IMMUNOLOGY

Prevalence of Skin Reactions to Aeroallergens in Asthmatics of Puerto Rico.

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ABSTRACT. In Puerto Rico, although a high prevalence of asthma has been reported, the sensitization rates to aeroallergens in these patients is unknown. The purpose of this study using a case control design, was to determine and compare the rates of sensitization to common aeroallergens in an asthmatic population of 576 asthmatics and 144 healthy controls. A skin prick test was conducted using standardized extracts of *Dermatophagoides farinae* (Df) and *D. pteronyssinus* (Dp), house dust (HD), cat hair and epithelium (CT), dog hair and dander (DG), grass pollen mix (PG), tree pollen mix (PT), weed pollen mix (PW), *Aspergillus* mix (AM), mold mixes A (MA) and B (MB), *Periplaneta americana* (PA) and Alternaria-Hormodendrum mix (AH). In addition, an extract from the domestic mite *Blomia tropicalis* (Bt) was also used. A wheal $\geq$ 3 mm^2 was considered a positive reaction. In addition, a standardized questionnaire was administered and a preliminary domestic mite identification survey was conducted. The analysis of the data showed that 85.8% of the asthmatics had at least one positive reaction and 61.6% of them had positive skin reactions to at least one mite species. Asthmatics reacted to domestic mites 6.19 times more than the control group ($p<0.0001$) and was the largest significant difference found in this study for any allergen tested. Preliminary identification of the acarologic fauna in southern Puerto Rico demonstrated that *Blomia tropicalis, Dermatophagoides pteronyssinus, D. farinae* and *E. maynei* are the dominant domestic mite species found in homes of asthmatic individuals. These results demonstrate that domestic mites are a very important source of sensitizing aeroallergens for asthmatic patients in Puerto Rico. Based upon the mite survey, *Blomia tropicalis* plays an important role in allergic sensitization, in addition to *Dermatophagoides pteronyssinus and D. farinae*. The skin prevalence to pollens and to molds may not reflect the true prevalence of sensitization to these allergens. Pollen identification and counts, and a survey of microflora of Puerto Rico are needed in order to identify and validate important allergens that eventually could be incorporated into a more appropriate panel for testing sensitization in susceptible individuals. *Key Words: skin test and asthma, Puerto Rico,*

In spite of significant improvement in the therapy of asthma, studies in the United States have suggested overall increases in hospitalization for asthma and in asthma mortality (1). Evidence supports that this rise in the prevalence of asthma is related to the increasing exposure of asthmatics to domestic mites (2-4). It has been estimated that asthma currently affects between 9 and 12 million persons in the United States (5). The increasing frequency of asthma and its severity is particularly affecting children and young adults (6). Asthma mortality in the United States has increased by 8.2 percent per year between 1968 and 1978 among children 5-14 years, and increased by 10.1 percent per year between 1979-1987 for the same age group (7). Therefore, it is of paramount importance that data regarding sensitization rates to common aeroallergens in patients with asthma be available for different geographical regions so that effective patient management and educational programs be implemented.

In the tropical environment of Puerto Rico, asthma can be considered a public health concern because the

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THIS WORK WAS SUPPORTED BY A GRANT IN AID FROM THE NATIONAL INSTITUTES OF HEALTH RR03050.

prevalence of this condition is higher than the continental United States. It has been estimated that 31.9% of the school children in San Juan, Puerto Rico have suffered from asthma and used bronchodilators at least once in their lives (8). It has been suggested that asthma has seasonal patterns (9). These patterns could be explained by the intensity of exposure to different outdoor and indoor pollutants. Of these, domestic mites (DM) might be of primary importance. In the Caribbean islands exposure to domestic mites has been identified as a risk factor for asthma and several species have been identified in house dust (10).

The objective of this research project was to define and compare the prevalence of skin reactivity to common aeroallergens in an asthmatic population with a non-asthmatic control group in southern Puerto Rico.

Material and Methods

Sample size. Sample size determinations were conducted in EPINFO 6.0 (Centers for Disease Control, Atlanta. A total of 395 asthmatics were required to be able to find a 20 per cent reactivity rate to any of the allergens used in this study with a 5% precision and 95% confidence. A total of 605,790 asthmatics were used as a reference population in the sample size calculation because of the prevalence of asthma in southern Puerto Rico has been reported to be 17.2% (11). An expected skin reactivity of 20 per 100 asthmatics was used in this calculation as it reflects the national prevalence of skin test reactivity (12). For this study, a total of 576 asthmatics were recruited. For comparison purposes, 144 controls were also recruited to be able to detect an odds ratio of at least 2.0 assuming a 20 per cent prevalence of skin reactivity to any of the allergens included in the study, a power of 80%, and a 95% confidence level.

Patient population. A total of 576 asthmatic patients were referred from routine medical visits to our laboratory, for skin (prick) testing by physicians in the area. These patients had a clinical history of asthma according to the American Thoracic Society (ATS) (13). None of the patients were under medications that could interfere with the skin tests. For the control population, a total of 144 non-allergic individuals were included in the present study. These volunteers were recruited from several sources including the student body of our Institution, at a local health fair, routine follow-up visits to a public hospital for other reasons than asthma and from patient companions during their follow-up visits. The control volunteers were individuals without self reported history of allergies and none of them were under medications that could affect the outcome of the test. Prior to testing, all the participants were asked to read and sign the informed consent forms. The skin testing was initiated on August 1992 and completed in August 1994.

Skin testing. A series of skin prick tests (SPT) were performed on the volar surface of both forearms by a well-trained technician, an experienced nurse, or a physician following standard procedures. Patients were tested using standardized commercial (Hollister Stier, Spokane, WA) glycerinated extracts including the house dust mites *Dermatophagoides pteronyssinus* and *D. farinae*, house dust, cat and dog dander and hair, pollens from weeds, grasses and trees, *Periplaneta americana, Aspergillus* mix, mold mixes A and B, and *Hormodendrum-Alternaria* mix. Extract from the domestic mite *Blomia tropicalis* was kindly provided by Dr. E. Fernández-Caldas. The composition of the allergen mixes to pollens was made with the assistance of a botanist. The general composition of the allergen mixes were as follows:

Tree 1:10 w/v: equal parts of White Ash, American Beech, River Birch, Black Walnut, Common Cottonwood, American Elm, Shagbark Hickory, Hard Maple, Red Oak, American Sycamore and Black Willow.

Weed 1:10 w/v: equal parts of Common Cocklebur, Kochia, Lamb's Quarters Marshelder, Small Povertyweed, True Marshelder, Ragweed Mix (Giant, Short), Rough Redroot, Pigweed, Russian Thistle, Sagebrush Mix, Dock/ Sorrel Mix and Western Waterhemp.

Southern Grass 1:10 w/v: equal parts of Kentuky bluegrass, Orchard Grass, Redtop, Timothy, Sweet Vernal Grass, Bermuda Grass and Johnson Grass.

Mold Mix A 1:10 w/v: equal parts of *Botytis cinerea, Chaetomium indicum, Epicoccum nigrum, Fusarium vasinfectum, Geotrichum candidum, Helminthosporidum interseminatum, Monilia sitophila, Mucor racemosus, Phoma herbarum, Penicillium mix, Pullularia pullulans, Rhizopus nigricans, Rhodoptorula glutinis* and *Saccharomyces cerevisiae.*

Mold Mix B 1:10 w/v: equal parts of *Trichophyton schentieini, Cephalothecium reseum. Hormodendrum cladosporioides, Neurospora crassa, Streptomyces griseous, Scopulariopsis brevacaulis, Curvularia spicifera, Penicillium roseum, Mycogone sp, Nigrospora spherica, Paecilomyces varioti, Spondylocladium sp, Stemphylium botyosum, Trichoderma viride* and *Tricosporon aquatile*

Aspergillus mix 1:10 w/v: equal parts of *A. fumigatus, A. nidulans, A. niger* and *A. terreus.*

Alternaria mix 1:10 w/v equal parts of *A. tenuis* and A. *cladosporioides.*

The skin reactivity was recorded at 15 minutes. The mean of the major diameter and its perpendicular for the wheal and flare were measured. The smallest reaction considered positive was a wheal of at least 3mm^2 in the

mean of diameters (14). Histamine diphosphate with base equivalent of 1 mg/ml was used as the positive control. The vehicle alone was used as negative control. For standardized prick tests, disposable polymethacrylate needles were used (Stallergenes, France).

Dust sampling. A small group of multi-mite skin positive asthmatics were selected for dust analysis. Dust from the 19 mattresses was collected using a modified hand-held vacuum cleaner at a rate of 2 min/m^2. The dust was stored at 4°C in sealed plastic bags until analyzed under the light microscope as previously described (15).

Data analysis. Data entry and analysis was carried out by using Stata (Stata Corporation, College Station, Texas) and EPIINFO 6.0 (Centers for Disease Control, Atlanta, GA). Frequency distributions were carried out for all variables. Arithmetic means and means differences for the major diameters of the prick tests were calculated. Student's t test was used to assess the mean differences between cases and controls. Pearson's correlation coefficients, and their 95 percent confidence limits were used to assess co-reactivity between allergens. The comparison of skin reactivity rates between cases and controls to different allergens was carried out by using 2x2 tables, the odds ratio (OR) were used as a measure of rate difference, Cornfield's 95 percent confidence intervals for the odds ratio (16 ,17). Chi square tests were used to assess the significance of the odds ratio. Age adjusted OR using the median were calculated by using the Mantel-Haenszel stratified analysis (18).

Results

Study population. The study population consisted of 720 volunteers (Table 1). Females comprised 71.6% and males 28.3%. A total of 576 were asthmatic with an age range from 2 to 84 years, 405 (70.3%) females and 171 (29.7%) males. In the controls (n=144), the age range was from 13 to 82 years of age and the female population was 77.1% and the male was 22.9%. In both groups, the proportion of females was higher than in males ($p<0.05$) for both groups, cases and controls in all age groups except for the age group of 10 years and younger.

Table 1. Age and Gender Distribution of Asthmatics and Non-asthmatics Tested by the Skin Prick Test

	Asthmatic cases				Non-asthmatic controls				Total
	Females		Males		Females		Males		
Age group	n	%	n	%	n	%	n	%	
0-9	20	4.9	43	25.1	-	-	-	-	63
10-19	57	14.1	29	17.0	16	14.4	5	15.2	107
20-29	65	16.0	11	6.4	26	23.4	6	18.2	108
30-39	93	23.0	21	12.3	26	23.4	6	18.2	146
40-49	70	17.3	28	16.4	22	19.8	9	27.3	129
50-59	52	12.8	16	9.4	10	9.0	4	12.1	82
60+	48	11.9	23	13.5	11	9.9	3	9.1	85
Total	405	70.3	171	29.7	111	77.1	33	22.9	720

Skin reactivity. Eighty five percent of the asthmatics had a positive skin reaction to at least one allergen, while 52.1% of the controls had skin reactivity (Table 2). The percent of positive skin reactions in the asthmatics slightly increased with age up to 20 to 29 years and then decreased thereafter (Figure 1). Domestic mites showed the highest frequency of skin reactivity with 61.6% having positive reactions to one or more house dust mite species (Table 2). A total of 41.8% asthmatics had positive skin reactions to *Dermatophagoides farinae*, 45.1% to *D. pteronyssinus* and 48.3% to *B. tropicalis* (Table 2). Skin reactivity in the asthmatic group to pets, molds, pollens and cockroach allergens was 24.3%, 23.3%, and 19.3% respectively.

Table 2. Prevalence of Positive Skin Reactivity by Allergen in the Asthmatic and Non-Asthmatics Controls

Positive skin reactivity to	Asthmatics %	Controls %
One allergen	85.8	52.1
Domestic mites	61.6	22.2
Dermatophagoides farinae	41.8	18.8
D. pteronyssinus	45.1	20.1
Blomia tropicalis	48.3	19.4
Pets	24.3	15.3
Dog dander	13.0	5.6
Cat hair	16.5	10.4
Molds	23.3	11.1
Mold Mix A	13.9	6.9
Mold Mix B	12.8	4.9
Aspergillus	8.7	0.0
Alternaria	8.7	0.0
Pollens	34.7	16.0
Pollen from trees	20.3	9.7
Pollen from weeds	18.1	6.3
Pollen from grasses	9.5	6.3
House dust	23.6	6.3
Periplaneta americana	19.3	11.8

The majority of the asthmatics (61.6%) were positive to at least one of the three mite species and exclusive reactivity to domestic mites was found in 15.1% of the asthmatics, to *B. tropicalis* was observed in 6.8% of the patients, 4.2% only to *D. farinae*, and 4% to *D.*

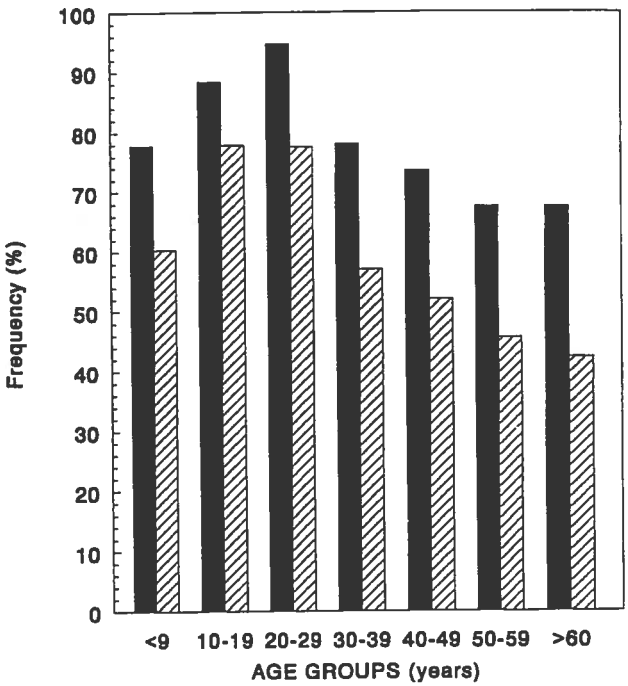

Figure 1. Bar graph of the age-specific prevalence of the skin test reactivity in the asthmatic population to any of the allergens tested, and to three domestic mite species: *Blomia tropicalis (Bt), Dermatophagoides pteronyssinus* (Dp) and *D. farinae* (Df). The solid bars represents the skin reaction of at least one allergen (SR+) and the hatched bars represent those patients with positive skin reactions to the three domestic mite species.

pteronyssinus (data not shown). The skin reactivity to mites followed a similar trend as the general skin reactivity; increasing with age up to groups 10 to 19 and 20 to 29 and then decreases in older age groups as shown in Figure 1. Skin reactivity in the asthmatics to pollen from trees, weeds, and molds had a much lower frequency in the population when compared to the skin reactivity to domestic mites (Table 2).

Table 3. Skin reactivity to groups of allergens between the asthmatics and non-asthmatic population in southern Puerto Rico.

Skin reactivity to	Asthmatics	Controls	Odds ratio Crude	Adjusted	95% CI*	P
At least one allergen			3.39	3.42	2.32-5.04	<0.0001
Yes	453	75				
No	123	69				
Mites			5.62	6.19	3.97-9.66	<0.0001
Yes	355	32				
No	221	112				
Molds			2.43	2.43	1.40-4.23	<0.001
Yes	134	16				
No	442	128				
Pollens			2.80	2.79	1.11-1.27	<0.0001
Yes	200	23				
No	376	121				

* Adjusted Ratio

Table 4. Skin reactivity to specific allergens between the asthmatic and non-asthmatic population in Puerto Rico.

Skin reactivity to	Asthmatics	Controls	Odds ratio Crude	Adjusted	95% CI*	P
Dermatophagoides farinae			3.12	3.35	2.11-5.34	<0.0001
Yes	241	27				
No	335	117				
D. pteronyssinus			3.26	3.49	2.22-5.48	<0.0001
Yes	260	29				
No	316	115				
Blomia tropicalis			3.86	4.11	2.61-6.48	<0.0001
Yes	278	28				
No	298	116				
Dog dander			2.54	2.56	1.2-5.45	0.01
Yes	75	8				
No	501	136				
Cat hair and dander			1.58	1.58	0.90-2.77	0.13
Yes	95	15				
No	481	129				
Mold mix A			1.98	1.97	0.99-3.92	0.37
Yes	80	10				
No	496	134				
Mold mix B			2.89	2.90	1.30-6.44	0.02
Yes	74	7				
No	502	137				
Aspergillus			NA	NA	NA	NA
Yes	50	0				
No	526	144				
Alternaria			NA	NA	NA	NA
Yes	50	0				
No	526	144				
Pollen from trees			2.37	2.38	1.32-4.29	0.01
Yes	117	14				
No	459	130				
Pollen from weeds			3.31	3.30	1.63-6.69	0.03
Yes	104	9				
No	472	135				
Pollen from grasses			1.58	1.58	0.76-3.28	0.09
Yes	55	9				
No	521	135				
House dust			4.82	4.81	2.39-9.69	0.002
Yes	136	9				
No	440	135				
Periplaneta americana			2.05	2.04	1.19-3.5	0.01
Yes	111	14				
No	465	127				

* Adjusted ratio

Comparison of the skin reactivity between the asthmatic and control groups demonstrated that asthmatics had 6.19 times more skin reactions to domestic mites than the controls (Table 3). Asthmatics were also more reactive than the controls to household dust, cockroach, dog hair, pollen from trees and weeds, mold mixes A and B, Aspergillus and Alternaria mix, but not at the magnitude of the mite allergens (Table 4). The adjusted OR analysis revealed that there is an interaction between age and atopy (Table 5). Asthmatics younger than 36 years of age 5.58 times more likely to have a positive reaction to at least one allergen than the controls, while there is no difference between asthmatics and controls older than 36 years. Similar results were obtained with reactors to cockroach (Table 5). Significant correlations ($P < 0.05$) between the reactivity to the three mite species and other indoor allergens such as house dust, cat hair, cockroach, mold mixes, and *Alternaria-Hormodendrum* (Table 6).

Table 5. Significative interactions between skin reactivity to allergens and age.

Positive skin reaction to	Odds ratio				
	Crude	<36 Yrs	>36 Yrs	Adjusted	P
At least 1 allergen	3.39	5.58	2.39	3.42	<0.0001
Periplaneta americana	2.05	3.56	1.14	2.04	0.01

Table 6. Correlation among mite allergens and other allergens in asthmatic patients in Puerto Rico.

		Domestic mite correlation with other allergens					
Allergen	DF	DP	BT	CT	MA	AH	PA
DF	1	32%	21%	6%	27%	20%	4%
	2	0.57	0.46	0.25	0.53	0.45	0.19
	3	0.47-0.65	0.34-0.56	0.0-0.48	0.25-0.71	0.08-0.71	0.17-0.52
DP	-	-	4%	13%	4%	5%	16%
	-	-	0.19	0.36	0.06	0.23	0.40
	-	-	-0.08-0.43	0.11-0.57	-0.24-0.36	0.03-0.41	0.22-0.56
BT	-	-	-	4%	20%	10%	6%
	-	-	-	0.19	0.45	0.31	0.24
	-	-	-	-0.8-0.43	0.09-0.70	0.12-0.49	0.03-0.43
CT	-	-	-	-	4%	2%	2%
	-	-	-	-	0.19	0.14	0.14
	-	-	-	-	-0.22-0.5	-0.19-0.44	-0.19-0.44
MA	-	-	-	-	-	3%	8%
	-	-	-	-	-	-0.18	0.29
	-	-	-	-	-	-0.44-0.10	0.12-0.44
AH	-	-	-	-	-	-	22%
	-	-	-	-	-	-	0.47
	-	-	-	-	-	-	0.22-0.66

DF: *Dermatophagoides farinae*, DP: *D. pteronyssinus*, BT: *Blomia tropicalis*, CT: cat dander, MA: mold mix A, AH: *Alternaria-Hormodendrum mix*, PA: *Periplaneta americana*
1. Determination coefficient percent: (r^2)100
2. Correlation coefficient: r
3. 95 percent confidence intervals for the correlation coefficient: 95% CI r

Domestic mite fauna. Species identification indicates that *D. pteronyssinus* was present in 36.8% of the samples analyzed and *D. farinae, Blomia tropicalis* and *E. maynei* were found in 31.5%, 26.3% and 5.2% of the samples respectively (Table 6). In addition, *D. siboney* was identified in one sample.

Table 7. Frequency and abundance of domestic mites in 19 mattresses of asthmatics in southern Puerto Rico.

Species	Mean1gr/dust	Range	Found in households	(%)
Dermatophagoides pteronyssinus	984	60-2500	7/19	36.8
Dermatophagoides farinae	478	50-2000	6/19	31.5
Blomia tropicalis	472	40-491	5/19	26.3
Dermatophagoides siboney	937	180-2250	4/19	21.0
Cheyletus malaccensis	46	40-50	3/19	15.8
Prostigmata	83	50-100	3/19	15.8
Tarsonemus spp	510	20-1000	2/19	10.5
Suidasia medanensis	250	NA	1/19	5.2
Euglyphus maynei	250	NA	1/19	5.2
Oribatids	40	NA	1/19	5.2

Discussion

The analysis of the epidemiological data demonstrates that in our study, domestic mites are very important sensitizing aeroallergens in Puerto Rican asthmatics when compared to non-asthmatic controls (adjusted OR 6.19, $p < 0.0001$). House dust, pollens and pets had the second and third highest frequencies of skin reactions in the asthmatics. House dust, the American cockroach and molds were also reactive but to a lesser extent. Domestic mites had the highest frequency of skin reactivity in relationship with all of the allergens tested. Demographic data indicated that male asthmatics predominate in the young age groups where as female asthmatics are more prevalent in the older ages. Asthmatics in the 20-29 years age category had higher skin reactions to at least one allergen and to the American cockroach than in the other age groups (Figure 1, Table 4). Skin reactivities to at least one allergen were detected in approximately 85.8% of the asthmatics as compared to 52.1% of the controls (Table 2). This latter prevalence rate in the control population is unusually high since it was expected that skin reactivity

to allergens in this population would be approximately 30 to 35% (19, 20).

Selection of the asthmatics included in this study was carried out by using a convenience sample rather than a probabilistic sampling procedure. All of the patients who participated in this study were under medical care including follow-up for their condition and none of them had the skin test previously performed. Therefore, our study population included atopic and non-atopic asthmatic individuals. The convenience sampling technique may generate problems in social economic strata representativeness of the source population which it has been reported as being positively associated with skin reactions (21). However, our sample population included patients seen in private practice and in community hospitals which provide medical attention to financially indigent persons. Controls were selected from potentially different sources by using again, a convenient or practical sampling. It was not possible to include controls in this study under the 10 years of age. Therefore, the asthmatic-control comparison may include some degree of age selection bias. When comparing cases with controls, age-adjusted odds ratios were calculated in an attempt to correct for further age differences.

There are population studies of skin tests that have been reported; its difficult to make valid comparisons between these surveys and ours. This is mainly because differences in antigen selection, commercial sources of allergens, methods of testing, and criteria for positive reactions. The latter has been recently standardized to a minimum of 3 mm^2 as a criterium of positivity. In despite of these restrictions, prevalence of skin reactions to domestic mites can be used for comparison purposes of their relative importance because of their world-wide distribution. The prevalence of skin reactivity to the extracts of the three species of domestic mites used in the Puerto Rican asthmatic population is not as high as the one observed in those asthmatics living in geographical regions in which temperature and humidity are ideal for the growth of the domestic mites throughout the year (22). For example, the prevalence of skin reactivity in asthmatics to *D. pteronyssinus* has been reported in the ranges of 100% in the Canary Islands, Spain (23); 91.2% in Sao Paulo, Brazil; 60.7% in Cartagena, Colombia; to 30.1% in New Zeland. In our study, the prevalence rates to this mite species reached 45.1%. Similar results were obtained for the domestic mite *D. farinae*. The reported ranges of the skin reactions to this mite are 97% in Caracas, Venezuela; 88.7% in Sao Paulo Brazil; 75% in Cartagena, Colombia; and 20.1% in Australia. The third domestic mite species analyzed was *B. tropicalis* and in the Puerto Rican asthmatic population, the skin reactivity was 48.3%. In Sao Paulo, Brasil the skin reactivity to this mite was 93.7%; in Caracas, Venezuela was 77.8%; in Córdoba, Argentina 58.0%; and in Mexico City 46.6%. These data suggest that in Puerto Rico the skin reactivity to *D. farinae* and *D. pteronyssinus* is in agreement with previous reports that suggest that the prevalence of positive skin reactions to pyroglyphid mite allergens among asthmatic patients is in the range of 40-100% (24). Our study also provides important evidence on the allergenicity of the domestic mite *B. tropicalis*. It was observed that there is a minimal difference in the patterns of sensibilization in our population to all of the mites species tested. This could be explained in part by a small degree of antigenic crossreactivity between *B. tropicalis* and the pyroglyphid mites *D. farinae* and *D. pteronyssinus* (25).

The skin prevalence to mites in the asthmatic population correlate with the description of a diverse domestic mite fauna present in dust collected from mattresses homes of asthmatics (Table 4). It was not surprising to identify the abundance of mite species present in dust from homes of asthmatics and this finding might have important implications for asthmatics in Puerto Rico since previous work has demonstrated that in tropical environments, the mite fauna is of clinical importance (26, 27). To our knowledge, the domestic mite fauna of Puerto Rico has not been previously identified. In our preliminary survey, the Pyroglyphid mites *Dermatophagoides pteronyssinus* and *D. farinae* were found in the dust of homes and these results are similar with those previous works in the Caribbean areas (28-31). In addition to these species, we found three additional mite species in the collected samples; *Blomia tropicalis, Euroglyphus maynei* and *Dermatophagoides siboney.* The domestic mite *Blomia tropicalis* was identified in 26% of the samples analyzed and the skin reactivity in the asthmatics to this mite species was of 45.1% (Table 2). The presence of this particular mite in dust from houses in tropical areas has been reported in geographical areas with tropical climate (32-36). The clinical significance of *B. tropicalis* in asthma has been provided by several authors (37). The other domestic mite species found in mattresses from asthmatics was *E. maynei*. This pyroglyphid mite has been identified in house dust in other surveys (38, 39). Although this particular mite species was not included in the prick test panel, it was found in 5.2% of the samples collected and could be also a potential source of allergens in domestic dust. This mite species has been shown to have had clinical relevance elsewhere (40). In the case of *Dermatophagoides siboney*, it was found in 21% of the samples. This mite species has been described only in Cuba (41). The presence of these three mite species needs to be confirmed in future studies since they could have clinical importance

by contributing to the allergen pool.

The selection of the extracts used to determine prevalence rate of the skin reactivities to pollens was based on the representation of plant families in Puerto Rico and the fact that there is a great deal of immunological crossreactivity between allergens from grasses; trees and some weeds (42-45). This allergen selection might impose some limits in the interpretation of the data, mainly because pollen counts are not being carried out in southern Puerto Rico for the identification of local species. In addition, validated allergen mixes containing extracts from plants of Puerto Rico are not available commercially, and by dilution of individual allergens present in the mixes. In our study, the pollen allergenic mixes contained some extracts from plants that are present in Puerto Rico, either with wide or limited distribution. In some instances, when the species were not present in Puerto Rico, the family would be represented by other species. The skin reactivity to pollens in general, including trees, weeds and grasses, was found in 34.7% of the asthmatics while 16.% in the controls (adjusted OR=2.79, p=<0.05%). In the case of pollen from grasses, the skin reactivity in the asthmatic population was 9.5% and in the controls was 6.3% (adjusted OR=1.58, p=<0.215). In our selection of the allergenic mixes, the grasses Red Top, Johnson Grass, Bermuda Grass were included and are widely distributed in Puerto Rico. The *Poa pratensins* (Kentucky Blue Grass) has limited distribution on the island and the Sweet vernal has not been described in Puerto Rico. The skin reactivity to pollen from weeds in the asthmatics was 18.1% as compared to 6.3% in the controls (adjusted OR=3.31, p>0.0001) and these allergens may represent a risk factor for the Puerto Rican asthmatic population. Regarding the trees, American sycamore, Beech, Hard Maple, Shagbark Hickory, *Betulia nigra* and the Read Oak are not present or do not have family representation in Puerto Rico. However, Black Willow is present on the island. The American Elm (*Ulmus americana*) is not present in the area, but is represented by several close related species. Of the extracts tested containing pollen from weeds, members of several families are represented in Puerto Rico. The families Compositae (Ragweeds), Amaranthaceae (Redwood), Polygonaceae (Red Sorrel) and Umbelliferae (Water Hemlock) are widely represented by several species. The weed Dock is present in the region but Sagebrush is not. The skin reactivity to these allergens was 20.3% in the asthmatics and was not significant when compared to the control group. Our results strongly suggest that pollen from trees and grasses may not be important source of sensitizing aeroallergens to Puerto Rican asthmatics. In contrast, pollen from weeds showed a three fold difference in sensitization in cases than in controls indicating that these allergens may be of clinical importance.

Skin reactivity to molds in general was relatively low. Asthmatics had 23.3% of positive reactions while the controls reached 11.1% (Table 2). These results should be interpreted with caution because the same limitations of the pollen allergenic mixes could be applied.

For many years, cockroach allergens have been implicated as sensitizing allergens (46). Since then, the clinical relevance has been demonstrated by many investigators. In our study, skin reactivity to the American cockroach was 19.3% of the asthmatics tested, while in the control group was 11.8%. Asthmatics were 2.04 times more frequently reactive to cockroach than controls (p=0.03). Although the skin reactivity was detected in a lower percentage of the asthmatics than that of domestic mites, the clinical importance of cockroach sensitivity can not be underestimated. In Puerto Rico, previous studies by Marchand reported a skin reactivity to *Periplaneta americana* was 48% of the asthma patients tested (47). However, the clinical relevance of the several species of cockroaches as a risk factor in asthmatics in Puerto Rico would have to be confirmed in future epidemiological studies.

The diverse domestic mite fauna found in southern Puerto Rico can lead to a high allergen load production by *D. pteronyssinus, D. farinae, D. siboney, Blomia tropicalis* and *Euroglyphus maynei*. Previous works have suggested that mite counts of 100 mites/g of dust may be associated with sensitization (48). In the present study, the mean mite count in dust from homes of asthmatics was 472 mites/gram of dust. At these levels, individuals can be sensitized by being exposed to high allergen levels as evidenced by the skin test results. Comparisons between asthmatic and the non-asthmatic control group confirmed that mites are an important known allergen playing a role in asthma in Puerto Rico. Mite sensitization in the asthmatics was found to be highly significant (p<0.001) and a four fold increase in frequency over the control group. A high level of correlation was found between several allergens in the asthmatics included in the study. The indoor allergens that showed strong correlations were between mites, molds and house dust.

Our study provides four major conclusions. First, because of the sensitization rates to *Dermatophagoides pteronyssinus, D. farinae* and *B. tropicalis*, domestic mites are very important sources of allergens for asthmatics in Puerto Rico. A mite survey of Puerto Rico is needed to define the acarofauna and to study the possible variations in the mite fauna within the island. Of importance would be also to perform studies to determine the seasonal variation of domestic mites and its possible

implication in the induction of clinical symptoms in the susceptible population. Second, the frequencies of skin reactivities to allergens other than domestic mites obtained in this study may be inconclusive since the indoor microflora in Puerto Rico has not been fully identified. An island wide study to identify the fungi and bacteria would provide valuable information regarding the species distribution allowing a better selection of allergenic extracts. Third, pollen identification and count would also provide a much needed information regarding the outdoor allergen composition. Once the diversity of indoor and outdoor components has been identified, the corresponding extracts would have to be validated in future epidemiological studies to define an adequate allergen panel that would reflect the sensitizing agents. Fourth, a survey of the cockroach species found in the island would have to be conducted to determine the number and frequency of species found in Puerto Rico.

We recommend that when performing the skin test, the inclusion of extracts from the domestic mites *Blomia tropicalis* and *Euroglyphus maynei* in addition to *Dermatophagoides pteronyssinus* and *D. farinae* in the allergen panel, may prove useful in the diagnosis of atopic conditions.

Resumen

En Puerto Rico se ha reportado una alta prevalencia de asma, sin embargo las tasas de sensibilización en estos pacientes a aeroalergénos es desconocida. El propósito *Dermatophagoides farinae* (Df) y *D. pteronyssinus* (Dp), polvo casero (HD), pelo y epitelio de gato(CT), pelo y escamas de piel de perro (DG), mezclas de pólenes de *Blomia tropicalis* (Bt) fué también incluido en la batería de alergénos. Una pápula igual o mayor que 3 mm^2 fué considerada como una reacción positiva. También se aplicó un cuestionario estandarizado a cada participante y se llevó a cabo un levantamiento preliminar de los ácaros domésticos en un número reducido de residencias de asmáticos. El análisis de los datos demonstró que el 85.8% de los asmáticos tuvo reacción positiva en la piel de por lo menos a un alergeno y el 61.6% reaccionó de forma positiva a una especie de ácaros domésticos. Asmáticos reaccionaron 6.19 veces más que los controles ($p<0.0001$) a los ácaros domesticos, siendo esta la diferencia la mayor de todos los alergénos. Identificación preliminar de la fauna acarológica en el sur de Puerto Rico demonstró que los ácaros domésticos *Blomia tropicalis, Dermatophagoides pteronyssinus, D. farinae, D. sibonei* y *E. maynei* fueron las espcies identificadas en el polvo de las casas de los asmáticos. Estos resultados demuestran que los ácaros domésticos son fuentes muy importantes de sensibilización por aeroalergénos en la población de asmáticos. Sin embargo, la prevalencia de reacciones a pólenes y a hongos puede ser que no refleje la verdadera tasa de sensibilización de estos alergénos. Identificación y contaje de pólen así como un levantamiento de la microflora de Puerto Rico son necesarios para poder identificar y validar alergénos importantes que eventualmente se pueden incorporar en las baterías de pruebas cutáneas para detectar sensitización en individuos susceptibles.

Acknowledgement

The authors wish to thank Mr. Juan Reckard of the Biology Department at the Pontificia Catholic University of Puerto Rico for his assistance in the taxonomy of the plants from which extract mixes were used and to Dr. Howard Mass, Professor at the Department of Physiology of the Ponce School of Medicine for his suggestions in the preparation of this manuscript.

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