Original Article

House Dust Mite Species and Allergen Levels in Galicia, Spain: a Cross-Sectional, Multicenter, Comparative Study

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Abstract. *Background and Objectives***:** Mites are important sources of allergens in Galicia, Spain. The objectives of this study were to identify the main mite species and to determine allergen levels in mattresses from different locations in Galicia.
*Material and Methods***:** Dust samples were collected with a portable vacuum cleaner for 2 minutes from 2 m^2 of the surface of the mattress used by mite allergic patients and controls. After collection, samples were immediately frozen. Mite species were collected, identified, and counted, and the results expressed as mites per gram of dust. Mite allergen levels (Der p 1 and Der f 1) were measured using monoclonal antibodies. All individuals answered a questionnaire about the characteristics of their homes.
*Results***:** A total of 332 dust samples were collected (112 from mite allergic patients and 220 from controls). Thirty-two species were identified. The mean age ± SD of all the participants was 32.4 ± 20.8 years and of the mattresses 7.6 ± 5.9 years. The geometric mean of the total mite counts was 910.2 mites per gram (896.2 mites per gram in the mattresses of mite allergic patients and 917.3 in the mattresses of control subjects; $P > .05$**).** The main species was *Dermatophagoides pteronyssinus*, which was present in 97.6% of the samples (geometric mean, 584.7 mites per gram). The geometric mean level of Der p 1 was 13.1 μg/g of dust: 11.9 in the mattresses of mite allergic patients and 13.8 in the mattresses of control subjects. The geometric mean level of Der f 1 was 1.1 μg/g: 1.5 in the mattresses of mite allergic patients and 0.8 in the mattresses of control subjects. Environmental risk factors associated with high mite counts included obvious signs of humidity in the bedroom and the age of the mattress.
*Conclusions***:** The mite fauna in Galicia is comprised of many species, several of which are known to be of allergologic importance. The total population is exposed to very high levels of mite allergens.

Key words: Galicia. Mites. *Dermatophagoides pteronyssinus*. *Chortoglyphus arcuatus*. *Euroglyphus maynei*. Allergens

Resumen. *Antecedentes y objetivos***:** Los ácaros son una fuente importante de alérgenos en Galicia. Los objetivos de este estudio fueron identificar las principales especies de ácaros y determinar los niveles de alérgenos en colchones en distintos lugares de Galicia.
*Material y métodos***:** Se obtuvieron muestras de polvo con un aspirador portátil durante 2 minutos en 2 metros cuadrados de superficie del colchón de pacientes alérgicos a los ácaros y controles. Tras su obtención, las muestras se congelaron inmediatamente. Se recogieron, identificaron y contabilizaron las especies de ácaros; los resultados se expresaron como ácaros por gramo de polvo. Los niveles de alérgenos de ácaros (Der p 1 y Der f 1) se determinaron con anticuerpos monoclonales. Todos los pacientes respondieron un cuestionario sobre las características de sus hogares.

Resultados: Se obtuvieron un total de 332 muestras de polvo (112 de pacientes alérgicos a los ácaros y 220 de controles). Se identificaron 32 especies. La edad media de todos los participantes fue de 32,4 ± 20,8 años y la de los colchones fue de 7,6 ± 5,9 años. La media geométrica (MG) del recuento total de ácaros fue de 910,2 ácaros/g (896,2 ácaros/g en los colchones de los pacientes alérgicos y 917,3 en los colchones de los controles; $p > 0,05$**).** La especie principal fue *Dermatophagoides pteronyssinus*, presente en un 97,6% de las muestras (MG: 584,7/g). La MG de Der p 1 fue de 13,1 mg por gramo de polvo; 11,9 en los colchones de los pacientes alérgicos y 13,8 en los colchones de los controles. La MG de Der f 1 fue de 1,1: 1,5 en los colchones de los pacientes alérgicos y 0,8 en los colchones de los controles. Los factores de riesgo medioambiental asociados con un recuento elevado de ácaros incluyeron signos evidentes de humedad en el dormitorio y la edad del colchón.
*Conclusiones***:** La fauna de ácaros en Galicia está formada por numerosas especies, algunas de las cuales son de importancia alergológica. La población total está expuesta a unos niveles muy elevados de alérgenos de ácaros.

Palabras clave: Galicia. Ácaros. *Dermatophagoides pteronyssinus*. *Chortoglyphus arcuatus*. *Euroglyphus maynei*. Alérgenos

Introduction

Mites represent the main source of allergens in humid areas and sensitization to their allergens is a major risk factor for asthma in exposed individuals [1-3]. Galicia is situated on the northwest corner of Spain. Its situation and geographic extension—from the sea to the central plateau of Spain—mean that it has both a maritime and an Atlantic climate. However, Galicia has some degree of climatic diversity. In the coastal strip, temperatures range from 8°C to10°C in the winter and 20°C to 25°C in the summer. The inner provinces of Lugo and Ourense have a drier climate, with greater differences between extreme temperatures in the summer and winter.

In recent years, there has been increasing interest in the study of the mite fauna in Galicia due to the high prevalence of positive skin tests to mites observed in patients with allergic respiratory symptoms. In general, the diagnosis of mite sensitivity is often hampered by the lack of knowledge of the mite fauna of a certain region. As for pollens, the determination of the main species in the environment is crucial for the accurate diagnosis and treatment of mite-induced sensitivities. Some studies have addressed the presence of different species in Galicia [4-6].

Sensitization to different mite species has also been the subject of several publications [4, 7-10]. These studies have demonstrated a high prevalence of cutaneous sensitivity to different mite species, including storage mites. Sensitivity to storage mites has been assessed in Lugo [8]. A total of 80 patients were studied. From this group, 16.3% were skin test positive exclusively to the storage mites *Lepidoglyphus destructor* and *Tyrophagus putrescentiae*, 41.3% to the storage mites and to the house dust mites *Dermatophagoides pteronyssinus* and *Dermatophagoides farinae*, and 34% exclusively positive to house dust mites. Based on these studies, *L destructor*, *T putrescentiae* and *Acarus siro* may be considered of clinical importance.

In Galicia, mite allergen levels have also been quantified in bedroom and living room dust samples from the homes of patients with allergic respiratory symptoms [8]. Mean Der p 1 levels ranged from 7.1 μg/g in the homes of house-dust and storage-mite sensitive patients to 3.6 μg/g in the homes of exclusively house-dust mite sensitized patients. Low levels of Lep d 2 were detected in these samples.

Based on the importance of mite sensitivity in this area and the limited information available about mite fauna in Galicia, a comprehensive study using a multicenter approach is required. The main objective of this study was to identify the mite species found in mattresses and to determine allergen levels at different locations in Galicia, Spain.

Material and Methods

Study Design and Patient Population

A prospective, cross-sectional, multicenter, comparative study was designed to investigate the mite fauna in mattresses from 4 provinces of Galicia. A total of 112 patients were initially recruited at the allergy services of the participating hospitals. All patients were skin tested with a battery of standardized allergen extracts, including mites, pollens, moulds, and animal danders. The inclusion criteria consisted of a positive skin test (wheal size > 3 mm) to at least 1 mite species and willingness to participate in the study. Patients were asked to collect a dust sample from their own mattress and 2 more from the mattresses of a neighbor and a family member who did not live in the same dwelling. A total of 332 samples were analyzed, of which 220 were controls. Samples collected in urban areas accounted for 75% of the total in La Coruña, 78% in Lugo, 70% in Ourense, and 40% in Pontevedra; the remainder were obtained in rural areas.

The mean age of the patients was 23.3 years, and that of the controls, 38.3 years. The main allergic symptoms in the patient population were rhinoconjunctivitis and/or asthma. The control population was selected by the patients, independently of their allergic status; 15% of these individuals presented clinical symptoms mainly compatible with rhinoconjunctivitis and 8% with asthma. All patients and controls completed a questionnaire containing questions about the age of the mattress and the home, signs of humidity in the bedroom, number of occupants, urban or rural habitat, etc.

Collection and Analysis of Dust Samples

Dust collection was carried out throughout 2002 and included all 4 seasons: 7% in the winter, 20.8% in the spring, 40.4% in the summer, and 31.8% in the autumn. Dust samples were collected by the patients with a portable vacuum cleaner for 2 minutes from 2 m^2 of the surface of the mattress. Before the samples were collected, the patients were instructed in performing the procedure by trained personnel. After collection, samples were immediately frozen to avoid mite proliferation. Once in the laboratory, dust samples were weighed and separated into 50 to 100 mg aliquots. Mites were isolated using the flotation method described previously [11]. Briefly, dust samples were suspended in 5 mL of saturated saline and individual mites were removed using a fine needle and placed into 2 drops of Hoyer's medium. Afterwards, they were counted and identified using standard keys and reference preparations [12]. The results are expressed as the number of mites per gram of dust.

Seasonal Variations in Mite Counts

Sequential dust samples were collected in 4 homes (1 in each province) to assess seasonal variations in mite counts. The entire surface of each mattress was sampled, sequentially, once a month for 1 year.

Determination of Der p 1 and Der f 1 Concentrations

Der p 1 and Der f 1 allergens were quantified using monoclonal antibody kits (Indoor Biotechnologies Ltd, Manchester, UK) according to the manufacturer's instructions.

Statistical Analysis

Statistical analysis consisted of nonparametric statistics, including geometric means, Spearman's rank correlation, Mann Whitney U, and χ^2 analysis. A P value of less than .05 was considered statistically significant. Analyses were performed using Statview for Windows ver. 4.53 (Abacus Inc., Berkeley, California, USA).

Results

The results of the questionnaire are shown in Table 1. A total of 32 mite species were identified. Only the most prevalent species are shown. Table 2 shows the prevalence of the different species in all 4 provinces. Mites were present in 99.4% of the samples. *D pteronyssinus*, the most common species in all 4 provinces, was present in 97.6% of the samples, followed by the predator mite *Cheyletus* species in 47.7%. Other important mites, based on prevalence, were *L destructor*, *Euroglyphus maynei*, and *Chortoglyphus arcuatus*. *D farinae* was only present in 5.2% of the samples. Table 3 shows the abundance of the different species. The mean abundance of *D pteronyssinus* was 584.7 mites per gram of dust: 569.5 in mattresses of allergic individuals and 597 in mattresses of controls ($P > .05$). Despite being present in a small percentage of mattresses, the geometric mean abundance of *D farinae* was 208.5, suggesting the localized importance of this mite. Other important mites based on abundance were *E maynei*, *C arcuatus*, *Glycyphagus privatus*, and *L destructor*.

Table 1. Characteristics of the Home Environments*

Age, y, mean ± SD	24.1 ± 19.4
Number of occupants, mean ± SD	3.9 ± 1.5
Homes with signs of humidity	28.2%
Bedrooms with signs of humidity	23.2%
Pets	**43.7%**
Dog	26.4%
Cat	21.1%
Heating	67.2%
Air conditioning	0.7%
Carpets in the bedroom	68.6%
Floor type	
Wood	59.7%
Tiles	29.0%
Cork	5.9%
Age of the mattresses, y, mean ± SD	7.6 ± 5.9
Age of the pillows, y, mean ± SD	6.3 ± 6.5
Encasings	2.7%
Recent acaricide treatment	0.8%

* Data are shown as the percentage of homes studied unless otherwise indicated.

Allergen Levels

Der p 1 and Der f 1 levels are shown in Table 4. Mean Der p 1 and Der f 1 levels in mattresses of mite-allergic patients were 11.9 and 1.5 μg/g of dust, respectively, compared with 13.8 and 1 μg/g in control mattresses ($P > .05$).

Seasonal Variations in Mite Counts

Marked differences were detected in the seasonal distributions of mites. Overall, there was a tendency toward higher numbers during the summer and early autumn months. Even though the main species in all 4

Table 2. Prevalence of Mites and Mite Species in the 4 Provinces*

Species	Total	La Coruña (n = 111)	Lugo (n = 84)	Ourense (n= 46)	Pontevedra (n= 91)
Total mites	99.4	100	100	98.6	100
Dermatophagoides pteronyssinus	97.6	98.2	98.8	93.3	100
Cheyletus species	47.7	50.5	51.8	31.5	63.8
Lepidoglyphus destructor	39.1	37.6	34.9	33.7	59.6
Eurogyphus maynei	36.7	27.5	41	56.2	12.8
Chortoglyphus arcuatus	35.5	45	38.6	1.1	72.3
Tarsonemus species	27.5	17.4	45.8	12.4	46.8
Glycyphagus privatus	12.2	7.3	22.9	7.9	14.9
Gohieria fusca	11.6	13.8	22.9	2.2	2.2
Tyrophagus putrescentiae	10.7	9.2	16.9	6.7	12.8
Nanacarus minutus	10.7	6.4	8.8	12.4	21.3
Glycyphagus domesticus	10.4	4.6	6	18	14.9
Acarus gracilis	8.0	0.9	3.6	15.7	17
Dermatophagoides farinae	5.2	5.5	4.8	6.7	2.2

* Data are shown as the percentage of samples containing a given species.

Table 3. Geometric Mean of Mite Counts*

Species	Total	La Coruña	Lugo	Ourense	Pontevedra
Total mites	910.2	1042.6	1072.3	488.7	1638.7
Dermatophagoides pteronyssinus	584.7	774.2	705.1	236.6	946.4
Dermatophagoides farinae	208.5	460	103.1	133.4	441.6
Euroglyphus maynei	129.7	120.5	146.8	131.7	82
Chortoglyphus arcuatus	111.7	98.6	94.2	147.4	155.7
Glycyphagus privatus	101.8	67.6	107.3	85.3	223.8
Lepidoglyphus destructor	92.7	100	81.9	100	87.1
Tarsonemus species	89.7	42.2	131.8	28.3	157.2
Glycyphagus domesticus	71.1	67.1	43.4	68.9	81.2
Gohieria fusca	52.5	50	59	33.6	59.3
Nanacarus minutus	48	3.5	74.9	59	36.1
Cheyletus species	47.6	48.4	54.6	32.3	54.3
Acarus gracilis	40.3	91.7	81.0	30.1	46.4
Tyrophagus putrescentiae	29.3	31.1	26.0	33.5	29.5

* Data are shown as the number of mites per gram of dust.

Table 4. Der p 1 and Der f 1 Levels in Samples of Mattress Dust in Galicia*

Der p 1	La Coruña	Lugo	Ourense	Pontevedra
Geometric mean, µg/g	16.7	13.53	8.13	18.5
Range, µg/g	0.45 – 102.7	1.15 – 78.1	0.47 – 91	2.5 – 58
> 2 µg/g, % of samples	98.1	94	61.5	100
> 10 µg/g, % of samples	69.8	66	34.1	78.3
Der f 1	**La Coruña**	**Lugo**	**Ourense**	**Pontevedra**
Geometric mean, µg/g	1.13	0.3	1.6	18.8†
Range, µg/g	0.06 – 55.3	0.03 – 19.4	0.4 – 14.5	NA
> 2 µg/g, % of samples	5.8	4.8	13.2	2.1
> 10 µg/g, % of samples	4.8	2.4	2.2	2.1

* NA indicates not applicable. †Present in only one sample.

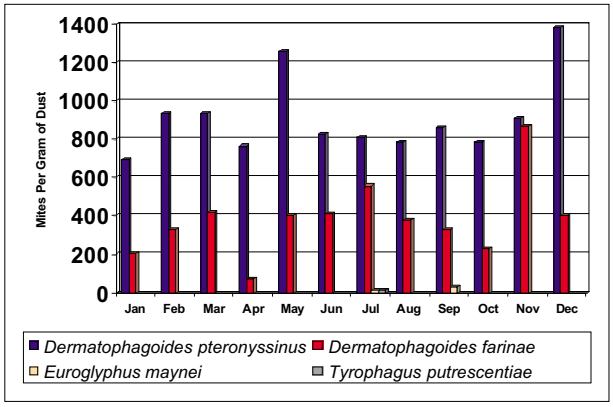

Figure 1. Seasonal variations in mite abundance in Pontevedra.

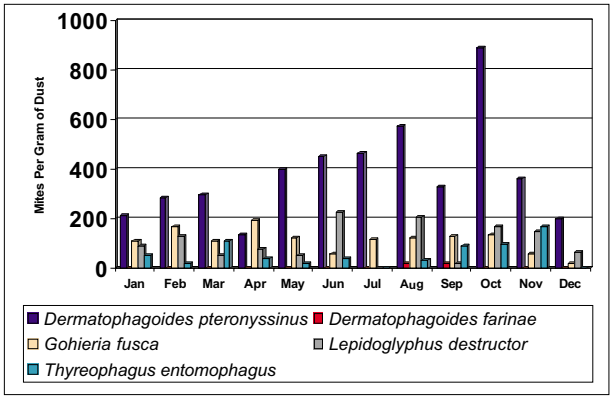

Figure 2. Seasonal variations in mite abundance in Ourense.

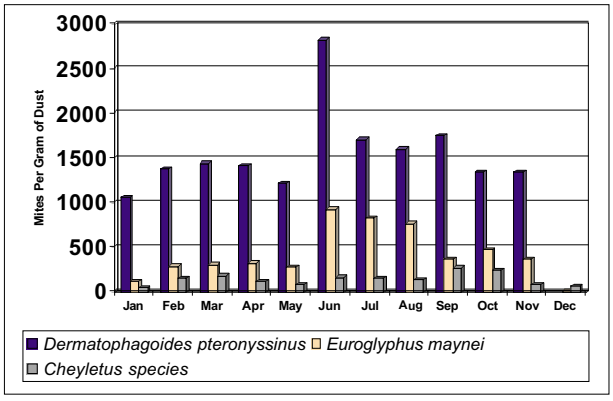

Figure 3. Seasonal variations in mite abundance in La Coruña.

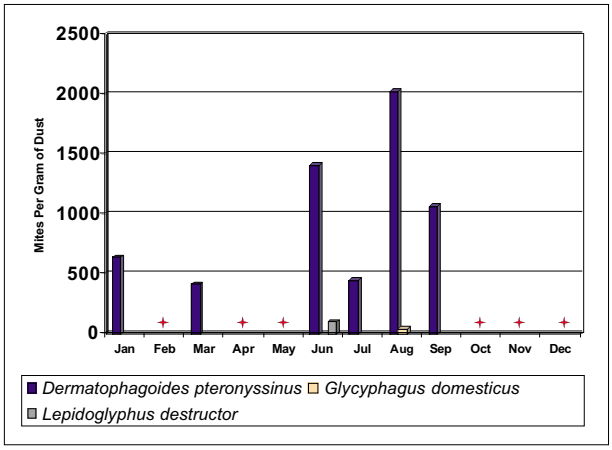

Figure 4. Seasonal variations in mite abundance in Lugo. Stars indicate insufficient sample.

mattresses was *D pteronyssinus*, other species also displayed the same pattern of seasonal changes. The seasonal variations of mite numbers and individual species are shown in Figures 1 to 4.

Statistical Analysis

There was a significant positive correlation between Der p 1 levels and *D pteronyssinus* counts (P = .0001) and a significant negative correlation between *D pteronyssinus* and *D farinae* counts (P = .006). The correlation between several environmental features and mite counts and allergen levels was assessed in order to identify potential risk factors. We detected a significant correlation between obvious signs of humidity and numbers of *D pteronyssinus* (P = .0004), *D farinae* (P = .009), *L destructor* (P = .0006), and *C arcuatus* (P = .0188). There was a greater abundance of the following species in rural environments than in urban ones: *E maynei* (P = .0017), *Glycyphagus domesticus* (P = .0214), *G privatus* (P = .0497), *C arcuatus* (P = .0001), and *Acarus gracilis* (P = 0.0389). Significant, positive correlations were also found between the age of the mattress and total mite counts (P = .004), *D pteronyssinus* (P = .0237), *L destructor* (P = .0237), *C arcuatus* (P = .0047), and *G privatus* (P = .0268). No significant differences were found in mite species or allergen levels between patients and controls.

Discussion

This study describes the presence of numerous mite species in mattresses of mite-allergic patients and control subjects in Galicia, a region where exposure to mite allergens poses a significant clinical problem. Our intention was to take a global approach to a problem that had been previously studied in a fragmented fashion. We found several species of mites that have not previously been reported in several areas of Galicia. Those species may play a role in mite-induced respiratory symptoms in this region of Spain. Furthermore, several aspects of the results deserve further attention, such as the low prevalence of *D farinae* in this area and the abundance of other species such as *Cheyletus* species, *E maynei*, *C arcuatus*, and *L destructor*. Other less abundant species, such as *G privatus* and *G domesticus* may also have local importance.

Overall, total mite counts were high. The geometric

mean number of total mites in all 4 provinces was 910.2 mites per gram of dust, ranging from 488.7 in Ourense to 1638.7 in Pontevedra. The data suggest that the climatic conditions in the province of Ourense, which is located inland and does not have a coastal area, are less conducive to mite proliferation than those in Pontevedra, which has a more humid Atlantic climate. The results confirm the dominant role of *D pteronyssinus* in Galicia, where it was found in high numbers in practically all dust samples. In this study we identified a significant negative correlation between the numbers of *D pteronyssinus* and those of *D farinae* (P = .006). Both mite species require high humidity for survival, and therefore, there is a positive correlation with signs of indoor humidity and mite counts of *D pteronyssinus* and *D farinae*. However, the biological response (fecundity, longevity, etc) of each species to humidity is not identical. The negative correlation between the numbers of these mite species is explained by biological competition for the food substrate and slight differences in environmental requirements, such as indoor humidity and temperature [13, 14].

The geometric mean abundances of 910.2 for total mites and 584.7 for *D pteronyssinus* are well above the numbers that have been suggested for sensitization and symptoms [15-17]. *L destructor* was the third most prevalent species in Galicia, confirming previous reports [4, 8]. The allergenic importance of *L destructor* is fairly well established and exposure to its allergens should be considered a risk factor for allergic symptoms.

Other species identified in Galicia have not been studied to the same extent as *D pteronyssinus*. *E maynei* is the source of important allergens in house dust in several regions of the world [18]. It contains allergens that are moderately cross-reactive with *D pteronyssinus* and to a lesser extent with *Blomia tropicalis* [19, 20]. The prevalence of *E maynei* was greater in the drier inland regions of Galicia (Lugo and Ourense), a finding that is in agreement with the data of Arlian et al [19]. Those authors suggested that the humidity requirements of *E maynei* are different from those of *D farinae* and *D pteronyssinus*. *E maynei* also seems to be an important mite species in the USA, where it has been identified in several states, especially in the south, where it was present in up to 35.7% of the homes [21]. Similar results have been obtained in Florida [22].

C arcuatus is another potentially important source of allergens in Galicia. It is a species that has been identified in farm dust, granaries, mills, and stored foods. It has also been described in house dust and mattress dust from numerous locations worldwide [23]. In Spain, it has been reported in cereal samples from Valladolid [24], and in house dust samples from Barcelona [25], Cádiz [26], Cantabria (11% of the dust samples) [27], Santiago de Compostela (26%) [4], La Coruña (11.5%) [6], Salamanca (1.3%) [28], and Tenerife (5%) [29]. Sensitization to the allergens of *C arcuatus* has been studied in occupationally exposed individuals in Valladolid [24], Seville (14.5% of the patients) [30], and Tenerife (12.7 %) [31]. An association between asthma and sensitization to this mite has also been suggested [32]. Sensitization to *C arcuatus* has also been demonstrated using in vivo and in vitro techniques in several Latin American countries [33], the United States [34], and Germany [35]. It has minimal cross-reactivity with *D pteronyssinus* [36, 37] and moderate cross-reactivity with other species [38]. Based on our findings, the allergenicity of *C arcuatus* deserves further evaluation in Galicia.

The levels of mite allergens can also be considered very high and consistent with those obtained in subtropical areas [32]. Our study is similar to the one conducted by Arbes et al [39] in the United States. Those authors analyzed Der p 1 and Der f 1 levels in 736 samples of mattress dust collected at different geographic locations. In that study, approximately half of the homes had levels of at least 2.0 μg/g—the proposed threshold for allergic sensitization—and approximately a quarter had levels of at least 10.0 μg/g—the proposed threshold for asthma. Independent predictors of higher levels were older homes, lower household income, musty or mildew odour, and higher bedroom humidity, among others. However, the overall geometric mean was 1.40 μg/g, much lower than in Galicia. In the study of Arbes et al, there were no differences in mite allergen levels collected during the winter, summer, or fall. In our study, we confirmed that, despite seasonal fluctuations, mite counts, and therefore mite allergen levels, remain high throughout the year.

Our results suggest that the total population of Galicia —allergic and nonallergic— is exposed to large quantities of mite allergens. Several risk factors were identified in this study, such as obvious signs of humidity, confirming previously published data [8, 40]. The age of the mattresses also seems to be a significant problem [41]. Patients should be advised of these risks and instructed in how to resolve the problems. Humidity problems in the home and an old mattress seem to be significant risks for mite-allergen exposure in Galicia and elsewhere [42]. Pet ownership did not influence mite counts.

We were also able to establish seasonal variations in 4 mattresses in Galicia. We detected a substantial increase in mite counts during the end of the summer and the beginning of the fall, probably due to an increase in temperature and humidity.

In conclusion, we have identified a rich mite fauna in Galicia. *D pteronyssinus* is the main species, but other species such as *Cheyletus* species, *E maynei*, *L destructor*, and *C arcuatus* are also common. It is noteworthy that only a small percentage of samples contained *D farinae*. Mite allergen levels can also be considered as very high. Seasonal variations in mite counts were also identified in 4 mattresses. Obvious signs of humidity in the home and the age of the mattress represent significant risk factors associated with high mite numbers and allergen levels. Considering the climatic conditions in Galicia, further studies should be conducted to establish the optimal methods to control and reduce exposure to mite allergens.

Acknowledgments

The authors would like to thank all the participants in

the study as well as all the family members and neighbors who collaborated in the collection of the dust samples.

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