

Differences in the presence of allergens among several types of indoor environments

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Summary. Exposure to indoor allergens can occur both at home and in public places such as schools and workplaces. To investigate and compare the presence of indoor allergens in different kind of environments (schools, offices and homes), dust samples were collected from furniture, desks, mattresses and floors with a standardized procedure. Samples were analyzed for Der p 1, Der f 1, Mite group 2 (mites) and Fel d 1 (cat) by monoclonal antibody ELISA assay. Mite allergens were detected with low frequencies in schools and workplaces and with high frequency in homes. Fel d 1 was found with high frequency in every examined environment. Homes rather than public places can represent the environment where people can easier incur in mite allergy. All environments could be at risk for cat allergen exposure.

Key words: school, home and workplace environments, indoor allergens, cat allergen, house dust mite allergens.

Riassunto (*Differenze nella presenza di allergeni indoor in diversi ambienti*). L'esposizione agli allergeni indoor può avvenire sia nelle abitazioni che nei luoghi pubblici. Per effettuare un confronto relativo alla presenza di allergeni indoor in differenti ambienti (scuole, uffici e abitazioni) sono stati raccolti campioni di polvere da mobili, scrivanie, materassi e pavimenti mediante procedura standardizzata. I campioni sono stati analizzati per il contenuto di Der p 1, Der f 1, Mite group 2 (acari) e Fel d 1 (gatto) mediante saggi ELISA. Gli allergeni degli acari sono stati riscontrati raramente nelle scuole e negli uffici, ma frequentemente nelle abitazioni. Fel d 1 è stato rilevato con elevata frequenza in ogni ambiente esaminato. In conclusione le abitazioni, piuttosto che i luoghi pubblici, possono rappresentare l'ambiente più a rischio per l'esposizione agli allergeni degli acari, mentre tutti gli ambienti possono costituire un rischio per l'esposizione agli allergeni del gatto.

Parole chiave: scuole, case e uffici, allergeni indoor, allergene del gatto, allergeni degli acari della polvere.

INTRODUCTION

In the last decades allergy and related symptoms have been increasing [1]. Since in developed countries people spend most part of the day in indoor environments [2], we can observe a significant number of subjects with symptoms induced by indoor allergen exposure. In fact, exposure and sensitization to house dust mites (HDM), the most common indoor allergen, have been suggested to be the main

cause of asthma [3, 4]. Furthermore, for dust mites sufficient evidence of a causal relationship between exposure and asthma development [5] has been provided. Another widespread allergen that could induce asthma symptoms is Fel d 1, the major cat allergen. A dose-response relationship between exposure and sensitization (threshold: 1-2 µg allergen/g of dust) to indoor allergens, as well as between exposure and symptom development (threshold: 8-10

$\mu\text{g/g}$), has been established [3]. Briefly, many studies have revealed that in asthmatic patients, sensitized to a particular allergen, asthma morbidity is directly related to the degree of exposure to that allergen [6]. For this reason an active surveillance of indoor environments could be really significant. The evaluation of indoor allergen levels in different environments where people spend much more time is an essential requisite for applying an active surveillance. While it is not realistic to make indoor environments allergen free, it is possible to apply some hygienic measures and periodical environmental controls.

Preliminary studies by our group (unpublished observation) reported the high and frequent presence of cat allergen rather than mite allergens in an indoor workplace. Prompted by these results and by other recently published studies [7], we sought to better investigate these issues by increasing the number of samples and by selecting different types of indoor environment. In particular, we sought to evaluate and compare the presence of such indoor allergens in the three most important environments where people spend time in the different stages of life, *i.e.* schools, homes and offices in a metropolitan area in the Center of Italy (137 samples) and in a smaller town in the North of Italy (158 samples).

Our final goal was to verify and to evaluate the allergen exposure during both the childhood (schools) and the adult age (offices) and ever at home, and the variation in allergen exposure in the different environments considered.

METHODS

Environment sampling

Several indoor environments (schools, workplaces and homes) were sampled. Two hundred and ninety-five samples from these environments were analyzed. We included in our analysis only samples containing > 30 mg of fine dust. Therefore we processed 134 samples from homes, 87 from offices and 74 from schools. Specific allergen avoidance measures (acaricide treatment, use of air cleaning systems with filters to remove particles carrying allergens, allergen-proof covers) have never been applied in all these environments.

Dust collection

In workplaces dust samples were collected from offices' floors and furniture (such as chairs and writing-desks), in schools from classrooms' floors and desks, and in homes from beds and floors of bedrooms and from floors and sofas of living rooms.

Trained technicians used a vacuum-cleaner Miele Electronic 1600 W (Gütersloh Westfalia Germany) to collect dust samples, equipped with a dust collection device (*Mitest* Dust Collector from Indoor Biotechnologies Inc., Cardiff, UK) which fits into the distal end of the vacuum extension tube. Dust collection was performed with a standardized procedure according to the protocol previously described

by Dreborg *et al.* [8]. Immediately after collection the samples were kept at $+4$ °C.

Samples extraction and ELISA assessment

The samples were extracted according to the Manufacturer's protocol (Indoor Biotechnologies, Cardiff UK) and the extracts were centrifuged for 20 minutes at $2\ 500\times g$ at $+4$ °C. The supernatants were stored at -20 °C until analysis. Dust samples were analyzed for Der p 1, Der f 1, Mite group 2 allergens (Mite g 2) and Fel d 1 with monoclonal antibodies-based ELISAs using quantitative ELISA Kits from Indoor Biotechnologies Inc. We considered positive any value higher than the quantitation limit (calculated as the mean value of 12 blanks plus six times the standard deviation) for any ELISA Kit (Der f 1= 0.07 $\mu\text{g/g}$; Der p 1= 0.2 $\mu\text{g/g}$; Mite g 2= 0.06 $\mu\text{g/g}$; Fel d 1= 0.1 $\mu\text{g/g}$).

Statistical analysis

For each allergen under study the median and the interquartile range of the values different from zero were calculated. Concentrations of different allergens were not normally distributed and were transformed using the logarithm base 10 scale in order to allow the use of parametric tests. The mean values of the transformed variables were then compared using the t-test when comparing two groups of values, and one-way ANOVA when comparing more than two groups. Frequencies of detectable samples were compared in a two by two way (workplaces vs schools, schools vs homes and workplaces vs homes) using the χ^2 test or Fisher exact test when appropriate. Comparisons were considered to be significant if $p < 0.05$.

In the distribution analysis, we imputed, for allergen concentrations below the lower detection limit, 0.5 times such a value, in order to show an overall distribution of allergen for all samples.

RESULTS

In the present study dust samples were collected from different environments. In each environment the floor together with the typical furniture were sampled.

Initially we considered two different urban types (a metropolitan area near the sea and a smaller continental city), but statistical analysis showed no statistical differences in allergen concentrations between the two different cities for each environment, therefore we consider all data as a single data set.

Environment 1: schools

As regards samples from schools, 74 samples were collected and detectable levels of Der f 1 were found in 4 samples (5.4%), Der p 1 in 2 (2.7%), and Mite g 2 in one sample only (1.3%), whereas Fel d 1 was found in 51 out of 74 samples (68.9%) (*Figure 1*).

Mite allergen concentrations are shown in *Table 1*, the highest value detected was 1.8 μg allergen/g of

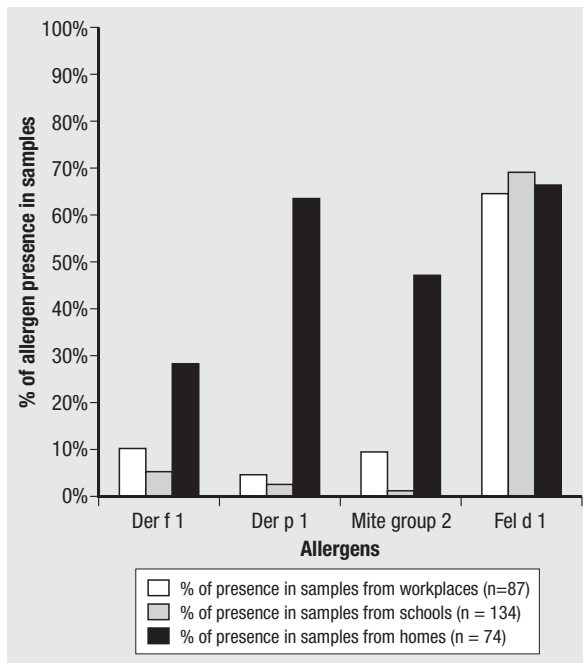


Fig. 1 | Percentage of allergen presence in samples analysed from studied environments.

dust (Der p 1), and none of the samples gave values over the cut-off for sensitization and/or symptom development (Table 2). On the contrary, Fel d 1 concentrations ranged from 0.2-25.1 $\mu\text{g/g}$, 13 samples out of 74 were over the threshold for sensitization, and 3 out of these 13 samples over the threshold for the development of symptoms (Table 2), the median value was 3.3 $\mu\text{g/g}$ (Table 1).

Environment 2: workplaces

As regards samples collected from the workplaces, 87 samples were analyzed. We found detectable levels of Der p 1 in 4 (4.6%) samples, whereas Der f 1 was found in 9 (10.3%) and Mite g 2 in 8 (9.2%) out of 87 samples. The frequency of Fel d 1 was higher, since this allergen was detected in 56 out of 87 (64.4%) samples (Figure 1).

Despite the low frequencies of mite allergens (<10%) their concentrations reached values up to 55.3 $\mu\text{g/g}$ (Mite g 2) (Table 1). Among mite allergens,

Der f 1 was the most common, its concentration values were over the threshold for sensitization in 4 out of 87 samples, and over the threshold for symptom development in 2 out of 87 (Table 2).

As regards cat allergen, Fel d 1 concentration reached a maximum peak of 320.8 $\mu\text{g/g}$ (median 25.4 $\mu\text{g/g}$, Table 1), 45 samples out of 87 were over the threshold for sensitization, and 20 out of these 45 samples were over the threshold for symptom development (Table 2).

Environment 3: homes

In homes we collected dust from beds (mattress), sofas, bookshelves and floors. One hundred and thirty-four dust samples were analyzed. Detectable levels of Der p 1 in 38 homes (28.3%), Der f 1 in 85 (63.4%), Mite g 2 in 63 (47.0%) and Fel d 1 in 89 out of 134 (66.4%) were found (Figure 1).

In these environments, high mite allergen concentrations were found in upholstered surfaces such as beds and sofas. Again, among mite allergens, Der f 1 was the most common and in 43 out of 134 samples its concentration was over the threshold for sensitization. Twenty-three out of these 43 samples overcome the threshold for symptom development (Table 2).

Similarly, the highest Fel d 1 values were detected in samples from upholstered furnishing (up to 82.8 $\mu\text{g/g}$), although the allergen was widespread in every place analyzed. The allergen concentration ranged between 0.1 and 82.8 $\mu\text{g/g}$ (Table 1) and values were in 52 out of 134 samples over the threshold for sensitization, in 22 out of those 52 over the threshold for symptom development.

Comparison of allergens distribution

Figure 2 gives an overall look at allergen concentration distribution for all samples. Significant differences in term of percentage of samples with a detectable allergen level were found between the three types of environments considered. In particular, when we compared the percentages of sites with a detectable concentration we found that homes had a significant higher concentration than both workplaces and schools for all allergens except Fel d 1. For Mite group 2 there was also a significant difference between workplaces and schools. Despite these frequency differences, no significant differ-

Table 1 | Summary statistics for allergen concentrations in the examined environments. Results reported only for rooms with detectable allergen concentrations. Median, interquartile range and max were expressed in μg of allergen per gram of dust

	Der f 1			Der p 1			Mite g 2			Fel d 1		
	Median	1 st -3 rd quartiles	Max	Median	1 st -3 rd quartiles	Max	Median	1 st -3 rd quartiles	Max	Median	1 st -3 rd quartiles	Max
Schools	0.6	0.4-1.2	1.7	NA	NA	1.8	NA	NA	0.2	3.3	1.4-7.4	25.1
Workplaces	2.5	2.0-7.9	34.0	2.6	1.6-4.2	4.8	0.7	0.6-3.2	55.3	4.3	2.3-25.4	320.8
Homes	2.3	0.9-8.7	42.6	1.2	0.7-5.6	39.0	1.1	0.3-2.4	19.2	4.9	1.4-10.4	82.8

NA: not available.

Table 2 | Percentage of samples with allergen levels within the risk threshold for sensitisation ($2\text{--}8\ \mu\text{g}$ allergen/g of dust) and above the threshold for elicitation of symptoms ($8\ \mu\text{g}$ allergen/g of dust), in the examined environments

	Der f 1		Der p 1		Mite g 2		Fel d 1	
	Samples within 2-8 $\mu\text{g}/\text{g}$	Samples over 8 $\mu\text{g}/\text{g}$	Samples within 2-8 $\mu\text{g}/\text{g}$	Samples over 8 $\mu\text{g}/\text{g}$	Samples within 2-8 $\mu\text{g}/\text{g}$	Samples over 8 $\mu\text{g}/\text{g}$	Samples within 2-8 $\mu\text{g}/\text{g}$	Samples over 8 $\mu\text{g}/\text{g}$
Workplaces	4/87 (4.6%)	2/87 (2.3%)	2/87 (2.3%)	0/87	1/87 (1.1%)	1/87 (1.1%)	25/87 (28.7%)	20/87 (23.0%)
Schools	0/74	0/74	0/74	0/74	0/74	0/74	10/74 (13.5%)	3/74 (4.0%)
Homes	20/134 (14.9%)	23/134 (17.2%)	10/134 (7.5%)	6/134 (4.5%)	18/134 (13.4%)	1/134 (0.7%)	30/134 (22.4%)	22/134 (16.4%)

ences were found when means of log transformed concentration values measured for each allergen in the three environments were compared. Fel d 1 was found in all the three types of environment, while HDM are much more frequently detected in homes than in public places.

DISCUSSION

Exposure and sensitization to indoor allergens have been associated with the development of asthma and other allergic diseases [9]. The most important remedy actions are to establish the degree of exposure and to evaluate whether allergen control measures are effective in a particular area [10].

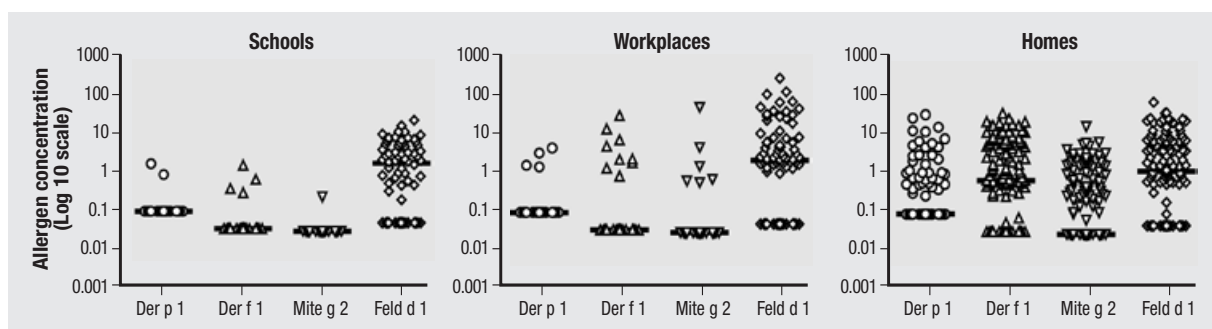
The aim of the present study was to identify which places (school, office, home) represent the major source of exposure to indoor allergens and to compare different indoor environments by monitoring allergen levels in dust samples. Furthermore, this is the first study aimed to compare the amount of four different indoor allergens in several types of indoor environments. We selected three types of indoor environments, *i.e.* public places (workplaces and schools) and private (homes) where people spend most time from childhood to adulthood. We initially compared the same kind of environments in two

towns selected as models with different extension areas, number of inhabitants and density of population as well as different atmospheric condition, that could potentially affect allergen exposition since climatic conditions can modify allergen levels [11].

The first interesting result from the present study is that differences between allergen concentrations in the same kind of environments of the two cities were not significant. Therefore, on the basis of these results, data from different schools, offices and homes were pooled together to carry out such analysis among these three environments.

Furthermore other important data are the relatively low frequency obtained for house dust mites in public places, both workplaces (offices) and schools. These results could be explained by the typical furnishings used in these environments, such as formica and others not upholstered materials, that are not an optimal microenvironment for house dust mites growth. Different results were obtained for domestic dwellings, in which house dust mites were detectable in a large number of samples and often with high concentrations.

Since mite allergens were scarcely present in public places and were considerably detected in houses, this study allowed us to confirm [12] homes rather than public places as the environment where peo-

**Fig. 2** | Allergen concentration distribution by type of environment. For analysis purposes, allergen concentrations below the lower quantitation limit were arbitrarily assigned 0.5 times such a value (see methods). House dust mite concentrations in samples from schools and workplaces mostly gave values under the quantitation limit.

ple can undergo sensitization and/or elicitation of symptoms by mite allergens. On the other hand, in our study, mite allergens showed a confined diffusion in domestic dwellings, although concentrations were very high due to the appropriate microenvironment. Our observations are supported by studies which reported home as the ideal microenvironment for house dust mites, representing a source of food associated with an optimal temperature and relative humidity [13]. In homes a reduction of mite allergen levels is possible by adopting some relatively simple measures such as new mattresses and increased ventilation of the bedrooms [14]. Moreover, several physical and chemical control methods are practicable in order to reduce the risk of exposure to mite allergens [15-22].

On the other hand, Fel d 1 was widely and almost equally widespread in offices, schools and domestic dwellings. Cat allergen was found in a large number of samples and very often in these environments the concentrations were higher than the threshold of both sensitization and elicitation of symptoms (1-2 µg allergen/g dust and 8-10 µg/g, respectively) [3]. Recently, Platts-Mills [23] suggested that the proteins that are less foreign (mammalian) will not induce a response in human beings until they are at high dose. From this point of view, the high frequency and concentrations obtained for Fel d 1 can constitute a considerable risk.

Results from the present study showed that Fel d 1 allergen only could play a significant role in sensitization and/or elicitation of symptoms in public places (offices and schools). Moreover, the high levels of Fel d 1 detected in cat-free environments such schools and offices, support the hypothesis that cat owners are an indirect surrogate for cat allergen exposure and their clothes are the main source for

diffusion of allergen [7, 24-27]. Consequently, being Fel d 1 ubiquitous, people could experience sensitization [28] and/or symptom elicitation in every place (public or not), independently from the type of place where they spend more time (according to their age and lifestyle) and from climatic condition or other characteristics of the city.

A recent study showed that a tendency to avoid pet keeping is clearly present in people with cat allergy [29]. But, as shown by our study and by data previously reported [25-27], we can confirm that pet's avoidance is not sufficient to avoid Fel d 1 exposure, and adequate allergen control methods are needed especially in public places. Although extensive washing is a simple, inexpensive, and extremely effective method of massively reducing mite as well as cat allergens from private environments (carpet, sheets, sofa) [17], this method is difficult to apply to public environments.

In conclusion, we can emphasize that, while it is relatively easy to carry out measures in order to reduce the risk of mite allergen exposure because they preferentially proliferate in confined environments like homes, this is quite difficult or not possible at all for cat allergen in public places. For this reason much more effort, including adequate environmental control and cleaning measures, is required to achieve an effective reduction of cat allergen levels.

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References

1. Busse WW. Mechanisms and advances in allergic diseases. *J Allergy Clin Immunol* 2000;105(6 Pt 2):S593-8.
2. Chapman MD. Indoor Allergens. In: Leung DYM, Sampson HA, Geha RS, Szefer SJ (Eds.). *Paediatric allergy - Principals and practice*. St. Louis: Mosby; 2003. p. 261-8.
3. Platts-Mills TA, Vervloet D, Thomas WR, Aalberse RC, Chapman MD. Indoor allergens and asthma: report of the third international workshop. *J Allergy Clin Immunol* 1997;100:S2-24.
4. Platts-Mills TA, Sporik RB, Chapman MD, Heymann PW. The role of domestic allergens. *Ciba Found Symp* 1997;206:173-85; discussion 185-9.
5. Illi S, von Mutius E, Lau S, Niggemann B, Gruber C, Wahn U, Multicentre Allergy Study (MAS) group. Perennial allergen sensitization early in life and chronic asthma in children: a birth cohort study. *Lancet* 2006;368(9537):763-70.
6. Custovic A, Taggart SCO, Francis HC, Chapman MD, Woodcock A. Exposure to house dust mite allergens and the clinical activity of asthma. *J Allergy Clin Immunol* 1996;98:64-72.
7. Arbes SJ, Cohn RD, Yin M, Muilenberg ML, Friedman W, Zeldin DC. Dog allergen (Can f 1) and cat allergen (Fel d 1) in US homes: Results from the national survey of lead and allergens in housing. *J Allergy Clin Immunol* 2004;114:111-7.
8. Dreborg S, Einarsson R, Lau S, Munir AK, Wahn U. Dust sampling for determination of allergen content. *Allergy* 1995;50(2):188-9.
9. Bemt L, Vries MP, Knapen L, et al. Influence of mattress characteristics on house dust mite allergen concentration. *Clin Exp Allergy* 2006;36(2):233-7.
10. Tobias KR, Ferriani VP, Chapman MD, Arruda LK. Exposure to indoor allergens in homes of patients with asthma and/or rhinitis in southeast Brazil: effect of mattress and pillow covers on mite allergen levels. *Int Arch Allergy Immunol* 2004;133(4):365-70.
11. Alvarez MJ, Olaguibel JM, Acero S, et al. Indoor allergens and dwelling characteristics in two cities in Spain. *J Investig Allergol Clin Immunol* 1997;7(6):572-7.
12. Arbes SJ Jr, Cohn RD, Yin M, et al. 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.
13. Arlian LG, Morgan MS. Biology, ecology, and prevalence of dust mites. *Immunol Allergy Clin North Am* 2003;23(3):443-68.

14. Zock JP, Heinrich J, Jarvis D, et al. Indoor Working Group of the European Community Respiratory Health Survey II. Distribution and determinants of house dust mite allergens in Europe: The European Community Respiratory Health Survey II. *J Allergy Clin Immunol* 2006;118(3):682-90.
15. Mahakittikun V, Boitano JJ, Tovey E, et al. Mite penetration of different types of material claimed as mite proof by the Siriraj chamber method. *J Allergy Clin Immunol* 2006;118(5):1164-8.
16. Arlian LG, Vyszynski-Moher DL, Morgan MS. Mite and mite allergen removal during machine washing of laundry. *J Allergy Clin Immunol* 2003;111(6):1269-73.
17. Tovey ER, Taylor DJ, Mitakakis TZ, De Lucca SD. Effectiveness of laundry washing agents and conditions in the removal of cat and dust mite allergen from bedding dust. *J Allergy Clin Immunol* 2001;108:369-74.
18. McDonald LG, Tovey E. The role of water temperature and laundry procedures in reducing house dust mite populations and allergen content of bedding. *J Allergy Clin Immunol* 1992;90(4 Pt 1):599-608.
19. Vyszynski-Moher DL, Arlian LG, Neal JS. Effects of laundry detergents on dermatophagoides farinae, dermatophagoides pteronyssinus, and euroglyphus maynei. *Ann Allergy Asthma Immunol* 2002;88:578-83.
20. Vanlaar CH, Peat JK, Marks GB, Rimmer J, Tovey ER. Domestic control of house dust mite allergen in children's beds. *J Allergy Clin Immunol* 2000;105:1130-3.
21. Bischoff ERC, Fischer A, Liebenberg B, Kniest FM. Mite control with low temperature washing. II. Elimination of living mites on clothing. *Clin Exp Allergy* 1998;28:60-5.
22. McDonald LG, Tovey ER. The effectiveness of benzyl benzoate and some essential plant oils as laundry additives for killing house dust mites. *J Allergy Clin Immunol* 1993;92:771-2.
23. Platts-Mills TA. The role of indoor allergens in chronic allergic disease. *J Allergy Clin Immunol* 2007;119(2):297-302.
24. Liccardi G, D'Amato G, Russo M, et al. Focus on cat allergen (Fel d 1): immunological and aerodynamic characteristics, modality of airway sensitization and avoidance strategies. *Int Arch Allergy Immunol* 2003;132(1):1-12.
25. Ritz BR, Hoelscher B, Frye C, Meyer I, Heinrich J. Allergic sensitization owing to "second-hand" cat exposure in schools. *Allergy* 2002;57(4):357-61.
26. Enberg RN, Shamie SM, McCullough J, Ownby DR. Ubiquitous presence of cat allergen in cat-free buildings: probable dispersal from human clothing. *Ann Allergy* 1993;70:471-4.
27. Bollinger ME, Eggleston PA, Flanagan E, Wood RA. Cat antigen in homes with and without cats may induce allergic symptoms. *J Allergy Clin Immunol* 1996;97:907-14.
28. Heinrich J, Bedada GB, Zock JP, et al. Indoor Working Group of the European Community Respiratory Health Survey II. Cat allergen level: Its determinants and relationship to specific IgE to cat across European centers. *J Allergy Clin Immunol* 2006;118(3):674-81.
29. Svanes C, Zock JP, Anto J, et al. Early Life Working Group Of The European Community Respiratory Health Survey. Do asthma and allergy influence subsequent pet keeping? An analysis of childhood and adulthood. *J Allergy Clin Immunol* 2006;118(3):691-8.