

Comparison of allergic responses to dust mites in U.K. bakery workers and Swedish farmers

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Summary

The IgE RAST response to *Dermatophagoides pteronyssinus* and four storage mites (*Lepidoglyphus destructor*, *Tyrophagus putrescentiae*, *Glycyphagus domesticus*, and *Acarus siro*) was examined in 251 U.K. bakery workers and compared with that previously found in 440 Swedish farmers. Storage mites are found commonly in stored hay and grain so both these groups potentially encounter them in their work. In neither group of workers was a positive RAST (≥ 0.35 PRU) to *D. pteronyssinus* correlated with a positive RAST to a single storage mite. As in the Swedish farmers, significant though not strong correlations were found in the U.K. bakers between positive RAST responses to *G. domesticus* and *L. destructor* and to *T. putrescentiae* and *L. destructor* ($P < 0.05$).

Homologous and heterologous RAST inhibition studies showed there was low cross-reactivity between storage mites and *D. pteronyssinus*. *L. destructor* showed the least inhibition by the other antigens, suggesting it possessed the fewest common allergens.

The most important difference in the IgE responses between the two groups was the much higher response to *D. pteronyssinus* in the U.K. bakers, which was not found in the Swedish farmers whose highest IgE response was to *L. destructor*.

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Introduction

Skin tests and nasal and bronchial challenge tests have identified pyroglyphid mites such as *Dermatophagoides pteronyssinus* as the most dominant cause of house dust allergy [1–4]. Non-pyroglyphid storage mites have been shown to be an important cause of allergic respiratory disease in farmers in Scotland [5,6], Finland [7] and Sweden [8]. Storage mites are found commonly in stored hay and grain [9–12] so both bakery workers and farmers potentially encounter them in their work. We have studied the IgE response by RAST to storage mites and to *D. pteronyssinus* in the workforce of a U.K. bakery [13] and compared these with similar responses in Swedish farmers where a high prevalence of allergy to storage mites with respiratory symptoms has been previously reported [8,14]. The IgE response to *D. pteronyssinus* was

much more prevalent in the U.K. bakery workers and we have exploited this difference to examine cross-reactivity between *D. pteronyssinus* and storage mites in these two populations.

Methods

Subjects

The U.K. study population was 318 current employees of a bakery in South-East England. This investigation was part of a cross-sectional survey of respiratory symptoms, lung function and sensitization to flour [13]. We compared our findings with 440 Swedish farmers who in an epidemiological survey [8] had given blood for RAST against *D. pteronyssinus* and the same four storage mites [8,14].

RAST

RAST specific IgE antibody measurements on sera of the 244 bakery workers were made to *D. pteronyssinus* (d1);

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wheat (f4); *Lepidoglyphus destructor* (d71); *Tyrophagus putrescentiae* (d72); *Acarus siro* (d70) and *Glyciphagus domesticus* (d73) (Pharmacia Diagnosis AB, Uppsala, Sweden). The Phadebas RAST reference system (Pharmacia) was included in all assays, which allowed expression of results in Phadebas RAST units (PRU). The assays were performed in duplicate according to the manufacturer's recommendations except that the first incubation of serum and disc was made for 16 hours to allow for maximum sensitivity.

A positive result was defined as ≥ 0.35 PRU/ml (approximately 2.3% isotope binding).

RAST correlation studies

Cross-reactivity between *D. pteronyssinus* and the four storage mites was first evaluated from the RAST results obtained in the 35 bakery workers who had a RAST ≥ 0.35 PRU to at least one of the four grain mites and/or *D. pteronyssinus* and compared with the response found in Swedish farmers. To estimate the strength of the relationships between the different species of dust mites, Kendall rank correlation coefficients of the RAST values in the bakery workers (positive to at least one of two species) were calculated.

RAST inhibition

Homologous and heterologous paper disc RAST inhibition assays were performed as previously described [15], using *D. pteronyssinus* and three storage mites and a serum pool of eight sera from the bakery workers.

Antigens

The *D. pteronyssinus* antigen was a lyophilized, dialysed whole culture mite extract prepared with no yeast in the growth medium (Miles Laboratories, Bridgend, U.K.). The *L. destructor*, *T. putrescentiae* and *A. siro* antigens were extracted by the Mayo Clinic, Rochester, Minnesota, U.S.A. from Allergon AB cultures and supplied to us in 50% glycerol.

Assay

Briefly the serum pool was titrated in the four RAST inhibition assays and the dilution at the top of the linear part of each dose-response curve used for the inhibition assay. The percentage isotope binding of the chosen serum dilutions were: *D. pteronyssinus* 6.4; *L. destructor* 7.1; *T. putrescentiae* 4.4 and *A. siro* 6.9.

In order to try and elucidate the inhibitory powers between *D. pteronyssinus* and *L. destructor* further, sera

Table 1. Number of RAST reactions ≥ 0.35 PRU against *D. pteronyssinus*, and four storage mites in the U.K. bakers (244) and Swedish farmers (440)

Antigen	RAST ≥ 0.35 PRU			
	U.K. bakers		Swedish farmers	
	n	%	n	%
<i>D. pteronyssinus</i>	35	14.3	37	8.4
<i>L. destructor</i>	12	4.9	51	11.6
<i>T. putrescentiae</i>	11	4.5	30	6.8
<i>A. siro</i>	5	2.0	26	5.9
<i>G. domesticus</i>	9	3.7	35	7.95
≥ 1 of 4 storage mite	16	6.55	53	12.0

with three types of different RAST response were studied in RAST inhibition assays:

- (1) A serum pool (five sera) from the U.K. bakery workers, RAST positive to both *D. pteronyssinus* and *L. destructor*.
- (2) A pool of two sera from the U.K. bakery workers RAST positive to *D. pteronyssinus* but negative to *L. destructor*.
- (3) A pool of two sera from Sweden RAST positive to *L. destructor* but negative to *D. pteronyssinus*.

Five fourfold inhibitory concentrations of both lyophilized extracts from 250 to 0.98 $\mu\text{g/ml}$ were tested with the three types of sera.

Results

RAST

RAST results on the 244 U.K. bakery sera to the six antigens were analysed and compared with those of the Swedish farmers taking the cut-off of ≥ 0.35 PRU as positive (approximately 2.3% isotope binding) (Table 1). Thirty-five (14.3%) bakery workers had a positive RAST (≥ 0.35 PRU) to *D. pteronyssinus* and 16 workers had a RAST of this level to one or more storage mites, all of whom were also RAST positive to *D. pteronyssinus*. In contrast the RAST response in the Swedish farmers was highest to one or more storage mites (12%) whilst only 8.4% had a positive RAST to *D. pteronyssinus*. Table 2 (upper right field) shows the number of ≥ 0.35 PRU RAST reactions against different combinations of storage mites and *D. pteronyssinus* in the bakery workers. The most frequent RAST positive response to a storage mite was to *L. destructor* (12) and the least frequent to *A. siro* (5).

Table 2. Number of positive RAST reactions (≥ 0.35 PRU) and correlation coefficients against different combinations of storage mites and *D. pteronyssinus* in the U.K. bakers.

	L.d	G.d	T.p	A.s	D.pt
L.d	12	9	7	1	12
G.d	0.48*	9	7	1	9
T.p	0.32*	0.18	11	5	11
A.s	-0.27	-0.38*	0.13	5	5
D.pt	0.098	0.12	-0.23	-0.12	35

L.d, *L. destructor*; G.d, *G. domesticus*; T.p, *T. putrescentiae*; A.s, *A. siro*; D.pt, *D. pteronyssinus*.

Upper right field shows number of positive RAST reactions. Lower left field shows correlation coefficient (r). * = $P < 0.05$.

Table 3. Percentage positive RAST reactions (≥ 0.35 PRU/ml) against different combinations of storage mite species and *D. pteronyssinus*: comparison of U.K. bakers and Swedish farmers.

	L.d	G.d	T.p	A.s	D.pt
L.d	75 (96)	56 (57)	44 (64)	6 (45)	75 (55)
G.d		56 (57)	44 (49)	6 (34)	56 (36)
T.p			69 (66)	31 (43)	69 (49)
A.s				31 (49)	31 (42)
D.pt					100 (70)

L.d, *L. destructor*; G.d, *G. domesticus*; T.p, *T. putrescentiae*; D.pt, *D. pteronyssinus*; A.s, *A. siro*; No, U.K. bakers ($n=16$); (No), Swedish farmers ($n=53$).

Comparing the 16 bakery workers with the 53 Swedish farmers who had a positive RAST (≥ 0.35 PRU/ml) to one or more storage mites, whereas all the U.K. workers had a positive RAST to *D. pteronyssinus* this was true of only 70% of Swedish farmers whose most frequent response was to *L. destructor* (96% as compared to 75% of UK workers) (Table 3).

RAST correlation studies

No significant correlation was found in the U.K. bakery workers or Swedish farmers between any storage mite and *D. pteronyssinus*. Results reported earlier [14] for the Swedish farmers (SF) are given for comparison. In the bakers there was a negative correlation ($r = -0.38$) (SF, $r = 0.06$) between *G. domesticus* and *A. siro* (Table 2 lower left field).

Significant correlations were found between *G. domesticus* and *L. destructor* ($r = 0.48$) and between *T. putrescentiae* and *L. destructor* ($r = 0.32$) in the bakers (SF, $r = 0.66-0.84$).

Figure 1a-c shows graphically the relationships between RAST values in the bakery workers against a selection of mites; the crossed lines indicate the positive RAST cut-off of 0.35 PRU. In Fig. 1a no correlation was seen between *D. pteronyssinus* and *L. destructor* ($r = 0.098$) (SF, $r = 0.18$). Figure 1b shows the positive correlation between *G. domesticus* and *L. destructor* ($r = 0.48$) (SF, $r = 0.84$) whilst Figure 1c shows the negative correlation between *G. domesticus* and *A. siro* ($r = -0.38$) (SF, $r = 0.41$).

RAST inhibition studies

The inhibitory capacities of *D. pteronyssinus* and three storage mites (*L. destructor*, *T. putrescentiae*, and *A. siro*) on the U.K. bakery serum pool are shown in Figure 2 (a-d). In the *D. pteronyssinus* RAST the three storage mites showed some inhibitory activity but the lines did not show a dose-dependent inhibition similar to the *D. pteronyssinus* self inhibition (Fig. 2a). The other mite antigens showed little inhibition of the *L. destructor* RAST (Fig. 2b). In the *T. putrescentiae* (Fig. 2c) and *A. siro* (Fig. 2d) RAST assays heterologous antigens gave dose-dependent curves of inhibition equal to that with the homologous antigen. To understand further the allergenic relationship between *D. pteronyssinus* and *L. destructor* we used three types of sera and found the only different reaction in these cross-inhibitions was between sera type 1 (RAST positive to both allergens) and sera type 2 (*D. pteronyssinus* positive and *L. destructor* negative) in the *L. destructor* inhibition of the *D. pteronyssinus* RAST (Fig. 3). With 250 $\mu\text{g/ml}$ *L. destructor* as inhibitor type 1 sera gave 42% RAST inhibition whilst type 2 sera gave only 16% RAST inhibition. Looking at both the *D. pteronyssinus* and *L. destructor* RAST inhibition results with 250 $\mu\text{g/ml}$ of inhibitor, *L. destructor* is seen to inhibit the *D. pteronyssinus* RAST (16 and 42%) more than *D. pteronyssinus* inhibits the *L. destructor* RAST (9% and 9%).

Discussion

In the present study we have compared the IgE antibody response to *D. pteronyssinus* and four storage mites in U.K. bakery workers with that previously found in Swedish farmers.

All the bakery workers who had a positive RAST to one or more storage mites also had a positive RAST to *D. pteronyssinus*. This finding could suggest cross-reacti-

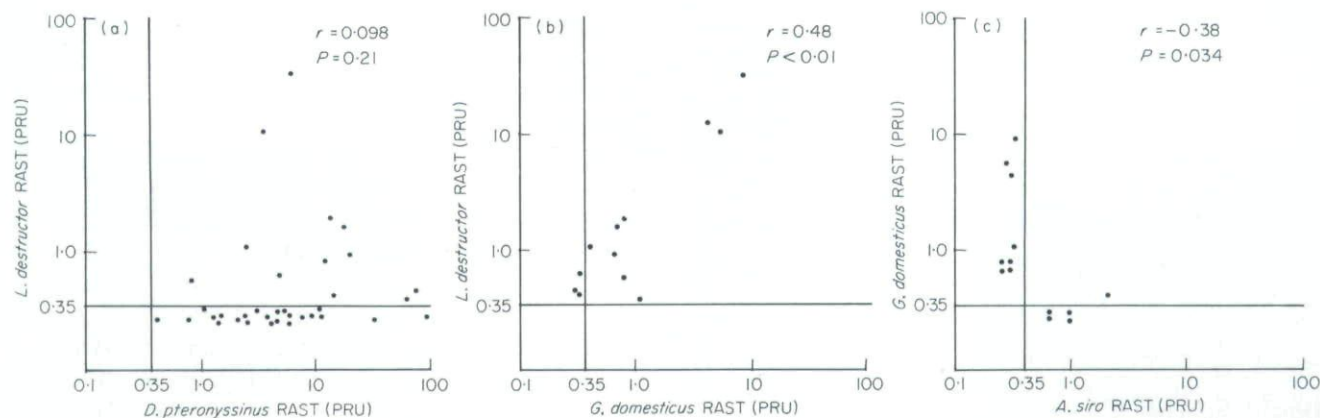


Fig. 1. RAST score correlations. (a) *L. destructor* and *D. pteronyssinus* ($n=35$). (b) *L. destructor* and *G. domesticus* ($n=12$). (c) *G. domesticus* and *A. siro* ($n=13$). The correlation coefficients (r) were calculated on all bakery workers with a positive RAST (≥ 0.35 PRU) to at least one of the two respective mites.

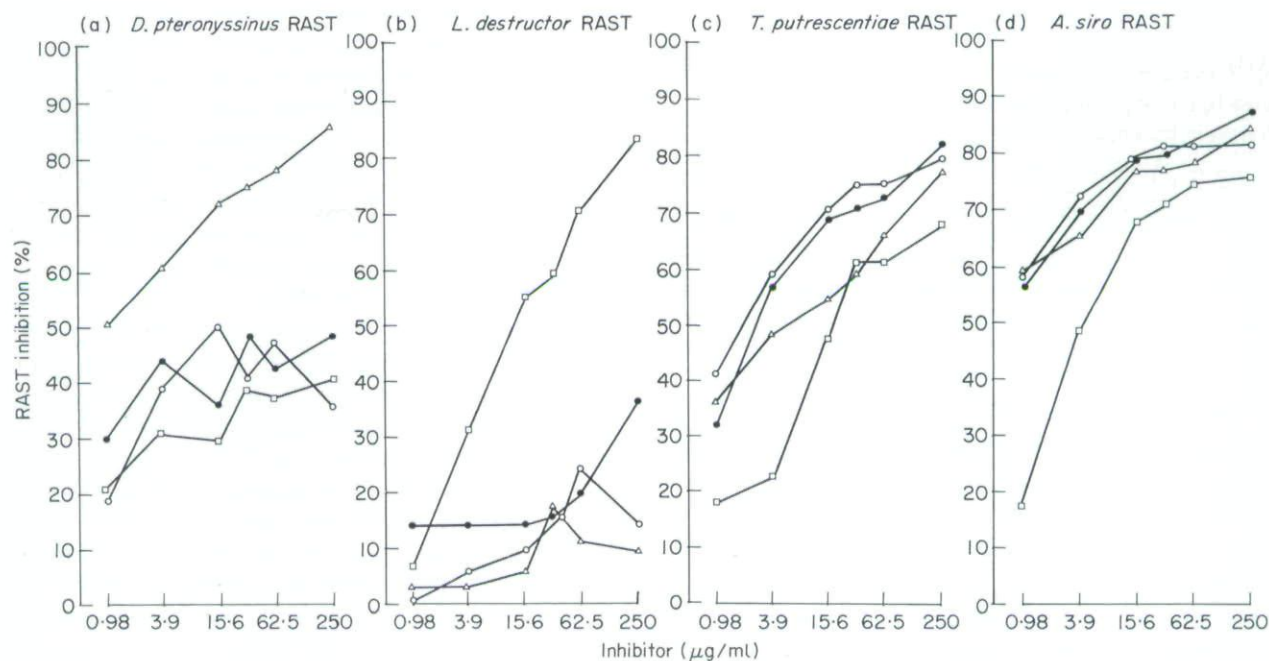


Fig. 2. Homologous and heterologous RAST inhibition assays with *D. pteronyssinus* (Δ), *L. destructor* ($\square$), *T. putrescentiae* ($\circ$) and *A. siro* ($\bullet$).

vity between this pyroglyphid and non-pyroglyphid mites as claimed by Griffin *et al.* [16] and by Luczynska *et al.* [17] but is not supported by further data in this paper nor reported earlier by van Hage-Hamsten *et al.* [18], as discussed later.

No significant correlation was found between the response to any storage mite and *D. pteronyssinus* in either the U.K. bakery workers or the Swedish farmers. In both groups significant RAST correlations were found between *G. domesticus* and *L. destructor* and between

T. putrescentiae and *L. destructor* indicating a strong allergenic relationship between these mites. *A. siro* appears to differ from these storage mites since there was not a positive correlation in either of the study groups between the RAST results for *A. siro* and *G. domesticus*.

RAST inhibition was used to examine cross-reactivity between the four mite species studied. The three storage mites showed some inhibitory activity in the *D. pteronyssinus* RAST but the inhibition lines were not parallel with *D. pteronyssinus* suggesting absence of immunological

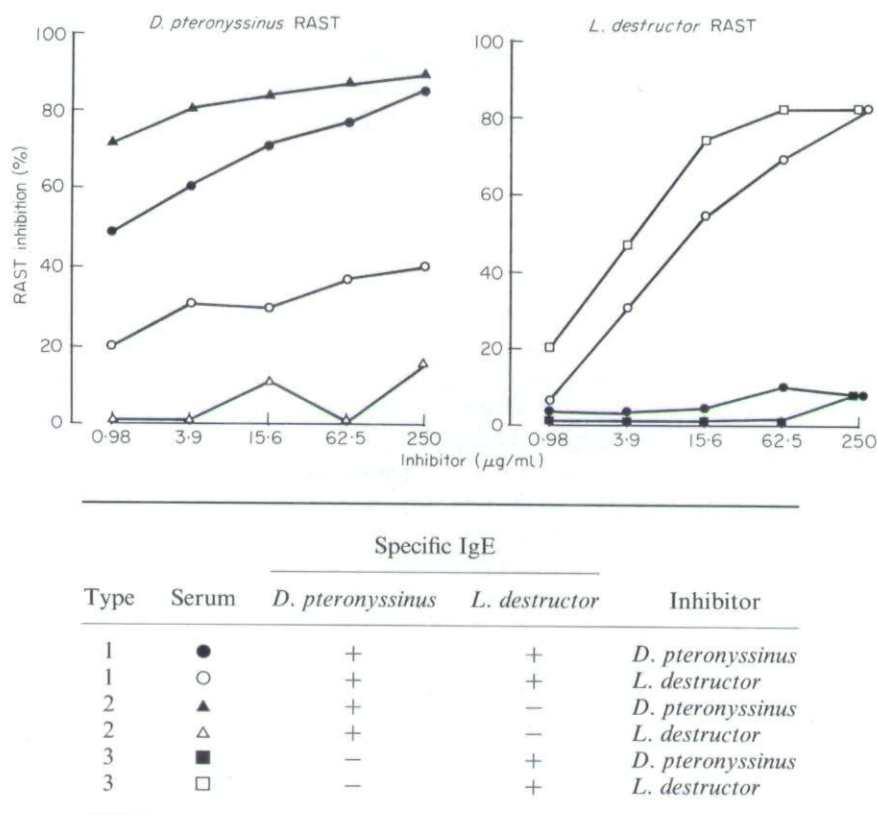


Fig. 3. Cross-RAST inhibition assays with *D. pteronyssinus* and *L. destructor* using three types of sera of varying hypersensitivities.

identity and allergenic cross-reactivity between *D. pteronyssinus* and these three storage mites. However, immunoblot studies of allergenic similarities of *D. pteronyssinus* and *L. destructor* have shown some allergens to be rather similar although others to be very different [19].

Extensive cross-reactivity was seen in the *T. putrescentiae* and *A. siro* RAST inhibition assays in that heterologous antigens gave dose-response curves of inhibition similar to that with the homologous antigen. This suggests that some *D. pteronyssinus* allergens are shared by *A. siro* and *T. putrescentiae*. In contrast, with *L. destructor* there was little inhibition by the other mite antigens, suggesting that *L. destructor* contains many unshared allergens. *L. destructor* and *D. pteronyssinus* were chosen for more detailed study because RAST inhibition showed that they had the greatest allergenic differences. Three types of sera with different antibody responses to these antigens were studied in RAST inhibition assays. In the *L. destructor* RAST Type 1 sera (RAST positive to both allergens) is seen to have specific antibodies to *L. destructor* without appreciable amounts of specific antibodies to *D. pteronyssinus*, since *D. pteronyssinus* is not inhibiting. In contrast, in the *D. pteronyssinus* RAST Type 1 sera is seen to have antibodies

specific to *D. pteronyssinus* which appear to recognize *L. destructor* (and also cross-react with *D. pteronyssinus*) since *L. destructor* has partial cross-reactivity.

In the type 2 sera (*D. pteronyssinus* positive, *L. destructor* negative) the primary sensitizing allergen was *D. pteronyssinus* since this allergen produced a dose-response curve of inhibition but *L. destructor* did not. By the same premise the primary sensitizing allergen of type 3 sera (*D. pteronyssinus* negative, *L. destructor* positive) was *L. destructor*. In the type 1 sera, which have antibodies to both allergens, primary sensitization was probably to *D. pteronyssinus* since in the *D. pteronyssinus* RAST the homologous allergen gave high inhibition whilst *L. destructor* gave much lower, partial inhibition.

There have been conflicting reports on the possibility of cross-reactivity between house dust mites and storage mites which may be further confused by poorly defined mite extracts used in some studies as well as the specificity of the sera used due to the nature of the primary sensitizing allergen. Griffin *et al.* [16] found limited cross-reactivity between *D. pteronyssinus* and storage mites and concluded that the two types of mite possess common as well as distinct allergenic determinants. Luczynska *et al.* [17] found more extensive cross-reactivity between *D.*

pteronysinus and storage mites in an urban population, although agreed with us that there was only limited cross-reactivity between *D. pteronyssinus* and *L. destructor*.

In contrast other groups have found no significant cross-reactivity between storage mites and *D. pteronyssinus*. These include Korsgaard *et al.* [20] looking at storage mites as a cause of bronchial asthma in Denmark, Georges *et al.* [21] studying asthmatic children with allergy to *D. pteronyssinus* in France and Ford and Platts-Mills [22] who considered multiple sensitization more likely than cross-reactivity. The detailed study of Swedish farmers by van Hage-Hamsten *et al.* [18] has provided the strongest evidence of lack of important cross-reactivity between *D. pteronyssinus* and the four storage mites. They found that *L. destructor*, *G. domesticus* and *T. putrescentiae* shared common or related allergens as we also found in our bakery workers. However, since some sera have IgE to storage mites and not to *D. pteronyssinus* and vice versa, storage mites and *D. pteronyssinus* must also possess distinct allergens.

Although there was a high immune response to storage mites in the bakery workers, *D. pteronyssinus* is probably the primary sensitizing allergen. Fourteen per cent had a positive RAST to *D. pteronyssinus* compared with 6.5% to one or more storage mites. There were 19 workers with RAST ≥ 0.35 PRU to *D. pteronyssinus* but not to storage mites and no workers with a positive RAST to storage mites and not to *D. pteronyssinus*. In contrast, in Swedish farming communities where there is considerable storage mite exposure, *L. destructor* appears to be the most important primary sensitizing mite allergen. Ninety-six per cent of Swedish farmers with specific IgE to a storage mite had a positive RAST to *L. destructor* [8] and the importance of this mite was also found by Cuthbert *et al.* [6] in Scottish farm workers and by Terho *et al.* [7] in Finnish dairy farmers.

House dust mite and storage mites flourish in a warm humid environment, ideally 90% relative humidity and at a temperature between 25 and 30°C. These conditions can occur in the environments of both the groups studied, making it unlikely that differences in occupational environmental conditions could account for the greater sensitivity to storage mite in the Swedish farmers and to *D. pteronyssinus* in the bakery workers.

Several workers have reported that mite growth is encouraged by damp housing conditions [23–25]. The differences we have observed could therefore be attributable to differences in housing conditions between the two countries. We are, however, unaware of any evidence which would suggest this.

The difference between the patterns of allergic response to house dust and storage mites probably reflect the greater occupational exposure among Swedish farmers to

storage mites and the greater environmental exposure to house dust mite in the U.K.

We have subsequently examined the immune response of salt-packing workers in the U.K. without flour exposure and found a similar prevalence of a positive RAST to storage mites as that found in the bakery workers [26]. These results imply that storage mites are widely distributed in the environment and are not of special significance in allergic responses in bakery workers exposed to flour. Further studies are required to determine which key allergenic determinants in storage mites are responsible for allergic disease and how they compare with those in *D. pteronyssinus*.

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