

## CLINICAL SCIENCE

## The hammock: a reservoir of allergens

Francisca X. M. Rego,<sup>1</sup> Pedro Giavina-Bianchi,<sup>1</sup> Jorge Kalil,<sup>1</sup> L. Karla Arruda,<sup>11</sup> Myrthes Toledo-Barros<sup>1</sup><sup>1</sup>Clinical Immunology and Allergy Division, Faculdade de Medicina da Universidade de São Paulo, São Paulo/SP, Brazil. <sup>11</sup>Clinical Immunology and Allergy Division, School of Medicine of Ribeirão Preto – São Paulo University, Ribeirão Preto, SP/Brazil.

**INTRODUCTION:** Asthma affects approximately 10% of the world's population. Sensitization to allergens is an important risk factor, and exposure to allergens is associated with disease severity.

**METHODS:** We performed skin tests to evaluate allergen sensitization to mites, cockroaches, cats, dogs, and molds in 73 asthmatic patients. Enzyme Linked Immunosorbent Assay was used to assay the mite and cockroach allergens found in dust from the bedding, hammocks, bedroom floors, living rooms, and kitchens of 29 patients and 14 controls.

**RESULTS:** Fifty patients (68.5%) had positive skin test responses. There were positive responses to *D. pteronyssinus* (52.0%), *B. tropicalis* (53.4%), *T. putrescentiae* (15.0%), *E. maynei* (12.3%), *L. destructor* (8.2%), *B. germanica* (20.5%), *P. americana* (21.9%), *Felis catus* (10.9%), *C. herbarium* (2.7%), *A. alternata* (4.1%), and *P. notatum* (1.3%). The exposure to mite and cockroach allergens was similar in the patients and the controls. The *Dermatophagoides pteronyssinus* Group 1 levels were highest in the beds and hammocks. The *Blattella germanica* Group 1 levels were highest in the kitchens, living rooms and hammocks.

**DISCUSSION:** The positive skin tests to mites, cockroaches and cats were consistent with previous studies. *D. pteronyssinus* was the most prevalent home dust mite, and hammocks were a source of allergens. To improve asthma prophylaxis, it is important to determine its association with mite allergen exposure in hammocks.

**KEYWORDS:** Asthma; Mite allergens; Cockroach allergens; Exposure; Sensitization; Skin tests; Allergen levels; Home dust; Hammock; Environmental control.

Rego FXM, Giavina-Bianchi P, Kalil J, Arruda LK, Toledo-Barros M. The hammock: a reservoir of allergens. Clinics. 2011;66(7):1199-1202.

Received for publication on March 10, 2011; First review completed on April 5, 2011; Accepted for publication on April 5, 2011

E-mail: saudesos@saudesos.com.br

Tel.: 55 11 30713189

## INTRODUCTION

Estimates indicate that 10% of the world's population suffers from asthma. In recent years, the frequency and severity of asthma have increased.<sup>1,2</sup> Despite the importance of genetic factors in the development of asthma, environmental factors, especially indoor aeroallergens, are probably the primary determinants of asthma expression and are essential for understanding the etiology of asthma. The majority of asthmatics are sensitized to at least one common allergen.<sup>3,4</sup>

The importance of sensitization to mite and cockroach allergens has been demonstrated in atopic individuals.<sup>5,6</sup> Sensitization to cockroach allergens is an important risk factor in underprivileged socioeconomic segments of the population and is associated with the severity of the disease.<sup>6</sup>

Allergen sensitization studies performed in several countries have shown that the most prevalent mite allergens are from *Dermatophagoides pteronyssinus* (Der p) and *Dermatophagoides farinae* (Der f).<sup>7,8</sup> Studies performed in

Brazil have revealed the substantial prevalence of Der p and *Blomia tropicalis* (Blo t).<sup>9-12</sup>

*Periplaneta americana* (Per a) is perhaps the most predominant species of domestic cockroach found in several areas of Brazil, while *Blattella germanica* (Bla g) is the most frequent species reported in the USA. According to studies performed in Brazil, 20–55% of the children and adults with asthma, rhinitis, or both have positive skin-prick tests for Per a or Bla g.<sup>13-15</sup>

The most abundant allergens found in domestic dust are derived from mite feces, the digestive tracts of cockroaches, cat dander, dogs, and mold spores. The mite allergens found in domestic dust are classified into two major categories: Group 1 (Der p 1 and Der f 1), and Group 2 (Der p 2 and Der f 2). Both these groups exhibit strong cross-reactivity.<sup>16</sup> The main cockroach allergens are from Group 1 (Bla g 1 and Per a 1) and exhibit strong cross-reactivity.<sup>17</sup>

Exposure to levels of  $\geq 2$  mcg of Der p 1/g of dust is thought to be a major risk factor for sensitization in atopic individuals, while the equivalent level in non-atopic children is approximately 50 mcg/g. An initial dust exposure of  $\geq 10$  mcg/g has been associated with the onset of acute asthma. However, subsequent epidemiological studies have not confirmed this finding.<sup>18</sup> A clear association between cockroach exposure and sensitization has been observed, especially in bedding, and 2 U/g and 8 U/g of

allergens in dust are sufficient to cause sensitization and asthma development, respectively<sup>19</sup>. In regions with low home dust mite and cockroach allergen levels, allergens derived from cats, dogs, and molds become the major sources of sensitization associated with asthma.<sup>20-21</sup>

We aimed to determine the sensitization to the most frequent aeroallergens in asthmatic patients in the city of Fortaleza, Brazil, to assay the mite and cockroach allergens in the home dust of patients with asthma, and to compare these exposure levels to those of healthy control subjects.

## MATERIALS AND METHODS

### Population and homes surveyed

We studied 73 patients (64 women) who were between 15 and 55 years of age and who had an asthma diagnosis based on the Global Initiative for Asthma (GINA) criteria. The majority of the patients were from socioeconomically disadvantaged families. We selected 15 healthy individuals (13 women) who were paired with patients according to their sex, age, and socioeconomic status. Informed consent was obtained from the patients, and the hospital ethics committee approved the study.

The patients and controls were selected based on a questionnaire that was completed during the first visit. The questionnaire included questions regarding any current disease symptoms, any personal and family history of allergic disease, and any factors that triggered the manifestation of symptoms. After completing the questionnaire, the subjects underwent skin-prick testing for aeroallergens using a previously described method.<sup>22</sup>

Within four months of the initial evaluation, dust samples were collected from the homes of the 29 asthmatic patients and the 14 controls. All of the homes were located in the city of Fortaleza, Brazil. The patient home visits were performed according to the study entry number and after the dust-collection consent. We also used a questionnaire to evaluate the conditions in the homes.

### Skin-prick tests

The skin-prick tests were performed using commercial extracts (Ifidisa-Aristegui) of the following allergens: *D. pteronyssinus* (Der p), *D. farinae* (Der f), *B. tropicalis* (Blo t), *T. putrescentiae* (Tp), *E. maynei* (Em), *L. destructor* (Ld), *C. herbarum* (Cla h), *A. alternate* (Alt a), *P. notatum* (Pen n), *A. fumigatus* (Asp f), *Blattella germanica* (Bla g), *Periplaneta americana* (Per a), animal dander (dog and cat), and grass pollen. A positive skin-prick test was defined as a mean wheal size of at least 3 mm after subtracting the negative control.

### Home dust collection

During the home visits, dust was collected from five sites: bedding, hammocks, bedroom floors, living rooms, and kitchens. Hammocks are used to sleep and rest in Brazil. We used a portable vacuum cleaner (Electrolux Compact Plus) fitted with an adapter and cloth filter. Between the collections, the adapter was cleaned with 70% alcohol. The dust samples were placed into separate plastic bags and stored at 4°C before processing according to standardized techniques.<sup>23</sup>

### Assay of mite and cockroach allergen levels in home dust

The dust extracts were assayed for Group-1 (Der p 1 and Der f 1), Group-2 (Der p 2 and Der f 2), Blo t 5, Bla g 1 and Bla g 2 mite allergens using a monoclonal antibody-based

Enzyme Linked Immunosorbent Assay (ELISA) technique (Indoor Biotechnologies, Inc., Charlottesville, VA, USA), as has been previously described.<sup>24-27</sup>

### Statistical Analysis

The statistical analyses were performed using Instat 2. The differences between the allergen levels the various sites were assessed using the Mann-Whitney test. The levels of several allergens were correlated using Pearson's and Spearman's rank-order coefficients. A *p*-value of <0.05 was considered to be statistically significant.

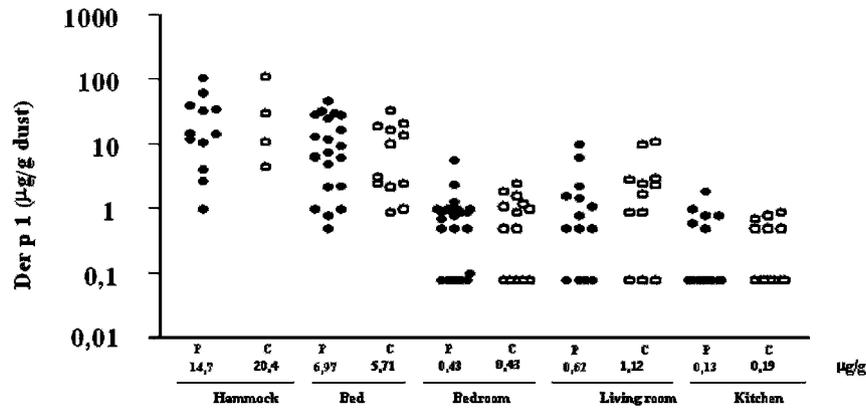
## RESULTS

A positive result to at least one of the allergens tested was found in 50 asthmatic patients (68.5%) and two controls (13.3%). Of these, 45 (90%) were sensitized to several allergens. The tests for Der p, Der f, and Blo t yielded the most frequent positive results.

Dust mite and cockroach allergens were assayed in 99 samples from 29 homes of patients with asthma (18 patients with positive skin-prick tests and 11 patients with negative skin-prick tests) and in 56 samples from 14 control-subject homes. No significant differences in the concentrations of most of the allergens were observed in the collected samples. However, we observed a significant difference in the Bla g 1 concentration in the bedding of the patients with asthma compared to the control group (*p*=0.003). Regarding the Group-2 allergen Der p 2, we noticed a significant difference between the samples collected in the kitchens of the asthma patients and those of the control subjects (*p*=0.04).

As shown in Figure 1, high levels of Der p were found in the hammock and bedding samples collected from the homes of the asthma patients and the control subjects, with no significant difference between them (*p*=0.10 and *p*=0.17, respectively). For all of the participants, the levels of Der p found in the hammock and bedding samples were significantly higher than those in the samples from the bedroom floors, living rooms and kitchens (*p*<0.0002 for patients and *p*<0.03 for controls). The levels of Der f in the dust samples collected from the homes of the patients and controls were considerably lower in all of the sites, with no significant differences among them. We noticed a strong correlation between the levels of the Group-1 and Group-2 allergens in the dust samples from the homes of the asthmatic patients and those of the control subjects (*n*=155; *r*=0.70; *p*<0.01). The levels of Blo t 5 were extremely low or undetectable in all of the samples.

In the patient and control homes, the Bla g 1 levels were the highest in the kitchen samples. Despite their significantly higher levels when compared to the bedding and bedroom floor samples (*p*<0.05), no significant difference was observed between them and the levels found in the hammocks and living rooms. Regarding the allergen concentrations in each home, Bla g 1 levels of ≥2 U/g were assayed in 10.3% of the samples from the homes of the patients and in 14.3% of the samples from the homes of the controls; Bla g 1 levels of ≥8 U/g were assayed in 51.7% of the samples from the homes of the patients and in 42.8% of the samples from the homes of the controls. The levels of Bla g 2 were extremely low or undetectable in the homes of both the patients and the controls. We found no significant correlation between the concentrations of Bla g 1 and Bla g 2 (*n*=153; *r*=-0.038; *p*=0.12).



**Figure 1** - The levels of Der p 1 in the dust samples collected from the homes of the asthma patients (dark circles) and the control subjects (blank circles).

We did not observe an association between exposure and a positive skin-prick test to either Der p or Bla g.

## DISCUSSION

We aimed to examine sensitization to the most frequent aeroallergens in asthmatic patients in the city of Fortaleza (CE), Brazil, and to assess the level of exposure by assaying the mite and cockroach allergens in their homes. Fortaleza is a city with an average annual relative air humidity of 78% and an average temperature of 26.8°C.

The association between asthma and sensitization to aeroallergens has been frequently reported, suggesting that sensitization to exogenous proteins inside the home represents a major risk factor for asthma and that there is a casual relationship between allergen exposure and asthma.<sup>18</sup> In asthmatic individuals, evaluating allergic responses is extremely important because it can point to an etiological diagnosis and have a direct impact on the implementation of preventive measures that are designed to decrease allergen exposure and, consequently, reduce the risk of developing acute asthmatic crises.<sup>1</sup>

This study evaluated 73 asthmatic patients. Of these, 50 (68.5%) were sensitized to at least one of the allergens tested. Studies in Brazil have reported between 67.2% and 80.3% positive skin prick tests to aeroallergens.<sup>4,14,28,29</sup> One of the most important factors that determines the prevalence of sensitization to aeroallergens is the age range of the study population, with higher rates observed during childhood. Our study involved adult patients, which might explain the increased number of chronic respiratory diseases of unknown origin, despite careful patient screening using a questionnaire that included questions about atopia. Regarding the control individuals, only two (13.3%) out of the 15 had positive skin-prick tests.

Our results agree with those of other studies conducted in the Northeast of Brazil that have reported the prevalence of sensitization to Der p, Blo t, Der f, and other mites. Sensitization to Der f is not associated with significant isolation of this mite in home dust, and it is probably caused by a cross-reaction with Der p (in contrast to the observations regarding Blo t 5).<sup>30</sup> According to most of the studies conducted in Brazil, investigating sensitization and exposure to Der f is not necessary because the prevalence of Der f is less than that of Der p. Furthermore, there is cross-reaction between both species.

We also noticed a substantial sensitization to cockroach allergens, which has been reported in a number of Brazilian studies. Cockroach allergens are currently the second-most-frequent cause of sensitizations after dust mites, with the positive skin-prick tests to cockroach extract mixtures varying between 23.6% and 24.1%.<sup>15,31</sup>

Cat owners represented 50% of the patients with positive skin-prick tests to this allergen. This finding highlights the importance of other sites as causes of sensitization.<sup>32</sup> The sensitization to cat allergens was similar to that reported in children in the Northeast of Brazil (between 9.2% and 15.2%).<sup>14,28</sup> Nevertheless, the results regarding sensitization to dogs were unexpected because, according to other national reports, sensitization to dog allergens varies between 3.4% and 23.7%.<sup>15,28</sup> The positive results for molds were low, and, in general, the sensitization reported in this study was lower than the sensitization reported in other studies. Studies performed in the Northeast of Brazil have reported 3.5% to 10.1% positive results for several molds.<sup>14,28</sup>

Our results show that the asthmatic patients and control individuals were exposed to similar levels of mite and cockroach allergens in their homes. This observation underlines the view that a genetic predisposal to exaggerated IgE synthesis is a key factor in sensitization. The differences in the allergen concentrations of Bla g in bedding and Der p 2 in kitchens (between the patients and the controls) is of no clinical relevance because the allergen levels were so low.

We found high levels of Der p 1 in the bedding of the asthmatic patients and the control subjects. We noticed a strong correlation between the Group 1 and Group 2, thereby confirming that *D. pteronyssinus* was the most prevalent mite in the homes surveyed in this study.

To the best of our knowledge, our study is the first to evaluate the allergen levels of dust samples from hammocks. Hammocks are common in the Northeast of Brazil. According to our results, hammocks are an important site of allergen accumulation and may play an important role in asthma sensitization and in the development of asthma symptoms in sensitized individuals. Because we found  $\geq 10$  mcg of Der p 1/g of dust in 75% of the hammock samples from the patients with asthma, the environmental control measures in this area should include frequently washing the hammocks used for rest and sleeping. The levels of the Der p 1 allergens found on the bedroom floors and in the living rooms and kitchens were low, probably

because the surveyed homes had good ventilation and no carpets. This result highlights the importance of sleeping places as sources of mite allergen exposure.

Surprisingly, despite the significant sensitization observed in the skin-prick tests, the Blo t 5 concentrations were close to the detection threshold. Studies performed in other Brazilian cities have also reported low concentration of Blo t 5 at several sites. Thus, we believe that the Blo t 5 assay may be inadequate for detecting *B. tropicalis* in the sites that we evaluated.

In the past 30 years, cockroach allergies have been recognized as an important cause of sensitization and asthma. Several studies have demonstrated that patients who were sensitized to cockroaches had been exposed to high levels of this antigen in their homes (especially in their bedding) and that a cockroach allergy is an important risk factor for hospital admission. A number of factors, including climate, the type of construction, home ventilation, hygiene, and socioeconomic status, have been associated with an increased sensitization to cockroaches. The allergen levels of Bla g 1 were higher than those reported for Bla g 2, possibly due to a cross-reaction between the Bla g 1 and Per a 1 allergens (*P. americana* is the most frequent cockroach in Brazil). The low levels of Bla g 2 we observed strengthen this hypothesis because this allergen is specific to Bla g.

In our study population of asthmatic adults, we observed no association between sensitization to Der p, Bla g, and/or Per a and exposure to mite and cockroach allergens. Our patients frequently reported childhood asthma, and it is possible that exposure during the initial years of life plays an important role in sensitization or that exposure to allergens outside the home environment is an important factor for developing sensitization.

The environmental control measures for patients who are allergic to mites and live in Fortaleza or other places where hammocks are used must consider hammocks to be possible sites for significant allergen accumulation. These findings may help implement more efficient prophylactic measures for allergic patients.

## REFERENCES

1. The Global Initiative for Asthma (Accessed 17/04/2010). Available from: <http://www.ginasthma.org/>.
2. Sears MR. Epidemiology of childhood asthma. *Lancet*. 1997;350:1015-20, doi: 10.1016/S0140-6736(97)01468-2.
3. Strachan DP. Hay fever, hygiene, and household size. *BMJ*. 1989;299:1259-60, doi: 10.1136/bmj.299.6710.1259.
4. Giavina-Bianchi P, Fidalgo S, Duarte AJS. Hipersensibilidade dos pacientes com asma e rinite na cidade de São Paulo. *Arch Argent Immunol Clin*. 1996;27:12.
5. Squillace SP, Sporik RB, Rakes G, Couture N, Lawrence A, Merriam S, et al. Sensitization to dust mites as a dominant risk factor for asthma among adolescents living in Central Virginia. *Am J Respir Crit Care Med*. 1997;156:1760-4.
6. Rosenstreich DL, Eggleston PA, Kattan M, Baker D, Slavin RG, Gergen P, et al. The role of cockroach allergy and exposure to cockroach allergen causing morbidity among inner-city children with asthma. *N Engl J Med*. 1997;336:1356-63, doi: 10.1056/NEJM199705083361904.
7. Baldacci S, Modena P, Carrozzi L, Pedreschi M, Vellutini M, Biavati P, et al. Skin prick test reactivity to common aeroallergens in relation to total IgE, respiratory symptoms, and smoking in a general population sample of northern Italy. *Allergy*. 1996;51:149-56.
8. Chew GL, Reardon AM, Correa JC, Young M, Acosta L, Mellins R, et al. Mite sensitization among Latina women in New York, where dust-mite allergen levels are typically low. *Indoor Air*. 2009;19:193-7, doi: 10.1111/j.1600-0668.2008.00578.x.
9. Bernd LAG, Baggio D, Becker AB, Ambrozio LC. Identificação e estudo da atividade sensibilizante de ácaros domésticos em Porto Alegre – RS. *Rev Bras Alerg Immunopatol*. 1994;17:23-33.

10. Serravalle K, Medeiros Jr M. Ácaros da poeira domiciliar na cidade de Salvador – BA. *Rev Bras Alerg Immunopatol*. 1998;22:19-24.
11. Sarinho E, Fernandez-Caldas E, Just E, Solé D. Ácaros da poeira domiciliar em residências de crianças asmáticas e controles da cidade de Recife, Pernambuco. *Rev Bras Alerg Immunopatol*. 1996;19:228-30.
12. Soplete MC, Silva DAO, Arruda LK, Chapman MD, Taketomi EA. Dermatophagoides farinae (Der f 1) and Dermatophagoides pteronyssinus (Der p 1) allergen exposure among subjects living in Uberlândia, Brazil. *Int Arch Allergy Immunol*. 2000;122:257-63, doi: 10.1159/000024407.
13. Santos ABR, Chapman MD, Aalberse RC, Vailes LD, Naspitz CH, Arruda LK. Cockroaches allergens and asthma in Brazil: identification of tropomyosin as a major allergen with potential cross-reactivity with mite and shrimp allergens. *J Allergy Clin Immunol*. 1999;104:329-37, doi: 10.1016/S0091-6749(99)70375-1.
14. Medeiros Jr M, Figueiredo JP. Sensibilização a aeroalérgenos em indivíduos com asma brônquica e/ou rinite crônica em Salvador, Bahia. *Rev Bras Alerg Immunopatol*. 1997;20:143-54.
15. Moreira MR, Mendonça MRMF, Gomes Jr EA, Souza GG, Gervásio AM, Soplete MC, et al. Sensibilização a alérgenos inalaváveis domiciliares em pacientes asmáticos em Uberlândia, MG. *Rev Bras Alerg Immunopatol*. 2001;24:11-21.
16. Arlian LG, Platts-Mills TAE. The biology of dust mites and the remediation of mite allergens in allergic disease. *J Allergy Clin Immunol*. 2001;107:S406-13, doi: 10.1067/mai.2001.113670.
17. Arruda LK, Vailes L, Ferriani VPL, Santos ABR, Pomés A, Chapman M. Cockroaches allergens and asthma. *J Allergy Clin Immunol*. 2001;107:419-28, doi: 10.1067/mai.2001.112854.
18. Platts-Mills TAE, Vervloet D, Thomas WR, Aalberse RC, Chapman MD. Indoor allergens and asthma: Report of the third international workshop. *J Allergy Clin Immunol*. 1997;100:S1-S24, doi: 10.1016/S0091-6749(97)70186-6.
19. Eggleston PA, Rosenstreich D, Lynn H, Gergen P, Baker D, Kattan M, et al. Relationship of indoor allergen exposure to skin test sensitivity in inner-city children with asthma. *J Allergy Clin Immunol*. 1998;102:563-70, doi: 10.1016/S0091-6749(98)70272-6.
20. Sporik R, Ingram JM, Price W, Sussman JH, Honsinger RW, Platts-Mills TAE. Association of asthma with serum IgE and skin test reactivity to allergens among children living at high altitude. Tickling the Dragon's Breath. *Am J Respir Crit Care Med*. 1995;151:1388-92.
21. Halonen M, Stern DA, Wright AL, Taussig LM, Martinez FD. Allergen sensitization and asthma in a desert environment. *Am J Respir Crit Care Med*. 1997;155:1356-61.
22. Dreborg S, Frew A. Allergen standardization and skin tests. *Allergy*. 1993;48:48-82, doi: 10.1111/j.1398-9995.1993.tb04698.x.
23. Tovey ER, Chapman MD, Wells CW, Platts-Mills TAE. The distribution of dust mite allergens in the homes of patients with asthma. *Am Rev Resp Dis*. 1981;124:630-5.
24. Chapman MD, Smith AM, Vailes LD, Arruda LK, Dhanaraj V, Pomés A. Recombinant allergens for diagnosis and therapy of allergic disease. *J Allergy Clin Immunol*. 2000;106:409-18, doi: 10.1067/mai.2000.109832.
25. Luczynska CM, Arruda LK, Platts-Mills TAE. A two-site monoclonal antibody ELISA for the quantification of the major Dermatophagoides spp. allergens, Der p I and Der f I. *Immunol Methods*. 1989;118:227-35.
26. Arruda LK, Vailes LD, Platts-Mills TAE, Fernandez-Caldas E, Montealegre F, Lin K, et al. Sensitization to *Blomia tropicalis* in patients with asthma and identification of allergen Blo t 5. *Am J Respir Crit Care Med*. 1997;155:343-50.
27. Pollart SM, Mullins DE, Vailes LD, Hayden ML, Platts-Mills TAE, Sutherland WM. Identification, quantification and purification of cockroach allergens using monoclonal antibodies. *J Allergy Clin Immunol*. 1991;87:511-21, doi: 10.1016/0091-6749(91)90010-L.
28. Rizzo MC, Solé D, Rizzo A, Holanda MA, Rios JBM, Wandalsen NF, et al. Etiologia da doença atópica em crianças brasileiras – estudo multicêntrico. *J Pediatr (Rio de Janeiro)*. 1995;71:31-5.
29. Feld L, Lima BC, Costa E. Sensibilização a alérgenos inaláveis em pacientes com alergia respiratória na cidade do rio de janeiro: comparação entre asma brônquica e rinite isolada. *Rev Bras Alerg Immunopatol*. 2001;24:54-64.
30. Chew FT, Yi FC, Chua KY, Fernandes-Caldas E, Arruda LK, Chapman MD, et al. Allergic differences between the domestic mites *Blomia tropicalis* and *Dermatophagoides pteronyssinus*. *Clin Exp Allergy*. 1999;29:982-8, doi: 10.1046/j.1365-2222.1999.00543.x.
31. Rosário Filho NA, Faria L, Riedi CA, Zulato AS. Sensibilização a baratas em crianças asmáticas: relação com a gravidade da doença. *Rev Bras Alerg Immunopatol*. 1999;22:151-5.
32. Almqvist C, Larsson PH, Egmar A, Hedrén M, Malmberg P, Wickman M. School as a risk environment for children allergic to cats and a site for transfer of cat allergen to homes. *J Allergy Clin Immunol*. 1999;103:1012-7, doi: 10.1016/S0091-6749(99)70172-7.