

Original article

Indoor mite allergen levels, specific IgE prevalence and IgE cross-inhibition pattern among asthmatic children in Haikou, southern China

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Keywords: mites; allergens; specific IgE; cross-inhibition

Background Haikou locates in tropical island with unique mite propagation. The aim of this study is to determine mite allergens levels in Haikou, and to investigate the prevalence of mite specific IgE-sensitization and IgE cross-reactivity between house dust mites.

Methods Allergen and antigen concentrations against six mite species were tested by enzyme-linked immunosorbent assay (ELISA). Specific IgE concentrations and cross-inhibitions were measured with ADVIA Centaur®.

Results Allergen or antigen *Dermatophagoides pteronyssinus* (Der p 1), *Blomia tropicalis* (Blo t) and *Tyrophagus putrescentia* (Tyr p) were detected in dust samples. *Dermatophagoides farinae* (Der f 1), *Lepidoglyphus destructor* (Lep d 2), and *Acarus siro* (Aca s) were found in very few samples. Specific IgE tests showed high prevalence of sensitizations against all tested mites with high IgE levels to Der p, Der f, and Blo t. Storage mites, Blo t, Tyr p, Lep d, and Aca s, could inhibit Der p from 0 to 50%. Storage mites could inhibit Der f between 30% and 100%. Der p IgE could be inhibited by Der f with up to 90%, and *vice versa*. Der p could inhibit Blo t from 40% to 80%. Blo t was able to fully inhibit IgE binding to Lep d, Tyr p, and Aca s compared to partial inhibition by Der p.

Conclusions Der p is the dominating mite and has the highest specific IgE prevalence among asthmatic children. Blo t represents an important source of storage mite sensitization and some patients may be independently sensitized to both Der p and Blo t. High prevalence of sensitization to Der f may be due to IgE-mediated cross-reactivity with Der p and Blo t.

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House dust mites are ubiquitous, they are one of the most important allergenic sources associated with allergic diseases, such as asthma and rhinitis. Such diseases have increased in worldwide prevalence over recent decades.¹⁻⁵ Apart from house dust mites (*Pyroglyphidae*), other mites mainly belonging to the Glycyphagidae family (*Lepidoglyphus* and *Blomia*) and the Acaridae family (*Tyrophagus* and *Acarus*) have also been shown to be important allergenic sources. Traditionally, these mites are named “storage mites” since they were initially found in rural regions mainly in relation to grain and foodstuff storage. However, storage mites are also found in urban environment, especially in regions with humid housing conditions.^{6,7} Both house dust mites and storage mite allergens play an important role in the pathogenesis of respiratory diseases,⁸ and are known to co-exist.⁹

The prevalence of storage mite sensitization and indoor allergen levels in China are, to a large degree, unknown. Recently, a multicentre study assessing the prevalence of skin prick test (SPT) sensitizations in patients with asthma and/or rhinitis in China showed high prevalence of sensitization to both house dust mites and the storage mite (*B. tropicalis*).¹ However, it is not clear if the high *B. tropicalis* SPT prevalence detected in both tropical and non-tropical regions of China is due to primary

sensitization or IgE mediated cross-reactivity to house dust mite allergens.

Hainan island has a population of over 8 million and is located in South China Sea in a tropical climate zone. Haikou is the capital city of this island. The average relative humidity is 79% with an average temperature of 24.9°C in 2009. The aim of this study was to determine mite allergens and antigen levels in household dust from beddings and living rooms in Haikou, and to investigate the prevalence of mite specific IgE-sensitization and IgE cross-reactivity between house dust mites (*Dermatophagoides pteronyssinus* (Der p), *Dermatophagoides farinae* (Der f)) and storage mites (*Tyrophagus putrescentia* (Tyr p), *Acarus siro* (Aca s));

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Lepidoglyphus destructor (Lep d); and *Blomia tropicalis* (Blo t)).

METHODS

Mite specific allergen levels in household dust samples

Two dust samples, one from beddings and one from living rooms, were collected at each household in Haikou in June or July 2009. Subjects had been living in their homes for at least 1 year before dust collection. Participants were asked not to replace their bed clothes for 2 weeks prior to dust collection. Dust was sampled by a trained technician using a hand-held vacuum cleaner equipped with a filter trap (ALK-Abelló, Horsholm, Denmark). Dust was collected from (1) sheets, pillowcases, pillows, quilts and mattresses from beddings; (2) sofas, carpets, furniture, cushions etc. from living rooms. The amount of dust was approximately 1 g per sample. Dust was stored at -18°C before extraction in 1:15 (dust to buffer w/v) ratio in 0.125 mol/L $\text{NH}_4(\text{HCO}_3)$ for 2 hours at room temperature by gentle shaking. The extracted solution was filtered through a 0.22 μm filter and stored at -18°C until analysis.

Allergen and antigen detection in extracted dust samples was performed using enzyme-linked immunosorbent assay (ELISA) with monoclonal antibodies against Der p 1, Der f 1, and Lep d 2, and polyclonal specific antibodies against Blo t, Aca s, and Tyr p (ALK-Abelló, Horsholm, Denmark), respectively. Detection limits were 0.009 $\mu\text{g/g}$ for Der p 1; 0.015 $\mu\text{g/g}$ for Der f 1; 0.012 $\mu\text{g/g}$ for Lep d 2; 1.5 AU/g for Blo t; and 3.0 AU/g for Aca and Tyr p. The results were expressed as medians and interquartile ranges.

Patients

Children clinically diagnosed with allergic asthma with/without rhinitis ($n=121$), aged 5–16 years old, from the Immunotherapy Center of People's Hospital in Haikou were included in the study. Patients with a positive SPT response to Der p and/or Der f (ALK-Abelló, Madrid, Spain) donated sera for specific IgE determination and IgE cross-reactivity experiments. The sera were stored at -18°C before performance. All subjects provided informed consents.

Specific IgE determination

Specific IgE levels against the six mite species, Der p, Der f, Blo t, Aca s, Lep d, and Tyr p, were measured using ADVIA Centaur® (Bayer Healthcare LLC, Tarrytown New York, USA), which is a reverse sandwich immunoassay using direct chemoluminescent technology. Allergens, calibrators, controls, and universal reagent

packs were produced by ALK-Abelló (Horsholm). The assay procedure was automatically performed and the results were expressed as kU/L. The sera with specific IgE higher than 100 kU/L were diluted and retested.

Specific IgE cross-reactivity experiments

Ten sera were selected for IgE inhibition studies based on having specific IgE concentrations >3 kU/L for Der p, Der f, Blo t, Aca s, Tyr p, and Lep d. Specific IgE cross-reactivity was measured using ADVIA Centaur® as IgE competition between allergenic extracts of the mites. An inhibitor solution (mite extract solution made from whole culture of each species) was added to biotinylated allergen extract at a final inhibitor concentration of 1.0 mg/ml. This concentration produces approximately 100% inhibition when measuring inhibition against the same allergen. Inhibitor allergen competes with biotinylated allergen, binding to serum IgE. Thus, readout of the specific IgE is reduced due to the competition. The inhibition is calculated as the ratio between IgE reduction and original IgE, Inhibition (%) = $\frac{((\text{sIgE RLU on buffer}) - (\text{sIgE RLU on inhibitor}))}{((\text{sIgE RLU on buffer}) - (\text{sIgE RLU on IgE basepool}))} \times 100$.

RESULTS

Mite allergen and antigen levels in dust samples

A total of 51 houses were visited and 102 dust samples were collected from the beddings and the living rooms in June or July 2009. The average relative humidity and out-door temperature during these two months were 77.0% and 29.1°C , respectively, as recorded at the Haikou Meteorological Bureau. Table 1 shows the mite allergen/antigen levels in the dust samples as measured by specific enzyme-linked immunosorbent assay (ELISA). The results for Der p 1, Der f 1, and Lep d 2 were expressed as $\mu\text{g/g}$ dust. For other storage mites, results were expressed as arbitrary units per gram dust (AU/g). Median values of Der p 1 from the beddings and the living rooms were 13.7 and 0.7 $\mu\text{g/g}$ dust, respectively. In contrast, the median value of Der f 1 allergen in samples from the beddings and the living rooms were lower than the detection limit. Median values of Blo t and Tyr p antigens from the beddings were 27.9 and 23.5 AU/g dust, respectively, and from the living room samples were 6.4 and 14.7 AU/g dust, respectively. Lep d 2 could only be detected in four and Aca s only in 10 out of the 102 samples at relatively low levels. Both house dust allergen and storage mite antigen levels were higher in samples from beddings compared to living room samples.

All dust samples from the beddings and 86.3% (44/51)

Table 1. Mite allergen and storage mite antigen levels in dust samples

Dusts	Median values per gram dust (inter quartile range)					
	Der p 1 ($\mu\text{g/g}$)	Der f 1 ($\mu\text{g/g}$)	Lep d 2 ($\mu\text{g/g}$)	Blo t (AU/g)	Tyr p (AU/g)	Aca s (AU/g)
Beddings ($n=51$)	13.7 (13.3)	<0.015	<0.012	27.9 (41.1)	23.5 (23.7)	<3.0
Living rooms ($n=51$)	0.7 (4.9)	<0.015	<0.012	6.4 (15.0)	14.7 (19.0)	<3.0

samples from the living rooms contained relatively high level of Der p 1. However, there were only 27.5% (14/51) dust samples from the beddings and 7.8% (4/51) from the living rooms with measurable Der f 1 at very low concentrations. All dust samples contained Blo t antigens. Tyr p antigens were found in all samples from beddings and 90.2% (46/51) samples from living rooms.

Specific IgE determination

A total of 121 serum samples were tested for specific IgE concentrations against Der p, Der f, Blo t, Lep d, Try p, and Aca s, as shown in Figure 1. The specific IgE prevalence against Der p and Der f were 99.2% and 95.0%, respectively. About 60% of the samples had high levels of Der p IgE, class 5 and 6, and about 50% of the samples had high levels of Der f IgE. All patients sensitized to Der f were also Der p IgE-positive.

The specific IgE prevalence for Blo t was 87.6%, Tyr p was 79.7%, Aca s was 52.9%, and Lep d was 67.8%. Specific IgE titer to Der p was generally higher than Der f; only 7.4% (9/121) of the patients had a higher IgE level to Der f than Der p. Approximately, 34.7% (42/121) showed high specific IgE concentrations (>17.5 kU/L) against Der p, Der f, and Blo t, simultaneously. Also 34.7% (42/121) patients had high specific IgE titers against Blo t (>Der p and/or Der f). For other storage mites, the specific IgE levels were generally lower.

Specific IgE cross-reactivity

Ten serum samples were selected for IgE cross-reactivity measurements based on specific IgE concentration >3.0 kU/L against all six mite species (Table 2). The specific IgE cross-reactivity among the six mite species is shown

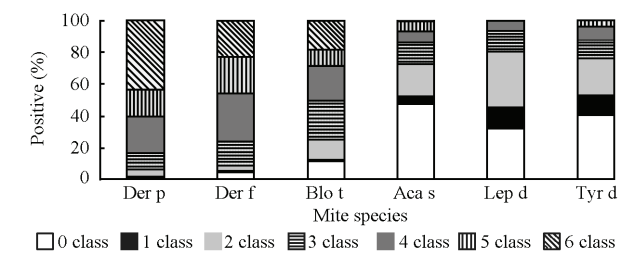


Figure 1. Specific IgE reactivity patterns against six mite species. Class 0: <0.35 kU/L; class 1: 0.35–0.7 kU/L; class 2: 0.7–3.5 kU/L; class 3: 3.5–17.5 kU/L; class 4: 17.5–50 kU/L; class 5: 50–100 kU/L; class 6: >100 kU/L.

in Figure 2. All mite species could fully inhibit themselves (about 100%) for the 10 selected sera except two sera for Der f. Storage mites, Blo t, Tyr p, Lep d, and Aca s, could not or only partly inhibit Der p with a degree less than 50%. For most sera, Der p could be inhibited by Der f in the range of 50%–90% and *vice versa*. All the storage mites could inhibit Der f from 30% to 100%, while they could only inhibit Der p from 0–50%. Der p could inhibit Blo t in the range between 40%–80%, while the other mites could inhibit Blo t in 45%–100%. Blo t was able to fully inhibit Lep d, Tyr p, and Aca s compared to only partial inhibition by Der p. Storage mites, Lep d, Tyr p, and Aca s could fully inhibit each other.

DISCUSSION

In this study, the levels of house-dust mite allergens and storage mite antigens were measured in 102 dust samples collected from 51 households. The levels of mite allergens were higher in the bedding samples compared to the living room samples, in agreement with a previous study.¹⁰ The major allergen Der p 1 was found in all dust

Table 2. IgE titers against 6 mite species of 10 selected sera for cross-reactivity experiments (kU/L)

Patient ID	Der p	Der f	Blo t	Aca s	Lep d	Tyr p
HN27018	26.8	101.0	261.3	93.8	29.5	93.5
HN27033	65.5	93.8	252.9	3.0	37.0	66.8
HN27045	41.5	44.1	62.5	22.6	6.2	20.3
HN27046	27.7	38.3	36.2	16.2	6.8	15.4
HN27053	71.6	97.7	129.9	54.4	27.6	56.4
HN27079	33.1	69.9	61.0	20.8	17.6	6.9
HN27087	572.8	215.6	181.1	50.9	10.7	41.9
HN27094	93.9	118.9	499.8	58.9	19.8	47.8
HN27103	6.5	176.3	312.8	123.7	42.7	94.1
HN27125	47.0	118.6	149.5	75.7	28.8	55.2

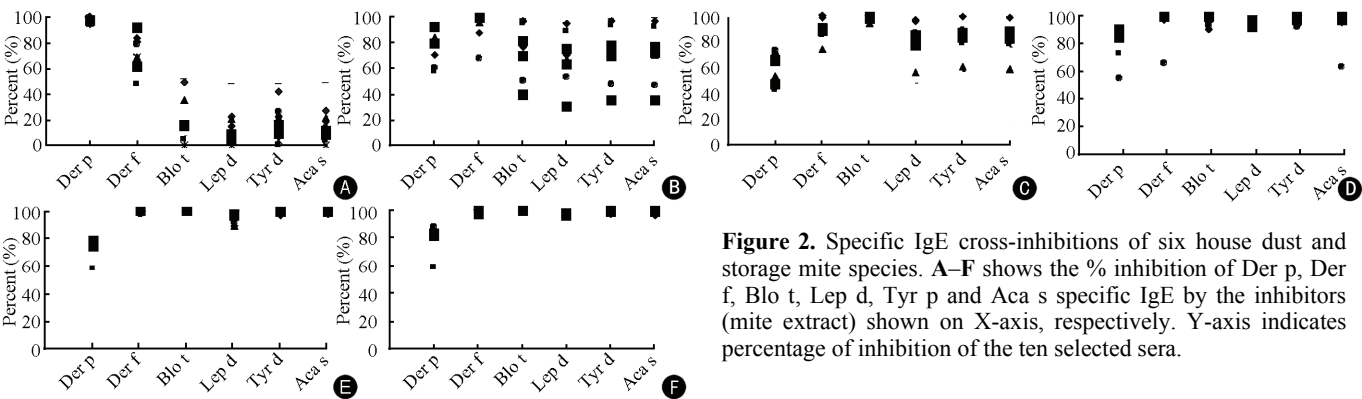


Figure 2. Specific IgE cross-inhibitions of six house dust and storage mite species. A–F shows the % inhibition of Der p, Der f, Blo t, Lep d, Tyr p and Aca s specific IgE by the inhibitors (mite extract) shown on X-axis, respectively. Y-axis indicates percentage of inhibition of the ten selected sera.

samples from the beddings and most samples from the living rooms with a median value of 13.7 and 0.7 $\mu\text{g/g}$ dust, respectively. Concentrations of house-dust mite allergen at 10 $\mu\text{g/g}$ dust are considered to be risk factors for sensitization and acute asthmatic attack, respectively.¹¹ The major allergen Der f 1 was only detected in very few samples and at low levels, indicating that Der f is not an important sensitizing indoor allergen source in this region. The city of Haikou is located in southern China experiencing a tropical moist monsoon climate with year round high relative humidity, which explains the lack of Der f 1 as Der f prefer drier climates compared to Der p.¹²

Of the storage mites, antigens specific to Blo t were detected in all household samples indicating that Blo t could be a source of indoor allergens and sensitization. However, there are no documented correlations between the levels of Blo t antigen/allergens in dust and the risk of sensitization and, furthermore, the concentration of storage mite antigens were measured in arbitrary units. No antigen from Aca s could be detected in the dust samples although both Blo t and Aca s favor warm and moist tropical environment.¹³ The lack of Aca s may be a result of competition with other mites. Antigens specific to storage mite Tyr p were found in almost all dust samples, while Lep d 2 was found in only four out of 102 samples.

The specific IgE results from this study show that patients had multiple sensitizations to mites. The highest prevalence of sensitization were house dust mite species Der p and Der f, in agreement with a previous SPT study from the southern region of China.¹ For the storage mite species, Blo t showed the highest prevalence of specific IgE reactivity and also the highest specific IgE titers indicating that Blo t may be an important source of allergens, co-existing with Der p in sub-tropical and tropical regions as shown in references.^{14,15} Patients also had high specific IgE prevalence against Lep d, Tyr p, and Aca s but with relatively low IgE titers. Ebner et al¹⁶ demonstrated that prevalence of sensitization to storage mites occurred predominantly in patients sensitized to allergens from house dust mites. The specific IgE prevalence and concentrations of storage mites in dust samples were relatively high in our study, especially for Blo t. To investigate if the measured storage mite specific IgE was due to true sensitization or due to IgE-mediated cross-reactivity, we performed IgE-inhibition experiments using ten sera and six different mite extracts. House-dust mite species Der p and Der f showed a large degree of IgE-mediated cross-reactivity, in agreement with other studies.^{17,18} Storage mites Lep d, Tyr p, and Aca s showed full serum IgE cross-reactivity indicating the presence of homologous storage mite allergens. The storage mites Lep d, Tyr p, and Aca s could not fully inhibit Blo t, but Blo t could fully inhibit binding of serum IgE to these three mites indicating the presence of Blo t specific IgE sensitization.

For the serum IgE inhibition between house dust mite and storage mite, there was a significant difference between the ability of Der p and Der f to inhibit binding of IgE to storage mites. Der f could inhibit binding of storage mite to 100% in almost all sera, whereas Der p showed a lower degree of inhibition, especially for Blo t. This indicates that the measured Der f IgE levels are mainly due to cross-reaction with Der p and Blo t, while Blo t could be a primary source of storage mite sensitization. This is in agreement with the finding that specific IgE against Blo t had the highest storage mite IgE titers and Blo t antigens were identified in all dust samples. In contrast to Der f, the binding of Der p to sera could only be inhibited by storage mite to maximum 50%, showing the presence of Der p specific IgE as well as Der p IgE cross-reacting with storage mite allergen.

The IgE cross-reactivity data, sensitization pattern and levels of mite antigens in dust samples, suggests that Der p is the primary sensitizing source among these patients and that Der p sensitization gives rise to Der p IgE that cross-reacts with Der f and storage mites. In addition, Blo t is probably a source for specific storage mite sensitization that could give rise to patients independently being sensitized to both Der p and Blo t. The inhibition data also point towards a somewhat higher degree of cross-reactivity between storage mites and Der f, compared to cross-reactivity between storage mites and Der p. This is in agreement with the low level of Der f 1 in Haikou dusts indicating that the measured Der f sensitization is mainly a cross-reaction due to IgE against homologue allergens of the mites.

From an allergy management perspective, the data suggest that specific immunotherapy using standardized Der p extracts would be effective for the majority of allergic patients in Haikou. However, some patients may be also specifically sensitized to Blo t in addition to Der p and identification of these patients requires diagnostic tools species specific for storage mites. From a treatment perspective, Der p vaccination could affect storage mite sensitized patients due to the unspecific anti-inflammatory effect of immunotherapy¹⁹ and likely presence of homologous allergens among Der p and Blo t, however, since therapeutic efficacy is correlated to the amount of specific allergen being administered,²⁰ some patients in Haikou may require additional treatment with Blo t specific immunotherapy.

The relationship between indoor mite allergen and specific IgE levels, and its influence on the severity and frequent exacerbation of asthma were very important for asthmatic patients' clinical treatment. However, there are still somethings that remain unclear in this work. Further studies with questionnaires and spirometry data are needed.

In conclusion, Der p is the dominating mite in house-hold dust samples and has the highest specific IgE prevalence

among asthmatic children in Haikou, southern China. In addition, Blo t represents an important source of storage mite sensitization and some patients may be independently sensitized to both Der p and Blo t. The levels and prevalence of Der f allergens in house-hold dust samples are low and the high prevalence of Der f IgE sensitization is most likely due to IgE-mediated cross-reactivity with Der p and Blo t.

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