

ORIGINAL ARTICLE

Exposure Levels of Asthmatic Children to Allergens, Endotoxins, and Serine Proteases in a Tropical Environment

Federico Montealegre, D.V.M., Ph.D.,^{1,*} Blanca Fernández, M.T.,¹
Alexie Delgado, R.T.,¹ Lisa Fernández, B.S.,¹ Ayleen Román, B.S.,¹
Domingo Chardón,¹ José Rodríguez-Santana, M.D.,²
Vivian Medina, R.T.,² Diego Zavala, Ph.D.,¹
and Manuel Bayona, M.D., Ph.D.³

¹Laboratory of Immunochemistry, Ponce School of Medicine, Ponce, Puerto Rico

²Pediatric Pulmonary Program, San Juan, Puerto Rico

³Department of Epidemiology and Biostatistics, School of Public Health,
University of North Texas, Fort Worth, Texas, USA

ABSTRACT

A cross-sectional study was conducted in Bayamón, Puerto Rico, to identify and quantify indoor allergens, serine proteases, and bacterial endotoxin present in homes of asthmatic children. A total of 126 dust samples from houses were obtained from the entire mattress and bedside floor. Most of the patients had detectable levels of mite, cockroach, cat, and dog allergens. Mold allergens were found only in bedside floor dust samples. Mouse allergens were not detected. Forty-two percent, 36.5%, and 1.8% of the patients demonstrated exposures to sensitizing levels of mite, *Blatella germanica* and cat allergens, respectively. The percentage of patients exposed to high levels of allergens capable of triggering asthma symptoms was 33.3% and 26.4% for mite and *Blatella germanica* allergens. Only dog allergen, bacterial endotoxin, elastase, and trypsin were associated with asthma symptoms. Eighty-nine percent of the asthmatic children were exposed to endotoxin concentrations greater than 100 EU/mg dust, and more than half of the patients were exposed to high levels of serine proteases. Our study indicates that indoor concentrations of allergens traditionally associated with asthma symptoms and severity may not be applicable in tropical environments and highly ventilated households. In fact, in the study population, endotoxins, dog allergen, and serine proteases may play a dominant role in the induction of asthma symptoms.

Key Words: Puerto Rico; Pediatric asthma; Allergen levels; Endotoxins; Serine proteases; Indoor environmental risk factors.

*Correspondence: Federico Montealegre, D.V.M., Ph.D., Ponce School of Medicine, P.O. Box 7004, Ponce, 00732-7004, Puerto Rico; Fax: 787-844-3270; E-mail: asthma@coqui.net.

INTRODUCTION

Exposures to medium and high concentrations of indoor allergens, such as house dust mites and cockroaches, are known risk factors for sensitization and clinical manifestations of asthma in children (1,2). However, these levels may depend on several variables including climate, geographical region, and the household architectural characteristics in which asthmatics reside. As a consequence, children may be exposed and sensitized to a single allergen or to several of them, and quantitative and qualitative differences in exposures may have relevance in the triggering of asthma symptoms or exacerbating asthma severity. For example, it is possible that in environments in which several indoor risk factors are present, the potential interaction between these factors could in fact be responsible for asthma symptoms, exacerbations, and possibly severity. The first step to understand how interactions between indoor environmental factors may have an impact on asthma is to identify and quantify the most important allergens and other risk factors present in the local area.

Puerto Rico has one of the highest prevalence rates of asthma in children in the United States (3). From a survey among volunteers in the City of Ponce, the estimated prevalence rate in the 1- to 10-year-old population is approximately 32% (4). Similar rates have been reported by other researchers in Puerto Rico (5,6). Causes for this high prevalence in asthma are unknown; however, data indicate that house dust mite fauna is diverse and composed of several species including *Dermatophagoides pteronyssinus*, *D. farinae*, *D. siboney*, *Blomia tropicalis*, and *Euroglyphus maynei* (7). Sensitization to allergens in Puerto Rican asthmatics also includes domestic mites and, to a lesser degree, cockroaches, cats, and dogs (8). Concentrations and exposure levels of these allergens are unknown.

We hypothesize that asthmatics living in highly ventilated households in a tropical environment are exposed to lower mite and cockroach allergen levels than those found in houses where low exchange rates exist such as in temperate or nordic climates. We further hypothesize that exposure to high levels of endotoxins and serine proteases and not to mite and cockroach allergens may be associated with asthma symptoms and severity. The clinical importance of measuring exposure to serine proteases is because cells from the respiratory track in humans express protease activated receptors (PAR), which are specific for this enzyme group capable of inducing bronchoconstriction (9,10). To address our hypotheses, in the municipality of Bayamón, Puerto Rico, we identified and determined the levels of allergens, bacterial endotoxins, and

serine proteases in a population of 126 pediatric asthma cases. In addition, we analyzed their possible relationship with asthma symptoms.

METHODS

Study Design, Sample Size, and Subjects

A cross-sectional study was conducted to quantify animal, plant, and fungal allergens, endotoxins and serine proteases, and to identify self-reported exposure to indoor environmental irritants present in homes of asthmatic pediatric cases in the municipality of Bayamón, Puerto Rico. Subjects were recruited by direct referral from a health care management organization. Bayamón is located 12 miles north of San Juan, with 180,000 inhabitants and an average annual temperature of 82°F (28°C) and 75% relative humidity. The accessible study group was composed of 6,000 pediatric asthma patients with a physician-based asthma diagnosis, according to the clinical criteria established by the American Thoracic Society (11). A total of 130 patients were required to detect with 95% confidence, a minimum of 10% of asthmatic households with at least one allergen present (12). Parents or legal guardians of all accessible patients were contacted by telephone, and 126 of them agreed to participate in the study. Patients from all socioeconomic strata were visited once at their home, and dust samples were first collected from the mattress and then from the floor around the bed, followed by an interviewer-based questionnaire given to the child's parents or legal guardians. This study was approved by the Internal Review Board (IRB) of the Ponce School of Medicine.

Home Visit Questionnaire

A standardized interviewer-administered 119-item instrument was used to collect information about demographics, asthma symptoms and severity, colds triggering asthma symptoms, asthma seasonality, tobacco exposures, and various indoor environmental risk factors. The questionnaire was standardized by using the methods provided in Kelsey et al. (13).

Briefly, the instrument was tested in 25 volunteers to assess its reliability by using a test-retest procedure. The percent agreement and the chance corrected agreement or Kappa statistics were calculated. All of the items had agreements greater than 95% and Kappa values more than 75%. Bedroom temperature and



relative humidity were recorded, and visual inspection for presence of molds and cockroaches in the sampling area was conducted by a trained technician.

Dust Sampling and Processing

Dust samples from the bedside floor and from the entire mattress were collected by using a vacuum cleaner (Medivac Health Products, Cheshire, England) at a rate of 2 min/m². Fine dust was placed on a plastic bag, labeled, and transported from the collection sites in a refrigerated container to the Laboratory of Immunochimistry at the Ponce School of Medicine for weighing and storage at -70°C, until extraction. Prior to extraction, the dust samples were sieved through a No. 45 mesh screen (355 µm) to remove large particles and fibers. After this, a total of 100 mg were mixed with 2 mL of PBS, pH 7.4, without KCL and supplemented with 0.05% Tween 80, vortexed and mixed in a refrigerated cabinet for 12 hrs on an orbital rotator (Scientific Industries, Inc., Bohemia, NY). The samples were centrifuged for 20 min. at 2500 rpm at 4°C. The resulting supernatant was removed, aliquoted in 100-µL fractions, and stored at -80°C for analyses.

Measurement of Levels of Mite, Cockroach, Cat, Dog, and Mold Allergens in Dust

Dust samples were analyzed for the presence of mite-derived allergens Der p 1, Der f 1, and Group 2, cockroach allergens Bla g 1 and Bla g 2, cat allergen Fel d 1, dog allergen Can f 1, mold allergen Alt a 1, and mouse allergen (Rat n 1). These allergens were detected by using a two-site ELISA following established protocols by the vendor (Indoor Biotechnologies, Charlottesville, VA). Allergens were expressed as micrograms of allergen per gram of dust to ensure the comparability with the available literature. Samples were analyzed in duplicate, and those with a coefficient of variation of 10% or greater were repeated.

Measurement of Bacterial Endotoxin

The endotoxin activity was measured as described by Milton et al., 1992 (14). The Limulus ameobocyte lysate (LAL) was obtained from BioWhittaker (Walkersville, MD), and reference standard endotoxin from the United States Pharmacopeia, Inc. (Rockville, MD). Reference standard and dust extract sample were serially diluted in endotoxin-free PBS and placed on an endotoxin-free 96-well, flat-bottomed polystyrene microplate (Associates of Cape Cod, MA); 50 µL of

LAL were added, and the microplate was agitated in a kinetic ELISA reader (BioWhittaker, Walkersville, MD). The optical density (OD) of each well was recorded at 405 nm every 15 secs for 120 min during incubation at 37°C. The endotoxin potency was computed by the parallel-line Klare bioassay method (15). The results were expressed as endotoxin units (EU) per milligram of dust.

Measurement of Serine Protease Activity

Trypsin activity was detected by dissolving a total of 10 mg of the substrate N-benzoyl-dl-arginine p-nitroanilide (BapNA) in 2 mL of DMSO. To this solution, a total of 18 mL of 0.046M Tris containing 0.0115M CaCl₂ buffer was added, and then a total of 100 µL was placed in the wells of a 96-well microtiter plate. Next, 100 µL of the dust extract was mixed and incubated at 37°C for 60 min. After this period, the reaction was analyzed on a Microtiter Plate Reader (Benchmark Plate Reader, Bio-Rad Laboratories, Hercules, CA) at OD 405 nm. The chymotrypsin assay was conducted under identical conditions as the trypsin assay but using the N-succinyl-ala-ala-pro-phe-p-nitroanilide substrate. Similarly, elastase was detected by using N-succinyl-ala-ala-pro-leu-p-nitroanilide (SA₃-pNA). The reaction was allowed to proceed for 60 min, and results were expressed in specific enzyme activity per minute/per gram of dust according to Exterkate (16).

Data Analyses

Stata version 7.0 (Stata Corporation, College Station, TX) was used to analyze the data. The continuous variables Der p 1, Der f 1, Fel d 1, and Bla g 2 were categorized by using cutoff points that had been previously suggested as threshold levels for sensitization and symptoms of asthma (17,18). The levels used were 2 and 8 µg Fel d 1 per gram of dust, 2 and 10 µg Der p 1 and Der f 1 per gram of dust, 2 and

Table 1. Distribution of the study population by age and gender.

Age group (yr)	Female(%)	Male(%)	Total(%)
1-5	27 (54)	23 (46)	50 (39.6)
6-10	18 (54.5)	15 (45.4)	33 (26.2)
>10	25 (58.1)	18 (42)	43 (34.3)
Total	70 (55.5)	56 (44.4)	126 (100)



Table 2. Indoor environmental risk factors.

Factor	N ^a	Study sample with factor (%)
Carpeting		
No	100	80.0
Living room	20	16.0
Child's bedroom	3	1.6
Living room and child's bedroom	2	2.4
Stove		
Electric	107	84.9
Gas	18	14.2
AC in child's room	76	60.8
Household dust exposure worsens asthma	92	73.6
Climatic changes worsen asthma	117	94.3
Asthmatic child exposed to strong odors	68	54.8
Asthmatic child exposed to powder detergents	46	36.8
Pets		
Any	67	54.0
Cats	7	5.6
Dogs	35	27.7
Birds	11	8.7
Rodents	4	3.1
Other	10	7.9
Presence of cockroaches in floor	6	5.8
Mold growth in bedroom	8	6.9
Mean temperature °C (SD)	31.1 (2.5)	
Mean humidity % (SD)	64.8 (13.4)	
Housing		
Single story	72	62.0
Two story	39	33.6
Apartment	5	4.3
Child's bedroom		
First floor	98	85.8
Second floor	16	14.0
Asthmatic shares bed with other children	13	11.1
Pet sleeps with child	1	0.86
House located in a street with:		
High traffic	36	31.0
Low traffic	74	63.7
Dead end	6	5.1

^aNumbers do not add up to 126 because of missing data.

8 U/g of dust for Bla g 1 and 80 ng Bla g 2 per gram of dust. For the endotoxins, a cutoff level of 100 EU/mg was used, a concentration which is capable of inducing wheezing in children (19). Other allergens or proteolytic enzymes without established cutoff levels

were categorized by using the geometric mean. Asthma severity was analyzed as a nominal variable with two categories, namely, severe or non-severe, according to the National Heart, Lung and Blood Institute (20). From interviews, a severe case of asthma was defined as a child with all of the following: 1) more than three visits to emergency departments due to asthma attacks in the 12 months prior to interview, 2) more than 3 hr of emergency room time, and 3) more than two hospitalizations due to an asthma attack per year. After the bivariate analysis, allergens detected in the bed or floor were combined into a single allergen burden according to their source. The Kruskal–Wallis equality of populations rank test was used to analyze continuous data (allergen levels, proteolytic enzyme, and endotoxin levels) and asthma clinical outcomes (i.e., severe asthmatic, and number of hospitalizations). The Mann–Whitney test was used to compare allergen levels found in mattresses and in floors. The t-test was used to compare the mean age of both sexes. By convention, $p < 0.05$ was considered significant.

RESULTS

Characteristics of the Study Population

The age of the participants ranged from 1 to 17 years with a mean age of 7.3 (SD±4.7 years). The median age for the study population was 12.6 years, and the female to male ratio was 0.80 and the mean age (±SD) found in females and males was similar (respectively, 7.2±4.8, and 7.4±4.7 years, pairwise $p=0.05$). The distribution of the study population by age and gender is summarized in Table 1.

Table 3. Cigarette and the asthmatic child.

Characteristic	N ^a	%
Parents are current smokers	34	27.2
Mother is current smoker	10	9.8
Father is current smoker	29	28.1
Parents smoke inside the home exposing child to ETS	9	9.5
Frequency of asthmatic child exposed to ETS		
No at all	59	57.2
Sometimes	28	27.2
Often	7	6.8
Too often	9	8.7

^aNumbers do not add up to 126 because of missing data.



Table 4. Total levels of environmental indoor irritants detected in household dust from asthmatic children.

Variable	Samples analyzed ^a	% Samples with detectable levels ^b	Geometric mean	Range
Mite allergens	113	95.6	4.30 µg/g dust	0–45.6
Cockroach (<i>B. germanica</i>)				
Bla g 1	112	94.6	13.82 U/g dust	0–58.8
Bla g 2	110	81.8	29.61 ng/g dust	0–3,530
Cat allergen	114	25.4	0.20 ng/g dust	0–2.4
Dog allergen	89	79.8	0.27 µg/g dust	0–17.04
Mold allergen (<i>A. alternata</i>)	69	42.0	0.13 µg/g dust	0–2.09
Mouse allergen	71	ND ^c	ND	ND
Trypsin	91	98.9	117.52 U/min/g dust	0–1,637.3
Chymotrypsin	89	98.9	511.67 U/min/g dust	0–2,232.1
Elastase	89	98.9	325.0 U/min/g dust	0–1511.0
Endotoxins	82	100	218.7 EU/mg dust	13.1–8410

^aNumbers do not add up to 126 samples because dust concentrations in households varied.

^bBed and floor pooled into a single concentration.

^cNot detected.

Self-Reported Indoor Environmental Risk Factors

Several indoor risk factors for asthma symptoms were assessed. These included whether the child had carpeting at home, type of stove used at home, presence of air conditioner in the child's bedroom, triggering of asthma symptoms after household dust exposure, climatic changes and asthma symptoms, exposures to strong odors and detergents at home, presence of pets, cockroaches and molds in the child's bedroom, housing characteristics, whether the child shares its bed with pets, house location, smoking habits, and environmental tobacco exposures. Eighty percent of the households had no carpets, a known risk factor for asthma. In addition, 73.6% of the parents indicated that household dust exposure worsen their children's asthma as well as climatological changes (94.3%). Dogs were the most common pets among the patients. The ETS exposure was found to be 41.7%. Tables 2 and 3 summarize the presence of the self-reported environmental factors in the asthmatic population.

Levels of Allergens, Serine Proteases, and Endotoxins

With the exception of cat, mold allergens, and most of the dust samples had detectable levels of allergens, endotoxins, and serine proteases as detailed in Table 4. The levels found ranged from nondetectable levels to extremely high levels. For example, the highest level for Bla g 2 was 3,530 ng/g dust, endotoxins was 8410-EU/mg dust and 2232 U/min/g

dust for chymotrypsin. We also compared the levels of allergens found in floors and mattresses (data not shown). Beds had significantly higher concentrations of Der p1 and Der f 1 than floors, but not Group 2 allergens. Twelve floor samples and four bed samples had Der p1 levels greater than 10 µg. High concentrations of Der f 1 were detected in seven floor samples and three from bed samples. High concentrations of Group 2 mite allergens were observed in only one sample from the mattress. The floors had significantly higher concentrations of Bla g 1 than the beds. Fel d 1 floor levels ranged from 0 to 0.33 ng/g of dust with a geometric mean of 0.13 ng/g dust. Fel d 1 bed levels ranged from 0 to 2.07 ng/g dust with a geometric mean of 0.20 ng/g dust. Figure 1 illustrates the frequency of asthmatic patients exposed to either clinically irrelevant, sensitizing, or those levels capable of triggering asthma symptoms.

Correlations Between Allergens, Serine Proteases, and Endotoxins

We found positive and strong correlation between mites, Bla g 1 and Bla g 2 allergens, between trypsin and elastase, and between chymotrypsin and elastase (data not shown).

Levels of Allergens, Serine Proteases, Endotoxins, and Asthma Symptoms

Table 5 summarizes the significant associations between allergens, endotoxins and serine proteases, and



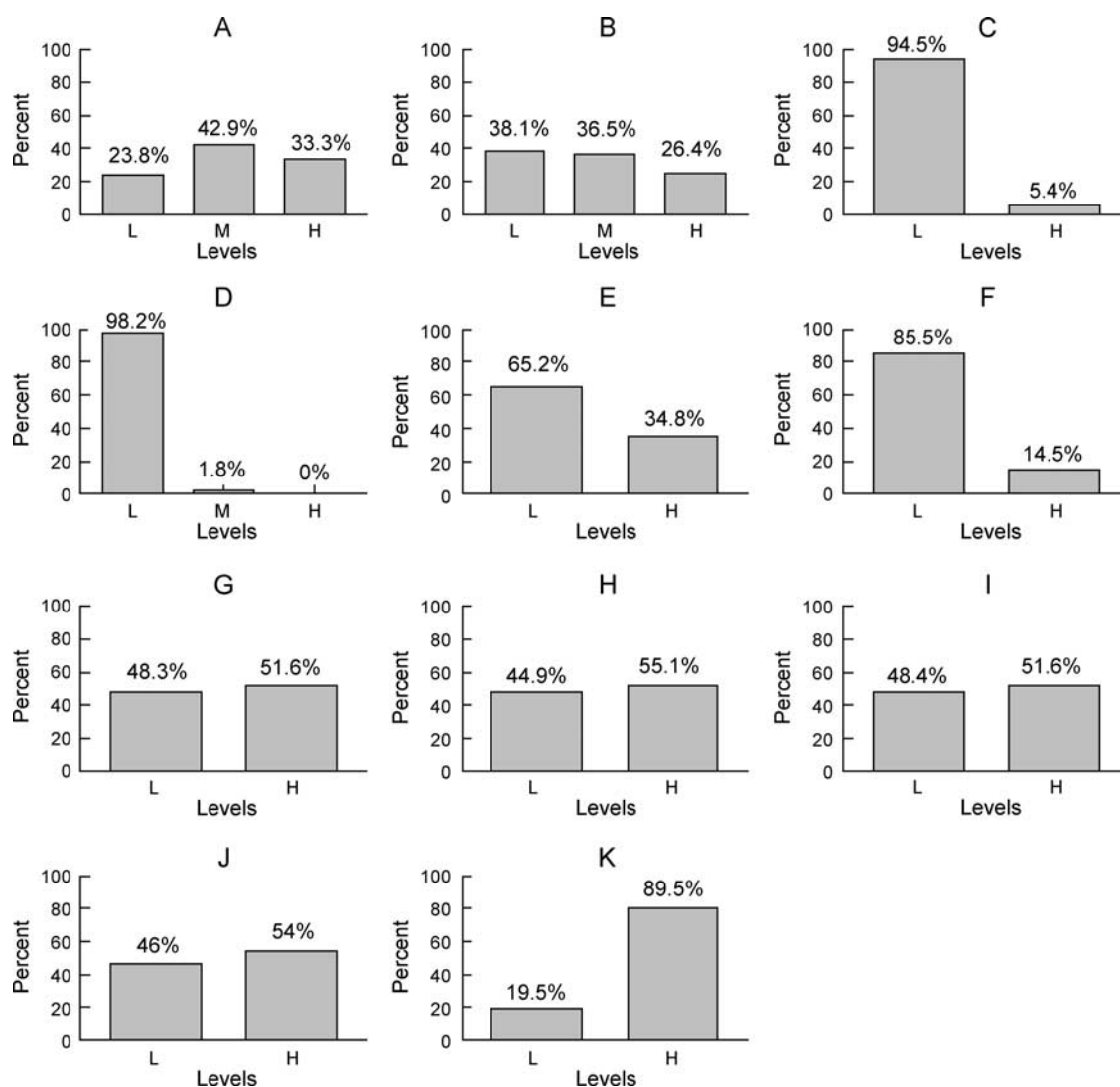


Figure 1. Frequency histogram of allergen, serine proteases, and endotoxin levels found in homes of asthmatic children in Bayamón, Puerto Rico. Mite, Bla g 1, Bla g 2, and Fel d 1 allergen levels were categorized into three cutoff levels: L=low or without clinical significance, M=medium or sensitizing, and H=high or associated with asthma symptoms or severity. Mold allergen, total proteolytic activity, and serine proteases were categorized according to their geometric mean: L=low or below the GM, and H=or higher than the GM. Bacterial endotoxins were categorized into two levels: L=low or below 100 EU/g dust and H=higher than 100 EU/g dust. A) mite allergens, B) cockroach allergen Bla g 1, C) cockroach allergen Bla g 2, D) cat allergen, E) dog allergen, F) mold allergen, G) nonspecific proteinase activity, H) trypsin, I) chymotrypsin, J) elastase, and K) bacterial endotoxin.

clinical asthma outcomes. We observed that Can f 1, endotoxins, and elastase, but not dust mites, cockroaches, and cat allergens had concentrations associated with asthma symptoms.

DISCUSSION

Dust mite and cockroach allergen levels in the present study were found not to be associated with

clinical symptoms of asthma. In contrast, exposures to Can f 1, endotoxins, and serine proteases had significant associations with asthma clinical outcomes. These findings suggest that regional differences may influence environmental risk factors and clinical outcomes, and more importantly, extrapolation of cutoff levels established in different geographical regions may not be applicable in others.

It would be logical to expect that in the tropical environment of Puerto Rico, the infestation levels of



dust mites and cockroaches, and consequently their allergen concentrations, would be high because of the ideal temperature and humidity required for their growth. Although our results show that nearly all of the households had measurable levels of dust mite allergens, these were lower than those observed in previous studies conducted in the Caribbean region and in semitropical areas in the continental United States (21,22). The dust mite allergen levels found in our study are in line with the available literature, which supports that there is great variability in dust mite allergen concentrations (23,24). In our study, half of the asthmatics were exposed to sensitizing levels, but only one-third of the asthmatic patients were exposed to high levels of mite allergens. Of these, very few severe asthmatics were exposed to these high allergen levels

associated with asthma attacks (data not shown). This observation was unexpected and raises the possibility that exposition to house dust mite allergen levels at the established threshold for asthma attacks may not be applicable in our study population. As in dust mites, cockroaches are also recognized as an important source of allergic sensitization, and high levels of these allergens are strongly associated with asthma symptoms and exacerbations and increased hospitalizations due to asthma (25–27). We detected very low levels of cockroach allergens in the study population, and only a few patients were exposed to allergen concentrations associated with asthma symptoms [Fig. 1(B)]. These results contrast those reported by Rosenstrich et al. (28), which showed that high levels of cockroach allergens are important risk factors for asthma

Table 5. Significant comparisons between clinical outcomes in the last 12 months and levels of indoor environmental irritants.

Asthma symptoms	Concentrations	<i>p</i> Value
Weekly asthma attacks.	Dog allergen (µg/g dust)	
1–5	75 ^a (3091) ^b	0.02
6–20	11 (649.6)	
Hospitalized due to asthma.	Dog allergen (µg/g dust)	
No	72 (2796)	0.05
Yes	8 (444)	
Frequency of asthma symptoms (any)	Dog allergen (µg/g dust)	
No	34 (1295)	0.04
A lot	55 (2709)	
	Endotoxin (U/g dust)	
No	46 (1597.50)	0.003
A lot	36 (1805.50)	
Time in emergency room before discharge (hrs)	Dog allergen (µg/g dust)	
≤4	55 (2097.5)	0.01
>4	34 (1907.5)	
Physician visits due to asthma	Endotoxin (U/g dust)	
≤3.5	51 (2352.5)	0.02
>3.5	31 (1050.5)	
Colds trigger asthma leading to emergency room visits	Dog allergen (µg/g dust)	
No	76 (3155.5)	0.002
A lot	13 (849.5)	
	Endotoxin (U/mg dust)	
No	69 (2712)	0.05
A lot	13 (691)	
Pump usage while in asthma treatment	Endotoxin (U/mg dust)	
≤2.81	46 (1530.5)	0.05
>2.81	27 (1170.5)	
Hospitalized due to asthma and colds	Elastase (U/mg/dust)	
No	81 (3476)	0.01
A lot	8 (529)	

^aNumbers do not add up to 126 because not all households had dust concentrations sufficient enough to conduct all of the environmental analyses.

^bSum of ranks.



symptoms and severity. It is possible, similar to house dust mites, that cockroach allergens are not important triggers for asthma symptoms in our population.

The exposure to animal allergens as a prerequisite for sensitization has been well documented (29–31). Early exposures to sensitizing levels of dog and cat allergens, in particular, in children less than 4 years of age, increase the risk of asthma (32). In the present study, very few patients under 4 years of age were exposed to sensitizing levels of animal allergens. In fact, dog allergen was not associated with the presence of pets, and its deposition in the bedroom may be linked with sources not directly related to pets present in the household. An important finding was that asthmatics with six or more weekly asthma attacks, hospitalizations due to asthma attacks, high frequency of asthma symptoms, long periods of emergency room time, and colds triggering asthma attacks leading to emergency room visits had significantly higher concentrations of dog allergens than their counterparts. This is indicative that Can f 1 allergen may contribute to the appearance of asthma symptoms. Exposures to high cat allergen concentrations were very low, and most of the asthmatics had clinically insignificant cat allergen exposures [Fig. 1(D)]. Mold exposures were also very low, and rat allergen was not detected in any of the samples tested and were considered together with cat allergens—not important for asthma symptoms.

Serine proteases and bacterial endotoxins were found in nearly all of the samples tested and were associated with clinical manifestations of asthma (Table 5). Our findings also show that elastase, chymotrypsin, and trypsin are closely associated with each other and may have a common source, which was not identified in the present study. These findings are specially important for asthmatics in general because proteolytic enzymes from the environment can be neutralized by the α_1 -protease inhibitor, α_1 -anti-chymotrypsin and secretory leukoprotease inhibitor (33,34). If proteolytic enzymes are not properly neutralized, they will gain access to the submucosa and, consequently, affect the cells of the immune system promoting an allergic response (35). Available data suggest that some asthmatics may have imbalances in the quantity and/or function of the antiprotease-environmental protease system resulting in asthma of variable severity (36). It is possible that asthmatics with abnormal levels or functional alterations in the α_1 -antiprotease and are exposed to environmental serine proteases will have their asthma symptoms worsen. This hypothesis needs to be evaluated.

Endotoxins are cell activators capable of inducing asthma symptoms and attacks by nonallergic mecha-

nisms (37–41), and one of the most important biological characteristics of endotoxin is its potency capable of inducing respiratory alterations at very low levels (42). In our survey, endotoxins were detected in all of the samples tested, and of these, the most had levels greater than 100 UE/mg dust, a value put forward as a trigger for asthma symptoms (43). In our study, high levels of endotoxins were not associated directly with asthma severity, but with high frequency of physician visits and high usage of inhaled anti-asthma therapy. Furthermore, subjects with endotoxin levels greater than the suggested cutoff limits had more asthma attacks leading to emergency room visits than their counterparts. The latter can be explained by the fact that endotoxins, perhaps in conjunction with viral infections or other agents, can induce asthma symptoms (44,45). Our results contrast recently published findings that suggest that exposure to high levels of endotoxins and presence of farm animals, such as those found in rural communities, prevent the development of atopic diseases, including asthma (46–48). As suggested by Douwes et al. (49), these results must be interpreted with caution because the reported protective effects are mostly related to atopy and marginally from asthma. Furthermore, most of these studies have been conducted in geographical locations and genetically distinct population than ours, and the underlying mechanisms for the protective effect in asthma are not fully understood.

In the present study, other indoor environmental risk factors were studied (Table 2). The presence of carpets is a well-known risk factor for asthma (50), but in our study population, the use of carpets is not a common practice. This observation could explain in part the low mite allergen levels found in the study population, and it can be concluded that carpets are not a significant risk factor for allergic sensitization or for asthma symptoms in our population. An important risk factor reported in our study was climatic change. The overwhelming majority of the interviewed subjects reported worsening of asthma when these changes took place. Climatic changes can influence asthma because it has been described as a known trigger for asthma exacerbations (51–53). In our study, there was a large number of asthmatics reporting a worsening of their condition with climate, especially in the months of November through January. A high percentage of asthmatics also experienced worsening of their condition while having colds. It is possible that climatological changes in conjunction with colds and endotoxins may play a very important role in the seasonality of the asthma epidemic in Puerto Rico. Further investigations should be focused to identify those agents involved



in triggering asthma and their relationship with bacterial endotoxins.

The low concentration levels of house dust mite and cockroach allergens found in our study can be explained in part by the housing conditions found in Puerto Rico, which can limit the infestation levels of these two important sources of allergens. We obtained the dust samples in highly ventilated housing, and it has been well documented that poor ventilation with low air change rates and humid houses lead to high dust mite counts (54). In fact, several studies have been conducted to determine whether improved ventilation influences mite infestation levels (55,56). In addition to the ventilated housing, the hygiene found in the households could explain the low prevalence of cockroach allergens. An interesting observation was that cockroach allergens were detected in homes across the economic spectrum and not only in low-income households, contrasting published data that indicate that cockroach infestations are mostly associated with low socioeconomic strata and public housing (57).

Evidence presented here indicates that bacterial endotoxins, serine proteases, dog allergen, and climatological changes are likely to influence asthma symptoms, and not dust mites and cockroaches. Our data also indicate that the established cutoff levels of exposures to mite, and perhaps cockroach allergens, may not be applicable in our study population. This observation is in agreement with emerging evidence that questions the applicability of the established cutoff levels for mite allergens exposures and clinical outcomes (58). It is possible also that environmental, total allergen burden, housing characteristics as well as genetical characteristics of the population may influence the lack of association between asthma symptoms and the cutoff levels of allergens exposures. We cannot exclude the importance of allergens derived from dust mites and cockroaches in allergic sensitization.

Our results also may have been influenced by the limitations of current technologies that can detect only some but not all allergens from mite or cockroach species present in tropical environments. Current dust, extraction protocol may not be the optimal method for allergen extraction from dust, and allergen concentrations may be underestimated. Additional limitations include those inherent to the study design.

In summary, the overall allergen burden detected in the sample population in Bayamón, Puerto Rico, is low, as well as the percent of asthmatics exposed to high levels of mite and cockroach allergens. Endotoxins, serine proteases, and dog allergens are important risk factors of asthma clinical symptoms. Our data support the hypothesis that the geographical and architectural

characteristics of the houses play an important role in the allergen burden with which asthmatics are exposed. Possibly, asthma symptoms in the study population are associated with chronic exposures to dog allergens, endotoxins, and serine proteases.

ACKNOWLEDGMENTS

Special thanks to Dr. Martin Hill of the Department of Pharmacology and Toxicology, Ponce School of Medicine, for critically reviewing this manuscript. This work was funded by a grant from the Environmental Protection Agency grant X-99276201-3 and a grant-in-aid from the National Institutes of Health RCMI-RR03050.

REFERENCES

1. Abramson M, Kutin JJ, Raven J, Lanigan A, Czarny D, Walters EH. Risk factors for asthma among young adults in Melbourne. *Respirology* 1996; 1(4):291–297.
2. Sarpong SB, Karrison T. Sensitization to indoor allergens and the risk for asthma hospitalization in children. *Ann Allergy Asthma Immunol* 1997; 79(5):455–459.
3. CDC. Self-reported asthma prevalence among adults—United States. *MMWR* 2000; 50(32):682–686.
4. Montealegre F, Bayona M. An estimate of the prevalence, severity and seasonality of asthma in visitors to a Ponce Shopping Center. *PR Health Sci J* 1996; 15(2):113–117.
5. Orengo JC, Loyo NI, Serrano RA, Mercado HE, Horta H. Prevalence of Respiratory and Allergic Problems Among School Children in the Cataño Air Basin. Presented at the Cataño Workshop, San Juan, Puerto Rico, 1996.
6. Pérez-Perdomo R, Suárez-Pérez E, Torres D, Morell C. Prevalencia de asma y utilización de servicios médicos en asegurados de una compañía de servicios de salud en Puerto Rico, 1996–1997. *Bol Asoc Med Puerto Rico* 1999; 91(7–12):91–97.
7. Montealegre F, Sepúlveda A, Quiñones C, Bayona M, Fernández-Caldas E. Identification of the domestic fauna of Puerto Rico. *PR Health Sci J* 1997; 16(2):109–116.
8. Montealegre F, Quiñones C, Michelen V, Bayona M, Fernández-Caldas E, Vázquez O, Colón F, Chardón D, García M. Prevalence of skin reactions



- to aeroallergens in asthmatics of Puerto Rico. *PR Health Sci J* 1998; 16(4):359–367.
9. Macfarlane SR, Seatter MJ, Kanke T, Hunter GD, Plevin R. Proteinase-activated receptors. *Pharmacol Rev* 2001; 53(2):245–282.
 10. Cicala C, Bucci M, De Dominicis G, Harriot P, Sorrentino L, Cirino G. Bronchoconstrictor effect of thrombin and thrombin receptor activating peptide in guinea-pigs in vivo. *Br J Pharmacol* 1999; 126(2):478–484.
 11. Standardization of spirometry—1987 update. Statement of the American Thoracic Society. *Am Rev Respir Dis* 1987; 136(5):1285–1298.
 12. Dean AG, Dean JA, Coulmbier D, Brendel KA, Smith DC, Burton AH, Dicker RC, Sullivan K, Fagan RF, Arner TG. Epi Info, Version 6: A Word Processing, Database, and Statistics Program for Epidemiology on Microcomputers. Centers for Disease Control And Prevention: Atlanta, GE, USA, 1994.
 13. Methods in Observational Epidemiology. Kelsey J, Thompson W, Douglas EA, eds. Oxford Press: New York, 1986:222.
 14. Milton D, Feldman DS, Neuberg RJ, Bruckner RJ, Greaves IA. Environmental endotoxin measurement; the kinetic Limulus assay with resistant-parallel-line estimation. *Environ Res* 1992; 57(2):212–230.
 15. Finney DL. Statistical Method in Biological Assay. Charles Griffin and Co., Ltd.: London, 1978, 69–104.
 16. Exterkate FA. Differences in short peptide-substrate cleavage by two cell envelope proteinases of *Lactococcus lactis* subs. *cremoris* are related to specificity. *Appl Microbiol Biotechnol* 1990; 33(4):401–406.
 17. Platts-Mills TAE, Thomas WR, Alberse RC, Vervoelet D, Chapman MD. Dust mite allergens and asthma: report of the second international workshop. *J Allergy Clin Immunol* 1992; 89(5):1046–1060.
 18. Eggleston PA, Rosenstreich D, Lynn H, Gergen P, Baker D, Kattan M, Mortimer KM, Mitchell H, Ownby D, Slavin R, Malveaux F. Relationship of indoor allergen exposure to skin test sensitivity in inner-city children with asthma. *J Allergy Clin Immunol* 1998; 102(4 Pt 1):563–570.
 19. Park JH, Gold DR, Spiegelman DL, Burge HA, Milton DK. House dust endotoxin and wheeze in the first year of life. *Am J Respir Crit Care Med* 2001; 163(2):322–328.
 20. National Asthma Education and Prevention Program. Expert Panel Report: Guidelines for the Diagnosis and Management of Asthma. NIH Publication No. 91-3042. Bethesda, MD: National Heart, Lung and Blood Institute, 1991.
 21. Barnes KC, Fernández-Caldas E, Trudeau WL, Milne DE, Brenner RJ. Spatial and temporal distribution of house dust mite (Astigmata, Pyroglyphidae), allergens *Der p1* and *Der f1* in Barbadian homes. *J Med Entomol* 1997; 34(2):212–218.
 22. Fernández-Caldas E, Codina R, Ledford DK, Trudeau WL, Lockey RF. House dust mite, cat and cockroach allergen concentrations in daycare centers in Tampa, Florida. *Ann Allergy Asthma Immunol* 2001; 87(3):196–200.
 23. Wickens K, Mason K, Fitzharris P, Siebers R, Hearfiels M, Cunningham M, Crane J. The importance of housing characteristics in determining *Der p1* levels in carpets in New Zealand homes. *Clin Exp Allergy* 2001; 31(6):803–805.
 24. Raunio P, Pasanen AL, Reiman M, Virtanen T. Cat, dog, and house-dust-mite allergen levels of house dust in Finnish apartments. *Allergy* 1998; 53(2):195–199.
 25. Litonjua AA, Carey VJ, Burge HA, Weiss ST, Gold DR. Exposure to cockroach allergen in the home is associated with incident doctor-diagnosed asthma and recurrent wheezing. *J Allergy Clin Immunol* 2001; 107(1):41–47.
 26. Rosenstreich DL, Eggleston P, Kattan M, Baker D, Slavin RG, Gergen P, Mitchell H, McNiff-Mortimer K, Lynn H, Ownby D, Malveaux F. The role of cockroach allergy and exposure to cockroach allergen in causing morbidity among inner-city children with asthma. *N Engl J Med* 1997; 337:1356–1363.
 27. Sears MR, Herbison GP, Holdaway MD, Hewitt CJ, Flannery EM, Silva PA. The relative risk of sensitivity to grass pollen, house dust mite and cat dander in the development of childhood asthma. *Clin Exp Allergy* 1989; 9(4):419–424.
 28. Rosenstreich DL, Eggleston P, Kattan M, Baker D, Slavin RG, Gergen P, Mitchell H, McNiff-Mortimer K, Lynn H, Ownby D, Malveaux F. The role of cockroach allergy and exposure to cockroach allergen in causing morbidity among inner-city children with asthma. *N Engl J Med* 1997; 336(19):1356–1363.
 29. Sears MR, Herbison GP, Holdaway MD, Hewitt CJ, Flannery EM, Silva PA. The relative risk of sensitivity to grass pollen, house dust mite and cat dander in the development of childhood asthma. *Clin Exp Allergy* 1989; 9(4):419–424.
 30. Ronchetti R, Bonci E, Cutrera R, De Castro G, Indinmemeo L, Midulla F, Tancredi G, Martinez



- FD. Enhanced allergic sensitization related to parental smoking. *Arch Dis Child* 1992; 67(4):496–500.
31. Wahn U, Lau S, Bergmann R, Kulig M, Forster J, Bergmann K, Bauer CP, Guggenmoos-Holzmann I. Indoor allergen exposure is a risk factor for sensitization during the first three years of life. *J Allergy Clin Immunol* 1997; 99(6 Pt 1):763–769.
32. Lindfors A, Wickman M, Hedlin G, Pershagen G, Rietz H, Nordvall SL. Indoor environmental risk factors in young asthmatics: a case-control study. *Arch Dis Child* 1995; 73(5):408–412.
33. Travis J, Salvesen GS. Human serum protease inhibitors. *Ann Rev Biochem* 1983; (52):655–709.
34. Hutchison DC. The role of proteases and anti-proteases in bronchial secretions. *Eur J Respir Dis Suppl.* 1987; 153:78–85.
35. Thompson PJ. Unique role of allergens and the epithelium in asthma. *Clin Exp Allergy* 1998; 28(suppl. 5):110–116.
36. Colp C, Talavera W, Goldman D, Green J, Multz A, Lieberman J. Profile of bronchospastic disease in Puerto Rican Patients in New York City: a possible relationship to α_1 -antitrypsin. *Arch Int Med* 1990; 150(11):2349–2354.
37. Michel O, Dentener M, Corazza F, Buurman W, Rylander R. Healthy subjects express differences in clinical responses to inhaled lipopolysaccharide that are related with inflammation and with atopy. *J Allergy Clin Immunol* 2001; 107(5):797–804.
38. Michel O, Kips J, Duchateau J, Vertongen F, Robert L, Collet H, Pauwels R, Sergysels R. Severity of asthma is related to endotoxin in house dust. *Am J Respir Crit Care Med* 1996; 154(6 Pt 1):1641–1646.
39. Michel O, Duchateau J, Plat G, Cantinieaux B, Hotimsky A, Gerain J, Sergysels R. Blood inflammatory response to inhaled endotoxin in normal subjects. *Clin Exp Allergy* 1995; 25(1):73–79.
40. Michel O, Ginanni R, Le Bon B, Content J, Duchateau J, Sergysels R. Inflammatory response to acute inhalation of endotoxin in asthmatic patients. *Am Rev Respir Dis* 1992; 146(2):352–357.
41. Thorn J, Rylander R. Inflammatory response after inhalation of bacterial endotoxin assessed by the induced sputum technique. *Thorax* 1998; 53(12):1047–1052.
42. Zock JP, Hollander A, Heederik D, Douwes J. Acute lung function changes and low endotoxin exposures in the potato processing industry. *Am J Ind Med* 1998; 33(4):384–391.
43. Park JH, Spiegelman DL, Gold DR, Burge HA, Milton DK. Predictors of airborne endotoxin in the home. *Environ Health Perspect* 2001; 109(8):859–864.
44. Reed CE, Milton DK. Endotoxin-stimulated innate immunity: a contributing factor for asthma. *J Allergy Clin Immunol* 2000; 108(2):157–166.
45. Gereda JE, Klennert MD, Price MR, Leung DY, Liu AH. Metropolitan home living conditions associated with indoor endotoxin levels. *J Allergy Clin Immunol* 2001; 107(5):790–796.
46. Riedler J, Braun-Fahrlander C, Eder W, Schreuer M, Waser M, Maisch S, Carr D, Schierl R, Nowak D, von Mutius E, ALEX Study Team E. Exposure to farming in early life and development of asthma and allergy: a cross-sectional survey. *Lancet* 2001; 358(9288):1129–1133.
47. Von Mutius E, Braun-Fahrlander C, Schierl R, Riedler J, Ehlermann S, Maisch S, Waser M, Nowak D. Exposure to endotoxin or other bacterial components might protect against the development of atopy. *Clin Exp Allergy* 2000; 30(9):1230–1234.
48. Braun-Fahrlander C, Riedler J, Herz U, Eder W, Waser M, Grize L, Maisch S, Carr D, Gerlach F, Bufe A, Lauener RP, Schierl R, Renz H, Nowak D, von Mutius E, Allergy and Endotoxin Study Team. Environmental exposure to endotoxin and its relation to asthma in school-age children. *N Engl J Med* 2002; 347(12):869–877.
49. Douwes J, Pearce N, Heederik D. Does environmental endotoxin exposure prevent asthma? *Thorax* 2002; 57:86–90.
50. Meijer G, van der Heide S, Postma DS, de Reus DM, Koeter GH, van Aalderen WH. House dust mite exposure in asthmatic and healthy children: the difference is carpeting. *Pediatr Allergy Immunol* 1995; 6(4):187–191.
51. Beer SI, Kannai YI, Waron MJ. Acute exacerbation of bronchial asthma in children associated with afternoon weather changes. *Am Rev Respir Dis* 1991; 144(1):31–35.
52. Ito S, Kawaoi T, Kondo Y, Hiruma F, Togo T, Takashima H, Kondo T. Outbreaks of asthma attack and meteorological parameters—comparison between two areas Arerugi. 1992; 41(4):475–484.
53. Garty BZ, Kosman E, Ganor E, Berger V, Garty L, Wietzen T, Waisman Y, Mimouni M, Waisel Y. Emergency room visits of asthmatic children, relation to air pollution, weather, and airborne allergens. *Ann Allergy Asthma Immunol* 1998; 81(6):563–570.
54. Ross MA, Curtis L, Scheff PA, Hryhorczuk DO,

- Ramakrishnan V, Wadden RA, Persky VW. Association of asthma symptoms and severity with indoor bioaerosols. *Allergy* 2000; 55(8):705–711.
55. Htut T, Higenbottam TW, Gill GW, Darwin R, Anderson PB, Syed N. Eradication of house dust mite from homes of atopic asthmatic subjects: a double-blind trial. *J Allergy Clin Immunol* 2001; 107(1):55–60.
 56. Harving H, Korsgaard J, Dahl R. House-dust mite exposure reduction in specially designed, mechanically ventilated healthy homes. *Allergy* 1994; 49(9):713–718.
 57. Leaderer BP, Belanger K, Triche E, Holford T, Gold DR, Kim Y, Jankun T, Ren P, McSharry JE, Platts-Mills TA, Chapman MD, Bracken MB. Dust mite, cockroach, cat, and dog allergen concentrations in homes of asthmatic children in the northeastern United States: impact of socioeconomic factors and population density. *Environ Health Perspect* 2002; 110(4):419–425.
 58. Munir AK, Einarsson R, Schou C, Dreborg SK. Mite (*Der p1*, *Der f1*), and cat (*Fel d1*), allergens in the homes of babies with a family history of allergy. *Allergy* 1993; 91(5):1067–1074.



Request Permission or Order Reprints Instantly!

Interested in copying and sharing this article? In most cases, U.S. Copyright Law requires that you get permission from the article's rightsholder before using copyrighted content.

All information and materials found in this article, including but not limited to text, trademarks, patents, logos, graphics and images (the "Materials"), are the copyrighted works and other forms of intellectual property of Marcel Dekker, Inc., or its licensors. All rights not expressly granted are reserved.

Get permission to lawfully reproduce and distribute the Materials or order reprints quickly and painlessly. Simply click on the "Request Permission/Order Reprints" link below and follow the instructions. Visit the [U.S. Copyright Office](#) for information on Fair Use limitations of U.S. copyright law. Please refer to The Association of American Publishers' (AAP) website for guidelines on [Fair Use in the Classroom](#).

The Materials are for your personal use only and cannot be reformatted, reposted, resold or distributed by electronic means or otherwise without permission from Marcel Dekker, Inc. Marcel Dekker, Inc. grants you the limited right to display the Materials only on your personal computer or personal wireless device, and to copy and download single copies of such Materials provided that any copyright, trademark or other notice appearing on such Materials is also retained by, displayed, copied or downloaded as part of the Materials and is not removed or obscured, and provided you do not edit, modify, alter or enhance the Materials. Please refer to our [Website User Agreement](#) for more details.

[Request Permission/Order Reprints](#)

Reprints of this article can also be ordered at

<http://www.dekker.com/servlet/product/DOI/101081JAS120033993>