms, compared to nonexposed subjects. Sensitized subjects exposed to elevated concentrations of *Der f* 1 or *Fel d* 1 were found to have the highest risk of asthma attacks and respiratory symptoms. No statistically significant association was found between exposure to endotoxins and respiratory health.

Indoor exposure to *Dermatophagoides farinae* antigen 1 and cat antigen d1 is a risk factor for respiratory symptoms in adults, and for cat antigen d 1 even in nonsensitized subjects. The risk is increased if subjects are exposed to a mixture of allergens or if they are sensitized in addition to high exposure.

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The prevalence of asthma and immunoglobulin-E (IgE)-mediated atopic diseases increased in many Western countries over recent decades [1]. Inhalant allergens play a major role in the pathogenesis of allergic asthma and allergic rhinitis. Indoor allergens from house dust mites, cat dander, cockroaches, and fungi are of particular importance. The house dust mites *Dermatophagoides pteronyssinus* and *D. farinae* are the predominant sources of inhalant allergens in most parts of the world [2].

Which individual indoor allergen plays the major role, is a question of area-specific exposure to a single allergen or a mixture of several allergens. As mites cannot survive in very dry climatic conditions, exposure to mites does not play a major role in the development of allergic sensitization in dry climates [3]. Cockroaches may be the predominant allergen exposure in USA inner cities [4, 5], but not in Germany [6].

There is increasing evidence for the impact of allergen exposure early in life on the development of allergic sensitization [7–9], but exposure to allergens later in life might be, at least in sensitized subjects, associated with the development of persistent inflammation in the airway wall, and consequently, with respiratory symptoms.

The aim of this paper was to study the relationship between asthma attacks and respiratory symptoms in

adults and exposure to mite and cat allergen content in settled house dust. As allergen exposure constitutes only one element of the indoor environment, and differences in allergen levels could reflect overall differences in the indoor environment, the role of endotoxins was also studied. Furthermore, the effect of mixtures of several indoor allergens and interactions between indoor allergen exposure and allergic sensitization was analysed.

Materials and methods

Study design

To enrich the population-based sample with atopic subjects, this study on indoor factors and genetics in asthma (INGA) was designed as a nested case-control study following a cross-sectional study performed from 1990–1992 in the two German cities of Erfurt and Hamburg within the European Community Respiratory Health Survey (ECRHS) [10–12]. The methods of the ECRHS are described in detail elsewhere [12]. On the basis of the study results of the ECRHS in 1990–1992, cases and controls were defined. Every subject willing to participate attended the centre again for a detailed questionnaire, including respiratory symptoms and determination of specific IgE. The study was performed from June 1995–November 1996 and an identical methodology was followed in the two centres. The study protocol had been approved by the local ethics committees and all subjects gave their informed written consent.

Study population

The study population consisted of a subset of the subjects who had participated within the ECRHS in Erfurt and Hamburg. Cases for the present study were defined as subjects fulfilling at least one of the following four conditions according to the results from the ECRHS (1990–1992): 1) asthma diagnosed by a physician according to the long questionnaire of the ECRHS [12]; 2) at least one positive specific serum IgE (>0.35 kU·L^{-1}, CAP system, Pharmacia and Upjohn, Stockholm, Sweden) against grass, birch, cat dander, *D. pteronyssinus* or *Cladosporium herbarum*; 3) at least one positive skin-prick test reaction (mean wheal diameter $\geqslant 3$ mm) against the allergens mentioned above; and 4) provocative dose causing a 20% fall in forced expiratory volume in one second (FEV_1) (PD_{20}) $\leqslant 2.0$ mg methacholine or positive bronchodilator test. The subjects of the control group did not show any of these conditions at the ECRHS.

Based on these criteria, 107 cases and 106 controls were recruited from a random list of 538 cases and 621 controls in Hamburg, and 115 cases and 109 controls out of 363 cases and 368 controls in Erfurt. Four-hundred and five of these subjects (204 in Erfurt and 201 in Hamburg, 205 cases and 200 controls) agreed to measurements of indoor exposure to mite (*D. pteronyssinus* antigen 1, *Der p* 1, and *D. farinae* antigen 1, *Der f* 1) and cat (cat antigen d1 *Fel d* 1) allergens and endotoxins in their apartments.

Questionnaire

The questionnaire used in the INGA study to asses a history of asthma attacks and respiratory symptoms within the past 12 months, smoking, and social status had been developed according to the validated long questionnaire with 71 items used in the ECRHS [12]. The validated German version of the original ECRHS questionnaire was shortened to 40 items without changing the questions or their wording.

Allergic sensitization

Allergic sensitization in the INGA study was assessed by measurement of specific IgE against *Alternaria alternata* (m6), *Aspergillus fumigatus* (m3), tree pollen (tx5), birch (t3), *C. herbarum* (m2), *D. pteronyssinus* (d1), *D. farinae* (d2), grass pollen (gx1, gx4), *Phleum pratense* (g6), weed pollen (wx3), *Blatella germanica* (i6), dog (e5), and cat dander (e1) using the IgE CAP system. Allergic sensitization was defined as at least one of the specific IgE listed above >0.70 kU·L^{-1}. This differs from the definition used for definition of cases, but the cut-off point of 0.70 kU·L^{-1} seemed to be more reliable.

Lung function

Spirometric measurements were performed using pneumotachograph-based electronic spirometers. For details, see RICHTER *et al.* [13].

Measurement of allergen and endotoxin content of house dust

The homes were visited from June 1995–November 1996 by trained personnel. In each apartment, dust samples were taken from the living room floor, from the bedroom floor, and from the mattress surface according to a standardized protocol [14]. All dust samples were taken using the same type of vacuum cleaner (Flüsterjet Vitall 371, Philips, Hamburg, Germany) by vacuuming an area of 1 m^2 for 2 min at any of the three locations. Dust was collected on cellulose filters using sampling nozzles and filter boxes (ALK Laboratories, Hørsholm, Denmark). Samples were stored in filter boxes at -20°C until extraction. Dust samples were not sieved before extraction.

Sample allergen content was measured by means of a monoclonal enzyme-linked immunosorbent assay (ELISA) [6] with standards UVA 93/03, UVA 93/02, and UVA 94/01 (Indoor Biotechnologies, Clwyd, UK). Allergen concentrations are expressed as ng·gram^{-1} of dust. The lower limit of detection was 10 ng·g dust^{-1} for *Der p* 1 and *Der f* 1, and 15 ng·g dust^{-1} for *Fel d* 1. Endotoxin content in living room floor dust was quantified using a chromogenic kinetic Limulus amoebocyte lysate test described in [15]. *Escherichia coli* endotoxin (lot no. 5L570, Bio Whittaker, Walkersville, UK) was used as the standard endotoxin. The potency of this standard was 14.5 endotoxin unit (EU)·g^{-1}. Endotoxin concentrations are expressed as ng·gram^{-1} of dust.

Definition of exposure

Exposure to allergens is considered of major importance while sleeping, since people come into closest contact with mites in their beds. Therefore, the analysis of the relationship between allergens in house dust and respiratory symptoms was restricted to allergen concentrations in mattress dust.

Allergen levels were classified into low and high, defining subjects exposed to >10 μg group I dust mite allergen per gram of dust or >8 μg cat allergen per gram of dust as highly exposed, which are the threshold levels supposed to increase the risk of sensitization and asthmatic symptoms [16, 17]. Exposure to endotoxins was categorized into three categories defining exposure to <1,100 ng·g dust^{-1} as low, 1,100–4,700 ng·g dust^{-1} (25th–75th percentile) as medium and >4700 ng·g dust^{-1} as high.

Statistical methods

Because of the lognormal distribution of the allergen and endotoxin levels, geometric means (GM) and 95% confidence intervals (CI) are presented to characterize distributions. Concentrations less than the detection limit were assigned a value of one-half of the detection limit. Correlations between allergens and endotoxins were expressed as Spearman correlation coefficients.

The definition of "case" in the present study was found to be inadequate as it was not a well-defined clinical entity. Consequently outcomes other than case/control were to be analysed (*i.e.* case/control data was not analysed as designed, but as cross-sectional data). In order to do this, it was necessary to show that a relationship between exposure and the respective outcome was not just a consequence of the selection process. This was approached by adjusting for "caseness".

Associations between allergen concentrations in mattress dust, endotoxin concentrations in living room floor dust and the potentially confounding factors of region, season of dust sampling, age, education, and active smoking were analysed by means of the Wilcoxon two-sample test and the Kruskal-Wallis test, respectively. For the age variable, tertiles were used as cut-off points.

Multiple logistic regression analysis was used to estimate the effects of mite and cat allergens in mattress dust and endotoxins in living room floor dust on the outcomes listed above. Adjusted odds ratios (OR) with 95% CI are presented. Statistical significance was set at a conventional 5% level, and marginal significance at a 10% level.

Results

Description of the study population

A description of the studied sample of subjects is given in table 1. Concerning respiratory symptoms, highest prevalence was observed for wheeze.

Table 1. – Demographic characteristics of study participants, prevalence of asthma attacks, self-reported respiratory symptoms, allergic sensitization, and baseline lung function

	Prevalence %	Frequency n/N
Demographic characteristics		
Place of residence		
Erfurt	50.4	204/405
Hamburg	49.6	201/405
Sex F	47.2	191/405
Age yrs*	38.5±6.7	
Education		
⩽8 grades	17.8	72/405
10 grades	41.5	168/405
⩾12 grades	40.7	165/405
Active smokers	38.5	156/405
Parameters of respiratory health		
Asthma attacks#	2.7	11/405
Wheeze#	13.1	53/405
Wheeze and breathlessness#	5.2	21/404
Wheeze w/o cold#	7.9	32/403
Shortness of breath at rest#	5.4	22/405
Breathlessness at night#	4.9	20/405
Cough at night#	8.6	35/405
Cough w/o cold in winter >3 months (habitually)	4.4	18/405
Allergic sensitization¶	31.8	126/396
Sensitization to mite	9.8	39/396
Der p+	9.6	38/396
Der f+	7.8	31/396
Sensitization to cat dander+	8.1	32/396
Current asthma medication	2.0	8/505
Baseline lung function n=394		
FEV1 L*	3.85±0.85	
FVC L*	4.60±1.02	

n/N: number of subjects/total number of subjects; F: female; *Der p*: *Dermatophagoides pteronyssinus*; *Der f*: *D. farinae*; FEV1: forced expiratory volume in one second; FVC: forced vital capacity; w/o: without. *: data are presented as mean± SD; #: past 12 months; ¶: at least one serum-specific immonoglobulin-E (IgE) ⩾0.70 kU·L^{-1} against *Alternaria alternata* (m6), *Aspergillus fumigatus* (m3), tree pollen (tx5), birch (t3), *Cladosporium herbarum* (m2), *D. pteronyssinus* (d1), *D. farinae* (d2), grass pollen (gx1, gx4), *Phleum pratense* (g6), dog dander (e5), cat dander (e1), weed pollen (wx3), and *Blatella germanica* (i6); +: serum-specific IgE ⩾0.70 kU·L^{-1}.

Prevalence of the remaining respiratory symptoms was <10%. Allergic sensitization (based on the specific IgE measurements conducted in the INGA study) to at least one of the allergens was found in 31.8% of the subjects. Sensitization to mites occurred more frequently than sensitization to cat.

Allergen and endotoxin concentrations in house dust

Table 2 presents the distributions of allergen and endotoxin levels for the different sampling locations. Highest concentrations of mite allergens were found in mattress dust and lowest concentrations in living room floor dust, whereas *Fel d* 1 concentrations were highest in living room floor dust and lowest in mattress dust. Levels of *Der f* 1 were generally higher than

Table 2. – Allergen and endotoxins concentrations in house dust samples from three different sampling locations expressed as ng per gram of dust

	Sampling location		
	Mattress	Bedroom floor*	Living room floor#
Total subjects n	405	365	405
Der p 1	169 (125–228)	55 (41–75)	26 (21–33)
Der f 1	421 (305–581)	188 (141–252)	83 (64–108)
Der p 1+*Der f* 1	1403 (1041–1890)	212 (165–273)	523 (392–700)
Fel d 1	424 (313–573)	469 (342–644)	575 (425–776)
Endotoxin	NM	NM	2274 (2030–2547)

Data are presented as geometric means (GM) with 95% confidence intervals (CI) in parentheses unless otherwise stated. *Der p* 1: *Dermatophagoides pteronyssinus* antigen 1; *Der f* 1: *D. farinae* antigen 1; *Fel d* 1: cat antigen d 1; NM: not measured. *: there were no separate bedrooms in 40 homes; #: rooms used as both bedroom and living room were defined as living rooms. Concentrations less than the detection limit were estimated by one-half of the detection limit.

levels of *Der p* 1. Weak, but statistically significant ($p<0.05$) correlations were found between *Der p* 1 and *Der f* 1 (r=0.35), *Der p1* and *Fel d* 1 (r=0.12), *Der f* 1 and *Fel d* 1 (r=0.15) and endotoxins and *Der p* 1 (r=0.20), *Der f* 1 (r=0.15), and *Fel d* 1 (r=0.21).

Allergen and endotoxin levels and potentially confounding factors

Concentrations of all indoor factors were significantly higher in Hamburg compared to Erfurt (*Der p* 1: GM 344 *versus* 84 $ng \cdot g^{-1}$, *Der f* 1: 1,114 *versus* 161 $ng \cdot g^{-1}$; *Der p* 1 plus *Der f* 1: 3,621 *versus* 551 $ng \cdot g^{-1}$; *Fel d* 1: 854 *versus* 213 $ng \cdot g^{-1}$; endotoxin: 2,713 *versus* 1,911 $ng \cdot g^{-1}$; $p<0.01$). There was a tendency towards higher *Der f* 1, *Der p* 1 plus *Der f* 1, and endotoxin levels in homes of subjects with the lowest educational level ($p<0.10$, data not shown).

Multiple logistic regression analysis

Results of multiple logistic regression analyses are shown in table 3. Statistically significant associations ($p<0.05$) were found between *Der f* 1 and the sum of *Der p* 1 and *Der f* 1 in mattress dust and wheeze and breathlessness, whereas a level of >8 μg *Fel d* 1 per gram of dust increased the risk of cough at night. No statistically significant association was found between endotoxin levels in living room floor dust and asthma attacks and respiratory symptoms; however, the risk tended to be higher in highly exposed subjects.

Distinguishing between exposure to single allergens and mixtures of several allergens, the risk of respiratory symptoms (except wheeze) was found to be highest among subjects exposed to elevated concentrations of at least two allergens (*Der p* and/or *Der f* 1 and/or *Fel d* 1). The respective ORs and 95% CIs are presented in figure 1 where estimable. Subjects exposed to elevated concentrations of at least two of the regarded allergens had a six- to seven-fold increase in the risk of wheeze and breathlessness and cough without cold in winter compared to non-exposed subjects. Because of sample size limitations, it was not possible to differentiate between different types of allergen mixtures.

Prevalence of asthma attacks and respiratory symptoms related to allergic sensitization are shown in figure 2. Sensitized subjects were found to have an increased risk of asthma attacks and respiratory symptoms compared to nonsensitized subjects. Interactions between allergic sensitization and exposure to elevated concentrations of *Der f* 1 and *Fel d* 1 are presented in table 4. Because of sample size limitations, it was not possible to classify sensitization into sensitization to related (*Der p* 1/*Der f* 1 or *Fel d* 1) and nonrelated allergens. Sensitized subjects exposed to elevated concentrations of *Der f* 1 or *Fel d* 1 were found to have the highest risk of asthma attacks and respiratory symptoms. In addition, sensitized subjects exposed to ⩽10 μg *Der f* 1 per gram of dust were found to have a five-times higher risk of wheeze and breathlessness ($p<0.05$), compared to nonsensitized subjects exposed to ⩽10 μg *Der f* 1 per gram of dust.

Nonsensitized subjects exposed to >10 μg *Der f* 1 per gram of dust were not found to have an increased risk of asthma attacks or respiratory symptoms compared to nonsensitized subjects exposed to ⩽10 μg *Der f* 1, but if subjects were sensitized in addition to high exposure, they were found to have an increased risk of asthma attacks ($p<0.05$), and wheeze and breathlessness ($p<0.05$). Sensitized subjects exposed to ⩽8 μg *Fel d* 1 per gram of dust showed a statistically significant increase in the risk of wheeze and breathlessness and breathlessness at night compared to nonsensitized subjects exposed to ⩽8 μg *Fel d* 1 per gram of dust. Nonsensitized subjects exposed to elevated concentrations of *Fel d* 1 had a significantly higher risk of breathlessness at night, and cough at night ($p<0.05$) compared to nonsensitized subjects exposed to low concentrations of *Fel d* 1. The risk of wheeze, wheeze and breathlessness, and shortness of breath at rest was strongly increased if subjects were exposed to >8 μg *Fel d* 1 per gram of dust in addition to sensitization.

Discussion

The aim of this study was to assess: first, whether a relationship existed between indoor exposure to mite and cat allergens and endotoxins and asthma attacks

Table 3. – Adjusted odds ratios (OR) and 95% confidence intervals (CI) for asthma attacks and respiratory symptoms comparing high to low exposure to *Dermatophagoides pteronyssinus* antigen 1 (*Der p* 1), *D. farinae* antigen 1 (*Der f* 1), *Der p* 1+*Der f* 1, and cat antigen d 1 (*Fel d* 1), and high and medium to low exposure to endotoxins

Symptoms	*Der p* 1	*Der f* 1	*Der p* 1+*Der f* 1	*Fel d* 1	Endotoxins	
	>10 μg·g dust^{-1}	>10 μg·g dust^{-1}	>10 μg·g dust^{-1}	>8 μg·g dust^{-1}	>1100–4700 ng·g dust^{-1}	>4700 ng·g dust^{-1}
Total subjects n	55	84	187	70	194	100
Asthma attacks[#]	ne	2.70 (0.74–9.84)	1.40 (0.39–5.06)	1.59 (0.35–7.08)	2.72 (0.30–25.00)	4.31 (0.46–40.78)
Wheeze[#]	1.46 (0.64–3.34)	1.19 (0.59–2.39)	1.37 (0.72–2.58)	1.77 (0.86–3.64)	0.93 (0.45–1.93)	0.91 (0.40–2.11)
Wheeze and breathlessness[#]	1.78 (0.52–6.06)	4.04* (1.53–10.64)	2.78* (1.06–7.28)	2.35 (0.84–6.54)	0.98 (0.27–3.56)	2.06 (0.57–7.50)
Wheeze without cold[#]	0.71 (0.20–2.54)	1.09 (0.45–2.65)	0.75 (0.32–1.75)	1.31 (0.52–3.28)	0.87 (0.36–2.13)	0.67 (0.23–1.98)
Shortness of breath at rest[#]	0.62 (0.13–2.89)	1.91 (0.71–5.17)	1.31 (0.50–3.45)	0.35 (0.07–1.64)	1.82 (0.47–6.96)	3.36 [¶] (0.84–13.50)
Breathlessness at night[#]	1.22 (0.30–4.98)	1.04 (0.34–3.24)	0.95 (0.34–2.69)	2.41 (0.81–7.15)	1.97 (0.49–7.91)	2.58 (0.59–11.31)
Cough at night[#]	0.62 (0.18–2.22)	2.10 [¶] (0.92–4.77)	1.12 (0.50–2.50)	2.74* (1.22–6.17)	0.61 (0.26–1.43)	0.78 (0.29–2.10)
Cough without cold in winter >3 months (habitually)	1.69 (0.43–6.62)	2.59 [¶] (0.92–7.31)	1.93 (0.68–5.43)	2.40 (0.79–7.24)	0.82 (0.24–2.77)	1.20 (0.33–4.35)

Data are presented as adjusted OR (adjusted for "caseness", season of dust sampling, region, sex, age, education and active smoking) with 95% CI in parentheses unless otherwise stated. ne: not estimable. [#]: past 12 months; Cut-off points were 10 μg·g dust^{-1} for *Der p* 1, *Der f* 1, and *Der p* 1+*Der f* 1, 8 μg·g dust^{-1} for *Fel d* 1, and 1100 and 4700 ng·g dust^{-1} for endotoxin; *: $p<0.05$; [¶]: $p<0.10$.

and respiratory symptoms in adults; second, the effect of mixtures of several allergens; and third, whether there was an interaction between allergen exposure and allergic sensitization.

Some of the respiratory symptoms were found to be associated with exposure to high levels of *Der f* 1 and *Fel d* 1, but not with *Der p* 1. Subjects exposed to elevated levels of more than one allergen, were found to have up to a seven-fold risk of respiratory symptoms. Sensitized subjects exposed to elevated levels of *Der f* 1 or *Fel d* 1 had an increased risk of asthma attacks and respiratory symptoms, compared to sensitized subjects exposed to low levels of *Der f* 1 and *Fel d* 1 and nonsensitized subjects exposed to elevated levels of *Der f* 1 or *Fel d* 1, respectively.

Allergen and endotoxin levels

The occurrence of mite species varies between countries, and even in the same country it may vary between regions. *D. pteronyssinus* and *D. farinae* were analysed in the present study. The concentrations of major mite allergen *Der f* 1 in floor and mattress dust were 2.5–3.5 times higher than *Der p* 1 levels. This is similar to findings of other German studies [18, 19] in which *D. farinae* was found to be the predominant species. Concentrations of *Der p* 1 and *Der f* 1 in mattress dust were 5- and 6.5-times higher than in living room floor dust. *Der p* 1 and *Der f* 1 levels in mattress dust were higher than in floor dust from bedrooms and living rooms. This is in accordance with the results of other studies [20–22].

Fig. 1. – Adjusted odds ratios (OR) and 95% confidence intervals (CI) for selected respiratory symptoms comparing low exposure to mite and cat allergens to high exposure to one single allergen and mixtures of several allergens. ◇: *Dermatophagoides pteronyssinus* antigen 1 (*Der p* 1) >10 μg·g^{-1} (total number of subjects (N)=32); ●: *D. farinae* antigen 1 (*Der f* 1) >10 μg·g^{-1} (N=55); ■: cat antigen d1 (*Fel d* 1)>8 μg·g^{-1} (N=49); ▲: *Der p* 1>10 μg·g^{-1} and/or *Der f* 1>10 μg·g^{-1} and/or *Fel d* 1>8 μg·g^{-1} (N=34). The horizontal line represents the null hypothesis that the respective OR=1.

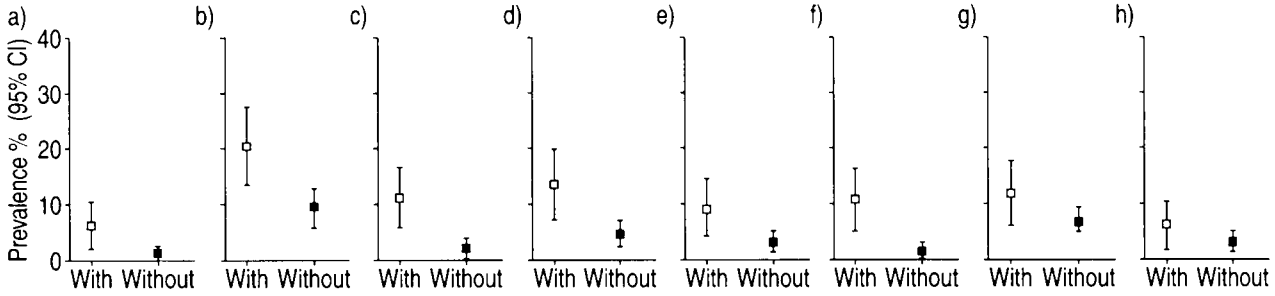

Fig. 2.–Prevalence and 95% confidence intervals (CI) of a) asthma attacks, b) wheeze, c) wheeze and breathlessness, d) wheeze without cold, e) shortness of breath at rest, f) breathlessness at night, g) cough at night, and h) cough without cold in winter, for subjects with (□) and without (■) allergic sensitization. Allergic sensitization refers to $\geqslant 1$ serum specific immunoglobulin-E (IgE) $\geqslant 0.70$ kU·L^{-1} against *Alternaria alternata* (m6), *Aspergillus fumigatus* (m3), tree pollen (tx5), birch (t3), *Cladosporium herbarum* (m2), *Dermatophagoides pteronyssinus* (d1), *D. farinae* (d2), grass pollen (gx1, gx4), *Phleum pratense* (g6), dog dander (e5), cat dander (e1), weed pollen (wx3), and *Blatella germanica* (i6).

Although only 15.6% of the subjects had a cat during the past 12 months, the major cat allergen *Fel d* 1 was found in 97.5% of the homes. This supports the fact that cat allergen is ubiquitous due to passive transport [23]. BOLLINGER *et al.* [24] found *Fel d* 1 levels capable of causing upper and lower respiratory symptoms in settled dust of homes without cats. Therefore, they concluded that the assessment of cat exposure should not be based solely on the presence or absence of a cat in the home. *Fel d* 1 levels >8 μg·g^{-1} were found in 11% of the homes where no cat was kept. Cat allergen content was lower in bedroom floor dust than in living room floor dust. This was also reported by CUSTOVIC *et al.* [25]. Endotoxin concentrations in living room floor dust were in line with the concentrations reported by MICHEL *et al.* [26].

Mite allergens and respiratory health

Since allergen exposure plays a major role in the pathogenesis of asthma and allergic sensitization in early childhood, or even prenatally, most studies include children. There are only a few studies on adults analysing the impact of allergen exposure on asthma and allergies. CHAN-YEUNG *et al.* [27] did not find a relationship between total allergen levels (sum of *Der p* 1 and *Der f* 1) and severity of asthma in adults either with or without house dust mite allergy, whereas CUSTOVIC *et al.* [28], as well as TUNNICLIFFE *et al.* [29], demonstrated a relationship between severity of asthma and exposure to *Der p* 1 in asthmatics sensitized to house dust mite. In the present study, the risk of asthma attacks and respiratory symptoms was not found to be increased continuously with exposure to mite allergens. For this reason, allergen levels were not used as continuous variables in the regression analysis, but were classified into two categories. The levels of 2 μg (data not shown) and 10 μg group I dust mite allergen, which have been proposed to increase the risk of acute attacks of asthma and symptoms, were used as cut-off values. Stronger effects (except for cough without cold in winter) were found using 10 μg as the cut-off value. Exposure to >10 μg mite allergen (*Der p* 1 plus *Der f* 1) and in particular, exposure to >10 μg *Der f* 1 per gram of dust, was found to be a risk factor for respiratory symptoms. Although *Der p* 1 and *Der f* 1 are highly cross-reactive, the effect estimates for the sum of *Der p* 1 and *Der f* 1 were weaker than the presented effects of *Der f* 1. No effect was found for *Der p* 1 alone, although the two antigens seem to be biologically rather similar. One possible explanation for this might be that *Der f* 1 is the predominant species in the region under study and that *Der f* 1 levels are much higher than *Der p* 1 levels. A relationship between presence of house dust mites and asthma-related respiratory symptoms in adults has also been demonstrated by BJÖRNSSON *et al.* [30]. In this Swedish study, the presence of house dust mites was assessed by applying the semiquantitative ACAREX test. Since this test is not species specific, it was not possible to clarify the role of different species of mites. ALVAREZ *et al.* [31] found asthma symptoms to be at least partially dependent on current exposure to *Der p* 1.

Cat allergens and respiratory health

ROOST *et al.* [32] found positive associations between the community prevalence of cat and the community prevalence of respiratory symptoms, physician-diagnosed asthma, and current asthma medication thus demonstrating the importance of cat allergens with respect to asthma and respiratory symptoms. *Fel d* 1 concentrations >8 μg·g^{-1} were found to be a risk factor for cough at night. The risk of respiratory symptoms was strongly increased when subjects were sensitized in addition to high exposure. The authors conclude that exposure to *Fel d* 1 levels >8 μg·g dust^{-1} is a risk factor for asthma attacks and respiratory symptoms in sensitized subjects. These findings are in line with the findings of NOERTJOJO *et al.* [33], who found cat owners to have a significantly higher risk of having current asthma and asthma-like symptoms compared to persons without cats. In the subset with positive skin-prick tests, they found that those who were allergic to cat dander had a significantly higher risk of current asthma than those not allergic to cat dander and not keeping a cat. Because of sample size limitations, it was not possible to do stratified analyses on cat-sensitized subjects in

Table 4. – Adjusted odds ratios (OR) and 95% confidence intervals (CI) for asthma attacks and respiratory symptoms comparing high to low exposure to *Dermatophagoides farinae* antigen 1 (*Der f* 1) and cat antigen d 1 (*Fel d* 1) related to allergic sensitization[#]

Symptoms	Sensitization[#] and *Der f* 1 ⩽10 µg·g^{-1}	No sensitization[#] and *Der f* 1 > 10 µg·g^{-1}	Sensitization[#] and *Der f* 1 >10 µg·g^{-1}	Sensitization[#] and *Fel d* 1 ⩽8 µg·g^{-1}	No sensitization[#] and *Fel d* 1 >8 µg·g^{-1}	Sensitization[#] and *Fel d* 1 >8 µg·g^{-1}
Total subjects n	95	50	31	106	48	20
Asthma attacks[¶]	2.28 (0.36–14.49)	1.69 (0.14–20.71)	7.94* (1.08–58.11)	3.35 (0.56–19.96)	2.22 (0.18–27.33)	4.65 (0.49–44.14)
Wheeze[¶]	1.42 (0.64–3.13)	0.68 (0.21–2.16)	2.26 (0.80–6.33)	1.81 (0.81–4.04)	2.14 (0.83–5.53)	3.35* (1.00–11.23)
Wheeze and breathlessness[¶]	4.92* (1.03–23.55)	3.28 (0.59–18.12)	20.12* (3.44–117.74)	5.57* (1.19–26.17)	3.48 (0.63–19.20)	14.24* (2.33–86.85)
Wheeze without cold[¶]	1.62 (0.60–4.42)	0.26 (0.03–2.15)	2.76 (0.77–9.85)	1.65 (0.59–4.61)	0.72 (0.15–3.56)	4.27* (1.15–15.90)
Shortness of breath at rest[¶]	2.44 (0.73–8.12)	1.33 (0.25–6.93)	4.25^{+} (0.94–19.14)	ne	ne	ne
Breathlessness at night[¶]	ne	ne	ne	7.30* (1.34–39.86)	10.29* (1.42–74.55)	9.21* (1.27–66.69)
Cough at night[¶]	1.35 (0.51–3.52)	1.42 (0.42–4.81)	2.55 (0.72–9.06)	1.89 (0.70–5.13)	3.78* (1.30–10.97)	2.72 (0.68–10.91)
Cough without cold in winter >3 months (habitually)	1.27 (0.33–4.92)	1.85 (0.42–8.14)	3.15 (0.60–16.50)	1.89 (0.48–7.42)	3.80^{+} (0.92–15.75)	2.99 (0.43–20.97)

Data are presented as adjusted OR (adjusted for "caseness", season of dust sampling, region, sex, age, education and active smoking) with 95% CI in parentheses unless otherwise stated. ne: not estimable. [#]: at least one serum-specific immonoglobulin-E (IgE) ⩾0.70 kU·L^{-1} against *Alternaria alternata* (m6), *Aspergillus fumigatus* (m3), tree pollen (tx5), birch (t3), *Cladosporium herbarum* (m2), *D. pteronyssinus* (d1), *D. farinae* (d2), grass pollen (gx1, gx4), *Phleum pratense* (g6), dog dander (e5), cat dander (e1), weed pollen (wx3), and *Blatella germanica* (i6); [¶]: past 12 months. Reference groups were *Der f* 1 ⩽10 µg·g^{-1} without allergic sensitization and *Fel d* 1 ⩽8 µg·g^{-1} without allergic sensitization. *: p<0.05; $^{+}$: p<0.10.

this study. In contrast, CHAN-YEUNG *et al.* [27] did not find a relationship between *Fel d* 1 and severity of asthma, either in adults with or without allergy to cats. Not only sensitized, but also nonsensitized subjects were found to have an increased risk of nocturnal breathlessness, nocturnal cough, and cough without cold in winter when exposed to >8 µg *Fel d* 1 per gram of dust. The reason for this is not yet clear. Active smoking was regarded as one possible factor explaining this dose-dependent effect in nonsensitized subjects, but smoking was not found to be associated with respiratory health, neither by itself nor as a cofactor in combination with a high allergen load.

Endotoxins and respiratory health

MICHEL *et al.* [26] found that the concentration of endotoxin measured in house dust is an important determinant of asthma severity in house dust mite-sensitized subjects. In the present study, endotoxins were not measured in mattress dust, but only in living room floor dust. Endotoxins in mattress dust were not found to affect respiratory health, neither by themselves nor as cofactors with high allergen levels (data not shown). One possible explanation for this lack of association might be that exposure to endotoxins, like exposure to allergens, is of major importance while sleeping, since people come into very close contact with it in their beds. A tendency towards higher frequencies of asthma attacks and respiratory symptoms was found among subjects exposed to elevated concentrations of endotoxins. Weak, but statistically significant, positive correlations were found between mite and cat allergens in mattress dust on the one hand and endotoxin concentrations in living room floor dust on the other. It is assumed that correlations would be stronger if both allergens and endotoxins were measured in mattress dust and that the correlation between allergens and endotoxins may explain the increase in risk of symptoms, even in nonsensitized subjects.

Mixtures of mite and cat allergens and respiratory health

Studies on the impact of exposure allergens in house dust on asthma-like symptoms focused either on mite or on cat allergen. Simultaneous exposure to elevated levels of more than one allergen was not reported in literature. In the present study, subjects exposed to elevated concentrations of at least two of the regarded allergens were found to have up to a seven-fold increase in the risk of respiratory symptoms in comparison with nonexposed subjects.

Conclusions

Indoor exposure to mite allergen (*Dermatophagoides farinae* antigen 1) and cat allergen (cat antigen 1) is a risk factor for asthma attacks and respiratory symptoms in adults, but not exposure to

Dermatophagoides pteronyssinus antigen 1. Subjects exposed to elevated levels of two or more of the aforementioned allergens had an increased risk of respiratory symptoms, compared to subjects exposed to elevated levels of a single allergen. Sensitized subjects were found to have an increased risk of asthma attacks and respiratory symptoms, which was strengthened if they were additionally exposed to elevated concentrations of *Dermatophagoides farinae* antigen 1 or cat antigen 1.

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