Original Article

Mite allergen exposure, sensitisation and clinical symptoms in Valdivia, Chile.

M. Calvo[1], E. Fernández-Caldas[2], P. Arellano[3], F. Marín[4], J. Carnés[2], A. Hormaechea[1]

[1] Department of Pediatrics, Faculty of Medicine, Austral University of Chile
[2] C.B.F. LETI, S.A, Tres Cantos, Madrid, España
[3] Surmedica Clinic, Valdivia, Chile
[4] Department of Public Health, Faculty of Medicine, Austral University of Chile

Summary. One hundred consecutive asthmatic paediatric patients were evaluated and skin tested with a battery of skin prick test reagents, including 8 different standardized house dust mite extracts. Asthma severity was graded according to the Global Initiative for Asthma (GINA) document in mild persistent (52 patients), moderate persistent (39) and severe persistent (9). Sixty patients had asthma and allergic rhinitis, 12 asthma and eczema, and 8 asthma, allergic rhinitis and eczema. The patient population was divided into 2 different socioeconomic groups (50 patients per group) based on a standardized, validated questionnaire. A dust sample was collected with an adapted vacuum cleaner from the mattress of each patient and analysed for Der p 1, Der f 1 and Der p 2 allergen content using monoclonal antibodies.

Eighty patients were skin test positive to at least one mite species. All positive skin test patients were positive to *Dermatophagoides pteronyssinus*, 99% to *D. farinae*, 92% to *Euroglyphus maynei*, 80% to *Lepidoglyphus destructor*, 73% to *Tyrophagus putrescientae*, 72% to *Blomia tropicalis*; 70% to *Acarus siro* and 68% to *Chortoglyphus arcuatus*. All patients with severe persistent asthma had a positive skin test to mites, 85% in the moderate group, and 73% in the mild group ($p<0.01$). 95% of patients with asthma and allergic rhinitis had a positive skin test to mites, 92% of patients with asthma and eczema and 100% of patients with asthma, allergic rhinitis and eczema; ($p<0.01$). Mean Der p 1, Der f 1 and Der p 2 allergen concentrations were 18.3, 0.6 and 5.6 μg/g of mattress dust, respectively. Mean Der p 1 allergen levels in the middle-low socioeconomic group were significantly higher than in the middle high group ($p < 0.01$).

There is a high rate of allergic sensitisation among pediatric asthmatic patients in Chile. More than one species are implicated, although sensitisation and exposure to *D. pteronyssinus* predominates. Mite allergic patients are exposed to high mite allergen concentrations, exceeding previously established risk levels for sensitisation and symptoms.

Key words: asthma, mites, *Dermatophagoides*, allergen levels, dust sample.

Introduction

The International Study of Asthma and Allergy in Childhood (ISAAC) demonstrated that bronchial asthma is frequent in many South American countries, including Chile [1,2]. Valdivia is located at sea level in the South of Chile at 40 degrees latitude. The mean yearly temperature is 12.2° C, the relative humidity oscillates between 84% and 92% and the yearly rainfall is approximately 2,300 mm. There is a high prevalence of asthma in this region, and the reported frequency of wheezing in children between 6 and 7 years of age in

the last 12 months is 20.5%, and in children between 13 and 14 years it is 11.5% [2].

A direct relationship has been shown between exposure to allergens of *Dermatophagoides spp*. and the development of allergic sensitisation and asthma [3,4]. It is widely accepted that one of the factors that best explains the increase in the current prevalence of asthma is sensitisation to dust mites, especially of the genus *Dermatophagoides*. A critical level for sensitisation has been suggested at a concentration of 2 μg of Der p 1/g of dust; higher levels, such as 10 μg of Der p 1/g of dust have been associated with respiratory symptoms [5].

Previous studies have shown that the mite fauna in Chile is rich in species. More than 20 species have been described in house dust samples collected throughout the country [6]. Sensitisation to several species could, therefore, be expected in the allergic population. Although the mite fauna in Chile is well established, there were no previous studies about allergen exposure in this country. Several studies have determined mite allergen levels in Latin America. Allergen levels were generally high, although some regional differences could be observed. These studies included cities such as Sao Paulo and Curitiba in Brazil, Cartagena in Colombia, five cities in Mexico, and Córdoba and Santa Fe in Argentina [7-12]. These studies did not take into consideration either the socioeconomic status of the patients recruited, or the severity of the disease.

Sensitisation to several species of mites is very common in Latin America. Several reports have shown that species such as *Blomia tropicalis* are important in this continent [12]. However, the great majority of these studies have only included adults. A study [12] showed that in Sao Paulo, Brazil, mite sensitisation among asthmatic children is above 90% for the mite species *D. pteronyssinus* and *B. tropicalis*. Taking into consideration the size of South America and its different climatic conditions, the extrapolation of these results to other countries seems difficult.

Although in developed countries, low socioeconomic status seems to be a risk factor for severe asthma, this possibility has not been sufficiently explored in South America. In the current situation, where the hygiene hypothesis seems to explain the increase in the prevalence of allergies, it is likely that children belonging to families with a low socioeconomic status could have a lower prevalence of allergic disease, or be somehow protected from suffering allergic diseases [13].

The aims of this study were to establish the frequency of sensitisation to different mite species in a group of pediatric patients, who were first evaluated for bronchial asthma in our outpatient clinics and to determine Der p 1, Der f 1 and group 2 allergen levels in mattress dust samples collected in their homes. The patients were classified according to disease severity and socioeconomic status.

Materials and methods

Patient Population

The patient population consisted of 100 consecutive patients who were diagnosed with bronchial asthma according to the Consensus of the Chilean Society of Respiratory Illnesses [14,15] and to the Global Initiative for Asthma (GINA) [16]. The criterion used in the selection of sample patients was as follows: the first correlative 50 patients from each group that accepted to take part in this study. The mean age of the patients (56 males and 44 females) was 9.14 ± 3.41 years (4.3 to 14.6 years). The asthma severity was graded according to the GINA document in mild persistent, moderate persistent and severe persistent. The patient population was divided into two different socioeconomic groups based on a standardized validated questionnaire. Fifty patients were allocated to each group. Signed consent was obtained from the parents before the children entered the study.

Skin prick tests

A battery of standardized skin test reagents was used to perform skin prick tests with lancet in the patient population. It included extracts of feathers, cat and dog dander, moulds, weed, and grass mixtures and standardized extracts of *D. pteronyssinus*, *D. farinae*, *E. maynei*, *Lepidoglyphus destructor*, *Tyrophagus putrescientae*, *Blomia tropicalis*, *Acarus siro* and *Chortoglyphus arcuatus* (CBF LETI, SA, Spain). A mean wheal ≥ 3 mm, in the absence of a positive reaction of the negative control, was considered positive. No patient presented a reaction in the negative control. The selection of the mite species for diagnosis was based on the data gathered from the Chilean mite distribution map [17].

Patients were classified as belonging to the middle-low or middle-high socioeconomic status according to a validated survey available through the National Institute of Food Technology (NIFT). This document is not included in this paper because of its length, but it is available upon request. The main issues considered were family income, educational level and ownership of electric devices.

Collection and analysis of dust samples

The mattress of each of the patients was vacuumed during one minute with a specially adapted vacuum cleaner [10]. Once the dust was collected, it was frozen at –20°C and stored until analysis. None of the patients had previously received special instructions on environmental control. The dust samples were analysed using monoclonal antibodies following the instruction of the manufacturer (Indoor Biotechnologies Ltd., Charlottesville VA, USA) [18].

Statistical Analysis

The statistical study consisted of the Kruskal-Wallis test for homogeneity of the sample, the U-Mann Whitney test, the student-t test for the comparison of averages and contingency tables (x^2 to study homogeneity).

Results

Patient population

The characteristics of the 100 patients included in the study are detailed in Table 1; 52% of the patients

Table 1. Characteristics of the patient population

CHARACTERISTICS		MLSES (n = 50)	MHSES (n = 50)	TOTAL (n = 100)	p
Age (Years)		8.63 ± 3.03	9.66 ± 3.76	9.14 ± 3.41	NS
Sex	Male	27 (54)	29 (58)	56 (56)	NS
(n - %)	Female	23 (46)	21 (42)	44 (44)	
Asthma	MP	23 (46)	29 (58)	52 (52)	
(n - %)	ModP	19 (38)	20 (40)	39 (39)	NS
	SP	8 (16)	1 (2)	9 (9)	
Asthma + AR (n - %)		24 (48)	36 (72)	60 (60)	< 0.01
Asthma + E (n - %)		7 (14)	5 (10)	12 (12)	NS
Asthma + AR + E (n - %)		4 (8)	4 (8)	8 (8)	NS

MLSES: middle-low socioeconomic status. MHSES: middle-high socioeconomic status. MP: mild persistent. ModP: moderate persistent. SP: severe persistent. AR: Allergic Rhinitis. E: eczema

Table 2. Skin prick tests of different allergen extracts in the two groups of patient divided by socio-economic status.

PRICK TEST	MLSES (n = 50) (n - %)	MHSES (n = 50) (n - %)	Total (n = 100) (n - %)	p
POSITIVE	36 (72)	44 (88)	80 (80)	< 0.05
D. pteronyssinus	36 (72)	44 (88)	80 (80)	< 0.05
D. farinae	35 (70)	44 (88)	79 (79)	< 0.05
Feathers	3 (6)	22 (44)	25 (25)	< 0.01
Cats	16 (32)	15 (30)	31 (31)	NS
Dogs	7 (14)	11 (22)	18 (18)	NS
Moulds	11 (22)	12 (24)	23 (23)	NS
Trees	6 (12)	6 (12)	12 (12)	NS
Grasses	13 (26)	21 (42)	34 (34)	NS
Weeds	7 (14)	5 (10)	12 (12)	NS
NEGATIVE	14 (28)	6 (12)	20 (20)	< 0.05

MLSES: middle-low socioeconomic status. MHSES: middle-high socioeconomic status.

Table 3. Skin prick tests of the different mites tested.

MITES	MLSES (n = 50) (n - %)	MHSES (n = 50) (n - %)	Total (n = 100) (N - %)	p
D. pteronyssinus	36 (72)	44 (88)	80 (80)	< 0.05
D. farinae	35 (70)	44 (88)	79 (79)	< 0.05
E. maynei	34 (68)	44 (88)	78 (78)	< 0.05
L. destructor	28 (56)	36 (72)	64 (64)	NS
T. putrescientae	24 (48)	34 (68)	58 (58)	< 0.05
B. tropicalis	22 (44)	35 (70)	57 (57)	< 0.01
A. siro	20 (40)	36 (72)	56 (56)	< 0.01
C. arcuatus	23 (46)	31 (62)	54 (54)	NS
NEGATIVE	14 (28)	6 (12)	20 (20)	< 0.05

MLSES: middle-low socioeconomic status. MHSES: middle-high socioeconomic status.

Table 4. Relationship between skin prick test and clinical symptoms.

PATHOLOGY		POSITIVE (n - %)	NEGATIVE (n - %)	p
Asthma	MP (52)	38 (73)	14 (27)	< 0.01
	ModP (39)	33 (85)	6 (15)	< 0.01
	SP (9)	9 (100)	0 (0)	< 0.01
Asthma + AR (60)		57 (95)	3 (5)	< 0.01
Asthma + E (12)		11 (92)	1 (8)	< 0.01
Asthma + AR + E (8)		8 (100)	0 (0)	< 0.01

MP: mild persistent. ModP: moderate persistent. SP: severe persistent. AR: Allergic Rhinitis. E: Eczema.

had mild persistent asthma, 39% moderate persistent asthma and 9% severe persistent asthma, graded according to the GINA guidelines. Sixty patients had asthma and allergic rhinitis (AR), 12 had asthma and eczema (E) and 8 had asthma, AR and E. Forty-eight % of the patients in the middle-low socioeconomic status (MLSES) had asthma and AR, in contrast to 72% of the patients in the middle-high socioeconomic status (MHSES) ($p < 0.01$).

Skin prick test

Skin pricks test results are shown in Table 2. Positive skin tests are more common in the MHSES ($p<0.05$). Skin prick test results of the 8 mite species are detailed in Table 3. Eighty patients had a positive skin test to at least one mite species. Eighty patients (80%) were skin test positive to at least one mite extract. All skin test positive patients had a positive skin reaction to *Dermatophagoides pteronyssinus*, 99% to *D. farinae*, 92% to *Euroglyphus maynei*, 80% to *Lepidoglyphus destructor*, 73% to *Tyrophagus putrescientae*, 72% to *Blomia tropicalis*, 70% to *Acarus siro* and 68% to *Chortoglyphus arcuatus*.

The relationship between positivity of skin prick test and clinical symptoms is analyzed in Table 4. It can be observed that, similarly to the general population, the fact that the skin prick test is positive is also found in each one of the pathologies presented by this population.

Table 5. Antigen concentration expressed in μg of allergen per g of dust in the two socioeconomic groups.

Mite Allergen		MLSES (n = 50) (n - %)	MHSES (n = 50) (n - %)	Total (n = 100) (n- %)	p
	< 2	2 (4)	13 (26)	15 (15)	< 0.01
Der p 1	2 - 10	15 (30)	12 (24)	27 (27)	NS
	> 10	33 (66)	25 (50)	58 (58)	NS
	Average	23.3 ± 22.6	13.3 ± 15.4	18.3 ± 19.9	< 0.01
Der p 2	< 2	15 (30)	27 (54)	42 (42)	< 0.05
	2 - 10	23 (46)	15 (30)	38 (38)	NS
	> 10	12 (24)	8 (16)	20 (20)	NS
	Average	6.9 ± 7.7	4.3 ± 7.0	5.6 ± 7.4	< 0.01
Der f 1	< 2	40 (80)	47 (94)	87 (87)	NS
	2 - 10	9 (18)	3 (6)	12 (12)	NS
	> 10	1 (2)	0 (0)	1 (1)	NS
	Average	0.8 ± 2.1	0.3 ± 1.3	0.6 ± 1.8	< 0.05

MLSES: middle-low socioeconomic status. MHSES: middle-high socioeconomic status.

Table 6. Allergen concentration expressed in μg of allergen per g of dust in patients with positive and negative skin prick test.

ALLERGEN	SKIN PRICK TEST		p
	Positive	Negative	
Der p 1	18.2 ± 20.6	18.7 ± 17.3	0.73 (NS)
Der p 2	5.4 ± 7.4	6.4 ± 7.5	0.44 (NS)
Der f 1	0.7 ± 1.9	0.1 ± 0.6	0.14 (NS)

Table 7. Der p 1 levels according to clinical symptoms.

PATHOLOGY		Der p 1 (μg/g of dust)	p
	Mild Persistent	16.6 ± 16. 6	
Asthma	Moderate Persistent	16.3 ± 14.6	0.59 (NS)
	Severe Persistent	37.0 ± 41.3	
Asthma	with Allergic Rhinitis	18.0 ± 18.6	0.94 (NS)
	Without AR	18.8 ± 21.9	
Asthma	with Eczema	22.7 ± 21.8	0.27 (NS)
	Without Eczema	17.7 ± 19.7	
Asthma	with AR with E	22.5 ± 25.3	0.59 (NS)
	Without AR without E	17.3 ± 17.6	

AR: Allergic Rhinitis. E: Eczema.

Allergen levels

Mite allergen levels are shown in Table 5. Der p 1 was the most common allergen in the mattress dust samples (mean: 18.3 μg/g of mattress dust). The mean level was lower in the MHSES group, although these differences were not significant between both groups.

Relationship between pathology and allergen levels

One hundred percent of patients with severe asthma had a positive skin prick test. This frequency decreased when the symptoms were less severe. Table 6 shows mite allergen levels and skin prick test results in both groups of patients. The concentration of Der p 1 contrasted regarding the pathology presented by the studied patients is described in Table 7. Although there was a tendency to higher mite allergen levels in mattresses of children with severe asthma and in mattresses of children with asthma associated with allergic rhinitis and eczema, the differences were not significant.

Discussion

In this study we describe a high prevalence of cutaneous sensitisation to different mite species in young asthmatic children in Valdivia, Chile. Allergen levels in 100 dust samples can be considered as very high. The criteria to demonstrate causality between an environmental exposure and a non-infectious illness were proposed by Bradford–Hill in 1965 [19]. The evidence relating these criteria allows us to state that mites are the most important causes of allergic asthma in Valdivia, Chile. Likewise, the abundant experimental evidence obtained from bronchial provocation challenges, that reproduces the asthmatic status in sensitised individuals and the result of environmental control studies to avoid the contact with mite allergens also show a consistency of association between sensitisation to mites, asthma and asthma severity [20,21].

In the last decade, much evidence has been gathered regarding the fact that exposure to mites allergens plays a major role in the development of bronchial hyperreactivity and asthma. However, a direct relationship between the level of exposure and the severity of asthma symptoms has not been proven yet.

In our study, 80% of the patients with asthma had a positive skin prick test to *D. pteronyssinus*, which turns it into the most important allergen in our area. All the patients studied had perennial asthma. None of them had seasonal asthma or a skin prick test exclusively positive to pollens. A high concentration of Der p 1 was detected in most cases. Therefore, *D. pteronyssinus* can be considered the most important source of indoor allergen in this geographical area of Chile [22]. In 85% of the mattress samples, concentrations above 2 mg of Der p 1/g of dust were detected (96% in MLSES and 74% in MHSES); in 58% of the cases, levels greater than 10 mg/g were measured (66% in MLSES and 50% in MHSES).

Recent studies of four different groups have confirmed a direct relationship between exposure to mites and sensitisation to their allergens [23,24,25]. It has also been shown that sensitisation to mites is a major risk factor for asthma in places with high levels of exposure [26,27,28]. In communities with low mite allergen levels, other indoor or outdoor allergens predominate in the sensitisation process [29,30].

The weather conditions in our city, with a high percentage of humidity (above 80% most of the year and with a rainfall exceeding 2300 ml/year) provide a great environment for the development of mites and sensitisation of genetically predisposed individuals. Based on our findings, the socioeconomical status does not constitute a significant variable when studying the prevalence of asthma in Chile. Overall, patients in the MLSES had significantly higher concentrations of mite allergens in their mattresses (Table 3). However, they were not significantly different when analysed for levels greater than 2 or 10 mg/g of dust. Although both groups were exposed to similar mite allergen concentrations, patients in the MHSES group had more frequently allergic rhinitis associated to their asthma ($p < 0.01$). This could be explained by different lifestyles, such as patients in the MHSES staying longer periods of time indoors and, therefore, resulting in more time of exposure to indoor allergens. This allows the speculation that above a certain mite allergen level, exposure time is more critical than the actual concentration of mite allergens. However, although the differences are not statistically significant probably due to the small sample size, severe asthma seems to be more frequent in the MLSES. According to the hygiene hypothesis, and as per our results, the highest concentrations of mite allergen were detected in families with MLSES. In these cases, the children showed less symptoms of allergic rhinitis ($p < 0.01$) and less positive skin tests ($p<0.05$), although the prevalence of severe asthma was greater. Children in the MLSES had probably been more frequently in contact with infectious agents in their early childhood due to the characteristic of their environment [31,32]. However, these observations deserve further evaluation and definitive conclusions cannot be drawn at this point in time.

The confirmation of a direct relationship between mite allergen concentration in the bedroom and the risk of sensitisation has been reported in many countries. Apart from several Latin America studies, publications in Germany [23], Australia [24,25], USA [26] and New Zealand [22] have found similar results. All geographical

areas in which allergen levels have been associated with the risk of sensitisation, have a common factor, that is a very high level of humidity [33,34]. This holds also true for Valdivia.

In conclusion, we have confirmed that the paediatric population in the city of Valdivia only experiences perennial asthma. The most common mite specie inducing sensitisation *is D. pteronyssinus*, which is also commonly present in house dust as evidenced by the high concentrations of Der p 1 detected in house dust and previous environmental studies. These levels could be considered very high as compared to the levels described as sensitising and able to produce symptoms. These allergen levels seem to be independent of the socioeconomic status of the patient. Mite allergen exposure and sensitisation constitutes, in genetically predisposed children, the most important factor of perennial allergic asthma.

References

1. The International Study of Asthma and Allergies in Childhod (ISAAC). Steering Commitee. Worldwide variations in the prevalence of asthma symptoms: the International Study of Asthma and Allergies in Childhood (ISAAC). Eur Respir J 1998, 12: 315-25.
2. Mallol J, Cortez L, Amarales L, Sánchez I, Calvo M, Soto S, Strickler A, Kyling A, Sanhueza I, Albornoz C. Prevalencia del asma en escolares chilenos. Estudio descriptivo de 24470 niños. ISAAC-Chile. Rev Med Chile 2000, 128: 279-85.
3. Sporik R, Holgate S, Platts-Mills T, Cogswell J. Exposure to house-dust mite allergen (Der pI) and the development of asthma in childhood. New Engl J Med 1990, 323: 502-7.
4. World Health Organization. Dust mites allergens and asthma: a worlwide problem. International workshop report. Bull WHO 1988, 66-69.
5. Platts-Mills T, Thomas W, Alberse R. Dust mite allergens and asthma: Report of a second international workshop. J Allergy Clin Immunol 1992, 89: 1046-60.
6. Artigas J, Casanueva M. Ácaros del polvo de las habitaciones en Chile (Acari). Ed Universitaria, 1983.
7. Arruda L, Rizzo M, Chapman M. Exposure and sensitization to dust mite allergens among asthmatic children in Sao Pablo, Brazil. Clin Exp Allergy 1990, 21: 433-9.
8. Sugisawa S, Rosario N, Baggio D, Suzuki M. Exposure to mites in public schools. J Allergy Clin Immunol 1994, 93: 265ª.
9. Puerta L, Fernández-Caldas E, Lockey R, Caraballo L. Mite allergy in the tropics: Sensitization to six domestic mite species in Cartagena, Colombia. J Invest Allergol Clin Immunol 1993, 3(4): 198-204.
10. Baena-Cagnani C, Fernandez-Caldas E, Patiño C. Comparison of mite allergens level in pillows and mattresses. J Allergy Clin Immunol 1993, 91: 352 [Abstract].
11. Neffen H, Fernández-Caldas E, Predolini N, Trudeau W, Sánchez-Cuerra M, Lockey R. Mite sensitivity and exposure in the city of Santa Fe, Argentina. J Invest Allergol Clin Immunol 1996, 6(5): 278-82.
12. Fernández-Caldas E, Baena-Cagnani CE, López M, Patiño C, Nefen HE, Sánchez-Medina M, Caraballo LR, Huerta López J, Malka S, Naspitz CK, Lockey RF. Cutaneous sensitivity to 6 mite species in asthmatic patient from 5 Latin American countries. J Investigational Allergol Clin Immunol 1993; 3 (5): 245-9.
13. Wills-Karp, Santeliz J, Karp CL. The germlesstheory of allergic disease: revisting the hygiene hypothesis. Nature Rev Immunol. 2001; 1: 69-75.
14. Boza L, Díaz P. Diagnóstico de asma en niños. Consenso chileno para el diagnóstico y manejo del Asma Bronquial en niños. Rev Chil Enf Respir 1995, 11:141-3.
15. Ceruti E, Díaz A, Pinto R. Clasificación según severidad y tratamiento del Asma Bronquial infantil. Consenso chileno para el diagnóstico y manejo del Asma Bronquial en niños. Rev Chil Enf Respir 1995, 11:145-8.
16. Global Iniciative for Asthma (Gina). Diagnosis and classification. Global strategy for asthma managemnet and prevention. NHLBI/WHO Workshop Report. NHI Publication No. 95-3659, 1995, 47-61.
17. Casanueva M, Artigas J. Distribución geográfica y estacional de los ácaros del polvo de habitación en Chile (Arthropoda, Acari). Gayona, Zool, 1985, 49 (3-4): 3-75.
18. Chapman M, Heymann P, Wilkins S, Brown M, Platts-Mills T. Monoclonal immunoassays for major house dust mite (Dermatophagoides) allergens Der p I and Der f I, and quantitative analysis of the allergen content of mite and house dust extracts. J Allergy Clin Immunol 1987, 80: 184-90.
19. Bradford-Hill A. Environment and disease: association or causation? Proc R Soc Med 1965,58: 265-300.
20. Platts-Mills T, Vervloet D, Thomas W, Aalberse R, Chapman M. Indoor allergens and asthma: Report of the Third International Workshop. J Allergy Clin Immunol 1997, 100, 6 (1): S2-S24.
21. Langley S, Goldthorpe S, Craven M, Morris J, Woodcock A, Custovic A. Exposure an sensitization to indoor allergens: Association with lung function, bronchial reactivity and exhaled nitric. J Allergy Clin Immunol 2003; 112:362-8
22. Wilkens K, de Bruyne J, Calvo M, Choon-Kook S, Jayaraj G, Lai CKW, Lane J, Maheshwari R, Mallol J, Nishima S, Purdie G, Siebers R, Sukumaran T, Trakultivakorn M, Crane J. The determinants of dust mite allergen and its relationship to the prevalence of symptoms of asthma in the Asia-Pacific region. Pediatr Allergy Immunol 2004; 15:55-61.
23. Kueher J, Frisher J, Meiner R. Mite exposure is a risk factor for the incidence of specific sensitization. J Allergy Clin Immunol 1994, 94: 44-52.
24. Peat J, Tovey E, Toelle B, Havy M, Gray E, Mahmic A, Woolcock A. House-dust mite allergens: a major risk factor for childhood asthma in Australia. Am J Respir Crit Care Med 1996, 153: 141-6.
25. Peat J, Tovey E, Mellis C, Leeder S, Woolcock A. Importance of house dust mite and Alternaria allergens in childhood asthma: an epidemiology study in two climatic regions of Australia. Clin Exp Allergy 1993, 23: 812-20.
26. Squillace S, Sporik R, Rakes G, Couture N, Lawrence A, Merriam S, Zhang J, Platts-Mills T. Sensitization to dust mites as a dominant risk factor for among adolescents living in Central Virginia – multiple regression analysis of a population-based study. Am J Respir Crit Care Med 1997, 156: 1760-1764.
27. Custovic A, Taggar S, Francis H, Chapman M, Woolcock A. Exposure to house dust mite allergens and the clinical activity of asthma. J Allergy Clin Immunol 1996, 98: 64-72.
28. Wickman M, Nordvall S, Pershagen G, Sundell J, Schwartz B. House dust mite sensitization in children and residential characteristics in a temperate region. J Allergy Clin Immunol 1991, 88: 89-95.
29. Sporik R, Ingram J, Price W, Sussman J, Honsinger R, Platts-Mills T. 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.
30. Platts-Mills T. Major risk factors according to age: The relevance of indoor allergens to the increase in asthma. In Neffen H, Baena-Cagnani C, Fabbri L, Holgate S, Byrne P (eds). Asthma- A link between environment, immunology and the airways. Seattle, Toronto, Bern, Göttingen. Hogrefe and Huber Publishers 1999, pp. 89-95.
31. Martinez F. Role of viral infections in the inception of asthma and allergies during childhood: could they be protective? Thorax 1994, 49:1189-91.
32. Burrows B, Sears M, Flannery E, Herbison G, Holdaway M. Relations of bronchial responsiveness to allergy skin test reactivity, lung function, respiratory simptoms and diagnoses in thirteen-year-old New Zealand children. J Allergy Clin Immunol 1995, 95: 548-56.
33. Sporik R, Holgate S, Platts-Mills T, Cogswell J. Exposure to house-dust mite allergen (Der p I) and the development of asthma in childhood: a prospective study. N Engl J Med 1990, 323. 502-7.
34. Arbes S Jr, Cohn R, Yin M, Muilenberg M, Burge H, Friedman W, Zeldin D. House dust mite allergen in US beds: Results from the first National Survey of Lead and Allergens in housing. J Allergy Clin Immunol 2003; 111:408-14.

Dr. Mario Calvo

P.O. Box 974
Valdivia, Chile
Tel.: + 56 63 22 95 70
Fax: + 56 63 22 95 70
E-mail: mcalvo@uach.cl

