

Occurrence of mites and fungi in the homes of patients with allergic manifestations

Francesca Palmas*, Sofia Cosentino, Valeria Meloni and Maria Elisabetta Fadda

Department of Experimental Biology, Section of Hygiene, University of Cagliari, Cittadella Universitaria, SS 554 KM 4.500, I-09133 Monserrato (CA), Italy

(*author for correspondence, e-mail: fpalmas@vaxca1.unica.it; fax: +39 070 6754197)

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Abstract

This survey was carried out to evaluate mites and moulds concentrations in the homes of patients with allergic manifestations and positive skin-test for mites and/or fungi. The home environments of 277 patients were evaluated by questionnaire, by sampling of airborne fungal spores and by determining the occurrence of mites and moulds in dust samples. Among the 277 patients examined, 83% reacted positively to house dust mites. The fungal allergen most frequently responsible for skin positivity was *Alternaria tenuis*. The prevalent airborne fungi were *Cladosporium*, *Penicillium* and *Alternaria*, followed by *Aspergillus* and *Aureobasidium*. The other genera were found in less than 50% of the homes. The presence of domestic mites in dust was documented in more than 85% of the homes sampled. The occurrence of fungi in dust was generally higher than in the air. The most common genera recovered were *Penicillium*, *Candida*, *Aspergillus*, *Aureobasidium*, *Alternaria* and *Cladosporium*. The homes investigated were divided into two groups, damp and dry, on the basis of the reported presence of wet or damp spots on inside surfaces and moulds growing inside the home. Our results showed that the concentration of mites and moulds in dust was significantly higher ($p < 0.01$) in damp homes as compared to dry ones.

In general, a good agreement between skin positivity and presence of the same allergens in patients' home environment was observed for mites and, among fungi, for *Alternaria*, *Cladosporium* and *Aspergillus*. Our results seem to confirm that the study of mycoaeroflora and the quantification of mites and moulds in dust samples of allergic patients' homes could lead to more precise diagnoses and therefore to better prophylactic and therapeutic programs for each individual patient.

1. Introduction

As living standards have improved in recent years, more energy-efficient, comfortable homes have been built that are much more airtight and insulated than older ones. As a result, indoor environmental factors such as mites, moulds and house dust have become an increasing problem, as has the number of people suffering from allergic diseases.

Mites and moulds have been known for many years to be the principal source of house dust allergens and consequently potential agents of allergic respiratory disease (Voorhost et al., 1967; Tarlo et al., 1988; Mishra et al., 1992).

Since Voorhost et al. first identified mites as an important component of house dust (Voorhost et al., 1964), numerous investigations have revealed that domestic mites are an important source of dust allergens in many different countries (Maunsell et al., 1968; Murray and Zuk, 1979; Arlian et al., 1982; Yi-Chung Chang and Kue-Hsiung Hsieh, 1989). Fungal spores have long been recognized as important environmental allergens as well (Gravesen, 1979; Wilken-Jensen and Gravesen, 1984; Malling, 1986; Summerbell et al., 1992), and they may also play a role in interaction with house dust mites (Sinha et al., 1970; Andersen, 1985).

The major ecological factor reported to affect mite numbers is indoor humidity of the home (Bronswijk, 1973; Platt-Mills et al., 1987). Murray and Zuk (1979) demonstrated that there are no viable mites in samples collected when relative humidity was less than 50%. The relative humidity has also an indirect influence on fungal growth, which is dependent on available water on surfaces (e.g. condensation or water leakage).

Since avoidance of exposure to allergens is extremely important in the treatment of allergic diseases, providing more precise information on levels of indoor allergens could be helpful in determining the exposure of occupants and therefore in formulating control measures in the home.

On these basis the present survey was undertaken to evaluate mites and moulds concentrations in the homes of patients with allergic manifestations and positive skin-test for mites and/or fungi.

2. Materials and methods

2.1 Patients

Among the subjects with suspected allergic disease examined in the Allergology and Immunology Centers of Cagliari, 277, which exhibited a positive skin test response to mites and/or fungi, agreed to take part in this study and to have their homes sampled for mites and fungi.

The inhalant allergens tested (Lofarma and Bayropharm), were: pollens (*Graminaceae* mix, *Urticaceae* mix, *Compositae* mix, *Olea europea*), animal dander, house dust mites (*Dermatophagoides pteronyssinus* and *D. farinae*) and fungal extracts of *Penicillium* mix, *Aspergillus* mix, *Alternaria tenuis*, *Cladosporium herbarum* and *Candida albicans*. In addition, extracts of *Aureobasidium pullulans*, *Cryptococcus* mix, *Mucor mucedo* and *Fusarium moniliformis* were assayed since in a previous study we have demonstrated the importance of these fungi in allergic sensitization (Palmas et al., 1997a).

Before skin testing a complete medical history was compiled and a physical examination was performed by physicians on each subject. Skin-prick tests were made as previously reported (Cosentino and Palmas, 1996).

Of the 277 patients selected, aging 4 to 63 years, 153 were males and 124 females; 72 had bronchial asthma, 98 rhinitis and 107 asthma and rhinitis. The symptomatology was mostly perennial with seasonal exacerbation in Spring and/or Autumn.

2.2 Environmental survey

The environmental survey was carried out in Spring–Autumn season. The home environments of all subjects selected were evaluated by questionnaire, by sampling of airborne fungal spores and by determining the occurrence of mites and moulds in dust samples.

Records were made of the following characteristics of each home: age, heating system, floor coverings, presence of air-conditioning system, presence of pets, plants, garden, wall-paper, wet or damp spots on inside surfaces, visible mould growth.

The airborne fungal spores were collected by the sedimentation method. This method is commonly employed by various investigators (Lumpkins and Corbit, 1976; Infante-Garcia-Pantaleon and Dominguez-Vilches, 1988) although volumetric air sampling is considered more reliable and accurate (Kozak et al., 1980).

Patients or patients' parents were instructed to expose a set of six petri plates containing three different culture media (potato dextrose agar, Sabouraud dextrose agar and corn meal agar) in the bedroom and living room of their homes. Three plates were exposed simultaneously in each room for 30 minutes, under calm conditions and during identical hours, as far as possible. After exposure the plates were sealed and returned to our laboratory on the next day; PDA and CMA were incubated at 25 °C for 3 to 7 days and observed daily; SDA plates were incubated at 37 °C for 48 hours. The total amount of airborne fungal spores in the rooms is given as colony-forming unit (cfu)/plate/30 min.

In order to evaluate mould concentration in dust samples, 0.1 gram of dust was placed into 9.9 ml of physiological saline and incubated at 25 °C for 24 h to allow the reactivation of the spores; then an aliquot of 0.1 ml was inoculated on PDA, SDA and CMA plates. The plates were incubated as above mentioned.

The presence of mites in dust was evaluated by sampling the bedroom and living room floor using a vacuum cleaner. About 1 m² of surface area of each site was vacuumed for one minute, following which each dust sample in the container of the vacuum cleaner was brushed completely into a plastic bag and analyzed within two days.

Isolation of mites was done according to the floatation technique of Hart and Fain (1987). Mites were counted under an inverted phase contrast microscope and the mite concentration was expressed as the number of mites (Acarine family) per gram of dust.

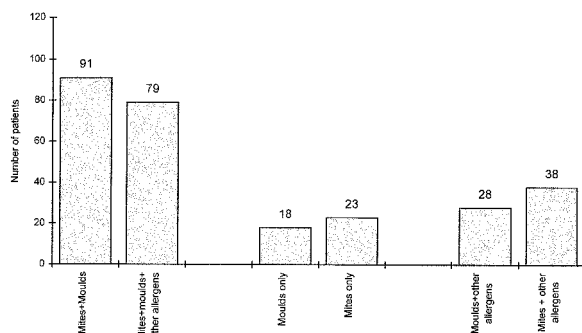


Figure 1. Distribution of sensitization to mites and moulds among the 277 patients studied.

No attempt was made to differentiate live from dead mites or to identify the mites to genus level; they can be referred to as belonging to the group of domestic mites (Colloff and Spieksma, 1992).

2.3 Identification of fungi

The yeasts were identified with the API 20C system and classified according to Barnett et al. (1990).

The moulds were identified by standard mycological techniques based upon gross cultural and microscopic morphology. The fungi that could not be identified in this manner were subcultured on potato dextrose agar and/or slide cultures for further study (Raper and Thom, 1949; Gilman, 1957; Raper and Fennel, 1965; Ainsworth et al., 1973; Emmons et al., 1977).

Statistical analyses were performed using the χ^2 test.

3. Results

The distribution of allergic sensitization to house dust mites and fungi in the 277 patients studied is reported in Figure 1. A detailed analysis of the data shows that 170 patients (61.4%) exhibited a positive skin test response to the moulds-mites association, 66 (23.8%) were sensitized to mites or moulds in association with other inhalant allergens and 41 (14.8%) showed single or multiple sensitivities to mites or moulds alone.

Table 1 reports the frequencies of positive skin responses to mites and moulds genera and their occurrence in the homes of the patients. The results are expressed as the percentage of homes in which fungi and domestic mites were isolated but similar results were observed when considering their concentrations.

Table 1. Frequencies of positive skin reactions and occurrence of mites and moulds in the patients' homes.

Allergens*	Positive skin reaction (%)	Frequency of isolation (%)	
		Air	Dust
Mites	83	N.D.	85.6
<i>Alternaria</i>	60	82	59
<i>Cladosporium</i>	43	91	52
<i>Aspergillus</i>	39	68	65
<i>Candida</i>	25	43	67
<i>Penicillium</i>	19	83	87
<i>Aureobasidium</i>	12	51	65
<i>Fusarium</i>	7	18	19
<i>Cryptococcus</i>	6	17	23
<i>Rhizopus</i>	4	14	16
<i>Mucor</i>	4	12	13

* Ranked in order of frequency with respect to allergens
N.D. = not determined

As can be seen, 83% of the patients studied reacted positively to house dust mites. The fungal allergen most frequently responsible for skin positivity was *Alternaria tenuis* (60%), followed by *Cladosporium herbarum*, *Aspergillus* mix, *Candida albicans*, *Penicillium* mix.

The prevalent airborne fungi were *Cladosporium*, *Penicillium* and *Alternaria*, followed by *Aspergillus* and *Aureobasidium*. The other genera were found in less than 50% of the homes.

As for dust, the presence of domestic mites was documented in more than 85% of the homes sampled. The occurrence of fungi in dust was generally higher than in the air. The most common genera recovered were *Penicillium*, *Candida*, *Aspergillus*, *Aureobasidium*, *Alternaria* and *Cladosporium*.

The remaining genera did not play a significant role either in sensitization or in airborne and dust prevalence.

Table 2 shows domestic mites and moulds concentrations in the dust of the homes sampled. The homes were divided into two groups, damp and dry, on the basis of the reported presence of wet or damp spots on inside surfaces and moulds growing inside the home. Damp and dry homes were then classified according to the concentration of mites and fungi in the dust samples.

Since the density of mites and moulds in dusts varied quantitatively among the rooms of a single house, the results from both sampling areas (bedroom and living room) were summed to provide a total for the whole house.

Table 2. Mites and moulds presence in the house dust of allergic patients. Mites and/or moulds dust concentration per gram of dust.

	Mites presence ^a			Moulds presence ^b		
	Damp homes	Dry homes	P	Damp homes	Dry homes	P
	(n = 105)	(n = 172)		(n = 105)	(n = 172)	
	%	%		%	%	
High concentration	42.9	29.1	<0.05	40	27.3	<0.05
Medium concentration	30.5	18.6	<0.05	48.6	43.6	N.S.
Low concentration	17.1	34.9	<0.01	11.4	29.1	<0.01
Absence	9.5	17.4	N.S.	0	0	

^a High >100; Medium = 51–100; Low ≤50

^b High >50; Medium = 11–50; Low ≤10

From the data it can be seen that the concentration of mites and moulds in dust was significantly higher ($p < 0.01$) in damp homes as compared to dry ones. It can be also noted that moulds were isolated in all homes sampled while mites were not recovered from 9.5% and 17.4% of damp and dry homes, respectively.

4. Discussion

The sensitization to mites and moulds varies considerably in different parts of the world presumably in consequence of the great variability of two important factors: their concentration in domestic indoor and the text extracts used (Berrens, 1970; Tarlo et al., 1988; Cosentino and Palmas, 1996).

Epidemiological studies have shown that living in an environment with high levels of mites greatly favors specific sensitizations (Murray, 1985). In subjects living at sea level, Charpin et al. (1988) have observed positive skin test to mites significantly more often than in those subjects who have always lived at higher elevations, where mite levels are low.

In addition, some authors have noted a connection between allergic symptoms and presence of high fungal concentrations in the home environments of allergic patients (Gravensen, 1981; Cosentino et al., 1993). On the other hand, as reported by various authors, a precise relationship between clinical symptomatology and mite or fungal allergy is still difficult to prove without more precise information on the type and quantity of these allergens in the patients' living environment (Tarlo et al., 1988; Mishra et al., 1992).

In our study, which is in agreement with previous data on skin-test incidence in our region (Ballero et al., 1984), the overall sensitization to mites was very high with respect to fungi (67% and 8.3% of positivity

for house dust mites and fungi respectively, on 3,594 cases examined) (data not shown).

Among the 277 patients which agreed to participate in the environmental survey, the sensitization to mites and moulds association was particularly high.

Among fungi, *Alternaria* and *Cladosporium* have been more frequently found to give positive reactions. In accordance with other studies (Chapman and Williams, 1984; Lowenstein et al., 1986; Cosentino and Palmas, 1996; Palmas et al. 1997a), *Alternaria* seems to play the major role.

As for the environmental survey, the majority of the homes sampled were characterized by high levels of domestic mites and moulds contamination. The occurrence of fungi in the homes of non allergic subjects living in the same area has been studied in previous investigations by our group, showing a different spore incidence in the homes of allergic and non allergic patients (Cosentino et al., 1993; Palmas et al., 1997b).

In this study, mites occurred in 85.6% of all dusts analyzed and an abundant mycoflora was also found in the air and in the dust samples of the homes.

Usually, mites and moulds concentration depends on interacting variables such as temperature and relative humidity. In our study, the reported home dampness seemed to influence the concentration of these two environmental allergens. In fact the number of mites and moulds per gram of dust was significantly higher in damp homes. According to certain authors (Korsgaard, 1989; Charpin and Vervloet, 1990; Summerbell et al., 1992) few mites or fungi are found in buildings where prevailing relative humidity is held at 45% for a temperature of 20 to 22 °C. This consideration is particularly important for Cagliari, where the warm and humid climate makes indoor dust

mites and moulds an important source of allergens (Ballero et al., 1984; Palmas et al., 1997b).

In general, a good agreement between skin positivity and presence of the same allergens in patients' home environment was observed for mites and, among fungi, for *Alternaria*, *Cladosporium* and *Aspergillus*. On the contrary, *Penicillium*, which has been found in high percentages in both air and dust, appears to be less important in sensitization. This may be due to several factors, such as inappropriate species used for the preparation of extracts and inadequate skin-test material (Mishra et al., 1992).

5. Conclusions

Our results seem to confirm that the study of mycoaeroflora and the quantification of mites and moulds in dust samples of allergic patients' homes could lead to more precise diagnoses and therefore to better prophylactic and therapeutic programs for each individual patient.

References

- Ainsworth G.C., Sparrow F.K. and Sussman A.S.: 1973, *The Fungi*. Academic Press, New York.
- Andersen A.: 1985, Microfungi in beds and their relation to house-dust mites. *Grana* **24**, 55–59.
- Arlian L.G., Bernstein I.L. and Gallagher J.S.: 1982, The prevalence of house dust mites, *Dermatophagoides* spp. and associated environmental conditions in homes in Ohio. *J. Allergy Clin. Immunol.* **69**, 527–532.
- Ballero M., Carluccio A., Anedda V. and Piu G.: 1984, Allergeni responsabili di manifestazioni cliniche IgE-mediate riguardanti la popolazione sarda. Osservazioni epidemiologiche e risultati terapeutici. *Folia Allerg. Immunol. Cl.* **31**, 133–144.
- Barnett J.A., Payne R.W. and Yarrow D.: 1990, *Yeasts: Characteristics and Identification*, 2nd edn. Cambridge University Press.
- Berrens L.: 1970, The allergens in house dust. *Progr. Allergy* **14**, 259–265.
- Bronswijk J.E.M., Van: 1973, *Dermatophagoides pteronissinus* (Trouessart 1897) in mattress and floor dust in a temperate climate (Acari: Pyroglyphidae). *J. Med. Ent.* **1**, 63–70.
- Chapman J.A. and Williams S.: 1984, Aeroallergens of the South-east Missouri area: A report of skin test frequencies and air sampling data. *Ann. Allergy* **52**, 411–418.
- Charpin D., Birnbaum J., Toumi M., Razzouk H., Lanteaume A. and Vervloet D.: 1988, Asthma and allergy to house dust mites in populations living at high altitude. *Chest* **93**, 758–761.
- Charpin D. and Vervloet D.: 1990, Dampness, mites and respiratory allergy. *Aerobiologia* **6**, 82–86.
- Colloff M.J. and Spieksma F.Th.M.: 1992, Pictorial keys for the identification of domestic mites. *Clin. Exp. Allergy* **22**, 823–830.
- Cosentino S. and Palmas F.: 1996, Occurrence of fungal spores in the respiratory tract and homes of patients with positive skin test to fungi. *Aerobiologia* **12**, 155–160.
- Cosentino S., Fadda M.E. and Palmas F.: 1993, Indoor airborne fungal spores in the home of allergic and non allergic subjects. *Proc. Int. Congr. Indoor Air '93* **1**, 159–163.
- Emmons C.W., Binford C.H., Utz J.P. and Kwon-Chung K.J.: 1977, *Medical Micrology*. Lea and Febiger, Philadelphia.
- Gilman J.C.: 1957, *A Manual of Soil Fungi*. The Iowa State University Press, Ames, Iowa.
- Gravesen S.: 1979, Fungi as a cause of allergy disease. *Allergy* **34**, 135–154.
- Gravesen S.: 1981, On the connection between the occurrence of airborne microfungi and allergy symptoms. *Grana* **20**, 225–227.
- Hart B.J. and Fain A.: 1987, A new technique for isolation of mites exploiting the difference between ethanol and saturated NaCl: Qualitative and quantitative studies. *Acarologia* **28**, 251–254.
- Infante-Garcia-Pantaleon F. and Dominguez-Vilches E.: 1988, Annual variation of *Cladosporium* spores in home habitats in Cordoba, Spain. *Ann. Allergy* **60**, 256–261.
- Kozak P.P., Gallup J., Cummins L.H. and Gillman S.A.: 1980, Currently available method for home mold surveys I. Descriptions of techniques. *Ann. Allergy* **45**, 85–89.
- Korsgaard J.: 1989, *House-Dust Mites and Humidity*. Workshop on Mite Allergy. XIV Congr. of the European Academy of Allergy and Clinical Immunol.
- Lowenstein H., Gravesen S., Larsen L., Lind P. and Schwartz, B.: 1986, Indoor allergens. *J. Allergy Clin. Immunol.* **78**, 1035–1039.
- Lumpkins E.D. and Corbit S.: 1976, Airborne fungi survey. II. Culture plate survey of the home environment. *Ann. Allergy* **36**, 40–48.
- Malling H.J.: 1986, Diagnosis and immunotherapy of mould allergy. IV. Relation between asthma symptoms, spore counts and diagnostic tests. *Allergy* **41**, 342–350.
- Maunsell K., Wraith D.G. and Cunniton A.M.: 1968, Mites and house dust allergy in bronchial asthma. *Lancet* **1**, 1267–1270.
- Mishra S.K., Ajello L., Ahearn D.G. et al.: 1992, Environmental mycology and its importance to public health. *J. Med. Vet. Mycol.* **30**, 287–305.
- Murray A.B. and Zuk P.: 1979, The seasonal variation in a population of house dust mites in a North American City. *J. Allergy Clin. Immunol.* **64**, 266–269.
- Murray A.B., Ferguson A.C. and Morrison B.J.: 1985, Sensitization to house dust mites in different climatic areas. *J. Allergy Clin. Immunol.* **76**, 108–112.
- Palmas F., Meloni V. and Fadda M.E.: 1997a, Fungi allergic pathologies: Correlation between allergological tests and presence of fungi in the upper respiratory tract. *Aerobiologia* **13**, 17–22.
- Palmas F., Meloni V., Fadda M.E. and Cosentino S.: 1997b, Fungal spores in the homes of subjects with positive skin-test for fungi. *Proc. Int. Congr. Healthy Building/IAQ '97*, pp. 437–442.
- Platts-Mills T.A.E., Hayden M.L., Chapman M.D. and Wilkins S.R.: 1987, Seasonal variation in dust mite and grass-pollen allergens in dust from the houses of patients with asthma. *J. Allergy Clin. Immunol.* **79**, 781–791.
- Raper K.B. and Fennel D.I.: 1965, *The Genus Aspergillus*. Williams & Wilkins, Baltimore.
- Raper K.B. and Thom C.: 1949, *A Manual of Penicillia*. Williams & Wilkins, Baltimore.
- Reynolds S.J., Streifel A.J. and McJilton C.E.: 1990, Elevated airborne concentration of fungi in residential and office environments. *Am. Ind. Hyg. Assoc. J.* **51**, 601–604.
- Sinha R.N., Bronswijk J.E.M. and Wallace H.A.H.: 1970, House dust allergy, mites and their fungal associations. *Can. Med. Assoc. J.* **103**, 300–301.
- Summerbell R.C., Staib F., Dales R., Nolard N., Kane J., Zwanenburg H., Burnett R., Krajden S., Fung D. and Leong D.: 1992,

- Ecology of fungi in human dwellings. *J. Med. Vet. Mycol.* **30**, 279–285.
- Tarlo M., Fradkin A. and Tobin R.S.: 1988, Skin testing with extracts of fungal species derived from the homes of allergy clinic patients in Toronto, Canada. *Clin. Allergy* **18**, 45–52.
- Voorhorst R., Spiekma FThM., Varekamp H., Leupen M.J. and Lyckema A.W.: 1967, The house dust mite (*Dermatophagoides pteronyssinus*) and the allergens it produces: identity with the house-dust allergen. *J. Allergy* **39**, 325–339.
- Voorhost R., Spiekma-Boezeman M.J.E. and Spiekma F.T.M.: 1964, Is a mite (*Dermatophagoides* sp) the producer of the house dust allergen? *Allerg Asthmaforsch* **6**, 329–335.
- Wilken-Jensen K. and Gravesen S.: 1984, *Atlas of Moulds in Europe Causing Respiratory Allergy*. Ask Publishing, Denmark.
- Yi-Chung Chang M.D and Kue-Hsiung Hsieh M.D.: 1989, The study of the dust house mites in Taiwan. *Ann. Allergy* **62**, 101–106.