

Guanine, mite, and cockroach allergens in Costa Rican homes

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Previous studies of schoolchildren in Costa Rica have shown an asthma prevalence of 23% and a high level of sensitization, particularly to mite allergens. As a continuation of these studies, some 400 dust samples were collected from various places in Costa Rica, and parts of these were analyzed for specific mite and cockroach allergens, as well as for the number of mites and amount of guanine. Guanine was quantified by a diazo, as well as an HPLC method, which were found to be highly correlated. The concentrations of guanine by the diazo method, Der p 1, Der f 1, and the number of mites were higher in bed dust than in bedroom floor dust, and it was possible to quantify mite allergens and guanine in almost all bed-dust samples. The mean levels were 2–3 times higher than the proposed risk level for elicitation of symptoms in mite-sensitive asthmatics. Bed and bedroom floor dust contained more guanine and mite allergen in humid (>2000 mm rain) than in drier places ($P<0.05$), but the number of mites in bed and bedroom floor dust was higher in less humid places ($P=0.01$). The guanine content in bedroom floor dust was higher in areas with a temperate climate than in areas with a warmer climate ($P<0.001$, Bartlett's chi square [BCS]), as was the number of mites ($P<0.01$, Kruskal-Wallis [KW], 0.04, BCS) and the Der p 1 concentration ($P=0.01$, BCS; $P=0.02$, KW). The Der f 1 concentration in bedroom floor dust was higher in a warmer than in a temperate climate ($P<0.001$, BCS). More guanine and mites were found in urban than in rural bed dust ($P=0.03$, KW). Dust samples from the metropolitan area (temperate climate) of Costa Rica contained higher levels of guanine ($P<0.01$) and Der p 1 ($P=0.07$) than the coastal areas, but very little Der f 1. In these samples, guanine and Der p 1 allergen were closely related, and 2 µg of the allergen was equivalent to 0.49 mg of guanine. Two-thirds of bed and floor samples collected on cotton filters contained Bla g 2 allergen at mean levels of 1.6 and 2.1 units/g dust, respectively. Cockroach allergen was, however, absent in all bed samples from the metropolitan area, but did occur in very high concentrations in the coastal bed dust samples collected with tighter polyester filters. In conclusion, the concentration of guanine and Der p 1 was very high in the bed dust of Costa Rican homes. Some factors, such as humidity, small houses for large families, and type of bedding, probably favored the heavy mite infestation, which is probably related to the widespread occurrence of bronchial asthma in this country.

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In a recent epidemiologic study, we found the prevalence of bronchial asthma among schoolchildren in Costa Rica to be 23% (1). Randomly selected subgroups from this study were subjected to allergy diagnostic analyzes *in vitro*, in a search for causes of this very high prevalence. The results indicated extensive sensitization to a panel of respiratory and alimentary allergens, with higher

levels for respiratory allergens in children classified as asthmatics than in nonasthmatics. Mite and cockroach were the most prevalent allergens, and all sensitized asthmatics had specific IgE to mite allergen (2).

In studies from various parts of the world where an increasing prevalence of asthma has been reported, mite (3–12) and cockroach (13–18) allergens have

been abundant and strongly associated with bronchial asthma, both as inducers and triggers. We have investigated the importance of these environmental factors in Costa Rica by taking dust samples from homes in this tropical country.

The dust samples were collected from various parts of Costa Rica during and after the diagnostic study of asthma and were analyzed for specific mite and cockroach allergens, as well as for the number of mites. We also applied two chemical analyses for quantification of guanine, which is reported to be a marker of the presence of allergenic mite feces and more or less closely correlated with the number of mites in house dust (19).

Material and methods

By established questionnaire criteria, a representative sample of 2682 Costa Rican schoolchildren from 98 educational centers were classified as asthmatic or nonasthmatic. From subsamples of these groups, blood was drawn, and house dust was collected by one nurse, or by a few carefully instructed medical students.

In total, some 400 house-dust samples were taken from coastal and central areas with different mean temperatures and rainfalls.

Sampling

Dust from the surface of the mattress sheet and adjacent floor was collected on cotton filters by vacuum-cleaning areas of approximately 1–2 m² for 5 min with a portable vacuum cleaner equipped with a plastic filter-holder at the outlet of the hose. After each sampling, the filter with its dust was transferred to a plastic bag and stored at 4°C until analysis. The yield was about 1 g or less, and considerable coarse material had to be removed by sieving.

Since sampling on cotton filters yielded only 0.5–1 g/sample as compared with a final sampling on polyester filters (as described below), we switched from extraction of only 50 mg (series 1 and 2) dust to extraction of all dust with its cotton filter in 10 ml of phosphate-buffered saline (PBS) plus 0.05% Tween 20 overnight. The water-soluble mite allergens were analyzed after further dilution of 1:5, while the insoluble guanine in the remaining material was dissolved with 10 ml of 2% KOH-20% methanol overnight for guanine determination. These samples may better reflect the total amount of mite allergen and guanine per vacuum-cleaned surface (series 3).

Finally, a series of 40 bed-dust samples (series 4) was taken by the senior allergist (L.B.) from middle-class homes in the two coastal areas of Costa Rica,

as well as in a central metropolitan area. These samples were collected on polyester filters (Munktell, Gryksbo, Sweden) with a diameter of 8 cm and a defined pore size of 5 mm from the mattress sheet, as well as the mattress surface below this sheet. This sampling was obviously more efficient since it usually yielded about 2 g of dust, of which 80% remained after sieving as a fine dust for antigen analyses and guanine determination by the same procedure as in series 1 and 2.

Analyses

Experienced acarologists at the School of Microbiology, University of Costa Rica, counted the total number of mites in the samples of series 2. Two aliquots of dust (50 mg dust each) were taken and were suspended in 25 ml of saturated saline solution, and 2 drops of a commercial liquid detergent were added (Brisol). The suspension was homogenized in a Vortex agitator (Model K550-G) and washed through a mesh of 45 µm (USA Standard Series no. 321). The retained mites were dyed with crystal violet (1% in alcohol at 95°C), passed to a square Petri dish, and counted at the surface, as well as at the bottom, with a stereoscopic microscope.

Group 1 major allergen of *Dermatophagoides pteronyssinus* (Der p 1), group 1 major allergen of *D. farinae* (Der f 1), and group 2 major allergen of *Blatella germanica* (Bla g 2) were assayed by the two-site ELISA described by Chapman et al., with monoclonal and polyclonal antibodies and standards produced by this research group (20).

The guanine determinations were performed as described by Le Mao et al. (19), with some modifications. Briefly, 50 mg of sieved fine dust was extracted by an alkaline alcoholic solvent (20% methanol and 2% KOH). The solution was kept at 30°C in a water bath for 2 min and then agitated for 30 s. After centrifugation, the supernatant was collected, and the wet dust was extracted another three times in the same way, but without heating. The four supernatants, each 0.5 ml, were mixed and used in the HPLC analysis of guanine as described below (21).

In the colorimetric test, the supernatant was first mixed with a solution of 16% Na₂CO₃ and then with a freshly prepared coupling solution (0.25% sulfanilic acid in 1 M HCl and 5% NaNO₂ in distilled water; the two solutions were mixed in a 1:1 ratio). Diazo ammonium compounds were formed at room temperature, and the intensity of yellow color was read after 20 min at 490 nm. For calculation of the guanine concentration in dust samples, a standard curve with guanine (Merck, Darmstadt, Germany, Art 4221) from 10 to 30 mg/ml was run at the same time.

The HPLC analyses were performed with 0.05 M $\text{NH}_4\text{H}_2\text{PO}_4$ /methanol (95/5, v/v) as elution buffer. The supernatant from the extraction was mixed 1:10 with elution buffer before injection into the sample loop. The absorbance was read at 254 nm. Guanine was run in the same way at a concentration of 1.25–20 mg/ml, and the peak height was measured to make a standard curve.

The analytic instruments were a V-max microplate reader (Molecular Devices Corp., Menlo Park, CA, USA) and a ConstaMetric III HPLC pump with a Spectro-Monitor III UV/Vis detector and an 8NVC18 5 m 100-mm column (Waters Associates, Milford, MA, USA).

Statistical analysis

The data were analyzed by the Epi Info Version 6, Public Domain Software for Epidemiology and Disease Surveillance (Centers for Disease Control, Epidemiology Program, Atlanta, GA, USA). The statistical analyses were done with frequencies, means, Bartlett's test for homogeneity of variance (Bartlett's chi-square [BCS] d.f.=1, *P* value, Mann–Whitney or Wilcoxon two-sample test (Kruskal–Wallis [KW] test for two groups), Kruskal–Wallis H (equivalent to chi-square), d.f.=1, *P* value, and Mantel–Haenszel or Yates correction).

Results

The mean values for guanine analyzed by the diazo and the HPLC methods, as well as the concentrations of Der p 1, Der f 1, and Bla g 2 and the number of mites, are given for bed and floor dust separately in Table 1. These results are expressed as weight/g fine dust in three of the sample series (1, 2, and 4) and per unit surface area in series 3. Almost all bed dust, but not bedroom dust samples, contained mites and measurable amounts of guanine, Der p 1, and Der f 1 allergens. In contrast, Bla g 2 allergen was lacking in some samples, from both bed and floor. Although high values and large variations were noted, there was no significant difference between asthmatic and nonasthmatic children in presence of guanine, number of mites, and Der p 1, Der f 1, or Bla g 2 allergens in their homes.

The samples of series 1 were analyzed by the diazo as well as the HPLC guanine methods, and a comparison of the results showed a very close correlation (*y*-intercept 7.57, *r*=0.91, *r*²=0.83) between the two assays, although the HPLC values were generally only 70% of the diazo values. All guanine results given below were obtained by the diazo method.

Table 1. Overall results of dust analyses for guanine, Der p 1, Der f 1, Bla g 2, and number of mites

Sample series	Site	1 (Weight/ g dust)	2 (Number/ g dust)	3 (Weight/ sample)	4 (Weight/ g dust)
<i>n</i>		140	150	150	40
Guanine (diazo)	Bed	10.5±7.5 mg	–	5.0±5.5 mg	7.9±5.8 mg
Guanine (diazo)	Floor	4.6±6.7 mg	–	2.7±2.2 mg	–
Guanine (HPLC)	Bed	7.3±6.4 mg	–	–	–
Guanine (HPLC)	Floor	2.6±5.4 mg	–	–	–
Der p 1	Bed	27.6±3.9 µg	–	9.6±10.7 µg	18.4±15.7 µg
Der p 1	Floor	12.5±3.2 µg	–	3.4±8.6 µg	–
Der f 1	Bed	0.8±0.8 µg	–	0.3±0.9 µg	2.2±17.4 µg
Der f 1	Floor	1.5±3.6 µg	–	0.3±1.5 µg	–
Number of mites	Bed	–	2554±2612	–	–
Number of mites	Floor	–	898±1642	–	–
Bla g 2, units/ g dust	Bed	1.6±2.0 µg	–	–	15.5±16.4 µg
Bla g 2, units/ g dust	Floor	2.1±3.7 µg	–	–	–

–: not done, mean value ± SD.

Differences in bed and bedroom floor dust

Very high levels of guanine and mite allergens and a high number of mites were found, particularly in bed dust (Table 1). Thus, the guanine content was twice and the number of mites thrice as high in bed as in bedroom floor dust. Furthermore, there was more than twice as much Der p 1 allergen in bed as in bedroom floor dust.

Der f occurred equally in bed and floor dust in sample series 3 and was more prominent in floor than in bed dust in series 1. However, all Der f levels in bed dust were only 2% of Der p 1 concentration and 10% of Der p 1 in floor dust, suggesting that *D. pteronyssinus* was the dominant species, particularly in the mite fauna of the beds.

Climatic and geographic differences in the occurrence of guanine and mites in dust

Both bed and bedroom floor dust contained significantly more guanine and Der p 1 plus Der f 1 allergens in areas with a rainfall above 2000 mm than in drier areas (*P*<0.05), as seen in Table 2. However, the number of mites in beds as well as in bedroom floor dust was higher in less humid places (*P*=0.01). Guanine in bedroom floor dust was higher in areas with temperate than in warmer climate (*P*<0.001, BCS), as was also the total number of mites (*P*<0.01, KW; *P*<0.04, BCS) and

Table 2. Effect of different rainfall on content of guanine, Der p 1, Der f 1, and number of mites in dust samples

	Bed dust			Bedroom floor dust		
	<2000	>2000	P	<2000	>2000	P
Rainfall (mm)						
Guanine (diaz)	8.4	12.7	0.02**	2.1	6.4	0.02**
Der p 1	22	33.6	0.06*	2.2	20.1	0.01**, 0.02*
Der f 1	4	7.7	0.01**	11.7	16.8	NS
No. of mites	2680	2144	0.03*	954	720	<0.001*, 0.04**

Guanine (mg/g dust). * Kruskal-Wallis H. Der p 1 (μg/g dust). ** Bartlett's chi-square. Der f 1 (μg/g dust). No. of mites (mites/g dust).

the Der p 1 concentration ($P=0.01$, BCS; $P=0.02$, KW). The Der f 1 concentration in bed dust was higher in temperate places ($P<0.01$, BCS; $P<0.04$, KW), as was the number of mites ($P<0.01$, KW; $P<0.04$, BCS), as seen in Table 3. On the other hand, the Der f 1 concentration in bedroom floor dust was higher in warmer than in temperate places ($P<0.001$, BCS; $P<0.06$, KW). There was no significant difference between the bed dust concentration of guanine in warmer and temperate areas.

More guanine and mites were found in urban than in rural bed dust ($P=0.03$, KW), as seen in Table 4. The number of mites in bedroom floor dust was also significantly higher in urban than in rural areas ($P<0.001$). However, bedroom floor dust guanine, as well as Der p 1 and Der f 1 concentration, was higher in rural than in urban areas ($P<0.01$, BC).

Comparison between mite allergens and guanine

The samples of series 4, which were collected from the beds in middle-class homes in the western and eastern coastal areas of Costa Rica (warm and humid), as well as in the metropolitan area (temperate and less humid), were almost as rich in guanine as the mean level in sample series 1. However, the metropolitan samples contained higher levels of Der p 1 than the coastal samples ($P=0.007$), but very little Der f 1 allergen. On the other hand, both eastern and western coastal samples contained significant amounts of Der f 1 ($P<0.001$), in addition to Der p 1 allergen. These results are shown in Table 5.

Since 2 μg/g fine dust of the Der p 1 and Der f 1 allergen has been proposed as a risk level for sensitization, we calculated the amount of guanine corresponding to 2 μg of mite allergens from the mean values of sample series 1, 2, and 4, as seen

Table 3. Effect of different temperatures on content of guanine, Der p 1, and Der f 1 and number of mites in dust samples

	Bed dust			Bedroom floor dust		
	<25°C	>25°C	P	<25°C	>25°C	P
Temperature						
Guanine (diaz)	10.4	12.7	NS	5.1	1.5	<0.0001**
Guanine (HPLC)	7.1	8.6	NS	2.9	0.2	<0.0001**
Der p 1	25.8	37.5	NS	12.9	12.5	0.01**, 0.02*
Der f 1	6.7	3.5	0.03**	7.7	59.9	<0.001**, 0.06*
No. of mites	3478	1460	<0.001**	1120	626	<0.001*, 0.04**

Guanine (mg/g dust). * Kruskal-Wallis H. Der p 1 (μg/g dust). ** Bartlett's chi-square. Der f 1 (μg/g dust). No. of mites (mites/g dust).

Table 4. Effect of different residency, urban and rural, on content of guanine, Der p 1, and Der f 1 and number of mites in dust samples

	Bed dust			Bedroom Floor dust		
	Urban	Rural	P	Urban	Rural	P
Guanine (diaz)	12.4	9.4	NS	3.7	5.3	<0.0001**
Der p 1	30	25	NS	8.3	16.7	<0.01**, 0.002*
Der f 1	4.9	7.1	0.008**	5.7	18.5	<0.001**
No. of mites	3144	2376	0.003*	960	863	<0.001**

Guanine (mg/g dust). * Kruskal-Wallis H. Der p 1 (μg/g dust). ** Bartlett's chi-square. Der f 1 (μg/g dust). No. of mites (mites/g dust).

in Table 6. For a further comparison, series 4 was divided into east and west coast samples, as well as metropolitan samples. As shown, the guanine amount corresponding to 2 μg of Der p 1 and Der f 1 allergens was somewhat higher than 0.6 mg, as has been suggested by several workers.

Detection of cockroach allergens

Two-thirds of the bed and floor samples of series 1 contained Bla g 2 allergen at mean levels of 1.6 and 2.1 units/g dust, respectively (Table 1). This allergen was absent in all the bed samples from the metropolitan area of series 4, but occurred in very high concentrations in the coastal bed dust samples of this series, as seen in Table 5.

Discussion

The concentrations of guanine, as well as Der p 1 plus Der p 2 allergens and number of mites, were very high in many Costa Rican homes, particularly in bed dust, although there were no differences between the homes of asthmatic and nonasthmatic

Table 5. Content of guanine, and Der p 1, Der f 1, and Bla g 2 allergens in dust samples from metropolitan and coastal areas of Costa Rica

	Geographic area				P
	Mean	East Coast	Metropolitan	West Coast	
Guanine (mg/g dust)	7.9±5.8	7.2	11.6	6.7	<0.01
Total guanine (mg)	13.7	21.3	11.9	10.2	NS
Der p 1 (µg/g dust)	18.4±15.7	13.3	31.9	15.1	<0.007
Der f 1 (µg/g dust)	2.2±17.4	2.7	0.4	2.8	<0.001
Bla g 2 (units/g dust)	15.5±16.4	21.9	0	19.0	<0.001

children. A similar finding has been reported by several authors (13, 22).

The levels of Der p 1 plus Der f 1 allergens, as well as the number of mites in bed dust, were 2–3 times higher than what has been calculated to provoke symptoms in mite-allergic patients, and 10–15 times higher than the risk level for sensitization (4–6, 8, 10, 11). These levels have been set at 2 and 10 µg of mite allergen, as assayed by the two-site mAb ELISA, corresponding to 100 and 500 mites and 0.6 and 3.0 mg of guanine/g dust, but other authors have reported that much lower levels of exposure to house-dust mites can stimulate T-cell responses and sensitize during early childhood (7, 23).

The equivalence estimations are arbitrary since the measurements have different specificities. However, our results with bed dust show similar equivalencies between mites, mite allergens, and guanine, but they also suggest that an increasing allergen/guanine ratio and a closer correlation to guanine can be expected if Der p 1 dominates the mite population, as in the metropolitan samples of series 4. A more mixed mite population, as may be expected, particularly in floor dust, gives a poorer correlation.

Furthermore, our Der f 1 results reflect *D. siboney* rather than *D. farinae*, since that species has been reported to be very common in some other tropical countries (24, 25). *D. farinae* is more likely to occur in drier inland areas (26). The close antigenic relationship between these two mite species has recently been reported, and the Der f 1 monoclonal assay may therefore be quite sensitive also to the *D. siboney* allergen (25).

The mite fauna in tropical and subtropical regions has been demonstrated to be different from that in the USA and Europe, and very high numbers and many species of mites have generally been found in house dust from tropical countries (27–35).

Table 6. Mean amount of guanine (mg) corresponding to 2 µg Der p 1 plus Der f 1 allergens in samples from different areas

	Bed dust	Floor dust
Series 1	0.75	0.65
Series 3	1.0	1.5
Series 4	0.79	n.d.
Metropolitan 4	0.71	n.d.
West coast 4	0.72	n.d.
East coast 4	0.91	n.d.

n.d.: not done.

Nevertheless, *D. pteronyssinus* seems to deliver an important bed-dust allergen in Costa Rica, as well as in other parts of the world. Other species, such as the storage mite *Blomia tropicalis*, which is reported to be very common in Costa Rica and in other areas of Latin America, are clinically important in individual cases and necessary to consider in diagnosis (27, 29, 30, 32, 33, 36, 37). However, an environmental survey of the mite allergen occurrence by screening guanine analysis may overcome the problems with unknown mite-allergen specificities and indicate the existence of allergenic mites for which there is no available immunoassay. It will reflect the total mite infestation regardless of species, and is, by the diazo method, simple and cheap to perform.

It should be remembered that spiders, as well as insects such as cockroaches, may also contribute to the guanine in floor dust. However, bed dust guanine is probably produced mainly by house-dust mites, since they are the main inhabitants of our beds. As regards time, sampling surface, and sampling device, this study is compatible with many other studies evaluating house-dust mites. In spite of the significantly different amounts of bed dust when sampled on cotton as compared with synthetic filters, the yield of guanine and mite allergen was in the same order of magnitude. On the other hand, cockroach allergen was much more efficiently collected on the polyester than the cotton filters, and a surprisingly high concentration of Bla g 2 allergens was found in bed dust samples from the coastal areas (series 4).

Some factors, such as humidity, probably favor mite infestation in Costa Rica. We found the highest levels of guanine and mite allergen in the coastal areas, where the rainfall is >2000 mm annually. The very heavy bed infestation is probably due to changes in bed furniture and home architecture. Costa Rica has a large middle class, which is gradually adopting a Western lifestyle, particularly in urban areas. The relatively small houses usually contain 2–3 small bedrooms, even for large families. The mattresses, which some decades ago were

commonly filled with a soft plant material ("paja") that had to be repeatedly renewed, have gradually been replaced by more permanent polyester foam or box-spring mattresses. In these materials, a mite population may propagate over many years without being disturbed by cleaning and drying.

The suggested process by which bed mite infestation in Costa Rican homes was increased may be consistent with the finding that Western-style bedding in some villages of Papua New Guinea resulted in a rapid parallel increase of mite infestation and incidence of bronchial asthma (38).

Reducing exposure to mites and other indoor allergens can play an important role in the management of bronchial asthma. Since we have identified and quantitated high levels of these sensitizing allergens in Costa Rican schoolchildren, our data will furnish a basis for preventive measures, including information about the necessity of improved bed hygiene, acaricidal treatment, and personal protection against mites.

Such a simple and safe acaricidal treatment of carpets as exposure to sunlight (39) may be effective also for mite infestation in mattresses and pillows. Since direct, strong sunlight is often available in Costa Rica, regular outdoor exposure of mite-infested bed material might be a good start for an intervention program.

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