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Mite allergen content in mattress dust of *Dermatophagoides*-allergic asthmatics/rhinitics and matched controls

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Summary

It has been suggested that the mites *Dermatophagoides pteronyssinus* and *D. farinae* are important indoor environmental factors facilitating both the sensitization of atopic subjects and asthmatic attacks of house dust-sensitive patients. Contradictory results have been reported about the current exposure to mites or their allergens among patients and control groups of atopic or non-atopic subjects. In order to determine whether there is a difference in mite exposure levels between *D. pteronyssinus*-sensitized asthmatics and/or rhinitis and control subjects we considered a case-control study of 70 patients with asthma and/or rhinitis and positive skin test reactions to *D. pteronyssinus* and twice as many control subjects who were matched as to age and sex. The first control subject for each patient was an immediate neighbour of the patient and the second was a patient arbitrarily chosen among hospitalized patients. Mite allergen exposure was measured in mattress dust collected under standardized conditions, by measuring *Der p* I + *Der f* I content and by performing a semiquantitative guanine determination (Acarex-test^R). The content of *Der p* I and *Der f* I was very high both in the homes of patients and those of healthy individuals: 69% of the total samples contained more than 10 μg *Der p* I + *Der f* I/g of dust. There was no significant difference between cases and controls with respect to *Der p* I, *Der f* I, *Der p* I + *Der f* I content and Acarex^R class distributions. The calculated odds-ratios associated with the Acarex test^R and the mite allergens did not differ significantly from the level 1·0. Our findings show that in a Rhine valley city such as Strasbourg with a high mite allergen load there is no difference in mite exposure between *D. pteronyssinus*-sensitized patients and controls who can be considered not to differ from the general population with respect to atopy.

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Introduction

House dust mites are an important determinant in the development of *Dermatophagoides pteronyssinus*-allergic asthma or rhinitis as suggested by studies showing the development of asthma in a community newly exposed to mites [1] and by clinical improvement when patients with asthma are removed from such exposure [2–4]. The lower prevalence of asthma at high altitudes compared with the prevalence in other areas was also attributed to the low levels of mite allergens found in mountain house dust samples [5]. Contradictory results have been reported in the literature concerning the current exposure to mites or their allergens among patients and control groups of atopic or non-atopic subjects. The purpose of this investigation was to measure levels of *Der p* I and *Der f* I (the group I allergen for the species *D. pteronyssinus* and *D. farinae*) and guanine levels, which give an index of the presence of all faecal mite antigens [6], in the homes of

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house dust mite-allergic asthmatics and/or rhinitics, and to compare these results with those from the homes of two groups of matched control subjects.

Materials and methods

Patients and control subjects

Seventy patients were selected from the patients referred to our outpatient clinic. All had asthma and/or rhinitis and positive skin-test reactions to *D. pteronyssinus*. Skin tests were performed intradermally (*D. pteronyssinus* extract, Institut Pasteur [7]) and were positive for 10^{-5} mg/ml (half sum of the cross-diameters of the weal > 12 mm). Radio allergosorbent tests were positive class 3 or 4. All patients had a documented history of asthma or rhinitis and perennial symptoms suggestive of house dust allergy requiring a continuous treatment. Most patients were adults (17 less than 15 years, eight less than 10 years). All lived in Strasbourg or the surrounding communities. No special avoidance procedures had been applied in their homes over the past year.

For each patient, two control subjects were selected who were matched as to age (± 5 yr) and sex. The first control subject was an immediate neighbour of the patient, the second was a patient arbitrarily selected from the list of patients referred to our University Hospital for non-allergic and non-respiratory reasons during the same month. Among these second controls we were unable to obtain data for 13 subjects who refused to participate. We made no attempt to investigate allergic status for the neighbours and hospital controls.

For each patient and control subject several data were recorded: age, family size, age and size of mattress, type of dwelling (i.e. apartment or individual home), type of heating and sampling season. The mean age of the whole population was 25 (s.d. 14·2, range 4–64). The indoor temperature and humidity were not measured at the time of sampling. The mean relative outdoor humidity for the sampling period was 80·72% (s.d. 9·44, range 63–94%). Using the mean outdoor relative humidity and mean outdoor temperature, we computed the mean absolute outdoor humidity (water vapour per kilogram of dry air) which was equal to 10·4 (s.d. 3·41, range 6·4–16·6).

Assessment of exposure

Dust was collected from mattresses under standardized conditions [8] by the same technician using the same vacuum cleaner (Philipps P95, 800 W) for 2 min/m^2. Dust collection was performed during the same month for each patient and his controls, between 26 October 1988 and 1 July 1990. Dust samples were sieved and weighed; one part of the sample was used to perform the Acarex-test^R [9] and another was used to prepare the aqueous extract for determination of *Der p* I and *Der f* I allergens.

According to the manufacturer's instructions Acarex test results were expressed in four increasing classes as follows: 0, guanine content <600 μg/g of dust; +, guanine content between 600 and 2500 μg/g of dust; + +, guanine content between 2500 and 10 000 μg/g of dust; + + +, guanine content of at least 10 000 μg/g of dust.

The evaluation of *Der p* I and *Der f* I allergen content was performed by a two-site monoclonal antibody ELISA following the procedure described by Luczynska *et al.* [10]. Monoclonal antibodies (5H8 specific for *Der p* I and 6A8 specific for *Der f* I), provided by Dr Chapman (University of Virginia, Charlottesville, U.S.A.), were coated onto a microtitre plate (Immulon MA98A, Dynatech, France). Results were expressed as micrograms of *Der p* I and *Der f* I per gram of dust according to the WHO International Standard [11]. Detection limits were 0·15 μg/g for *Der p* I and 0·05 μg/g for *Der f* I, respectively. The inter-assay coefficient of variation ranged from 5 to 20% and that of the intra-assay was 4%.

Statistical methods

The simple contingency tables were analysed by χ^2. The differences in means of *Der p* I, *Der f* I and *Der p* I + *Der f* I between patients and controls were assessed by non-parametric methods taking matching into account (i.e. Wilcoxon signed rank test). Parametric methods using a transformation of the quantities into logarithms (to normalize distributions) and comparison of these by a Student test for matched sample were also done.

Multivariate analysis is based on conditional logistic regression which takes matching into account [12]. The matching variables define strata and are never used as co-variables in the regression, resulting in better estimates of the odds-ratios.

We did not combine the two control groups because the matching variables were different. The climatic variables were more likely to be similar for the neighbour group than for the hospital control group.

Results

The Acarex^R class distributions for the 70 patients, the 70 control neighbours and the 57 hospital-recruited controls are shown in Fig. 1. Although there was a trend towards fewer Acarex classes + + and + + + among hospital controls, there was no significant difference between the three groups ($\chi^2 = 8{\cdot}49$, $P = 0{\cdot}2$) (Fig. 1).

The mean values, standard error of mean, median and range of *Der p* I, *Der f* I and *Der p* I + *Der f* I contents of the mattress dusts are shown in Table 1. There was no

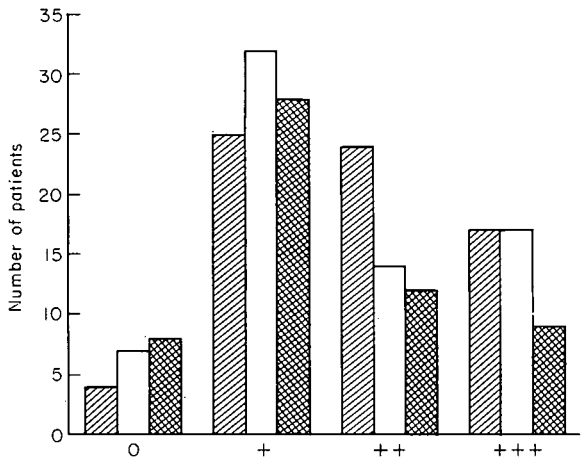

Fig. 1. Acarex^R class distributions for the ▨, patients, □, control neighbours and ▩, hospital-recruited controls.

significant difference between cases and control neighbours with respect to *Der p* I ($P=0{\cdot}22$), *Der f* I ($P=0{\cdot}43$) and *Der p* I + *Der f* I ($P=0{\cdot}23$), nor between cases and hospital controls ($P=0{\cdot}079$, $P=0{\cdot}87$, $P=0{\cdot}227$, respectively).

Most samples contained antigens from both *D. pteronyssinus* and *D. farinae*. Only one sample had no detectable mite antigen while five samples had a single species, in three cases only *D. pteronyssinus* and in two cases only *D. farinae*. In all, 128 mattress samples had a group I allergen content greater than 10 μg/g of dust (52 from patients, 76 from control subjects). Only three patients had a level less than 2 μg *Der p* I + *Der f* I/g of dust, whereas low levels were observed in seven control neighbours and 10 hospital controls. These data are in accordance with the results of the Acarex test^R where only 19 samples were class 0 (four patients, seven control neighbours and eight hospital controls).

The calculated odds-ratios associated with the Acarex-test^R results did not differ significantly from 1 (Table 2). The same was observed when exposure to mite allergens was considered (Table 3). The co-variables (i.e. season, type of heating, age and size of mattress, family size) did not differ significantly between patients and controls. The types of dwelling did not differ between patients and control neighbours but differed significantly between patients and hospital controls.

Discussion

This study shows that there is a large mite load in the Strasbourg area both in the homes of patients allergic to house dust mites (and using no preventive measures or acaricides) and those of healthy individuals. With respect to the semiquantitative determination of guanine, less than 10% of the house dust samples were class 0. In a previous study we found that only class 0 could predict *Der p* I + *Der f* I values below 2 μg/g [13]. Moreover, 69% of the samples have mite allergen levels as high as 10 μg/g of house dust and 90% of the samples have a level greater than 2 μg/g of house dust. These amounts are generally regarded as thresholds for the induction of sensitization in atopic children [14,15] and for the development of respiratory symptoms in sensitized persons [16]. In a prospective study using children with one parent who had asthma or hay fever, Sporik *et al.* [17] found geometric mean values of *Der p* I from mattress dust to be 18·4 and 10·6 μg/g in measurements performed in 1979 and 1989, respectively; almost all children with asthma at the age of 11 had been exposed to *Der p* I levels greater than 10 μg/g of dust at 1 year of age.

The comparison of antigen contents of house dust samples from different studies is not easy [18].

(1) The amounts of mite allergens measured do not exactly reflect the real amounts of inhaled allergens. The contents of house dust mite allergens have only been measured in mattress dust and not in house dust samples from other origins. We have no data concerning either the amount of mite allergens vacuum-extracted from the mattresses which would have been effectively rendered airborne by disturbances, or the real quantity of inhaled allergens. Moreover, measurement of house dust mite allergens in dust samples is more representative of the total amount of mite allergens possibly accumulated over several years.

(2) Procedures may differ. It is worth noting that our study had a constant sampling site (i.e. mattresses) and procedure (all samples being performed by the same technician). The allergen level can be determined on crude dust or on sieved dust. Nevertheless, all data are usually given in allergen concentration per gram of dust. Moreover, several authors express their overall results in geometric means after logarithmic transformation. A logarithmic transformation is used to normalize the distribution and allows (although not always) the use of parametric methods based on normal distributions. Geometric means (obtained as usual means on logarithmic transformation of the data) have no physical or concrete interpretation in our case.

(3) The populations used in the studies differ. No cross-sectional epidemiological study is so far available for our region. Nevertheless, among 3962 outpatients referred to our clinic for asthma or rhinitis between 1968 and 1984, 60·4% were sensitized to house dust

Table 1. Quantitative variables for *Der p* I, *Der f* I and *Der p* I + *Der f* I in mattress dust

	Patients ($n=67$)*	Control neighbours ($n=67$)*	Hospital controls ($n=56$)†
Der p I			
Mean (μg/g)	28·2	22·68	21·1
Range	0·174–176·0	0·05–144·0	0·043–232·25
s.e.m.	4·13	3·49	5·53
Median	15·4	13·1	8·74
Der f I			
Mean (μg/g)	18·2	22·68	21·1
Range	0·029–175·5	0·003–242·0	0·009–101·99
s.e.m.	4·27	3·49	5·53
Median	1·65	13·1	8·75
Der p I + *Der f* I			
Mean (μg/g)	46·4	38·8	33·07
Range	0·229–287·5	0·071–309·93	0·127–255·5
s.e.m.	6·37	6·39	6·55
Median	31·65	20·53	13·74
Geometric mean	24·38	15·64	10·44

* In three instances too little of the house dust samples remained after the Acarex-test^R so that house dust extracts could not be prepared for *Der p* I and *Der f* I determination.

† In one instance too little of the house dust samples remained after the Acarex-test^R so that house dust extracts could not be prepared for *Der p* I and *Der f* I determination.

and mites [19]. This prevalence among a selected population can also be compared with those of other French regions, even if the methodologies used to obtain the prevalence estimates differed: 18·2% among asthmatics living in the Alps and 49·2% in asthmatics living in Marseille [20]. For group I allergen the highest values found by Charpin *et al.* [5] were 106 μg/g in Martigues and 32·4 μg/g in Briançon; the value was 287·5 μg/g in the Strasbourg area. Lau *et al.* [15], in Berlin, found 210 μg/g to be the highest value for *Der p* I and 500 μg/g for *Der f* I. However, these authors found very low median values, indicating that half of the samples contained less than 0·6 μg/g mite allergen in atopic children; this contrasts with our study especially with respect to *Der p* I for which we found a median of 15·4 μg/g in allergic patients. In an earlier study performed on samples from a group in which 50% of subjects were sensitized, Wood *et al.* [21] found very low *Der p* I and *Der f* I levels, as demonstrated by the highest values found (i.e. 25 μg/g *Der p* I and 30 μg/g *Der f* I) and very low medians (i.e. 0·15 μg/g *Der p* I and 0·3 μg/g *Der f* I).

(4) Cross-comparability between studies may also depend on the different antibodies used to quantify *Der p* I allergens: species-specific rabbit antibody [21] or various monoclonal non-cross-reacting antibodies [5,15].

We did not attempt to evaluate the allergic status of the control groups, although we eliminated patients referred to the hospital for an allergic disease. Therefore, our groups should not differ from the general population in terms of frequency of atopy. The *D. pteronyssinus*-allergic patients and controls in this study did not differ significantly in terms of the guanine content of mattress house dust samples nor group I allergen levels. These results are in accordance with those reported by Wood *et al.* [21], who found no difference among asthmatics and non-asthmatics recruited among consecutive patients, nor among positive *D. farinae*-sensitized patients and *D. farinae*-unsensitized patients. However, this last study was not a matched case-control study. The only matched case-control study was performed by Korsgaard in 1983 [22], in which much higher concentrations of house dust mites were found in dust from the patients' homes than in dust from the homes of control subjects. However, this

Table 2. Calculated odds-ratio associated with Acarex-test[R] results

	Conditional logistic regression		
	Odds-ratio	Confidence interval (95%)	*P*
Patients vs neighbours	1·159	0·935–1·436	0·17
Patients vs University Hospital controls	1·219	1·012–1·64	0·19

study focused on the total number of mites and did not take into account mite allergens, since this determination was not available in 1983. Assessing antigen exposure by measuring major allergens in mattress house dust samples seems to be a more appropriate method than measuring mite content. Recent studies have pointed out that group I, group II and group I+group II allergens as well as quantitative guanine measurements showed poor correlations with total mite counts [23,24]. On the other hand, a significant correlation is found between quantitative guanine determinations and the group I allergens [13,24]. In spite of the differences in assessing mite allergen exposure, the discrepancies between the case control study performed by Korsgaard [22] and ours merit further discussion. Both use identical statistical analyses. Our patient population is nearly threefold larger, with only two matched controls. A third one, as in the Danish study, would not have given greater statistical weight to the conclusions [12]. In the Danish study, housing conditions were not included in the matching criteria and it turned out that the patients' dwellings were older. In our study we chose as the first control subject a neighbour, as being more likely to have a microclimate and dwelling similar to that of the patient; in fact, the type of dwelling differed significantly only between patients and hospital controls. It must be pointed out that univariate analysis showed a trend both for Acarex-test[R] and major allergen measurements to be lower in the hospital controls, but without statistical significance. This could be a consequence of the differences in dwellings, since other co-variables (i.e. type of heating, family size, age and size of mattress) did not differ significantly. Another major difference between the Korsgaard [22] study, our study and those from Hart and Whitehead [25] and Young *et al.* [26] might be the fact that under Danish climatical conditions the number of houses with a low mite infestation is greater in Aarhus than in Strasbourg. In regions such as the Strasbourg area where the level of 10 μg *Der p* I+*Der f* I is a frequent lower limit, no or very little difference between patients and controls can be found. Hart and Whitehead [25] and Young *et al.* [26] found that mite-sensitized patients did not differ significantly from either non-atopic or atopic subjects in the number of mites present in their homes. The results of a more recent study [27] suggest that differences in bed mite allergen levels of siblings with a comparable genetic tendency to atopy play a significant role in determining the development of house dust mite allergy. In contrast, there was no difference in the mattress levels of *Der p* I between the house dust mite-atopic and non-atopic sibling pairs. This is in agreement with our own findings.

Our results show that in a Rhine valley city such as Strasbourg and the surrounding communities, where there is a very high mite load, there is no difference in mite

Table 3. Calculated odds-ratios associated with exposure to mite allergens

		Conditional logistic regression		
		Odds-ratio	Confidence interval (95%)	*P*
Der p I	Patients vs control neighbours	1·008	0·994–1·022	0·25
	Patients vs University Hospital controls	0·997	0·983–1·01	0·62
Der f I	Patients vs control neighbours	1·004	0·992–1·016	0·47
	Patients vs University Hospital controls	1·01	0·992–1·013	0·24
Der p I+*Der f* I	Patients vs control neighbours	1·005	0·996–1·013	0·24
	Patients vs University Hospital controls	1·002	0·992–1·012	0·71

allergen exposure between *D. pteronyssinus*-sensitized patients and controls. Thus, the high levels of mite allergens may increase the risk of sensitization in the atopic population only.

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