

# Mite allergen levels and acarologic analysis in house dust samples in Uberaba, Brazil

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## Summary

Mite allergen exposure has been widely related to sensitization and development of allergic diseases. This study intended to evaluate the degree of allergen exposure in Uberaba, Brazil, through the measurements of Der f 1 and Der p 1 allergen levels associated with the acarologic analysis in house dust samples. A total of 240 dust samples were collected from 60 houses through vacuuming sofas and bedding, during the months of March and July 2000. Indoor temperature and relative humidity were also measured. Mites were counted and identified under light microscopy and allergen levels were measured by two-site monoclonal antibody ELISAs. The major mite family was Pyroglyphidae (39.4%), having *D. pteronyssinus* as the most frequent species (15.6%), followed by *D. farinae* (12.3%) and *E. maynei* (7.9%). The family Glycyphagidae was less commonly found (4.8%), with *Blomia tropicalis* (4.4%) as its majoritary member. The highest levels of Der f 1 and Der p 1 allergens were found in bedding samples in March (31.7 and 0.9 µg/g of dust, respectively), with Der f 1 levels significantly higher than Der p 1 ( $p < 0.0001$ ). There was a significant positive correlation between the mite number and allergen levels. These results indicate that *Dermatophagoides* sp are the most frequent mites in our region followed by *E. maynei*. Therefore, the knowledge of the local mite fauna would improve the means of investigating the association between allergen exposure and sensitization, allowing the addition of new mite extracts in diagnostic tests.

**Key words:** Acarologic analysis - Allergen exposure - Der f 1 allergen - Der p 1 allergen - House dust mite

## Introduction

Mites are present in house dusts all over the world and represent common sources of allergens. The role of mite allergen exposure in sensitization and development of allergic diseases, particularly asthma and rhinitis, has been recognized in many parts of the world [1, 2].

The allergen exposure degree can be estimated by the number of mites and the concentration of their allergens detected in the house dust [3]. House dust mites (HDM) have a worldwide distribution, however there are differences in the mite number and allergen concentration in different locations and seasons [4]. Considerable variation within and between different homes has shown that factors such as age of home, floor level, ventilation, orientation, or living habits of the occupants, may contribute to the differences in indoor

humidity that influence the growth conditions of mites [4, 5].

In temperate regions, *Dermatophagoides pteronyssinus*, *D. farinae*, and *Euroglyphus maynei* (family Pyroglyphidae) are the most commonly species found [6, 7] while in tropical and subtropical areas, *Blomia tropicalis* (family Glycyphagidae) and *D. pteronyssinus* are the most frequent species and *D. farinae* is rarely found [8].

In Brazil, previous reports have shown that *D. pteronyssinus* and *B. tropicalis* are the more prevalent mites [9-11] while *D. farinae* was less frequent [12]. However, Sopenete et al. [13] have demonstrated Der f 1 allergen levels  $\geq 2$  µg/g of dust in 84-91% of the house dust samples in Uberlândia, MG, Brazil.

The aims of this study were to evaluate the degree of mite allergen exposure in the city of Uberaba, MG,

Brazil, through the measurements of Der f 1 and Der p 1 allergen levels and the mite microscopic identification in house dust samples, and to analyze the variations in the mite allergen levels in two different seasons.

## Materials and methods

### Houses and dust sampling

Sixty houses, whose inhabitants had or not a history of atopy, were randomly selected for a mite indoor environmental study in the city of Uberaba, MG, Brazil.

Sampling visits were made during the months of March (warm and humid summer) and July (dry and mild winter) 2000. Dust samples were collected by vacuuming the entire surface of sofa and bedding (mattresses, pillows and bedclothes); 60 dust samples from sofa and 60 from bedding were collected each month, totaling 240 dust samples over the period analyzed. The collection was performed with a household vacuum cleaner (Arno, SA, São Paulo,

Brazil), which was modified to incorporate a plastic adaptor with cellulose filters. These filters containing the dust samples were identified separately according to the site of collection, placed in plastic bags, and stored at  $-20^{\circ}\text{C}$  prior to assays. Local temperature and relative humidity were measured at the moment of dust sampling using a thermohygrometer (Hygrotherm, TFA, Germany).

### Acarologic analysis and extraction of house dust mite allergens

First, large particles of dust were removed by sieving through a 1.0 mm mesh screen, and then a total of 100 dust samples (50 mg) were submitted to the acarologic analysis. Mites were removed from dust samples by flotation on saturated NaCl solution. All the mites found were kept on glass slides containing 2 drops of Hoyer-Berlese fluid [6]. After clarification, the mites were identified and counted as previously described [14- 16]. The remaining dust was sieved through a 0.3 mm mesh screen (Standard Sieve Series A.S.T.M., USA). Samples of 100 mg were extracted overnight at  $4^{\circ}\text{C}$  in 2 mL borate-buffered saline (pH 8.0), centrifuged, and the supernatants were stored at  $-20^{\circ}\text{C}$  prior to immunoassays for the detection of mite allergen levels.

### ELISA for measuring levels of mite allergens

ELISAs for Der f 1 and Der p 1 allergens were carried out as described by Sopelete et al. [13], by using the respective capture antibodies: monoclonal antibody (mAb) anti-Der f 1 (6A8) or mAb anti-Der p1 (5H8) at  $1\ \mu\text{g}/\text{well}$  in 0.06 M carbonate buffer (pH 9.6). The detection antibody consisted of biotinylated mAb anti-Der f 1 and anti-Der p 1 (4C1) at  $1\ \mu\text{g}/\text{well}$ . Reference standards containing known levels of each allergen were included in each plate, in duplicate, to obtain control curves in two-fold serial dilutions ranging from 250 to 0.5 ng/mL for Der f 1 and Der p 1. Absorbance results were expressed in  $\mu\text{g}/\text{g}$  of dust as described by Platts-Mills & De Weck [17]. The limits of allergen detection were  $0.002\ \mu\text{g}/\text{g}$  of dust for Der f 1 and  $0.004\ \mu\text{g}/\text{g}$  of dust for Der p 1.

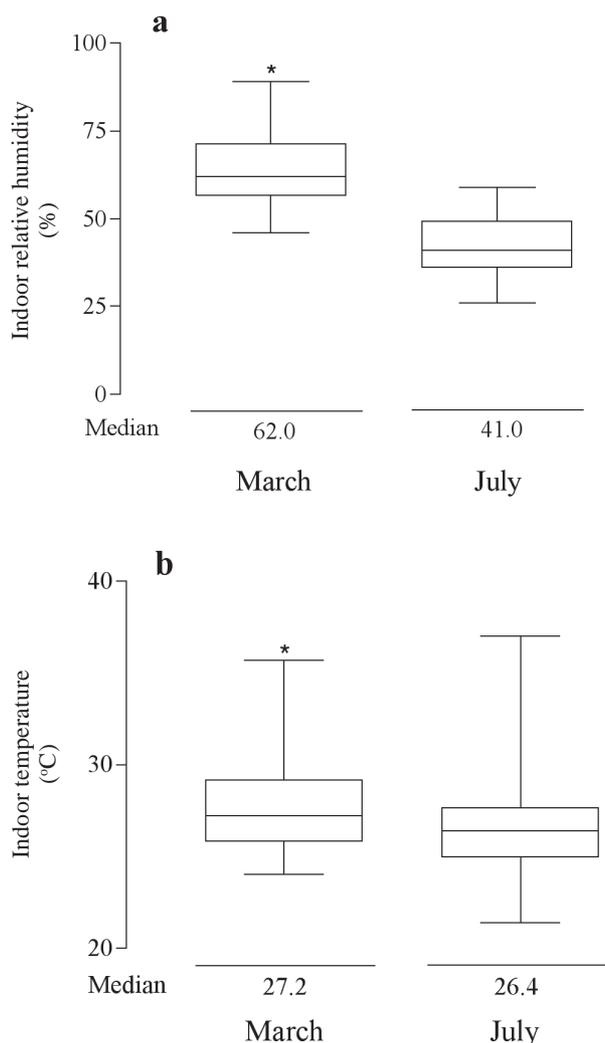


Figure 1. Indoor relative humidity (a) and temperature (b) values measured in homes when dust samples were collected ( $n = 240$ ), during the months of March and July 2000, in Uberaba, MG, Brazil. The comparisons between median values were made by the Student's *t* test.

\*  $p < 0.0001$

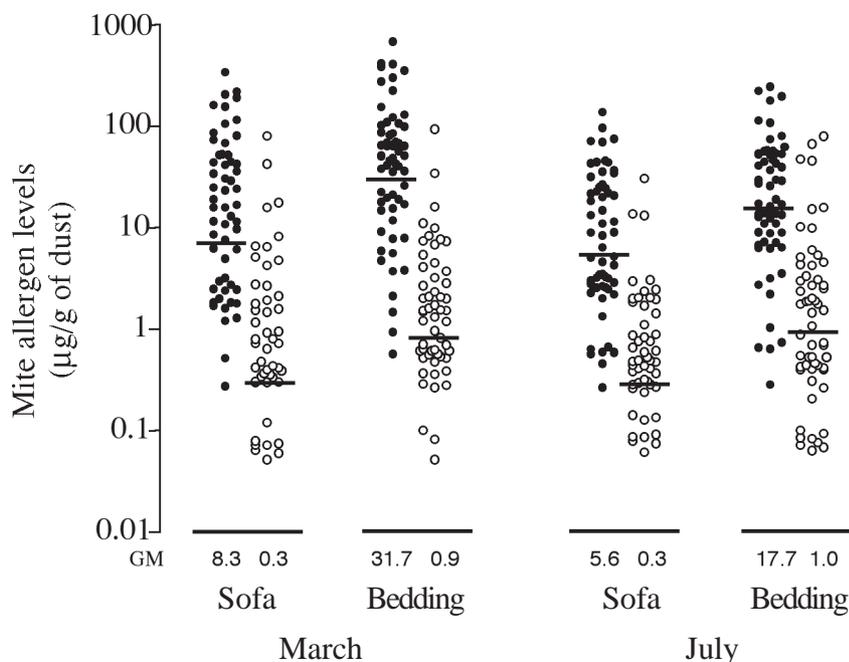


Figure 2. Levels of Der f 1 (●) and Der p 1 (○) allergens found in house dust samples collected from sofa and bedding (n = 240) in Uberaba, MG, Brazil, during the months of March and July 2000. The horizontal bars indicate the geometric means (GM).

## Statistical analysis

As the allergen concentrations followed a non-Gaussian distribution, calculations were performed on log-transformed data. Geometric means (GM) with 95% confidence interval (CI) were obtained for the allergen levels and the differences between the means were analyzed by the Mann-Whitney and Wilcoxon tests. The frequency of microscopically identified mite species was expressed in percentage. The correlation between mite number and allergen levels was carried out by the Spearman test. The median values obtained from the indoor temperature and relative humidity in the different seasons were compared by using the Student's t test. Values of  $p < 0.05$  were considered statistically significant.

## Results

The values of indoor temperature and relative humidity ranged from 24 to 35.7°C (median = 27.2°C) and 46 to 89% (median = 62.0%) in March, and from 21.4 to 37°C (median = 26.4°C) and 26 to 59% (median = 41.0%) in July, respectively (Figure 1). The median values of relative humidity and temperature were significantly greater in March than in July ( $p < 0.0001$ ).

The levels of Der f 1 allergens were significantly higher in bedding dust samples in March (GM: 31.7 µg/g of dust; 95% CI: 19.3-52.1 µg/g) than July (GM: 17.7 µg/g of dust; 95% CI: 12.0-26.0 µg/g) ( $p < 0.01$ ). In addition, mean levels  $> 2$  µg of Der f 1/g of dust were

also detected in sofa samples, with the highest levels in March (GM: 8.3 µg/g of dust; 95% CI: 4.1-16.7 µg/g;  $p < 0.01$ ) (Figure 2). For Der p 1 allergen, the greatest levels were also found in bedding samples in March (GM: 0.9 µg/g of dust; 95% CI: 0.5-1.6 µg/g) and in July (GM: 1.0 µg/g of dust; 95% CI: 0.6-1.7 µg/g), with no statistically significant differences between them ( $p > 0.05$ ). In sofa samples, Der p 1 mean levels found in March (GM: 0.3 µg/g of dust; 95% CI: 0.1-0.6 µg/g) were similar to those detected in July (GM: 0.3 µg/g of dust; 95% CI: 0.2-0.5 µg/g) ( $p > 0.05$ ). Der f 1 levels in bedding and sofa samples were significantly higher than those observed for Der p 1 ( $p < 0.0001$ ) in both months, while bedding samples showed Der f 1 and Der p 1 levels significantly higher than sofa samples ( $p < 0.05$ ). The mean coefficients of intra- and inter-assay variation were 3.2% and 18.3% for Der f 1 and 2.4% and 18.8% for Der p 1, respectively.

From 100 dust samples selected for the mite microscopic analyses, 92% revealed at least one species of mite. A total of 825 mites were found in these dust samples and the mite number in the surveyed homes varied between 0 and 1,140 mites/g of dust. The major family observed was Pyroglyphidae (39.4%), with *D. pteronyssinus* as the most frequent species (15.6%), followed by *D. farinae* (12.3%) and *E. maynei* (7.9%) (Table 1). The family Glycyphagidae was less commonly found (4.8%), showing *Blomia tropicalis* (4.4%) as its majoritary member. As shown in table 1, other mites were found with in very low frequencies, while eggs (9.1%), larvae (6.7%), and nymphs (31%) were observed with relatively high frequencies.

Table 1. Mite species and number in house dust samples (sofa or bedding; n = 100) in Uberaba, MG, Brazil

Mite species	Count (%)
<b>Astigmata</b>	
Acaridae	
<i>A. siro</i>	12 (1.5)
Glycyphagidae	40 (4.8)
<i>B. kulagini</i>	4 (0.4)
<i>B. tropicalis</i>	36 (4.4)
Pyroglyphidae	326 (39.4)
<i>D. farinae</i>	102 (12.3)
<i>D. microceras</i>	26 (3.2)
<i>D. neotropicalis</i>	4 (0.4)
<i>D. pteronyssinus</i>	129 (15.6)
<i>Euroglyphus maynei</i>	65 (7.9)
<b>Cryptostigmata</b>	
Oribatidae	13 (1.7)
<b>Mesostigmata</b>	
Uropodina	6 (0.7)
<b>Prostigmata</b>	
Cheyletidae	
<i>Cheyletus sp</i>	12 (1.5)
<b>Tarsonemidae</b>	
<i>Tarsonemus sp</i>	9 (1.1)
Eggs	75 (9.1)
Larvae	55 (6.7)
Nymphs	256 (31)
Unidentified	21 (2.5)
<b>Total</b>	<b>825 (100)</b>

There was a significant positive correlation between the mite number and allergen levels for *D. farinae* ( $r = 0.31$ ;  $p = 0.0023$ ) (Figure 3a) and *D. pteronyssinus* ( $r = 0.49$ ;  $p < 0.0001$ ) (Figure 3b) in the house dust samples analyzed.

## Discussion

Many factors may influence the mite growth and proliferation. Differences in the seasonality and geographic distribution of the house dust mite species as well as allergen levels within and among different homes are hence attributed to variations on the environment components, such as the building characteristics (age of houses, building material, and size

of the dwelling) which affect the indoor climatic conditions (temperature and humidity), and the differences in the individual living habits (keeping of pets, carpets or mattresses, and cleaning routes) [18, 19]. HDM incorporate water molecules from the microenvironment through their cuticle and therefore require a high ambient humidity to counterbalance the excessive water loss [7]. In countries with seasonal variations in climate, the HDM number is influenced by the corresponding seasonal fluctuations, with the highest numbers in the period of higher humidity [20, 21].

In the present study, the maximum levels of Der f 1 and Der p 1 allergens were found in bedding samples in both months. These results agree with previous studies that found the highest level of mite allergen in the bedroom dust samples [13, 22], thus confirming that bedding, due to the close contact with people for longer periods, is the main source of mite allergen exposure. In addition, our results showed that the uppermost allergen levels were also found in the season (March) with higher relative humidity.

Regarding the mean allergen levels, both Der f 1 and Der p 1 allergens were found at sensitization levels ( $\geq 2$   $\mu\text{g/g}$  of dust), but only Der f 1 was detected at high levels ( $\geq 10$   $\mu\text{g/g}$  of dust). Such occurrence should be considered when analyzing the sensitization of asthmatic patients, given the fact that IgE antibodies to mite allergens of either *Dermatophagoides* species are highly cross-reactive.

Studies carried out in the United States reported that *D. farinae* and *D. pteronyssinus* were the predominant mite species with variation in their relative prevalence depending on geographic localization and climate differences [7, 23]. In our study, we also found *D. farinae* and *D. pteronyssinus* to be the more prevalent species in Uberaba, which agrees with the reports of high prevalence of *D. farinae* in Uberlândia, Brazil (13), a city located 100 km away from Uberaba, which presents the same tropical climate, characterized by dry and mild winters. These findings are also supported by significant positive correlations between Der f 1 and Der p 1 allergen levels and the mite number of these species found in analyzed dust samples, particularly *Dermatophagoides pteronyssinus*.

It should be emphasized that *E. maynei* was relatively frequent on mite microscopic identification, and since the three allergen (Eur m I, Der f 1 and Der p 1) amino acid sequences taken together present between 76-78% identity [22, 24], a probable cross-reactivity between them could not be ruled out. On the other hand, *B. tropicalis* was detected in lower frequency on acarologic analysis and our previous assays were unable to detect Blo t 5 allergen levels when using a two-site monoclonal antibody based ELISA (unpublished data), suggesting that climate conditions of this region could be unfavorable for the development of this mite species.

In conclusion, the results here reported show that *Dermatophagoides sp* are the most frequent mites in

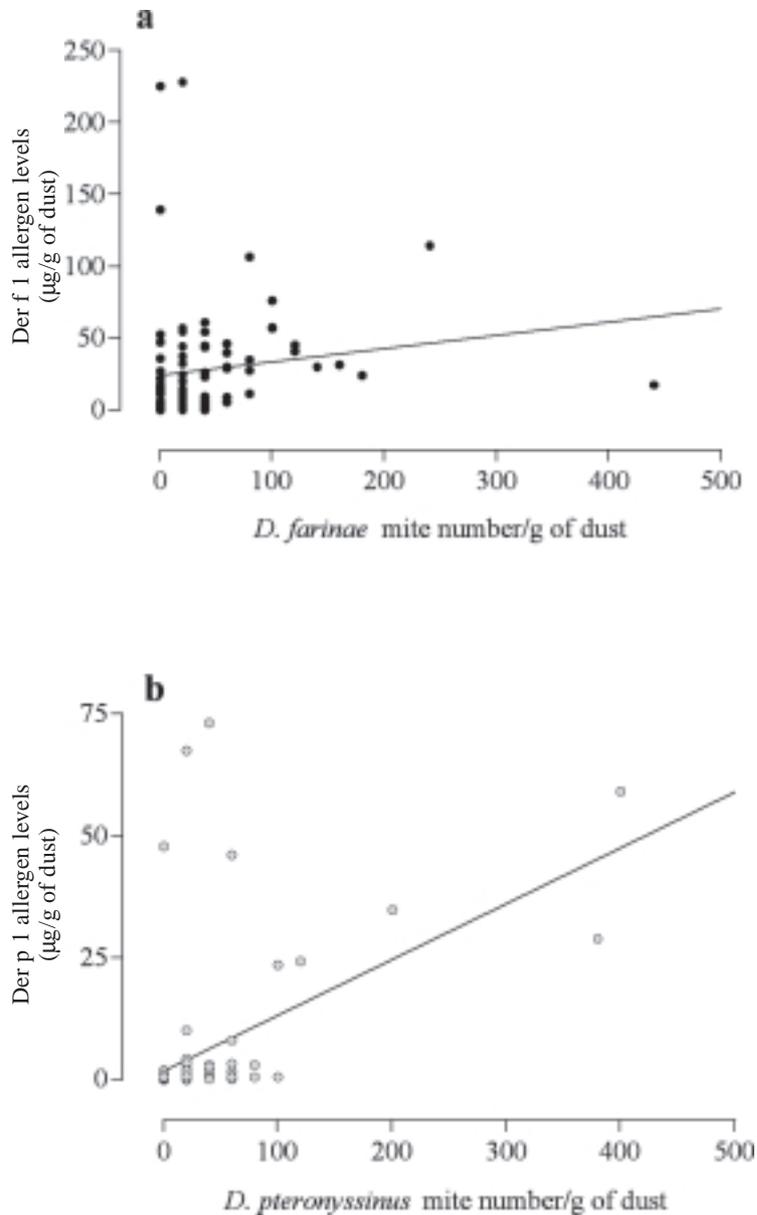


Fig. 3. Correlations between the mite number and allergen levels for *D. farinae* (a) and *D. pteronyssinus* (b) in house dust samples collected from sofa and bedding (n = 92) in Uberaba, MG, Brazil. Correlation coefficients were obtained in a ( $r = 0.31$ ,  $p = 0.0023$ ) and b ( $r = 0.49$ ,  $p < 0.0001$ ) by the Spearman test.

our region followed by *E. maynei*. Thus, mite allergen extracts used in skin prick tests should include the latter for evaluating sensitization of atopic patients. Additionally, the acarologic analysis for mite identification and count is subject to seasonal fluctuations and observer's experience, and by itself could give only an underestimate idea of the mite allergen exposure. Based on the significant positive correlation between mite number and allergen levels, we believe that the measurements of allergen levels in house dust by quantitative two-site monoclonal antibody ELISA still represents one of the most rapid, easy, sensitive and specific methods for evaluating allergen exposure.

## Acknowledgments

We thank all people for consenting the collection of house dust samples and Dr Manoel Medeiros Jr for assistance with the material to identify the mites. This study was supported by Brazilian financial agencies (CAPES, CNPq, and FAPEMIG).

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