

Original article

Distribution of dust-mite allergens (Lep d 2, Der p 1, Der f 1, Der 2) in pig-farming environments and sensitization of the respective farmers

Background: The aim of the present study was to determine the distribution of mite allergens in pig-farming environments in comparison to urban homes and the relationship between exposure to mite allergens and sensitization to the respective allergens in 100 pig farmers with work-related respiratory symptoms.

Methods: The concentration of storage mite (Lep d 2) and house-dust-mite (Der p 1, Der f 1, and Der 2) allergens in dust collected from five different sampling sites (floor, wall, grain mill, transit area between confinement house and farmer's kitchen, and farmers' mattresses) was studied in relationship to the respective sensitization rates. Allergen concentrations in the mattresses were compared to those determined in mattresses from 22 urban dwellers.

Results: Median concentrations of Der p 1 and Der 2 in the mattresses of the farmers were significantly higher than in the urban dwellers' samples (53.4 µg/g dust vs 1.05 µg/g dust, $P=0.001$; 19.6 µg/g dust vs 2.2 µg/g dust, $P<0.0001$, respectively). Allergen concentrations in the transit areas were strongly related to bedroom exposure. In a multiple logistic regression model, a weak but significant relationship between Der p 1 exposure and sensitization to Der p 1 was found. Despite these findings, the prevalence of sensitization to mite allergens in the farmers (18%) was comparable to the prevalence in the general population.

Conclusions: Allergen exposure at the workplace is strongly related to the concentration of allergens in farmers' beds. Exposure to domestic mite allergens should be taken into account when assessing occupational exposure to allergens and the respiratory health of farmers.

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Farmers who work in barns or with animals frequently develop obstructive airway diseases (1, 2). In randomly selected farming populations, the prevalence of sensitization to storage mites is from about 5% (3) to 6% (4), with considerably higher rates in asthmatic farmers (3). Studies involving farmers from Sweden (5), Finland (6), and Denmark (3) have found *Lepidoglyphus destructor* to be the most important species inducing storage mite allergy, and comparable results have been reported in the UK (7, 8). High numbers of storage mites are present in crops (3, 9), but storage mites have also been found in the farmers' mattresses, with a median number of 60 mites (*Acarus siro*, *Tyrophagus putrescentiae*, *L. destructor*, or any storage mite) per gram dust (3).

Besides being rather time-consuming, counting mites as an index of allergen exposure may not optimally reflect the biologically relevant allergen concentrations at the workplace. The study of Campbell et al. (10) was the first to investigate the prevalence of a number of

potential allergens in barns. The major findings were that *L. destructor* allergens were abundantly present, and that in some barns allergens reactive with antisera to *Dermatophagoides* sp. could be demonstrated. No such data, however, are available regarding the distribution of these allergens in animal confinement houses where pigs or poultry are kept.

The main *L. destructor* allergen, Lep d 2, has been purified and characterized (11, 12), and it has become possible to measure Lep d 2, as well as group 1 and group 2 allergens of *Dermatophagoides* (13–17), by ELISA.

This study aimed

- 1) to assess the concentrations of *L. destructor* (Lep d 2) and *Dermatophagoides* (Der p 1, Der f 1, and Der 2) allergens in farming environments
- 2) to study the relationship between allergen concentration and sensitization to the respective allergens

in pig farmers with work-related respiratory symptoms.

Additionally, we determined the antigen concentrations in the mattresses of the farmers and of 22 urban dwellers.

Material and methods

Selection of farms

All pig farmers claiming compensation for suspected occupational airway disease at the local farm organization in Lower Saxony (northern Germany) in the years 1994–8 participated in a large-scale study of agricultural airway disease (2). The respective 100 pig farmers complained of work-related respiratory symptoms (69% shortness of breath, 76% cough without cold, and 44% wheezing), but no selection was made as to the presence or absence of atopy. The mean age of the farmers (86 men, 14 women) was 47 ± 11 years (age $\pm$ SD). The farms were quite representative of commercial pig farms, with an average of 423 ± 343 pigs per farm and 118 ± 90 pigs in an individual pig house.

Dust-sampling sites

Dusts were collected from five different sites on each farm by the same technician. In the swine confinement houses, samples of approximately 20 g were taken from the floor, wall, and grain mill with a metal spoon. In the transit area, an enclosed walkway or room usually located between the confinement house and the farmer's kitchen, floor dust was collected with a 700-W vacuum cleaner on a paper filter (pore size 6 μ m, ALK-Laboratories, Hørsholm, Denmark). Likewise, bedroom dust was collected by vacuuming a 1 m² area of the mattress for 2 min by standard methods (18). These sampling sites were chosen in order to get representative samples of the allergen exposure on the farm. The dust was stored at -20°C in sealed plastic bags until extraction and allergen analysis.

Additionally, 22 dust samples from the mattresses of randomly selected urban dwellers were taken by the same method.

Dust extraction and analysis of allergen content

The dust samples were sieved through a 1-mm mesh sieve, and dust was extracted for 2 h at room temperature in 0.1 M phosphate buffer containing 0.01% thimerosal and 0.05% bovine serum albumin. The extracts were centrifuged (4000 rpm, 15 min), passed through a 0.45- μ m pore size filter (Acrodisc GHP, Gelman Sciences, Rossdorf, Germany), and stored in aliquots at -20°C . Extracts were mailed on dry ice to the Research Department of Alergia e Immunología Abelló, Madrid, Spain, and the measure-

ments were performed on dust extracts by monoclonal antibody ELISA for Lep d 2, Der p 1, Der f 1, and *Dermatophagoides* group 2 allergens (Der 2) (13, 19). Allergen concentrations were expressed as micrograms per gram of sieved dust. Detection limits were 0.002 μ g/ml for Lep d 2, and 0.005 μ g/ml for Der p 1, Der f 1, and Der 2, which was equivalent to 0.02 μ g/g dust for Lep d 2 and 0.05 μ g/g dust for Der p 1, Der f 1, and Der 2 when at least 1 g of dust was available.

RAST

Specific IgE antibody measurements on sera from the farmers were made to *Dermatophagoides pteronyssinus* (d1), *D. farinae* (d2), *L. destructor* (d71), swine epithelium (e83), cow dander (e4), horse dander (e3), dog epithelium (e2), cat dander (e1), chicken feathers (e85), *Aspergillus fumigatus* (m3), *Penicillium notatum* (m1), and *Cladosporium herbarum* (m2) with RAST (Pharmacia and Upjohn AB, Uppsala, Sweden). The Phadebas RAST reference system (Pharmacia) was included in all assays, allowing expression of results in Phadebas RAST units (PRU). The assays were performed in duplicate according to the manufacturer's recommendations. A positive result was defined as ≥ 0.35 PRU/ml.

Statistical methods

Computations were completed with the aid of a statistical package for personal computers (Statistica© 5.1, StatSoft, Tulsa, OK, USA). Because the data were not normally distributed, differences between groups were analyzed by the Mann–Whitney U-test. Because of the wide range of the mite concentrations, the square roots of the crude data were used. Additionally, logistic regression was used to test dose-response relationships between the square root of the allergen concentrations and sensitization to the respective allergens. In order to estimate the quality of the models, chi-square values ($-2 * \log[L_0] - \log[L_1]$) for three (*L. destructor*, *D. farinae*) and four (*D. pteronyssinus*) degrees of freedom, as well as the corresponding *P* values, were calculated.

Results

Distribution of mite allergens

In the swine confinement buildings and transit areas, Lep d 2 was ubiquitously distributed with the highest concentrations in the grain mills (Table 1). The concentrations of house-dust mites (Der p 1, Der f 1, and Der 2) were low at all sampling sites in the confinement houses. Only in the transit area was Der p 1 detected in most (80%) of the dust samples, and more than 50% of these samples contained Der 2 mites.

Table 1. Mite allergen concentration in farming and urban environments

Allergen concentration (µg/g) median 25th, 75th percentile range	Confinement house						Transit area		Bedroom (mattress)			
	Floor			Wall			Floor		Farmer		Urban resident	
		% pos		% pos	Grain mill	% pos		% pos		% pos		% pos
Lep d 2	0.06 0.02, 0.53 0.0–54.1		0.07 0.0, 0.36 0.0–48.6		0.52 0.12, 3.69 0.0–25.69		0.17 0.07, 0.40 0.0–62.39		0.04 0.0, 0.12 0.0–2.34		0.0 0.0, 0.06 0.0–0.89	
Der p 1	0.0 0.0, 0.0 0.0–2.55	77	0.0 0.0, 0.0 0.0–0.0	74	0.0 0.0, 0.0 0.0–3.33	93	0.2 0.07, 1.00 0.0–10.01	91	53.40 13.6, 190.0 0.0–774.0	63	1.05 0.0, 10.0 0.0–51.0	41
Der f 1	0.0 0.0, 0.0 0.0–0.1	3	0.0 0.0, 0.0 0.0–0.49	0	0.0 0.0, 0.0 0.0–0.0	5	0.0 0.0, 0.0 0.0–0.62	80	0.2 0.0, 0.9 0.0–116.5	99	1.68 0.18, 17.0 0.0–67.0	73
Der 2	0.0 0.0, 0.0 0.0–0.39	2	0.0 0.0, 0.0 0.0–300.0	1	0.0 0.0, 0.11 0.0–0.64	0	0.07 0.0, 0.33 0.0–8.88	22	19.64 3.9, 57.7 0.0–312.0	65	2.2 0.9, 8.5 0.0–19.0	82
		15		12		36		56		99		86

% pos: percentage of samples above detection limit of assay.

In the mattresses of the farmers, the median concentration of Lep d 2 was 0.04 µg/g, compared to a median of 0 µg/g in the group of urban dwellers ($P=0.15$, Table 1). The median concentrations of Der p 1 and Der 2 in the mattresses of the urban population were significantly lower than that of the farmers' group. Der f 1 was found in 82% of urban beds with a median of 1.7 µg/g dust, a finding which was not significantly different from that of farmers' beds ($P=0.14$).

A univariate regression model was carried out to get information about the relationship between allergen concentrations in the transit areas and in the farmers' mattresses. As shown in Fig. 1, the concentrations of house-dust mites and storage mites in the transit areas were significantly correlated with the concentrations of these mites in the beds of the respective farmers ($P\leq 0.01$). This relationship was strongest for Der p 1.

RAST results

RAST results were available for 99 farmers, and the distribution of IgE antibodies to house dust and storage mites is shown in Fig. 2. Of the 37 sensitized farmers, 14 had IgE antibodies to both *D. pteronyssinus* and *D. farinae*. In total, 37% of the farmers were sensitized to at least one allergen (Table 2). Of the 15 farmers with IgE to at least one animal allergen, only two were positive to pig epithelium. One of the farmers was sensitized to pig epithelium alone, while the other also had specific IgE to *C. herbarum*. Forty-one percent of the sensitized farmers reacted to mold, and six of them were also sensitized to animals, while three farmers were also positive to house dust or storage mites.

Relationship between allergen concentration and sensitization

The median Lep d 2 concentrations in dust samples from the confinement house were slightly higher in farmers sensitized to *L. destructor* than in farmers not

sensitized to *L. destructor*, a finding which was statistically significant only for the floor samples (0.56 µg/g vs 0.05 µg/g; $P<0.05$). The median Der p 1 allergen concentrations in the mattresses of the farmers sensitized to *D. pteronyssinus* was about three times higher than in samples from RAST-negative subjects (110.3 µg/g vs 40.4 µg/g, $P=0.09$). There was no significant difference in allergen concentration between sensitized and nonsensitized farmers in samples taken from other locations. No differences could be observed when comparing farmers sensitized to *D. farinae* and farmers not sensitized (*D. farinae*-positive vs *D. farinae*-negative subjects: 0.14 vs 0.26 µg/g dust). The Der 2 content of mattresses of farmers sensitized to *D. pteronyssinus* or *D. farinae* did not differ significantly from mattresses of farmers who were not sensitized (28.6 vs 18.7 µg/g dust) (Table 3).

In addition to the univariate comparison between mite concentrations at different sampling sites of sensitized and nonsensitized farmers, logistic regression models were carried out (see Table 4 for mite concentrations in farmers' beds). In these models, no dose-response relationship between storage mite concentrations or Der f 1 concentrations and sensitization to either Lep d 2 or Der f 1 could be confirmed. Both models failed to reach the level of significance. Der p 1 concentrations in farmers' beds were shown to be significant predictors of sensitization to Der p 1 in the respective farmers (OR [95% CI]: 1.30 [1.01–1.67]; $P<0.05$).

Discussion

The four main findings of this study were as follows:

- 1) The median concentrations of Der p 1 were 50-fold higher in farmers' beds than in the beds of the group of urban dwellers (53.40 µg/g vs 1.05 µg/g,

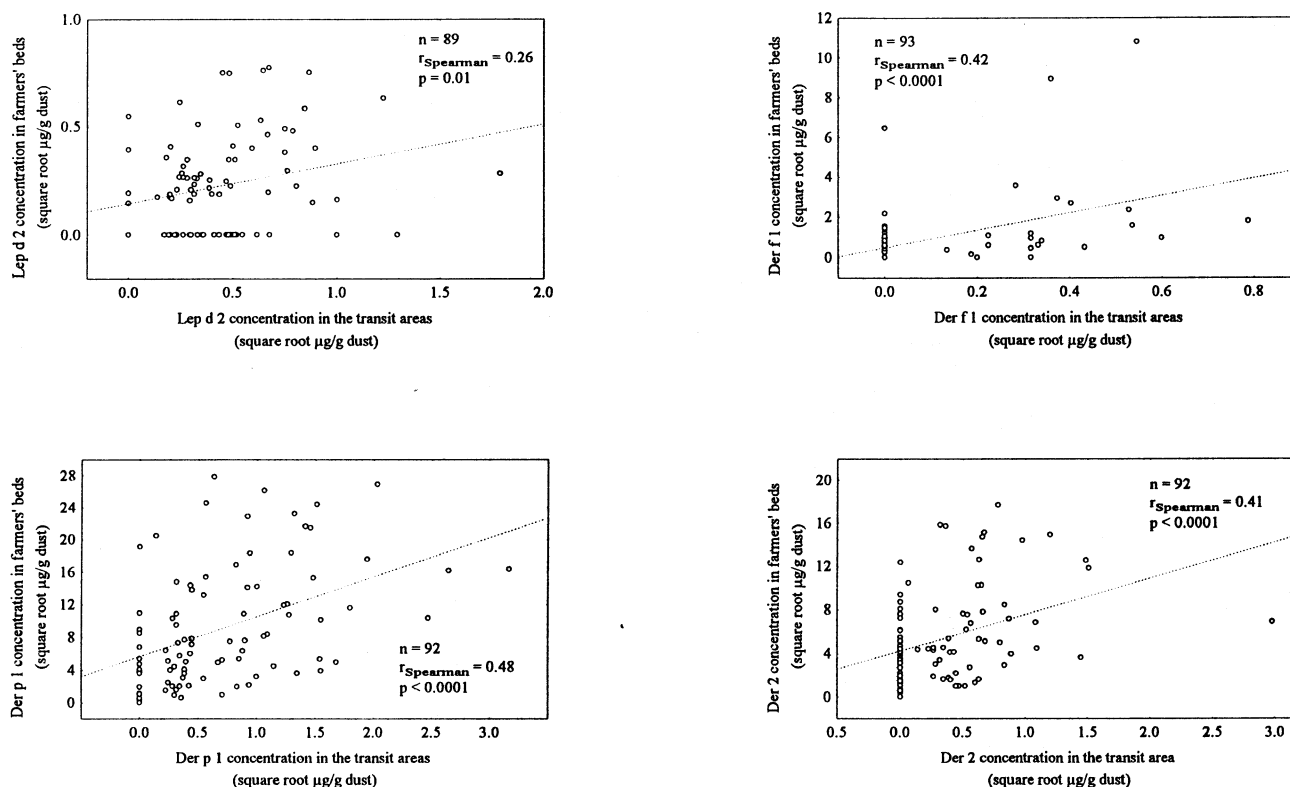


Figure 1. Spearman rank correlation between mite concentrations in transit areas and mite concentrations in farmers' beds.

- $P=0.001$), and higher than concentrations reported from many other places around the world (20–24).
- Mite concentrations in the transit areas were shown to be related to dust and storage mite concentrations in farmers' beds.
 - In a multivariate model, a dose-response relationship was seen between Der p 1 concentrations in farmers' beds and sensitization to *D. pteronyssinus*.
 - Despite high Der p 1 concentrations in dust samples taken from farming and home environments, farm-

ers did not show a higher prevalence of sensitization than the general population in Germany (18%).

L. destructor was found to be abundantly distributed. Since storage mites feed on molds, they can flourish on moldy grain. Our findings are consistent with this observation, since we found the highest concentrations of Lep d 2 (median 0.52 $\mu\text{g/g}$) in the grain mill. Echechipia et al. (19) detected Lep d 2 in 23% of house-dust samples in an urban population, with low levels (0.05–0.20 $\mu\text{g/g}$) in 10%, moderate levels (0.21–2.00 $\mu\text{g/g}$) in 6%, high levels (2.01–10.00 $\mu\text{g/g}$) in 3%, and very high levels (> 10 $\mu\text{g/g}$) in 4%. While Lep d 2 in our

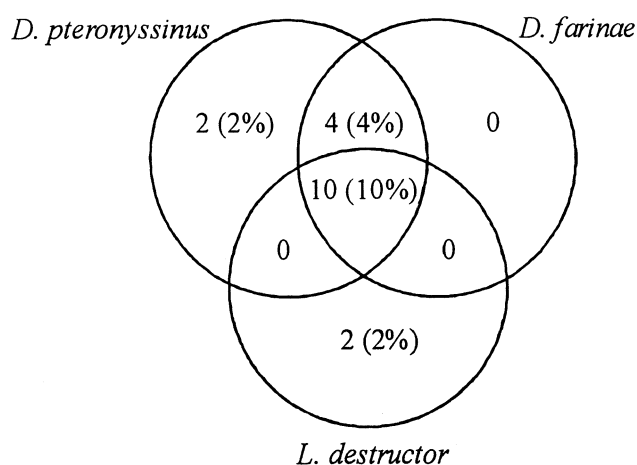


Figure 2. Distribution (number, %) of positive RAST to dust mites.

Table 2. Number and percentage of RAST-positive sera to different allergens tested

Allergens	RAST results (n=99)				
	Class 1	Class 2	Class 3	Class 4	Sum
<i>Dermatophagoides pteronyssinus</i> (d1)	2 (2%)	7 (7%)	3 (3%)	4 (4%)	16 (16%)
<i>D. farinae</i> (d2)	3 (3%)	5 (5%)	3 (3%)	3 (3%)	14 (14%)
<i>Lepidoglyphus destructor</i> (d71)	1 (1%)	2 (2%)	9 (9%)	0	12 (12%)
Swine epithelium (e83)	1 (1%)	0	1 (1%)	0	2 (2%)
Cow dander (e4)	1 (1%)	1 (1%)	0	0	2 (2%)
Horse dander (e3)	3 (3%)	0	0	0	3 (3%)
Dog epithelium (e2)	2 (2%)	0	0	0	2 (2%)
Cat dander (e1)	1 (1%)	1 (1%)	0	0	2 (2%)
Chicken feathers (e85)	6 (6%)	0	0	0	6 (6%)
<i>Aspergillus fumigatus</i> (m3)	2 (2%)	2 (2%)	0	0	4 (4%)
<i>Penicillium notatum</i> (m1)	7 (7%)	1 (1%)	0	0	8 (8%)
<i>Cladosporium herbarum</i> (m2)	9 (9%)	0	0	0	9 (9%)

Table 3. Mite allergen concentration in farming environments for farmers not sensitized to mites in comparison to sensitized farmers

Allergen concentration (µg/g) Median (range)	Swine confinement house			Transit area Floor	Bedroom (mattress) Farmer
	Floor	Wall	Grain mill		
Lep d 2					
RAST (<i>L. destructor</i>) negative	0.05 (0.0–54.1)	0.06 (0.0–48.6)	0.48 (0.0–25.7)	0.19 (0.0–62.4)	0.04 (0.0–2.3)
RAST (<i>L. destructor</i>) positive	0.56 (0.0–29.1)*	0.27 (0.0–13.2)	0.98 (0.0–10.5)	0.13 (0.0–1.7)	0.02 (0.0–0.6)
Der p 1					
RAST (<i>D. pteronyssinus</i>) negative	0.00 (0.0–2.6)	0.00 (0.0–0.0)	0.00 (0.0–3.3)	0.20 (0.0–10.0)	40.4 (0.0–774.0)
RAST (<i>D. pteronyssinus</i>) positive	0.00 (0.0–0.1)	0.00 (0.0–0.0)	0.00 (0.0–0.0)	0.32 (0.0–7.0)	110.3 (0.8–722.0)
Der f 1					
RAST (<i>D. farinae</i>) negative	0.00 (0.0–0.1)	0.00 (0.0–0.5)	0.00 (0.0–0.0)	0.00 (0.0–0.6)	0.26 (0.0–116.5)
RAST (<i>D. farinae</i>) positive	0.00 (0.0–0.0)	0.00 (0.0–0.0)	0.00 (0.0–0.0)	0.00 (0.0–0.4)	0.14 (0.0–2.5)
Der 2					
RAST (<i>D. pteronyssinus</i>) negative	0.00 (0.0–0.4)	0.00 (0.0–300.0)	0.00 (0.0–0.6)	0.08 (0.0–2.2)	18.7 (0.1–312.0)
RAST (<i>D. pteronyssinus</i>) positive	0.00 (0.0–0.3)	0.00 (0.0–0.1)	0.00 (0.0–0.3)	0.00 (0.0–8.9)	28.6 (0.0–247.0)

$P < 0.05$, Mann–Whitney U-test.

study was detected in 41% of the urban beds, none of the samples were above 2 µg/g. Forty-one percent were found to have low concentrations, and 9% had moderate concentrations of *L. destructor*. In contrast, 16% of farmers' beds had concentrations of Lep d 2 of 0.2–2 µg/g, but only one farmer's mattress had high concentrations (> 10 µg/g).

The results for Der p 1 agree with the investigations of Iversen et al. (3), who also found very high concentrations of house-dust mites in the mattresses of farmers (median count was 1480 living mites/g dust, *D. pteronyssinus*, *D. farinae*, any other house-dust mite). Exposure to more than 2 µg/g of group 1 mite allergen or 100 mites per gram dust is considered to increase the risk of sensitization and symptoms; exposure to more than 10 µg/g or 500 mites per gram dust is thought to increase the risk of acute asthma attacks (18, 25). Wahn et al. (26) proposed even lower thresholds. They suggest a low allergen exposure below concentrations of 0.4 µg Der p 1/g dust and a significant allergen exposure in the range of 0.4–2 µg Der p 1/g dust. In our study, only 13% of the mattresses were below the level of 2 µg/g. Antigen levels equal to and above 2 µg/g but below 10 µg/g were measured in 11% of the mattresses. A possible explanation could be the way farmers were recruited for the study. All subject farmers complained of work-related respiratory symptoms. Thus, one might spec-

ulate that the less "hygienic" conditions causing a higher concentration of mites are overrepresented in the sample. Furthermore, it is possible that *D. pteronyssinus* finds better growth conditions in a rural environment than in urban homes. Indoor temperature and humidity have the greatest influence on the growth of house-dust mites, and reports suggest that 7 g/kg is the level of absolute humidity above which excess mite growth will occur (27). Homes with a higher number of occupants, those located on the first floor, and private buildings tend to have a higher concentration of Der p 1 and Der 2 antigens (28–30). Older mattresses, use of a cover or under blanket, a higher weight of sampled dust, and a higher ratio of inhabitants per m² were significantly associated with increased concentrations of Der f 1. On the other hand, lower Der f 1 concentrations were found when interior spring mattresses were used (31). However, Der f 1 was the only allergen which tended to be higher in urban mattresses than in farmers' mattresses. Which of these factors were responsible for our results will be elucidated in future studies.

We could show a significant relationship between mite concentrations in transit areas and farmers' beds. Thus, among farmers, mite concentrations at occupational locations are related to bedroom exposure. Allergen avoidance in the workplaces of symptomatic farmers alone may be insufficient.

Table 4. Multiple logistic regression models using RAST results as dependent variable and mite concentration in bed of farmer, age, and sex as independent variables

OR (95% CI)	<i>n</i>	Chi square (DF ⁺)	<i>P</i>	Square root Lep d 2	Square root Der p 1	Square root Der f 1	Square root Der 2	Sex	Age
RAST (<i>L. destructor</i>) positive (1) vs negative (0)	93	1.71	0.63	0.57 (0.04, 8.17)	–	–	–	0.64 (0.07, 5.91)	0.97 (0.92, 1.03)
RAST (<i>D. pteronyssinus</i>) positive (1) vs negative (0)	94	10.23	0.04	–	1.30 (1.01, 1.67)	–	0.69 (0.44, 1.06)	0.40 (0.04, 3.76)	0.95 (0.90, 1.00)
RAST (<i>D. farinae</i>) positive (1) vs negative (0)	94	4.28	0.23	–	–	0.57 (0.21, 1.52)	–	0.57 (0.06, 5.25)	0.97 (0.91, 1.02)

⁺Degrees of freedom: three for *L. destructor* and *D. farinae*; four for *D. pteronyssinus*.

Only two serum samples contained IgE antibodies to *L. destructor* alone. The majority of samples (10%) contained IgE antibodies to all three mites tested. This may be due to cross-reactivity between these two families of Acaridae mites, as shown by Luczynska et al. (32) and Griffin et al. (33), who performed RAST inhibition studies. However, van Hage-Hamsten et al. (5) suggested that each of the storage mites and *D. pteronyssinus* possess unique allergens. Ventas et al. (11) also found that the major allergen of Lep d 2 is not responsible for allergenic cross-reactivity between *L. destructor* and *D. pteronyssinus*. One explanation is that most individuals were exposed to both storage mites and house-dust mites and became sensitized to both. Our findings demonstrate the complexity of the immunologic responses to the different mite species. Nevertheless, there was a tendency for higher Lep d 2 concentrations in the swine confinement houses in farmers sensitized to *L. destructor* that was significant for the floor samples. In contrast, concentrations of Der p 1, Der f 1, and Der 2 tended to be higher mostly in the mattresses of sensitized farmers. Due to the different habitat of the mite species, it might be that the sites of sensitization to house-dust mites and storage mites are different. However, when the logistic regression model was repeated, using sensitization to *L. destructor* as dependent variable and mite concentrations in the confinement houses as predictor variables, no dose-response relationship was seen (data not shown). One reason might be the low number of sensitized farmers in the present study.

A relationship between exposure and sensitization to Der p 1 has been shown in many other investigations (25, 26, 34) and was confirmed in a multiple logistic regression model in our study. Because of the high Der p 1 and Der 2 levels in farmers' beds, it might have been expected that these farmers would have a higher sensitization rate than the urban population; however, the prevalence of sensitization to house-dust mites among the farmers was not significantly higher than that of a randomly selected urban population (35) in the

same region of Germany (farmers vs urban dwellers aged 20–44: 24% vs 19%). In general population samples in the European Health Survey, Burney et al. (36) reported that 7–35% of adults aged 20–44 were sensitized to house-dust mites, whereas 24% of the farmers in this age group were sensitized to house-dust mites.

Our results are compatible with observations on sensitization rates among children growing up on farms. Children living on a farm had a significantly lower sensitization rate than children living in a rural environment but not on a farm (37, 38). Therefore, another possible explanation for the lack of an association between sensitization and exposure in our study might be a protective effect of dietary or environmental factors which seem to be relevant to farming environments, such as endotoxin. It may well be that "lifestyle" factors responsible for an increase in sensitization to common allergens in urban dwellers, such as spending most of the time in well-insulated buildings, may not be true of farmers and their children growing up on farms.

In conclusion, in farmers sensitized to any mite allergen, avoidance of workplace exposure may not be sufficient to control symptoms due to domestic exposure because of house-dust mites in mattresses. Further studies are needed on indoor factors associated with high Der p 1 and Der 2 antigen concentrations in farmers' mattresses, and the reason for similar sensitization rates in farmers exposed to higher allergen levels than those of the total population.

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References

1. ZEJDA JE, DOSMAN JA. Respiratory disorders in agriculture. *Tuber Lung Dis* 1993;**74**:74–86.
2. NOWAK D. Prevalence and risk factors for airway diseases in farmers – a new EC multicentre project. *Ann Agric Environ Med* 1994;**1**:81–82.
3. IVERSEN M, KORSGAARD J, HALLAS T, DAHL R. Mite allergy and exposure to storage mites and house dust mites in farmers. *Clin Exp Allergy* 1990;**20**:211–219.
4. VAN HAGE-HAMSTEN M, JOHANSSON SGO, HÖGLUND S, TÜLL P, WIRÉN A, ZETTERSTRÖM O. Storage mite allergy is common in a farming population. *Clin Allergy* 1985;**15**:555–564.
5. VAN HAGE-HAMSTEN M, JOHANSSON SGO, ZETTERSTRÖM O. Predominance of mite allergy over allergy to pollens and animal danders in a farming population. *Clin Allergy* 1987;**17**:417–423.
6. TERHO EO, VOHLONEN I, HUSMAN K, et al. Sensitization to storage mites and other work-related and common allergens among Finnish dairy farmers. *Eur J Respir Dis* 1987;**87 Suppl 152**:165–174.
7. CUTHBERT OD, BROSTOFF J, WRAITH DG, BRIGHTON WD. "Barn allergy": asthma and rhinitis due to storage mites. *Clin Allergy* 1979;**9**:229–236.
8. BLAINEY AD, TOPPING MD, OLLIERS S, DAVIES RJ. Respiratory symptoms in farm workers: role of storage mites. *Thorax* 1988;**43**:697–702.

9. CUTHBERT OD, JEFFREY IG. Barn allergy: an allergic respiratory disease of farmers. *Sem Respir Med* 1994;**14**:73–82.
10. CAMPBELL AR, SWANSON MC, FERNANDEZ-CALDAS E, REED CE, MAY JJ, PRATT DS. Aeroallergens in dairy barns near Cooperstown, New York and Rochester, Minnesota. *Am Rev Respir Dis* 1989;**140**:317–320.
11. VENTAS P, CARREIRA J, POLO F. Purification and characterization of *Lep d 2*, a major allergen from the mite *Lepidoglyphus destructor*. *Clin Exp Allergy* 1992;**22**:454–460.
12. VARELA J, VENTAS P, CARREIRA J, BARBAS JA, GIMENEZ-GALLEGO G, POLO F. Primary structure of *Lep d 2*, the main *Lepidoglyphus destructor* allergen. *Eur J Biochem* 1994;**225**:93–98.
13. HEYMANN P, CHAPMAN M, AALBERSE R, FOX J, PLATTS-MILLS T. Antigenic and structural analysis of group II allergens (*Der f 1 I* and *Der p 1 I*) from house dust mites (*Dermatophagoides* spp.). *J Allergy Clin Immunol* 1989;**83**:1055–1067.
14. VENTAS P, CARREIRA J, POLO F. Cuantificación del alérgeno mayoritario de *L. destructor* (*Lep d 2*) mediante anticuerpos monoclonales. *Rev Esp Alergol Immunol Clin* 1990;**5 Suppl** 3:88.
15. VENTAS ALGUACIL P, LOMBARDEO VEGA M, DUFFORT GUALTIERI O, CARREIRA GAREA J. Cuantificación de los alérgenos *Der p 1* y *Der f 1* de los ácaros del polvo doméstico y *Fel d 1* de gato mediante un ELISA en fase sólida. *Rev Esp Alergol Immunol Clin* 1990;**5**:71–75.
16. LUCZYNSKA CM, ARRUDA LK, PLATTS-MILLS TAE, MILLER JD, LOPEZ M, CHAPMAN MD. A two-site monoclonal antibody ELISA for the quantitation of the major *Dermatophagoides* spp. allergens, *Der p 1* and *Der f 1*. *J Immunol Methods* 1989;**118**:227–235.
17. PLATTS-MILLS TAE, POLLART SM, LUCZYNSKA CM, CHAPMAN MD, HEYMAN PW. Indoor allergens, quantification and characterization in relation to asthma. *Allergologie* 1989;**12**:63–67.
18. PLATTS-MILLS TAE, THOMAS WR, AALBERSE RC, VERVOLOET D, CHAPMAN MD. Dust mite allergens and asthma: report of a second international workshop. *J Allergy Clin Immunol* 1992;**89**:1046–1060.
19. ECHECHIPIA S, VENTAS P, AUDICIANA M, et al. Quantitation of major allergens in dust samples from urban populations collected in different seasons in two climate areas of the Basque region (Spain). *Allergy* 1995;**50**:478–482.
20. HENDERSON AJW, KENNEDY CTC, THOMPSON SJ, CARSWELL F. Temporal association between *Der p 1* exposure, immediate hypersensitivity and clinical severity of eczema. *Allergy* 1990;**45**:445–450.
21. OWEN S, MORGANSTERN J, HEPWORTH J, WOODCOCK A. Control of house dust mite antigen in bedding. *Lancet* 1990;**335**:396–397.
22. SPORIK R, HOLGATE ST, PLATTS-MILLS TAE, COGSWELL JJ. Exposure to house dust mite allergen (*Der p 1*) and the development of asthma in childhood. *N Engl J Med* 1990;**332**:502–507.
23. KALRA S, CRANK P, HEPWORTH J, PICKERING CAC, WOODCOCK AA. Absence of seasonal variation in concentrations of the house dust mite allergen in south Manchester homes. *Thorax* 1992;**47**:928–931.
24. MARKS GM, TOVEY ER, TOELLE BG, WACHINGER S, PEAT JK, WOOLCOCK AJ. Mite allergen (*Der p 1*) concentration in houses and its relation to the presence and severity of asthma in a population of Sydney schoolchildren. *J Allergy Clin Immunol* 1995;**96**:441–448.
25. LAU S, FALKENHORST G, WEBER A, et al. High mite-allergen exposure increases the risk of sensitization in atopic children and young adults. *J Allergy Clin Immunol* 1989;**84**:718–725.
26. WAHN U, LUN S, BERGMANN R, et al. Indoor allergen exposure is a risk factor for sensitization during the first three years of life. *J Allergy Clin Immunol* 1997;**99**:763–769.
27. PLATTS-MILLS TAE, DE WECK AL. Dust mite allergens and asthma – a worldwide problem: report of an international workshop. *J Allergy Clin Immunol* 1989;**83**:416–427.
28. WICKMAN M, NORDVALL SL, PERSHAGEN G, KORSGAARD J, JOHANSEN N. Sensitization to domestic mites in a cold temperate region. *Am Rev Respir Dis* 1993;**148**:58–62.
29. VAN STRIEN RT, VERHOEFF AP, BRUNEKREEFF B, VAN WIJNEN JH. Mite antigen in house dust: relationship with different housing conditions in The Netherlands. *Clin Exp Allergy* 1994;**24**:843–853.
30. CHAN-YEUNG M, BECKER A, LAM J, et al. House-dust mite allergen levels in two cities in Canada: effects of season, humidity, city and home characteristics. *Clin Exp Allergy* 1995;**25**:240–246.
31. KUEHR J, FRISCHER T, KARMAUS W, et al. Natural variation in mite antigen density in house dust and relationship to residential factors. *Clin Exp Allergy* 1994;**24**:229–237.
32. LUCZYNSKA CM, GRIFFIN P, DAVIES RJ, TOPPING MD. Prevalence of specific IgE to storage mites (*A. siro*, *L. destructor* and *T. longior*) in an urban population and crossreactivity with the house dust mite (*D. pteronyssinus*). *Clin Exp Allergy* 1990;**20**:403–406.
33. GRIFFIN P, FORD A, ALTERMAN L, et al. Allergenic and antigenic relationship between three species of storage mites and the house dust mite, *Dermatophagoides pteronyssinus*. *J Allergy Clin Immunol* 1989;**84**:108–117.
34. PLACIDO JL, CUESTA C, DELGADO L, et al. Indoor mite allergens in patients with respiratory allergy living in Porto, Portugal. *Allergy* 1996;**51**:633–639.
35. NOWAK D, HEINRICH J, JÖRRES R, et al. Prevalence of respiratory symptoms, bronchial hyperresponsiveness and atopy among adults: West and East Germany. *Eur Respir J* 1996;**9**:2541–2552.
36. BURNEY P, MALMBERG E, CHINN S, JARVIS D, LUCZYNSKA C, LAI E. The distribution of total and specific IgE in the European Community Respiratory Health Survey. *J Allergy Clin Immunol* 1997;**99**:314–322.
37. VON EHRENSTEIN O, VON MUTIUS E, ILLI S, HACHMEISTER A, VON KRIES R. Reduced prevalence of atopic diseases in children living on a farm. *Epidemiology* 1998;**9 (Suppl)**:S118.
38. BRAUN-FAHRLANDER C, GASSNER M, GRIZE L, et al. Prevalence of hay fever and allergic sensitization in farmers' children and their peers living in the same rural community. SCARPOL team. *Clin Exp Allergy* 1999;**29**:28–34.