

Skin Tests and Conjunctival and Bronchial Challenges with Extracts of *Blomia tropicalis* and *Dermatophagoides pteronyssinus* in Patients with Allergic Asthma and/or Rhinoconjunctivitis

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Key Words

Blomia tropicalis · *Dermatophagoides pteronyssinus* · Canary Islands · Allergens · Conjunctival and bronchial challenges · Asthma

Abstract

Background: *Blomia tropicalis* and *Dermatophagoides pteronyssinus* are important mite species in Tenerife, Canary Islands, Spain. Several studies have demonstrated a variable degree of allergenic cross-reactivity in vitro. However, only a few have addressed their allergenic cross-reactivity using challenge tests. **Objective:** The objective of this study was to conduct conjunctival and bronchial challenge tests with *B. tropicalis* and *D. pteronyssinus* extracts in a group of 42 patients with allergic asthma and/or rhinoconjunctivitis sensitised to house dust mites (31 females, 11 males; mean age 21.7 ± 7.02 years). **Methods:** Prick tests using standardised extracts and specific IgE determinations using the CAP system were performed. Bronchial and/or conjunctival challenges were conducted using freeze-dried extracts of both mite species. A patient was considered sensitive to a mite species if she/he had a positive prick and/or CAP test result. A total of 32 conjunctival and 15 bronchial

challenges were performed with both mite species. **Results:** Prick tests were positive to *B. tropicalis* in 23 patients (54.7%) and to *D. pteronyssinus* in 41 (97.6%). One patient (2.4%) was exclusively sensitive to *B. tropicalis*. The CAP test was positive for *B. tropicalis* in 28 patients and for *D. pteronyssinus* in 41. Conjunctival challenges to *B. tropicalis* were positive in 20 patients (18 sensitised and 2 non-sensitised) and negative in 12 (5 sensitised and 7 non-sensitised patients). Conjunctival challenges with *D. pteronyssinus* were positive in all 31 *D. pteronyssinus*-sensitised patients who underwent conjunctival challenges. Bronchial challenges with *B. tropicalis* were positive in 9 sensitised patients and negative in 6 patients (2 sensitised and 4 non-sensitised). Bronchial challenges with *D. pteronyssinus* were positive in all patients except 1, who only reacted to *B. tropicalis*. **Conclusions:** Allergens of the mite species *B. tropicalis* induce positive conjunctival and bronchial challenges in *B. tropicalis*-sensitised individuals. Our results suggest that although there is a low to moderate degree of in vivo cross-reactivity between *B. tropicalis* and *D. pteronyssinus*, *B. tropicalis* seems to be a relevant source of allergens in areas where patients are exposed.

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Introduction

There has been a significant increase in the prevalence of allergic rhinitis and asthma over the last decade. It has been suggested that exposure to indoor allergens and other factors have significantly contributed to this overall increase. In the subtropical island of Tenerife, Canary Islands, Spain, mites are the most important source of allergens. We have previously established that the Pyroglyphid mite species *Dermatophagoides pteronyssinus* and *Dermatophagoides farinae* are the most abundant in house dust. However, several other species are also frequently identified in house dust samples collected in this island, including *Blomia tropicalis* [1]. Most of the patients evaluated in the Allergy Clinics in the Canary Islands are sensitised to *D. pteronyssinus* and *D. farinae*, but co-sensitisation with other mite species, such as *Blomia* spp., is also frequent [2–4]. Similar results have been obtained in other subtropical and tropical regions of the world, where *B. tropicalis* is endemic [5, 6].

In vitro cross-reactivity studies between whole extracts of *B. tropicalis* and other mite species have demonstrated that these mites share common as well as species-specific allergens [7–10]. Puerta Llerena et al. [8] demonstrated a greater degree of cross-reactivity between *B. tropicalis* and *Lepidoglyphus destructor* than between *B. tropicalis* and *Dermatophagoides* spp. Arlian et al. [9] demonstrated that the majority of the allergens present in *B. tropicalis* are species-specific. Using immunoelectrophoresis, 3 allergens are common with *D. farinae* body and faeces extracts, and 2 and 1 with body and faeces extracts of *D. pteronyssinus*, respectively. Morgan et al. [10] demonstrated corresponding IgE-binding proteins of 105, 75, 57, 18 and 14 kD in extracts of *Euroglyphus maynei* and *B. tropicalis*. However, the majority of IgE-binding proteins did not show corresponding bands in both extracts. The authors concluded that *E. maynei* and *B. tropicalis* are the source of both species-specific and cross-reactive allergens, and that most allergens in each extract were species-specific.

In recent years, several allergens of *B. tropicalis* have been cloned and sequenced [11–19]. Some of them have shown sequence homology with purified allergens of *D. pteronyssinus* such as Blo t 5, a homologue of Der p 5 [12, 14], Blo t 13, a fatty acid-binding protein [13, 15], Blo t 11, homologous to paramyosin [16], Blo t 10, homologous to tropomyosin and Der p 10 [17], Blo t 3, a trypsin-like protease [18], and Blo t 1, homologous to cysteine proteases [19]. All these studies have confirmed a low to moderate degree of cross-reactivity.

Several studies have focused on the in vitro cross-reactivity of purified Blo t 5 and Der p 5 [6, 12, 14, 20, 21] and Blo t 10 and Der p 10 (tropomyosin) [17]. Most group 5 studies demonstrated low to moderate cross-reactivity at the molecular level. There is less information about group 10 allergens. Yi et al. [17] concluded in their study that Blo t 10 and Der p 10 are highly conserved molecules (shared 95% amino acid identity) and that they are cross-reactive allergens, although unique IgE epitopes do exist.

Few studies have been conducted to address the allergenic cross-reactivity between *D. pteronyssinus* and *B. tropicalis* using challenge studies. The objective of this study was to conduct skin tests and conjunctival and bronchial challenges with *B. tropicalis* and *D. pteronyssinus* extracts in a group of patients with rhinitis and/or asthma sensitised to house dust mites. This study was conducted with standardized extracts, which makes the results more reproducible. The clinical significance of sensitisation to *B. tropicalis* and the allergenic cross-reactivity with *D. pteronyssinus* was evaluated.

Patients and Methods

Patient Population

The patient population consisted of 42 individuals with positive skin tests to house dust mites; 31 were female and 11 were male; mean age was 21.7 ± 7.02 years (range 11–38 years).

Two patients had asthma alone, 12 had rhinoconjunctivitis alone and 28 had both rhinoconjunctivitis and asthma. The patients were referred by their general practitioner to the Allergy Department and had a clinical history of allergic asthma and/or rhinoconjunctivitis of at least 1 year of evolution. They had previously received treatment with antihistamines, inhaled steroids and/or β -agonists. Their clinical symptoms were compatible with mite sensitisation, as demonstrated by a positive prick test and/or specific IgE determination. A patient was considered sensitive to a mite species when the result of the prick and/or CAP test was positive. Conjunctival challenges were performed in 27 patients, bronchial challenges in 10 and bronchial and conjunctival tests in 5 patients. The study was approved by the ethics committee of our hospital, and informed consent was obtained from each patient prior to the study.

Prick Tests

Skin prick tests were conducted with standardized extracts of *D. pteronyssinus* and *B. tropicalis* extracts at a concentration of 100 histamine equivalent prick units (HEP)/ml (C.B.F. LETI, S.A., Madrid, Spain). These extracts had been previously standardised using mite-allergic patients from the Canary Islands. The Nordic and European Guidelines for allergen standardisation were followed [22, 23]. A wheal of 3 mm in the absence of a reaction in the negative control was considered positive.

CAP

Specific IgE to both mite species was determined by the CAP System (Pharmacia).

Conjunctival Challenge Tests

Conjunctival challenges were conducted using a standardized procedure of serial dilutions following the method described by Möller et al. [24]. Briefly, 1 drop of increasing concentrations of the extract was applied to the conjunctival sac. A reaction was considered positive when the patient developed erythema and pruritus of the conjunctiva. The same physician evaluated all reactions 20 min, 3 and 6 h after the test was conducted. Saline solution was used as a control in the other eye. The test was started at the concentration of 0.1 HEP/ml. It was stopped in the presence of an obvious positive reaction in the eye where the allergen was placed, or when the maximum concentration of 100 HEP/ml had been administered. Sixty-four tests were conducted in 32 patients suffering from rhinoconjunctivitis (32 with *D. pteronyssinus* and 32 with *B. tropicalis*).

Bronchial Challenge Tests

Spirometry and bronchial challenges were conducted according to the general guidelines for standardisation of bronchial challenge tests with allergens [25, 26]. Vital capacity, FEV1 and FEV25–75 were recorded in a Datospir 2000 Admate DP-130 set (Sibel, S.A., Barcelona, Spain). The bronchial challenge tests were performed using a nebulizer attached to a dosimeter (Mefar MB3, Bovezzo, Italy) following standardised procedures [27]. Tests were conducted using lyophilised and reconstituted extracts of *D. pteronyssinus* and *B. tropicalis*. All these extracts belonged to the same batch.

All medications which could interfere with the results of the challenges were stopped prior to the tests. The patient's clinical condition was stable at the time of the challenge. A baseline spirometry was performed to confirm that the FEV1 was above 70% of the predicted value. The challenge began with 5 inhalations of the diluent. After 10 min, a new spirometry was performed to corroborate the FEV1 value, and the study was continued. The FEV1 value obtained after this second spirometry was the reference value. The allergen challenge was conducted by administering 5 breaths of the lowest concentration (0.001 HEP/ml of the respective mite extract). After 10 min, a new spirometry was conducted. Increasing 10-fold concentrations were administered, and spirometries were conducted after each increase in dose. The test was stopped after a fall in FEV1 of 20% or greater had occurred, or the maximum concentration of 10 HEP/ml was administered. The test was always conducted first with *D. pteronyssinus*. The *B. tropicalis* challenge was conducted 4 weeks later and the patient had to meet the same entry criteria. The same methodology was used. In the event that the test was positive, the patient received 2 inhalations of salbutamol and 60 mg of intravenous methyl prednisolone. All patients remained under observation during 24 h. A total of 30 challenges were conducted in 15 asthmatic patients (15 with *D. pteronyssinus* and 15 with *B. tropicalis*).

Results

Skin Test and CAP Results

All 42 patients had a positive prick test to mite: 41 were positive to *D. pteronyssinus* and 23 to *B. tropicalis*. The CAP test was positive for *D. pteronyssinus* in 41 patients and negative in 1, who was positive to *B. tropicalis*. Twenty-eight patients were positive to *B. tropicalis*.

Overall, the following results were obtained, considering as positive criteria a positive skin test and/or CAP test: 30 patients were sensitive to *B. tropicalis*, including 21 by prick test and CAP, 2 by prick test only (negative CAP) and 7 by CAP only. The 41 *D. pteronyssinus*-sensitive patients had a positive skin and CAP test.

Conjunctival Challenges

A total of 64 challenges were performed in 32 patients using extracts of *D. pteronyssinus* and *B. tropicalis*; 51 out of these 64 challenges were positive (79.7%). Conjunctival challenges to *B. tropicalis* were positive in 20 patients out of 32 (62.5%; 18 sensitised and 2 non-sensitised) and negative in 12 patients (37.5%; 5 sensitised and 7 non-sensitised).

Conjunctival challenges with *D. pteronyssinus* were positive in all 31 *D. pteronyssinus*-sensitised patients who underwent conjunctival challenges (100%). The results are shown in table 1. The positive predictive value for conjunctival tests is 90% with *B. tropicalis* and 100% with *D. pteronyssinus*; the kappa concordance between this test and the prick test for *B. tropicalis* was 0.8 ($p < 0.001$). Patients who had a positive conjunctival challenge had a significantly higher specific IgE level ($p = 0.003$) and skin test reactivity ($p < 0.0001$) to *B. tropicalis* than those with a negative test (Mann-Whitney). This result was confirmed by contingency table analysis. Patients with a specific IgE level above the median (1.05 kU/l) had a significantly greater chance of having a positive conjunctival challenge test (Fischer exact p value = 0.001). Regarding prick tests, patients with a wheal size greater than the median (10.3 mm²) had a significantly greater chance of having a positive conjunctival challenge test (Fischer exact p value < 0.0001).

Bronchial Challenges

A total of 30 bronchial challenges were conducted including both mite species; 23 out of these 30 challenges (76.6%) were positive. Nine out of 11 *B. tropicalis*-sensitised individuals (81.8%) reacted to *B. tropicalis*. Six individuals were negative to *B. tropicalis*, including 2 sensitised and 4 non-sensitised patients.

Fourteen out of 15 *D. pteronyssinus*-sensitive individuals (93.3%) reacted to the *D. pteronyssinus* extract. Patient 7 in table 2 had a positive skin test with *D. pteronyssinus* and *B. tropicalis*. However, the bronchial challenge test was only positive for *B. tropicalis*. The results are shown in table 2. The positive predictive value for bronchial challenge tests is 100% with *B. tropicalis* and 100% with *D. pteronyssinus*. The kappa concordance between this

Table 1. Results of the prick tests, conjunctival challenges (CC) and specific IgE values (CAP) to *D. pteronyssinus* (Dpt) and *B. tropicalis* (Bt)

Patient No.	Prick Dpt	Prick Bt	CC Dpt	CC Bt	CAP Dpt kU/l	CAP Bt kU/l
1	positive	positive	positive	positive	>100.0	22.00
2	positive	positive	positive	positive	>100.0	5.60
3	positive	positive	positive	positive	76.4	0.89
4	positive	negative	positive	negative	18.1	<0.35
5	positive	negative	positive	negative	>100.0	0.70
6	positive	positive	positive	positive	>100.0	18.00
7	positive	negative	positive	negative	18.2	<0.35
8	positive	negative	positive	negative	13.1	1.15
9	positive	negative	positive	positive	33.4	<0.35
10	positive	positive	positive	negative	2.3	<0.35
11	positive	positive	positive	positive	14.1	9.42
12	positive	positive	positive	positive	18.6	3.58
13	positive	positive	positive	positive	77.8	33.80
14	positive	positive	positive	positive	68.1	5.93
15	positive	positive	positive	positive	32.7	6.74
16	positive	positive	positive	positive	64.7	15.20
17	positive	positive	positive	positive	93.0	0.80
18	positive	positive	positive	positive	9.9	7.34
19	positive	negative	positive	positive	12.0	<0.35
20	positive	positive	positive	positive	50.0	1.10
21	positive	positive	positive	positive	69.6	8.60
22	positive	negative	positive	negative	15.3	<0.35
23	positive	negative	positive	negative	23.1	<0.35
24	positive	negative	positive	negative	31.1	<0.35
25	positive	positive	positive	positive	6.3	5.50
26	positive	negative	positive	negative	2.3	<0.35
27	positive	negative	positive	negative	1.1	0.80
28	positive	positive	positive	positive	1.0	<0.35
29	positive	negative	positive	negative	9.2	5.95
30	positive	negative	positive	negative	19.6	<0.35
31	negative	positive	negative	positive	<0.35	0.59
32	positive	positive	positive	positive	19.0	4.88

Patients 1–5 underwent a bronchial and a conjunctival challenge.

test and the prick test for *B. tropicalis* was 0.73 ($p = 0.003$). There was a significant correlation (Spearman rank) between PD20 and CAP levels for *B. tropicalis* ($p = 0.020$). However, this correlation was not significant for the CAP values for *D. pteronyssinus* and PD20 for *B. tropicalis* ($p = 0.81$). Figure 1 shows the percentage of patients who had a positive bronchial challenge at the different concentrations used. Patients who had a positive bronchial challenge test had a significantly higher specific IgE level ($p = 0.002$) and skin test reactivity ($p < 0.034$) to *B. tropicalis* than those with a negative test (Mann-Whitney). Contingency table analysis revealed that patients with a specific IgE level above the median (1.05 kU/l) had a significantly greater chance of having a positive bron-

chial challenge test (Fischer exact p value = 0.0014). This value was not significant for skin test data.

Discussion

In this paper, we describe a high prevalence of positive conjunctival and bronchial challenge tests to *B. tropicalis* in *B. tropicalis*-sensitised individuals. To the best of our knowledge, this is the first study to describe positive bronchial challenge tests with extracts of this mite species. In the present study, 9 of the 11 asthmatic patients (81.8%) who were sensitised to *B. tropicalis* had a positive bronchial challenge, suggesting the clinical significance of this

Fig. 1. Percentage of patients who had a positive reaction to bronchial challenge at a given allergen concentration of *B. tropicalis* and *D. pteronyssinus*.

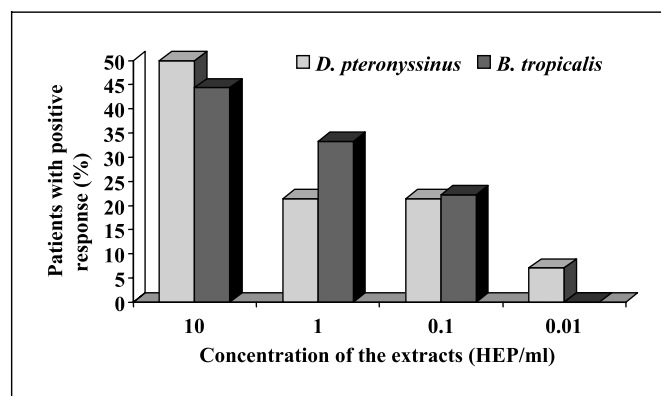


Table 2. Result of the prick tests, bronchial challenges (BC) and specific IgE determinations (CAP) to *D. pteronyssinus* (Dpt) and *B. tropicalis* (Bt)

Patient No.	Prick Dpt	Prick Bt	BC Dpt	BC Bt	CAP Dpt kU/l	CAP Bt kU/l
1	positive	positive	positive	positive	>100.0	22.00
2	positive	positive	positive	positive	>100.0	5.60
3	positive	positive	positive	positive	76.4	0.89
4	positive	negative	positive	negative	18.1	<0.35
5	positive	negative	positive	negative	>100.0	0.70
6	positive	positive	positive	positive	67.5	9.13
7	positive	positive	negative	positive	0.5	1.97
8	positive	positive	positive	positive	>100.0	16.20
9	positive	negative	positive	positive	7.7	1.30
10	positive	negative	positive	negative	16.4	1.00
11	positive	positive	positive	positive	83.0	13.40
12	positive	negative	positive	negative	43.3	<0.35
13	positive	negative	positive	negative	17.4	<0.35
14	positive	negative	positive	negative	58.8	<0.35
15	positive	negative	positive	positive	50.7	1.14

Patients 1–5 underwent a bronchial and a conjunctival challenge. All patients had asthma.

sensitisation. In a previous study, we also demonstrated a high prevalence of positive bronchial and conjunctival challenges to *Blomia kulagini* [4]. Although there are no published studies about the cross-reactivity between *B. tropicalis* and *B. kulagini*, a significant cross-reactivity should be expected.

It has been demonstrated that in subtropical and tropical regions, house dust mites are the main perennial source of allergens. Sensitisation to mites of the family Pyroglyphidae predominates, but sensitisation to other species seems to be a common phenomenon [28, 29]. In most cases, the high prevalence of sensitisation to multi-

ple mite species correlates with the presence of a rich domestic mite fauna in the environment [30].

The allergenicity of *B. tropicalis* has been reported in several subtropical areas of the world [31, 32]. The presence of *B. tropicalis* in house dust has been reported to range from 16 to 96%, and the described rates of sensitisation from less than 40 to 93% [33].

Most of the studies that have investigated in vivo sensitisation to *B. tropicalis* are based on prick tests [34]. In vivo studies using inhalation tests to investigate the clinical significance of *B. tropicalis* and its cross-reactivity with *D. pteronyssinus* are scarce. Stanaland et al. [35]

demonstrated that 83% of *B. tropicalis*-sensitive patients in Florida had a positive nasal challenge with a *B. tropicalis* extract. This result is very similar to the one obtained in the present study. Other studies in Brazil and Singapore have demonstrated the allergenicity of *B. tropicalis* in vivo using nasal challenges [36, 37]. In Brazil, a group of *D. pteronyssinus*- and *B. tropicalis*-sensitive patients was evaluated; 90% of the patients had a positive nasal challenge to *D. pteronyssinus* and 60% to *B. tropicalis* [36]. The study conducted in Singapore included 20 adults with persistent allergic rhinitis, 5 of whom had a history of asthma [37]. Significant increases in subjective and objective nasal symptoms, together with a significant increase of tryptase and LTC₄ concentrations in nasal secretion, were found in all patients after each challenge with *B. tropicalis*. This study suggested that a nasal challenge with *B. tropicalis* may also provoke a concomitant asthmatic response during the late-phase reaction.

Our study demonstrates that patients who are sensitised to two mite species may only react to one of them. This had been previously suggested for even closely related species such as *D. pteronyssinus* and *D. farinae* [38]. In our study, this held true for patient number 7 (table 2), who only experienced a positive bronchial challenge to *B. tropicalis*. In some cases, it may, therefore, be necessary to conduct challenge tests in order to better define the relevant allergen from a clinical point of view and start the appropriate specific immunotherapy treatment.

In general, challenges were only positive when skin and/or specific IgE tests were positive. There was a significant correlation between PD₂₀ and CAP levels for *B. tropicalis*, but the correlation was not significant between CAP levels for *D. pteronyssinus* and PD₂₀ for *B. tropicalis*, suggesting the overall specificity of the reaction. Bronchial challenges were negative in 6 out of 8 patients

who had a negative skin test to *B. tropicalis*. Patients 5 and 10 had a negative skin test to *B. tropicalis* (wheal <3 mm), but had low levels of specific IgE and presented a negative bronchial challenge to *B. tropicalis*. Discrepancies also occurred in patients 9 and 15, who had a negative skin test to *B. tropicalis* and a positive one to *D. pteronyssinus*, low levels of specific IgE to *B. tropicalis* and higher levels to *D. pteronyssinus*. However, both patients had a positive bronchial challenge test to both species.

Similarly, 11 out of 13 patients with a negative skin test with *B. tropicalis* had a negative conjunctival challenge test with *B. tropicalis*. However, these 11 patients were sensitised to *D. pteronyssinus* and had a positive challenge test with this mite species. There were discrepancies in patients 9 and 19, who had a negative skin test and low levels of specific IgE to *B. tropicalis*, a positive skin test and higher levels of specific IgE to *D. pteronyssinus* and experienced a positive conjunctival challenge with both species. In these and the previous cases, low levels of specific IgE or weakly positive skin tests might reflect cross-reactivity with *D. pteronyssinus*.

Our in vivo study confirms previous observations of in vivo and in vitro cross-reactivity using whole extracts as well as purified allergens. The overall data suggest that although there is some in vitro and in vivo allergenic cross-reactivity between *B. tropicalis* and *D. pteronyssinus*, clinical symptoms induced by the inhalation of *B. tropicalis* and *D. pteronyssinus* allergens seem to be species-specific, although some patients may react to common allergens. Due to the wide distribution of *B. tropicalis*, the high rate of sensitisation detected in many countries and the specificity of the allergic reactions, the inclusion of *B. tropicalis* in the diagnosis and treatment of specific IgE-mediated allergic reactions to this mite species seems warranted.

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