

DR JERÓNIMO CARNÉS (Orcid ID : 0000-0003-4050-2577)

DR DAVID CALZADA (Orcid ID : 0000-0001-5563-9942)

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Environmental exposure of Der p 23 in household dust samples.

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Authors: Martín-López L.¹, López-Matas M.A.¹, Calzada D.¹, Benjumeda-Maira A.², Martín E.²,
García E.³, Carnés J.¹

¹ R&D Allergy & Immunology Unit, LETIPharma S.L.U., Tres Cantos, Madrid, Spain.

² Allergology Section. Department of Internal Medicine. Hospital Dr. José Molina Orosa, Lanzarote, Spain.

³ Endocrinology Section. Department of Internal Medicine. Hospital Dr. José Molina Orosa, Lanzarote, Spain.

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Corresponding author:

Jerónimo Carnés, PhD

Research & Development Allergy and Immunology Unit

LETIPharma S.L.U.

Calle del Sol, 5. Tres Cantos 28760, Madrid (Spain)

Tel.: +34 91 771 1790

Fax: +34 91 804 0919

E-mail: jcarnes@leti.com

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Author contributions:

LM contributed to design of the work, acquisition, analysis and interpretation of data, drafting the manuscript and final approval and agrees to be accountable for all aspects of the work.

MALM, DC contributed to design of the work, analysis and interpretation of data, drafting and revising the manuscript critically, final approval and agrees to be accountable for all aspects of the work.

AB, EG, EM contributed to acquisition of data, revising the manuscript critically and final approval and agrees to be accountable for all aspects of the work.

JC contributed to the design of the work, interpretation of data, drafting and revising the manuscript critically, final approval and agrees to be accountable for all aspects of the work.

Data sharing: The data that support the findings of this study are available from the corresponding author upon reasonable request.

To the editor,

Dermatophagoides pteronyssinus (DPT) is the most common source of indoor airborne allergens worldwide. To date, 30 allergens have been described, with Der p 1 and Der p 2 being the most relevant in mite respiratory allergies¹. In recent years, Der p 23, a peritrophin-like protein that is mainly present in the posterior midgut and the outer membrane of mite faeces, has also been identified as a potent allergen, despite its low concentrations^{1,2}. Previous studies have demonstrated that Der p 23 elicits a positive sensitisation rate of 75% in patients with house dust mite (HDM) allergies, particularly in children with persistent moderate-to-severe asthma²⁻⁵. Different studies have investigated the correlation between the levels of HDM allergen exposure and risk of developing allergic sensitisation and, consequently, allergic symptoms^{3,6}. However, the levels of Der p 23 to which patients are environmentally exposed remain unknown. Therefore, the aim of this study was to quantify the Der p 23 levels in household dust samples and to investigate the correlations with Der p 1 and Der p 2 levels and the number of adult mites.

Twenty-nine dust samples were collected in Lanzarote (Canary Islands, Spain), a region with a subtropical climate and an average relative humidity of approximately 68%, from mattresses, couches, and carpets by vacuuming the entire surface, using a dust collector adapter to a vacuum cleaner (Dustscreen® Collector, Heska Ltd., Bern, Switzerland). After collection, the dust samples

were frozen at -20°C for 48 h and weighed; 50 mg of each dust sample was then extracted in 1 mL of phosphate-buffered saline at 4°C over the course of 18 h under constant shaking. The dust extracts were then centrifuged for 5 min at 13,800 g, and the supernatants were recovered. The Der p 1, Der p 2, and Der p 23 levels were quantified in the supernatants using commercial ELISA kits (Indoor Biotechnologies, Charlottesville, VA, USA) following the manufacturer's instructions (standard limit of detection: EL-DP1, 2 ng/mL for Der p 1; EL-D2, 0.8 ng/mL for Der p 2; and EPC-DP23, 0.39 ng/mL for Der p 23). The results are expressed as µg of allergen/g of dust. Additionally, HDMs present in the samples were identified and counted using a binocular microscope. Statistical analysis was performed and graphs were created using GraphPad Prism 9 software (San Diego, CA, USA). The Wilcoxon test was used to compare the major allergen levels. D'Agostino and Pearson normality tests were used to determine the normality of the number of adult DPTs. Correlation analysis among the different variables measured in the dust samples was performed using Spearman's rank order correlation.

This study is, to the best of our knowledge, the first to quantify Der p 23 in dust samples, and provides useful information regarding the concentration of this allergen in the environment. Der p 23 was detected in all samples with the exception of two, in which the levels were below the limit of detection. The allergen quantification results are shown in Figure 1. The mean level of Der p 23 was 1.85 ± 2.62 µg/g of dust (range 0.3-11.45 µg/g of dust), which was approximately four-fold lower than the mean levels of Der p 1 and Der p 2 (7.74 ± 9.36 and 7.35 ± 10.36 µg/g of dust, respectively). The content of Der p 1, which is abundant in feces¹, was in accordance with previous findings⁷ and was similar to that of Der p 2, which is associated with mite bodies but can also be found in faecal pellets¹. Significant correlations were observed among the quantified allergens (Figure 2a). The highest correlation was found between Der p 23 and Der p 1 ($r = 0.92$), while the lowest correlation was found between Der p 23 and Der p 2 ($r = 0.84$). It is important to consider that the study of mite allergens that patients are directly exposed to is not without drawbacks, primarily because there is still some uncertainty regarding the aerodynamic behaviour of particles carrying dust mite allergens. However, it is generally considered that these measurements should be performed using dust samples because they are more reliable than air samples. Furthermore, mite allergens fall rapidly because they are typically carried on faecal pellets and are associated with mite bodies⁶.

Previous studies have demonstrated that the capacity of Der p 23 to exacerbate mite allergic symptoms is very high, and this does not correlate with its abundance^{1-3,5}. However, its sensitisation has been related to asthma, mainly in children⁴. In Der p 1, 2 µg/g of dust may lead to sensitisation to dust mites⁶⁻⁷, but the sensitisation threshold for Der p 23 has not yet been established. It has been hypothesised that low Der p 23 levels could be sufficient to promote sensitisation, supporting its status as a major DPT allergen and its immunogenic capacity as a major sensitizer¹⁻². However, the overall evidence from observational and intervention studies suggests that the relationship between allergen exposure and the development of sensitisation and allergic disease is complex and likely influenced by other factors, such as individual genetic predisposition, distinct geographic areas, allergen structure, other allergens, and environmental factors^{3,5-6}. In a comprehensive analysis of molecular sensitisation profiles to HDM allergen molecules, Der p 23 was demonstrated to be a frequent sensitiser (64%), but this seroprevalence is considered to be influenced by geography³. Further, a recent study conducted in a region close to where our samples were collected found a high prevalence of Der p 23 sIgE (85%) in patients with moderate-to-severe persistent asthma⁸. However, one limitation of our study is the lack of information regarding the clinical status of the persons residing in the houses where dust samples were collected and the relationship with the exposure to the different levels of allergens detected. The relationship between Der p 23 sensitisation and its clinical impact is not clear. Therefore, future studies should investigate this additional analysis, which may improve our knowledge regarding the clinical impact of Der p 23.

DPT was the most frequent mite and was identified in 28 dust samples (96.5%), followed by *Cheyletus* sp. (41.4%), *D. farinae* (31%), *Blomia tropicalis* (10.3%), and *Lepidoglyphus destructor* (3.4%). The majority of HDMs in each sample were DPTs, with the number of adults ranging from 0 to 9,139 DPTs/g of dust and a mean of $2,716 \pm 2,831$ adults. The ranges were lower in the other species identified: 0-472 mites/g of dust for *B. tropicalis*, 0-377 mites/g for *Cheyletus* sp., 0-199 mites/g for *D. farinae*, and 0-97 mites/g for *L. destructor*. A significant positive correlation was observed between the Der p 23 levels and the number of adult DPTs ($r = 0.75$). Similarly, the levels of Der p 1 ($r = 0.86$) and Der p 2 ($r = 0.82$) also correlated positively with the number of adult DPTs (Figure 2b). There is a strong relationship between the mite allergen concentration in dust and the number of mites⁶. DPT is reportedly the most abundant domestic mite in the Canary

Islands⁹, as was observed in the samples analysed in this study. We found significant correlations between the number of adult DPTs and Der p 23 levels, and between the number of adult DPTs and Der p 1 and Der p 2 levels. Therefore, to know the number of adult DPTs may be a useful information when evaluating the environmental exposure to Der p 23 and each patient necessities regarding allergy treatment.

In conclusion, this study is the first to quantify Der p 23 levels in dust samples. This allergen was detected despite a low number of mites in the samples. Additionally, significant correlations were observed between Der p 23 and Der p 1 and Der p 2 levels, and the number of adult DPTs. These findings could improve our understanding of the levels of Der p 23 that patients are exposed to, contributing to a more precise diagnosis of HDM allergy. Further studies investigating the relevance of this allergen, its clinical impact, and the importance of appropriate representation in allergen immunotherapy are also required in the near future.

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Figure legends:

Figure 1. Scatter plot with the individual quantification values of major allergens (MAs) Der p 1, Der p 2, and Der p 23 in 29 dust samples

Bars correspond to median values. Significant differences were observed (***) $p < 0.001$. ns: Non-significant differences.

Figure 2. Correlations among the different variables measured in the dust samples

A: Correlations between the quantities of each pair of major allergens (MAs) (μg of MA/g of dust). B: Correlations between the numbers of adult mites in the dust samples and the quantities of each of the three MAs (μg of MA/g of dust). Spearman's r and p -values are shown in each graph.

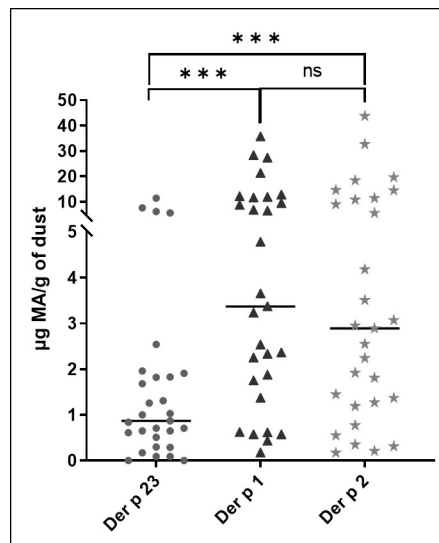


Figure 1

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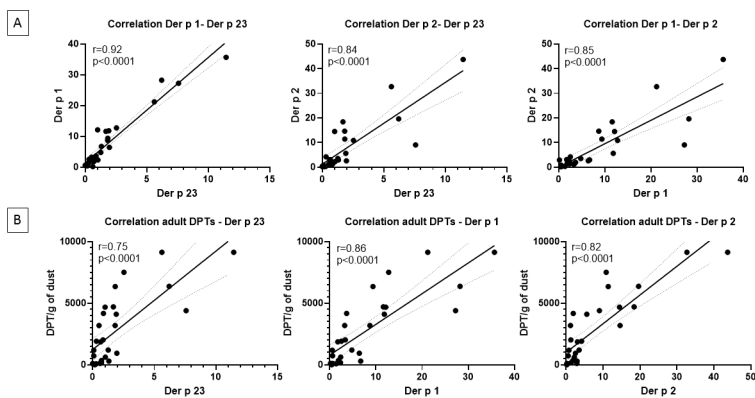


Figure 2

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