Mixed *Dermatophagoides farinae* and *Alternaria alternata* extract: stability and immunogenicity for use in veterinary allergy immunotherapy

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Introduction

The presence of **proteolytic enzymes in mite and fungus allergenic extracts** has been related with the absence of stability of some extracts used for immunotherapy (1, 2). However, no studies have been performed in veterinary allergy despite the frequent use of these allergens in immunotherapy treatments (3).

Objective

To determine the stability and immunogenicity of a mixture of Dermatophagoides farinae (50%) and Alternaria alternata (50%) extracts using serum samples from a dog population sensitized to both allergens.

Materials and methods

Canine serum samples

Serum samples from 14 atopic dogs with positive IgE to *D. farinae* and *A. alternata*, were obtained and pooled.

Allergen preparations

Extracts of *D. farinae* and *A. alternata* or a mixture of both allergens (50%-50%) were prepared in phenolated saline solution and stored at 5ºC

Stability study

The stability of the individual or mixed extract solutions was examined at time 0 and after 1, 2, 4 and 8 weeks. Total protein content was determined by the Bradford method. Individual allergens Der f 1, Der f 2 and Alt a 1 were quantified using commercial ELISA kits (Indoor Biotech). The protein profile of the extracts was determined by SDS-PAGE. Specific IgE elicited by the *D. farinae* and *A. alternata* or mixed extracts in the pool of sera were determined by direct ELISA.

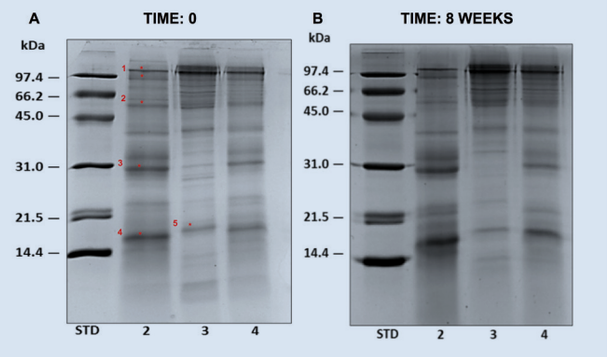

Figura 1. SDS-PAGE of 8 µg of D. farinae (2), A. alternata (3) and the mixture of both extracts (4) at time 0 (A) and after 8 weeks of storage (B). STD: Low range MW standard. 1: Der f 15; 2; Der f 18; 3: Der f 1; 4: Der f 2; 5: Alt a 1.

Results

Protein contents

In all extract samples both, individual and mixed allergens, **protein contents remained stable** during the storage period and no differences between individual or mixed extracts were observed.

Allergen quantification

Over a 8-week storage period, **individual allergens content did not change:** Der f 1 and Der f 2 allergen ranged from 20 to 28 µg/mg in the *D. farinae* extract and Alt a 1 levels were ≤1 µg/mg in the *A. alternata* extract. As expected, in the mixed extract preparation, levels of all three allergens were approximately the half of the individual extracts content and maintained stable.

Protein profiles

The most prominent bands in the *D. farinae* extract appeared at 30 and 14 kDa corresponding to the allergens Der f 1 and Der f 2, respectively. In the *A. alternata* extract, Alt a 1 was identified as a band at about 15 kDa. **In the mixed extract , bands were identified as in the individual extracts** but at lower intensities, as expected (Figure 1) and no changes were observed along the time.

Immunogenicity

Values of IgE against *D. farinae* and *A. alternata* in the pool of sera were determined and **no relevant differences were observed along the eight weeks** of the study between the mixed or individual extracts (Figure 2).

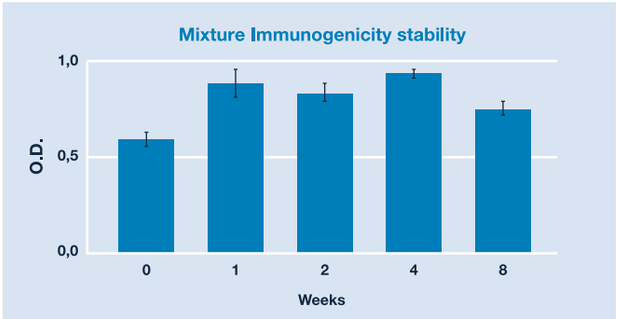

Figura 2. IgE levels of the pool of sera against D. farinae, A. alternata in the mixture extract along the storage period.

Conclusion

Both the individual and the mixed extracts of *D. farinae* and *A. alternata* showed **a stable protein and immunologic profile**, over the course of eight weeks of storage at 5ºC.

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