

ELISA method for detection of mite allergens in barn dust: comparison with mite counts

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ELISA (enzyme-linked immunosorbent assay) inhibition with a monoclonal antibody (mAb) (42B6) to *Lepidoglyphus destructor* was used to detect and quantify the storage-mite allergens in 30 dust samples collected from barns. Regarding the mite fauna, microscopic inspection of the barn dust and mite counts showed that *L. destructor* infested all 30 barns investigated (range 430–195 400 mites/g dust). In 29/30 barns, *L. destructor* constituted more than 70% of the Astigmata species. *Acarus siro* was found in 26 samples, the highest value being 16 155 mites/g. No *Dermatophagoides* species were found. As to mites of the suborder of Prostigmata, species belonging to seven different families were detected. Besides the predominant *L. destructor*, allergens derived from other storage mites such as *Glycyphagus domesticus*, *A. siro*, and *Tyrophagus putrescentiae* have previously been assessed by this ELISA method. The correlation between number of mites and concentrations of mite antigen as measured by ELISA was assessed by linear regression ($r^2=0.83$). Thus, inhibition of mAb 42B6 in ELISA would seem to offer a simple and reliable method to detect levels of dust-mite species belonging to the Acaridae and Glycyphagidae families.

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Mites are considered to be a major cause of asthma throughout the world (1). In the late 1970s, *Tyrophagus putrescentiae* was shown to cause “barn allergy” in the Orkney Islands of the UK (2). Previous studies in farming populations in Sweden have shown that the mite *Lepidoglyphus destructor* is the main cause of IgE-mediated sensitization (3, 4). *L. destructor* flourishes mainly in storage barns and other farming environments but is also found in urban areas in Sweden (5) and Spain (6).

Several studies have established a dose-response relationship between allergen exposure and sensitization to mites. These studies were used to define a threshold above which exposure was likely to result in sensitization; for example, 2 µg Der p 1/g dust (7). To inform the allergic patient about high-risk areas and to ascertain the efficiency of allergen avoidance strategies, allergists must know allergen levels in indoor dust. No data are available on storage-mite allergens. Basically, there are three different methods of determining exposure to mites; microscopic counting, analysis of guanine content,

and specific allergen assay with immunochemical methods such as radioimmunoassay (RIA) and enzyme-linked immunosorbent assay (ELISA). Counting is time-consuming and requires expert knowledge of mite morphology to distinguish among different species. Guanine determination suffers in specificity because substances other than those of mites can contribute to the amount obtained (8). Therefore, the most common assays are based on detection of allergens by antibodies (9, 10).

In the present study, we have used an inhibition-ELISA method to measure dust-mite allergens found in farming environments. The monoclonal antibody (mAb) used in this assay, 42B6, has been shown to immunoprecipitate a band of 39 kDa (11). This inhibition-ELISA assay has previously been shown to detect mites of Glycyphagidae and, to a lesser extent, mites of Acaridae origin (11). IgE antibodies to the 39-kDa allergen detected by 42B6 are present in roughly half of the sera from sensitized patients (5). To evaluate the accuracy of

the assay, we here also counted the different mite species in each sample.

Material and methods

Thirty barn dust samples were collected from 30 farms on Gotland, a Swedish island in the Baltic Sea with a high prevalence of mite allergy (12). The sampling took place in winter. The samples were collected from the center of stored hay and on or close to the floor. About 2 l of hay dust was placed in plastic bags, which were carefully sealed and mailed to the laboratory. No later than 3 days after collection, each sample was sieved three times with a 1-mm mesh laboratory sieve (Endecotts Ltd). The dust was divided into two portions: one for extraction and one for mite counting.

Barn dust extracts

Extraction of fine barn dust samples was performed in 0.15 mol NaCl containing 0.02% NaN_3 at 1/20 (w/v) for 18 h at room temperature. The extracts were then centrifuged (6600 *g* for 10 min), filtered (0.8 μm), and stored at -20°C until analyzed. In addition, an extract of *L. destructor* purchased from Allergon AB (Ängelholm, Sweden) was prepared from "raw material" as described previously (13). Briefly, the extract was prepared in 0.15 mol NaCl at 1/10 w/v for 18 h at room temperature followed by centrifugation (6000 *g* for 10 min) and filtration (0.8 μm). This extract (referred to here as "standard extract") was used to coat ELISA plates and as an inhibitory control reagent for construction of standard curves.

Protein determination

Protein concentrations were determined by BCA Protein Assay Reagent according to the manufacturer's instructions (Pierce Chemical Company, Rochford, IL, USA). The protein concentration in the 30 extracts ranged from 4.1 to 11.3 mg/ml.

Mite counting

Mites were identified and counted by the Service Centre for Taxonomic Zoology, Swedish Museum of Natural History, Stockholm. Lactic acid was added, and the samples were incubated in 4-cm petri dishes at 50°C for 24 h. The mites were identified and counted under a stereomicroscope (Wild) at $\times 24$ and 50 magnification. Unidentified mites were first transferred into microculture slides and washed in 80% ethanol. Subsequently, they were transferred into a drop of Hoyer's solution on microslides and identified by transmission micro-

scope with phase contrast (Leitz) at a magnification of $\times 500$. Identification was done according to Hughes (14), and the numbers were recorded as mites per gram of sieved hay dust.

Monoclonal antibody

The monoclonal antibody (mAb) used, 42B6, which binds to a 39-kDa allergen (5, 11), has been used previously in ELISA inhibition (11). In addition to *L. destructor*, other storage mites such as *Glycyphagus domesticus*, *Acarus siro*, and *T. putrescentiae* have been detected by this antibody (11). The antibody was purified from tissue culture medium according to standard methods using Protein G (Pharmacia, Uppsala, Sweden).

ELISA-inhibition assay

To analyze the mite content in the dust sample, we used the inhibition-ELISA assay. Microtiter plates (Nunc, Roskilde, Denmark) were coated with *L. destructor* extracts (1 $\mu\text{g}/\text{ml}$ standard extract). The mAb 42B6 was preincubated with dust sample extracts for 1 h at room temperature. The final concentration of the mAb was 0.3 $\mu\text{g}/\text{ml}$, and the samples were diluted 100 times. In parallel, the mAb was preincubated with various protein concentrations of the standard extract (0.04–10 μg). These mixtures were subsequently transferred to the *L. destructor*-coated wells and incubated for 2 h. Next, alkaline-phosphate-conjugated rabbit antimouse immunoglobulin (Dako A/S, Glostrup, Denmark) diluted 1/1000 was added and allowed to incubate overnight. Finally, the substrate, *p*-nitrophenyl phosphate (Sigma, St Louis, MO, USA), was diluted in 1 M diethanolamine buffer, pH 9.8, and added. Absorbance at 405 nm was measured 30 min later with an ELISA plate reader. A standard curve constructed from the standard extract was used to calculate the amount ($\mu\text{g}/\text{ml}$) of *L. destructor* protein in the dust samples.

Statistics

Linear regression was calculated for association between mite counts and ELISA determinations.

Results

Mite fauna

Mites of the suborder Astigmata belonging to nine different genera were found (Table 1). In agreement with previous investigations, *L. destructor* was the main species in the barn dust found in all 30 samples collected, constituting in 29 of these more than 70% of the total Astigmata mite count. The greatest

Table 1. Various Astigmata mite species in dust from 30 farms

Species	No. of farms	Maximum no. (mites/g dust)	Mean ¹
<i>Acarus siro</i>	26	16 155	1350
<i>Aleuriglyphus ovatus</i>	1	640	640
<i>Carpoglyphus munroi</i>	2	30	20
<i>Chortoglyphus arcuatus</i>	15	2160	438
<i>Diamesoglyphus intermedius</i>	6	120	50
<i>Gymnoglyphus longior</i>	1	40	40
<i>Glycyphagus domesticus</i>	25	2020	230
<i>Lepidoglyphus destructor</i>	30	195 400	27 350
<i>Tyrophagus longior</i>	16	1450	290
<i>Tyrophagus palmarum</i>	4	160	90
<i>Tyrophagus putrescentiae</i>	9	540	140
<i>Tyrophagus similis</i>	1	10	10
Other <i>Tyrophagus</i> spp	9	1100	280

¹ Dust samples lacking given species were not included in determination of mean value.

number of any other Astigmata species was in sample no. 23 where *Tyrophagus* species constituted 22%. As noted in a previous study (3), *Dermaphagoides* species were not found in the barn dust. However, here the Pyroglyphidae mite species *Gymnoglyphus longior* was found in one sample (Table 1).

Regarding the suborder Prostigmata, mites belonging to seven different families were detected. Of these, Cheyletus and Tydeus were found in small numbers in 29 and 28 dust samples respectively. Tarsonemus was detected in 26 dust samples, in two of which over 50 000 mites/g dust were also found, in these two instances greatly exceeding *L. destructor* and other Astigmata species. Small numbers of mites of the suborder Mesostigmata were also found in all samples, whereas about half the samples contained small numbers of Cryptostigmata (data not shown in Table 1).

ELISA-inhibition assay

The total mite count and the ELISA results correlated well (linear regression, $r^2=0.83$) (Figs. 1 and 2). Furthermore, the dust samples were subdivided into five categories by mite content. In the group of four samples with the highest mite counts, the mean ELISA value was 219 (Table 2). The mean ELISA values declined, as expected, in the categories of samples with fewer mites. No. 6 was assessed six times with different dilutions ranging from 1/100 to 1/8100 to estimate the reproducibility of the assay sample (Fig. 2).

Discussion

It is well established that farm workers exposed to barn dust frequently develop asthma, cough, and

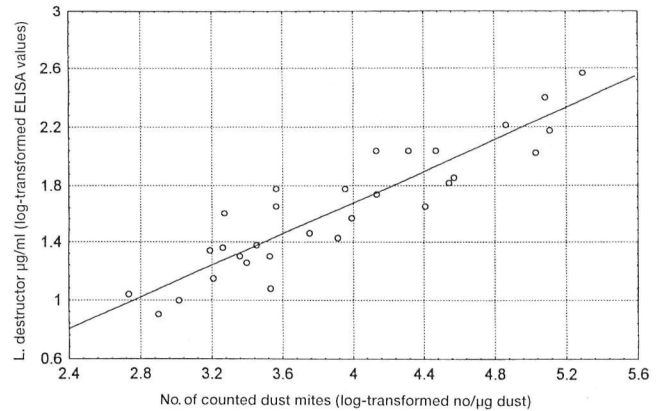


Fig. 1. Pair-wise comparison between number of *L. destructor* in each dust sample and ability of sample to inhibit ELISA. Linear regression was performed with log-transformed ELISA values vs. log-transformed number of counted *L. destructor* of each sample: $r^2=0.83$, $n=30$.

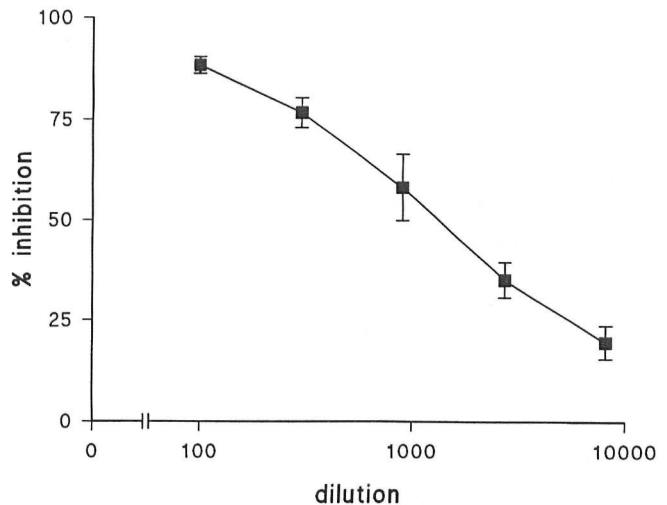


Fig. 2. Sample no. 6 was run in six consecutive experiments. x-axis gives dilution of dust sample; y-axis gives percent inhibition of ELISA obtained (see Materials and methods section for details). Values are given with standard deviation.

Table 2. Samples classified according to number of mites: correlation with inhibition ELISA

No. of dust mites/g dust	No. of samples	Mean ELISA ¹	Range ELISA
0–1000	2	9.5	8–11
1000–10 000	15	28.2	10–60
10 000–100 000	9	85.4	37–110
> 100 000	4	219	106–370

¹ Values correspond to µg/ml of *L. destructor* extract giving same degree of inhibition.

rhinoconjunctivitis (1, 2, 12, 15). Exposure is believed to be due to inhalation of airborne mite allergens, and here, fecal pellets are important for sensitization to other dust mites. A number of assays designed to measure mite allergens have been developed (9, 10). In the present study, we have

attempted to develop a simple method of analyzing dust for the presence of *L. destructor* and other storage mites. These mites, about 0.3 mm long, feed on straw and grain dust. Unlike Pyroglyphidae species (for example, *D. pteronyssinus* and *D. farinae*), they survive in the relatively harsh winter climate.

IgE antibodies to the 39-kDa allergen detected by the mAb 42B6 are present in roughly half of the sera from sensitized subjects (5). Previous studies have revealed Lep d 2 (formerly Lep d 1), as well as the 39-kDa allergen, in fecal pellets and the mite gastrointestinal tract (12). In addition, mAb 42B6 also stained the cytoskeleton (16). 42B6 was chosen because, as well as detecting *L. destructor*, it also detects the other major storage mites. Thus, the inhibition ELISA detected mites of Acaridae and Glycyphagidae origin (11). Although the allergen detected by 42B6 is not the most common allergen of *L. destructor*, it seemed to allow a good estimate of the number of storage mites. However, 42B6 fails to detect Pyroglyphidae species such as *D. pteronyssinus* and *D. farinae*. As the latter species are not found in barn dust samples, this is not a problem in the area under investigation. However, where Pyroglyphidae species are present, complementary assays will be needed.

The number of mites correlated well with the ELISA values. In our inventory, counting revealed tremendously high concentrations of mites in some barns. Mites were found in all barns, *L. destructor* being predominant. The results agree well with our previous study of the mite fauna in Swedish barn dust, which showed that *L. destructor* is the most important mite species. *A. siro* is the second most common species, infesting more than 80% of the farms. In Danish farms, Iversen et al. also found *A. siro* to be a major mite species in stored hay (4). As regards Pyroglyphidae mites, only a few specimens of *G. longior* were found on one farm. Several species of the suborder Prostigmata were also found. Among these, species of the families Tydeidae and Tarsonomidae were most frequent, i.e., in 28 and 26 of 30 dust samples, respectively. To our knowledge, there are no reports of the ability of *Tarsonemus* spp and *Tydeus* spp to cause allergic disorders. In investigations in England and Wales (17), *Tydeus* spp have been found. *Tarsonemus* spp have been reported to infest stored hay in Iceland (18) and Finland (19).

A number of methods have been used to collect dust samples. The reproducibility is reported to be good for samples collected in paper bags as well as for those collected on filters (20, 21). Some studies claim good correlation with airborne levels of mite allergens, while others detect measurable levels only after vigorous disturbance (22). Immunoassays of

mite antigens in barns have only been reported once before (23). In that study, aeroallergens were analyzed with RIA. Unavoidably, the different barn samples are a more heterogeneous mixture than that found in private homes. Presumably, it might include materials that could be inhibitory in the assay. However, we found no indication of this when the samples were diluted 1:100. Thus, the inhibition ELISA employed in this work would seem to offer a simple and reliable method to detect levels of dust mites of the Acaridae and Glycyphagidae families.

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