

Daycare centers and schools as sources of exposure to mites, cockroach, and endotoxin in the city of São Paulo, Brazil

Vera E. V. Rullo, MD,^a M. Candida Rizzo, MD,^a L. Karla Arruda, MD,^b

Dirceu Solé, MD,^a and Charles K. Naspitz, MD^a São Paulo and Ribeirão Preto, Brazil

Background: Public places, including schools, have been identified as sources of exposure to allergens derived from mites, cockroach, cat, and dog and to endotoxin.

Objectives: The purposes of this study were to assess and compare exposure to allergens and endotoxin in 4 types of public child-care facilities in Brazil and to investigate whether the presence of children and the performance of cleaning procedures could have an influence on allergen and endotoxin levels.

Methods: We have analyzed dust from bedding, floors, chairs, and tables of daycare centers (DCs), preschools, kindergartens, and elementary schools (ESs). Major allergens from mites, cockroach, cat, and dog were quantitated by means of ELISA, and endotoxin content was determined by using the Limulus Amebocyte Lysate assay.

Results: Group 1 mite allergens were greater than 2 µg/g in 67% of DC and preschool samples and in 8.9% and 2.2% of kindergarten and ES samples, respectively. The presence of bedding in DCs and preschools accounted for increased levels of mite allergens in these settings. Levels of Bla g 1 were higher in ES floors compared with those found in DC and preschool floors. Low levels (<1 µg/g) of Fel d 1 e Can f 1 were found in most samples. Levels of endotoxin in DCs and preschools were 3 times higher than in ESs.

Conclusions: DCs and schools in Brazil should be considered as important sources of exposure to dust mites and cockroach allergens and to endotoxin. Recommendations for mite allergen avoidance should include appropriate care of bedding in DCs and preschools. (*J Allergy Clin Immunol* 2002;110:582-8.)

Key words: Allergens, endotoxin, schools, daycare, mites, cat, dog, cockroach

Environmental exposure to indoor allergens during early childhood has been associated with an increased risk of sensitization.^{1,2} Although there is a strong association between allergen sensitization and asthma, other concurrent events in early life, including viral infections, pattern

Abbreviations used

DC: Daycare center

ES: Elementary school

EU: Endotoxin units

of intestinal microflora, exposure to endotoxin and other bacterial cell-wall products, and infection with intestinal parasites, might play a role in the development of the asthmatic phenotype.³⁻⁵ For dust mite and cockroach allergens, there is good evidence for a dose-response relationship between exposure and sensitization and development of asthma.⁶⁻⁸ On the other hand, decreased sensitization has been associated with exposure to very high levels of the cat allergen Fel d 1 in the home, indicating that different dosing of allergen exposure might be critical for modulating sensitization to cat.^{9,10} Epidemiologic studies have also shown that children raised in a house with a cat are less likely to have asthma.^{11,12}

Avoidance programs starting early in life, addressing high-risk infants, have shown that sustained low levels of mite allergens can be achieved in homes¹³ and that this reduction was associated with a decrease of wheezing in the first year of life.¹⁴ Although the home environment has been the focus of primary and secondary interventions, previous studies have shown that public areas, including daycare centers (DCs) and schools, can also be a relevant source of exposure to allergens from mites, cat, dog, and cockroach.¹⁵⁻²⁵

Endotoxins are cell-wall components of the outer membrane of gram-negative bacteria, which are present in house dust. It has been suggested that exposure to endotoxin early in life could be protective for the development of atopic diseases in childhood by modulating immune responses toward a T_H1 phenotype.^{26,27} Epidemiologic studies have shown a lower risk of allergic sensitization and development of hay fever among children who grow up on a farm.^{26,28} Increased concentrations of house dust endotoxin have been detected in homes of farming families and in households where children had regular contact with farm animals compared with concentrations found in homes of nonfarming children.²⁶ Gereda et al,²⁹ studying children with a high risk of asthma, have shown that endotoxin exposure early in life seems to protect against allergen sensitization and that levels of endotoxin in house dust correlated with the presence of IFN-γ-producing T lymphocytes. Exposure to high levels of endotoxin

From ^athe Division of Allergy, Clinical Immunology, and Rheumatology, Department of Pediatrics, Paulista School of Medicine, Federal University of São Paulo, São Paulo, and ^bthe Division of Immunology, Allergy and Rheumatology, Department of Pediatrics, School of Medicine of Ribeirão Preto-University of São Paulo, Ribeirão Preto.

Supported by FAPESP grant 98/06810-2 and CAPES.

Received for publication March 27, 2002; revised June 18, 2002; accepted for publication June 19, 2002.

Reprint requests: L. Karla Arruda, MD, Department of Pediatrics, School of Medicine of Ribeirão Preto-University of São Paulo, Av. Bandeirantes, 3900, Ribeirão Preto, SP, CEP 14049-900, Brazil.

© 2002 Mosby, Inc. All rights reserved.

0091-6749/2002 \$35.00 + 0 1/81/127511

doi:10.1067/mai.2002.127511

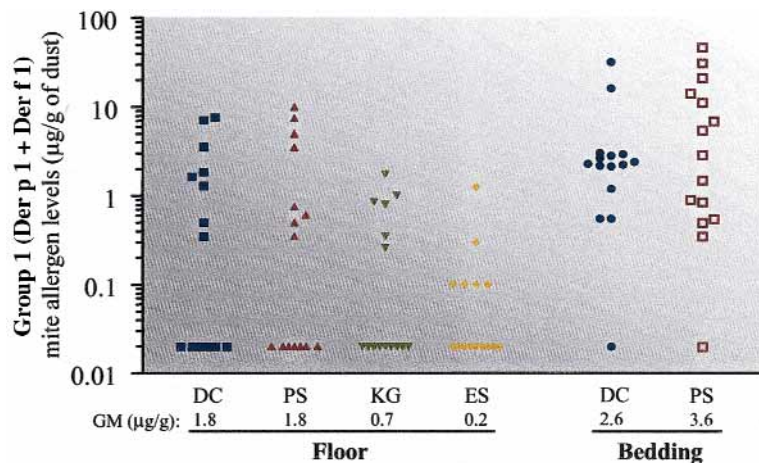


FIG 1. Group 1 mite allergens in DCs and schools. Levels of group 1 mite allergens (Der p 1 and Der f 1) in dust samples collected in the morning from DCs (n = 15), preschools (PS, n = 15), kindergartens (KG, n = 15), and ESs (n = 15) were quantitated by means of ELISA. There were no significant differences between mite allergen levels surveyed in the morning and in the late afternoon (data not shown).

3 months after birth was also protective against the development of atopic eczema in the first 6 months of life.³⁰ On the other hand, endotoxins have strong proinflammatory properties and are capable of inducing airway inflammation and worsening of asthma.³¹⁻³³

Our previous studies in Brazil revealed that 80% and 55% of children with asthma, rhinitis, or both were allergic to mites and cockroach, respectively.³⁴ High levels of mite allergens in homes have been consistently found in different areas in Brazil, including São Paulo,^{35,36} whereas cat and dog allergen levels have been low. The purposes of the present study were to assess and compare exposure to allergens from mites, cockroach, cat, and dog and to endotoxin in 4 types of public child-care facilities in São Paulo, Brazil, and to investigate whether the presence of children or the performance of cleaning procedures in these facilities could contribute to allergen loads, endotoxin loads, or both. We hypothesized that DCs and schools could be relevant sources of exposure to allergens and endotoxin. The results of our study might help in the design of environmental control measures targeted at modifying exposure to these components.

METHODS

Study sites and dust collection

In this cross-sectional study we have evaluated allergen and endotoxin exposure in 60 public child-care facilities located in the southern districts of São Paulo City, Brazil. Dust samples were obtained from 15 DCs (attended by 0- to 2-year-old children), 15 preschools (attended by 2- to 4-year-old toddlers), 15 kindergartens (attended by 4- to 7-year-old children), and 15 elementary schools (ES; attended by 7- to 14-year-old children). All public DC, preschool, kindergarten, and ES facilities in the study area were surveyed. Kindergartens and ESs comprised 15% of all public schools in the city of São Paulo. Dust samples were collected with a vacuum cleaner (Electrolux) with an airflow rate of 45 L/s in the morning and late afternoon. Morning samples were obtained before arrival of the children, and afternoon samples were obtained after the children had left school and after cleaning. Floor dust was collected from all sites (n

= 120) by vacuuming an area of 1 m² for 2 minutes. The type of floor covering was vinyl or tile in all buildings, and no carpets or rugs were present. In DCs and preschools additional samples were collected from bedding by vacuuming bed coverings and mattress surfaces for 2 minutes in all rooms (n = 60). All mattresses in DCs were encased in vinyl covers. In kindergartens and ESs dust samples from tables and chairs were also collected (n = 120) by vacuuming 5 m² of the horizontal surface of tables and chairs for 5 minutes.³⁷ A total of 300 dust samples were analyzed for allergen contents, and 157 dust samples were analyzed for endotoxin levels.

Dust extracts were prepared by using standard procedures.³⁸ Briefly, fine dust was obtained by sieving samples through a 355-µm-diameter mesh screen to remove large particles and fibers. One hundred milligrams of fine dust was resuspended with a vortex mixer with 2 mL of borate-buffered saline containing 0.1% Tween 20, pH 8.0. Samples were rotated for 2 hours at room temperature and centrifuged for 20 minutes at 2500 rpm at 4°C. After centrifugation, supernatants were removed and stored at -20°C for subsequent analysis of allergen content.

Measurements of major allergens from mites, cockroach, cat, and dog and quantitation of endotoxin in dust samples

Group 1 *Dermatophagoides* species allergens (Der p 1 and Der f 1), group 2 *Dermatophagoides* species allergens, the cat allergen Fel d 1, the dog allergen Can f 1, and the cockroach (*Blattella germanica*) allergens Bla g 1 and Bla g 2 were measured by using mAb-based ELISAs, as previously described.³⁹⁻⁴³ Reference standards containing known levels of each allergen were used to make control curves, and results were expressed as micrograms per gram of dust. Cockroach assays were quantitated on the basis of reference German cockroach extract (UVA 93/04; 97/02), and results were expressed as arbitrary units of Bla g 1 per gram of dust and Bla g 2 per gram of dust. All reagents were obtained from Indoor Biotechnologies Inc.

For endotoxin quantitation, dust samples were resuspended in pyrogen-free water at 25 mg of dust per milliliter, diluted to 1:40,000, and sonicated for 60 minutes with intermittent vortexing. Endotoxin levels were determined by using the turbidimetric kinetic limulus assay (Endosafe), according to the manufacturer's instructions, and quantitation was performed by using the endotox-

Asthma, rhinitis,
other respiratory
diseases

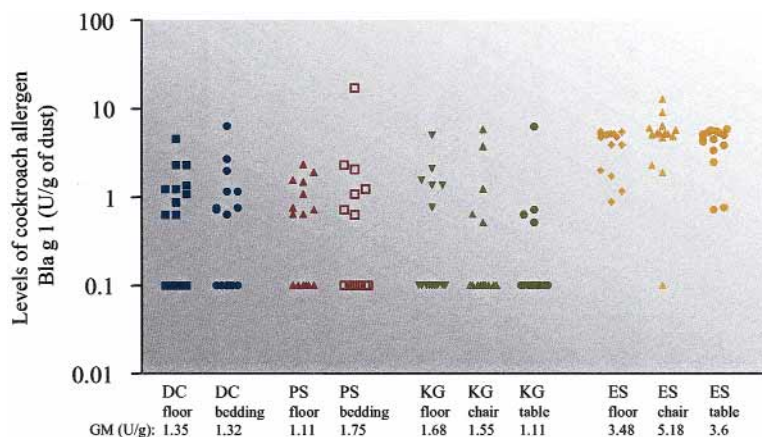


FIG 2. Cockroach allergen Bla g 1 in DCs and schools. Levels of cockroach allergen Bla g 1 in dust samples collected in the morning from DCs ($n = 15$), preschools (PS, $n = 15$), kindergartens (KG, $n = 15$), and ESs ($n = 15$) were measured by means of ELISA. There were no significant differences between cockroach allergen levels surveyed in the morning and in the late afternoon (data not shown).

in reference standard developed by the US Food and Drug Administration.⁴⁴ Serial dilutions of each sample were run in duplicate, and endotoxin concentrations were quantified in endotoxin units (EU) per milligram of dust.

Statistical analysis

The Fisher test was used to compare proportions of samples containing sensitizing levels of allergens. The Kruskal-Wallis test was used to compare allergen and endotoxin levels in samples collected from bedding, floors, chairs, and tables at different child-care facilities. The Mann-Whitney test was used to compare levels of allergens and endotoxin collected from bedding samples in DCs and preschools. The Wilcoxon test was used to compare levels of allergens collected in morning samples and afternoon samples. The GraphPad InStat program was used for statistical analysis. A P value of less than .05 was considered significant.

RESULTS

Fig 1 shows levels of group 1 (Der p 1 and Der f 1) mite allergens in morning samples. At all sites, comparisons of levels of group 1 allergens in morning and afternoon samples showed no significant differences. In afternoon samples geometric means (GMs) of group 1 allergen levels in DC and preschool bedding samples were 2.6 $\mu\text{g/g}$ (range, <0.02-16.2 $\mu\text{g/g}$) and 6.3 $\mu\text{g/g}$ (range, 1.3-30.7 $\mu\text{g/g}$), respectively, whereas DC, preschool, kindergarten, and ES floor samples were 1.3 $\mu\text{g/g}$ (range, <0.02-10.5 $\mu\text{g/g}$), 1.9 $\mu\text{g/g}$ (range, 0.35-5.5 $\mu\text{g/g}$), 0.07 $\mu\text{g/g}$ (range, <0.02-0.25 $\mu\text{g/g}$), and 0.5 $\mu\text{g/g}$ (range, 0.06-4.7 $\mu\text{g/g}$), respectively (data not shown). Higher levels of group 1 mite allergens were detected in DC and preschool floors compared with those found in ES floors. In addition, levels of group 1 allergens were significantly increased in DC and preschool bedding samples compared with those found in ES floor samples (Fig 1). Group 2 mite allergens showed similar distribution to group 1 allergens (data not shown).

Levels of the cockroach allergen Bla g 1 in morning samples are shown in Fig 2. Similarly to mites, levels in morning samples were not significantly different from

those in afternoon samples at all sites. Bla g 1 levels were significantly higher in ES floors compared with those found in DC and preschool floors both in morning samples (GM: 3.4 U/g vs 1.4 U/g and 1.1 U/g, respectively; Fig 2) and afternoon samples (GM: 4.7 U/g vs 1.3 U/g and 0.9 U/g, respectively; data not shown). Levels of Bla g 1 in chair and table surfaces were also increased both in morning samples (GM: 5.2 U/g and 3.6 U/g, respectively; Fig 2) and in afternoon samples (GM: 4.7 U/g and 3.7 U/g, respectively; data not shown). Bla g 2 stayed at levels less than the detection limit in most samples or were consistently low, and none of the samples contained more than 2 U/g dust of this allergen (data not shown).

A significantly higher proportion of samples collected from DCs and preschools contained detectable group 1 mite allergens compared with samples from kindergartens and ESs. Similarly, 66.7% of samples from DCs and preschools contained group 1 levels equal or greater than the proposed sensitization threshold level of 2 $\mu\text{g/g}$ dust, whereas only 8.9% and 2.2% of samples from kindergartens and ESs, respectively, presented these levels (Fig 3, A and B). By contrast, a higher proportion of samples obtained from ESs had detectable cockroach allergen Bla g 1 and levels of Bla g 1 equal to or greater than the proposed sensitization threshold of 2 U/g dust⁷ compared with kindergarten, preschool, and DC samples (Fig 3, C and D).

Animal allergens were remarkably low in all samples. Cat allergen ranged from undetectable to 1.1 μg of Fel d 1/g of dust, and dog allergen ranged from undetectable to 3.3 μg of Can f 1/g of dust. None of the samples contained levels comparable with those usually found in homes with cats or dogs (>8 μg of Fel d 1/g of dust or >10 μg of Can f 1/g of dust, respectively).⁴⁵ No significant differences were found in levels of cat and dog allergens surveyed in the morning and in the late afternoon.

Endotoxin levels of greater than 10 EU/mg were found in most samples. In morning samples significant differences were observed between floor endotoxin levels in

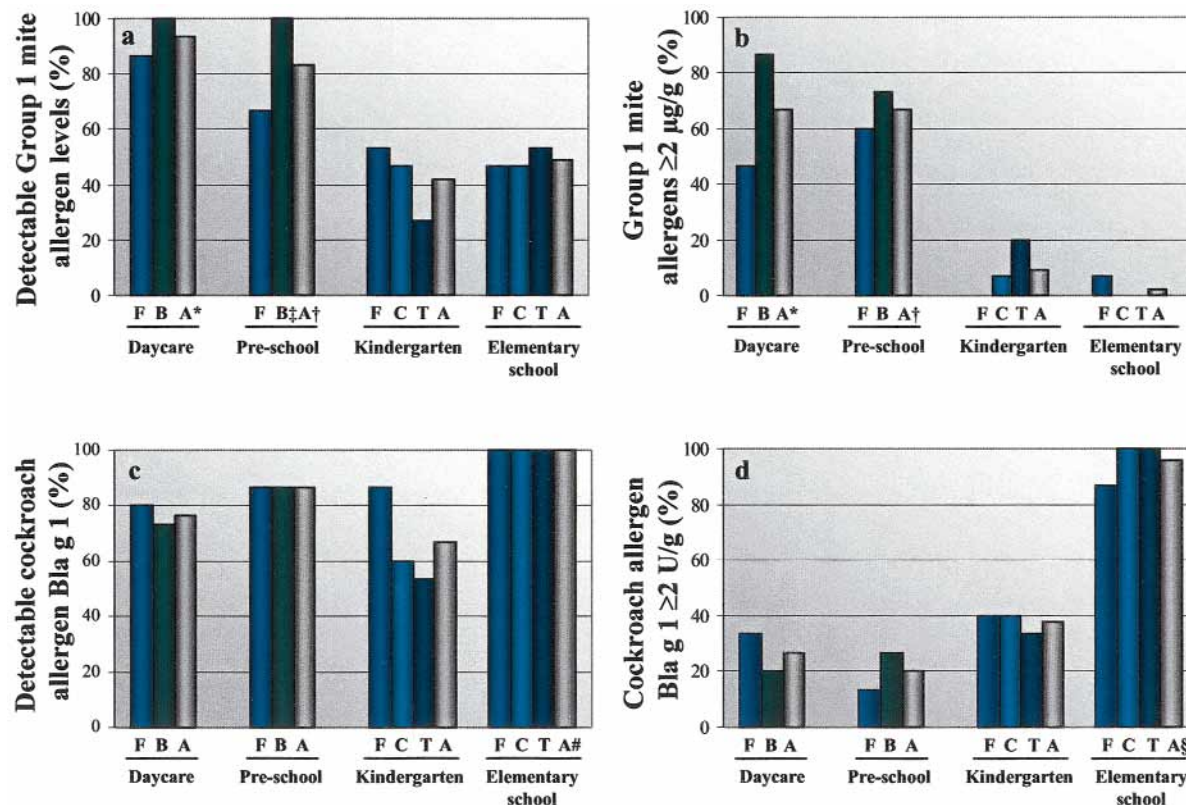


FIG 3. Mite and cockroach allergens in DCs and schools. The percentages of dust samples containing detectable group 1 mite allergens (a), with group 1 mite allergens at greater than sensitizing threshold levels ($>2 \mu\text{g/g}$) (b), with detectable cockroach allergen Bla g 1 levels (c), and with Bla g 1 levels of greater than sensitizing threshold levels ($>2 \text{ U/g}$; d) are shown for floor (F), bedding (B), chairs (C), tables (T), and any site (A) in DCs, preschools, kindergartens, and ESs. The highest value (either morning or afternoon) was chosen for this analysis. * $P < .001$: DC > kindergarten and ES; † $P < .001$: preschool > kindergarten and ES; ‡ $P < .05$: bedding > floor in preschools; § $P < .05$: ES > DC, preschool, and kindergarten; # $P < .0001$: ES > DC, preschool, and kindergarten (Fisher test).

preschools and ESs (GM: 27.4 EU/mg and 10.1 EU/mg of dust, respectively; Fig 4), whereas in afternoon samples significant differences were detected between floor endotoxin levels in DCs and ESs (GM: 32.2 EU/mg and 13.3 EU/mg of dust, respectively; data not shown). No significant differences in endotoxin levels were found in samples collected in the morning or afternoon.

DISCUSSION

It has been suggested that the onset of childhood asthma might be influenced by environmental factors during early life.¹⁻⁵ Our results revealed that bedding and floors from DCs and preschools are important sources of exposure to mites. It is likely that bedding in DCs and preschools were breeding sites for mites and reservoirs for mite allergens. All mattresses were encased in vinyl covers; however, washing bedding at least weekly in hot (130°F) water, which has been shown to kill mites and remove most allergen, has not been performed. On the other hand, floors, tables, and chairs from kindergartens and ESs presented exposure at less than sensitizing levels

of group 1 mite allergens in most cases. It is well established that hard surfaces, such as those in chairs and tables, and vinyl floors not covered with carpets or rugs do not support extensive mite growth. A study carried out in France²⁰ previously showed that mite allergen levels in dust from DCs were low and were below those found in schools. On the other hand, it has been recently reported that mite allergen concentrations of greater than those associated with sensitization were found in 40% of DCs surveyed in Tampa, Florida.¹⁵ These results are in keeping with our finding that group 1 mite allergens were equal or greater than $2 \mu\text{g/g}$ dust in approximately 70% of DCs and preschools attended by children 0 to 4 years old. These children would be at risk for sensitization to mites.

In our study ESs were a source of significant exposure to cockroach allergen. Although the cockroach allergen Bla g 1 could be detected in all facilities, a higher degree of exposure was found among children 7 to 14 years old attending ESs. Almost all ESs surveyed presented levels equal to or greater than 2 U/g dust, a level that has been associated with sensitization,^{7,46} and a proportion of these facilities contained levels of Bla g 1 of greater than

Asthma, rhinitis,
other respiratory
diseases

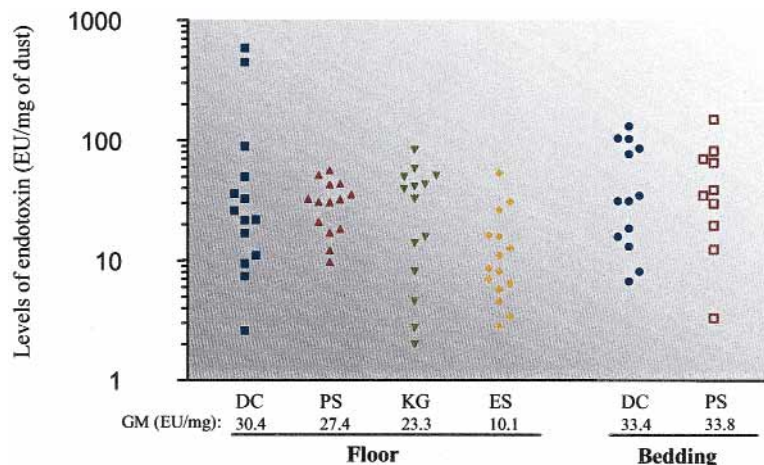


FIG 4. Endotoxin in DCs and schools. Levels of endotoxin in dust samples collected in the morning from DCs ($n = 15$), preschools (PS, $n = 15$), kindergartens (KG, $n = 15$), and ESs ($n = 15$) were determined by using the Limulus Amebocyte Assay and quantitated by means of ELISA. There were no significant differences between endotoxin levels surveyed in the morning and in the late afternoon (data not shown).

8 U/g, which has been regarded as the threshold for worsening of symptoms.^{46,47} Schools have been previously recognized as important sources of exposure to cockroach allergens by Sarpong et al,²⁵ who studied urban primary schools in Baltimore. Food-related areas presented higher allergen content compared with classrooms, and the overall levels of Bla g 1 were similar to those found in our study.²⁵ The relationship between exposure to cockroach allergen and development of sensitization and symptoms of asthma has been clearly established.⁴⁸⁻⁵⁰ Our results suggest that exposure to cockroach allergens in DCs and schools in our area might be associated with an important risk of sensitization and worsening of symptoms in students, teachers, and other school workers and indicate that DCs and schools should be thoroughly inspected for the presence of cockroaches.

Very low levels of cat and dog allergens were found in DCs and schools in our study, and these levels were similar to or lower than those found in houses without dogs or cats.^{42,51} Higher levels of Fel d 1 and Can f 1 have been reported in Swedish DCs¹⁹ and on chairs and floors in Swedish schools,^{16,23,24} probably as a result of high rates of passive transferring of these allergens in children's and teachers' clothing. Our results might reflect considerable differences in indoor climate (temperature and humidity), ventilation rates, cleaning routines, type of chairs and tables used in schools, and lower rates of pet ownership, habit of keeping pets indoors, or both in our community.

Studies on the role of exposure to endotoxin in the environment have yielded apparently conflicting results. Exposure to high levels of endotoxin has been linked to either protection against allergen sensitization or increased wheezing and worsening of established asthma.²⁶⁻³³ It is not clear whether there would be a threshold level of endotoxin exposure above which protection against allergic sensitization would occur. In addition, caution should be taken in comparing levels in different studies, which might have

been carried out with different endotoxin detection methods. In a European study levels of endotoxin as high as 649 EU/mg and 143 EU/mg have been reported in stables and kitchen floors of farming families, respectively, and were significantly higher than those found in homes of nonfarming families (39 EU/mg).²⁶ In the study by Gereda et al,²⁹ mean levels of house dust endotoxin associated with sensitization and protection were 93 EU/mg and 207 EU/mg, respectively. On the other hand, endotoxin exposure equal or greater than 100 EU/mg in the first 3 months of life was associated with an increased risk of any wheeze and repeated wheeze during the first year of life for children with a familial predisposition to allergy or asthma, suggesting a proinflammatory role of endotoxin in these children.³³ In keeping with this, we have previously reported that endotoxin levels in the home showed a direct correlation with symptoms in asthmatic children with mite allergy living in São Paulo.³¹ The median level of endotoxin in dust, collected over a year, was 10.8 EU/mg.³¹ In the present study few dust samples contained levels as high as 100 EU/mg; however, mean levels of endotoxin in DCs, preschools, and kindergartens were 3 times higher than those previously detected in homes in São Paulo,³¹ indicating that schools are at least as important as the home environment as a source of endotoxin exposure. In the present study we speculate that frequent diaper changes in DCs and preschools could contribute to higher levels of endotoxin in these facilities compared with levels found in ESs.

Unexpectedly, we found no significant differences in allergen or endotoxin levels in dust samples collected in the morning, before arrival of children at school, and in the afternoon after children had left the building and after cleaning. These results suggest that current cleaning procedures carried out in these facilities might not have been sufficient for decreasing mite and cockroach allergen and endotoxin levels and that the presence of children throughout the day had no effect on allergen levels.

In conclusion, we have shown that DCs and schools in Brazil should be considered as important sources of exposure to dust mites and cockroach allergens and to endotoxin. It has been reported that children attending DCs in the first 6 months of life were less likely to have asthma and frequent wheezing later in childhood⁵²; however, the study has not addressed the issue of allergen or endotoxin exposure, which might be different from that encountered in DCs in Brazil or other areas of the world. Our results indicate that DCs and schools should be considered as targets for environmental interventions aimed at decreasing allergen exposure. In particular, recommendations for mite allergen avoidance should include appropriate care of bedding in DCs and preschools.

We thank Ellen Oliveira Dantas, MD, and Albertina Rosa Borges, MD, for their contributions to the study; Katia Tobias for excellent technical assistance; Ana Paula Trombone for assistance in the preparation of the manuscript; Prof Elias Rodrigues de Paiva for help with statistical analysis; and the members of the institutional boards of the schools participating in the study for permission to collect dust samples.

REFERENCES

- Platts-Mills TAE, Rakes G, Heymann PW. The relevance of allergen exposure to the development of asthma in childhood. *J Allergy Clin Immunol* 2000;105:S503-8.
- Lau S, Illi S, Sommerfeld C, Niggemann B, Bergmann R, von Mutius E, et al. Early exposure to house-dust mite and cat allergens and development of childhood asthma: a cohort study. Multicenter Allergy Study Group. *Lancet* 2000;356:1392-7.
- Martinez FD. Maturation of the immune responses at the beginning of asthma. *J Allergy Clin Immunol* 1999;103:355-61.
- Wahn U, von Mutius E. Childhood factors for atopy and the importance of early intervention. *J Allergy Clin Immunol* 2001;107:567-74.
- Björkstén B, Sepp E, Julge K, Voor T, Milkelsaar M. Allergy development and the intestinal microflora during the first year of life. *J Allergy Clin Immunol* 2001;108:516-20.
- Sporik RB, Holgate ST, Platts-Mills TAE, Cogswell J. Exposure to house dust mite allergen (Der p 1) and the development of asthma in childhood: a prospective study. *N Engl J Med* 1990;323:502-7.
- Eggleston PA, Rosenstreich D, Lynn H, Gergen P, Baker D, Kattan M, et al. Relationship of indoor allergen exposure to skin test sensitivity in inner-city children with asthma. *J Allergy Clin Immunol* 1998;102:563-70.
- Sporik R, Squillace SP, Ingram JM, Rakes G, Honsinger RW, Platts-Mills TA. Mite, cat, and cockroach exposure, allergen sensitization, and asthma in children: a case-control study of three schools. *Thorax* 1999;54:675-80.
- Platts-Mills TAE, Vaughan J, Squillace S, Woodfolk J, Sporik R. Sensitization, asthma and a modified Th2 response in children exposed to cat allergen: a population-based cross-sectional study. *Lancet* 2001;357:752-56.
- Custovic A, Hallam CL, Simpson BM, Craven M, Simpson A, Woodcock A. Decreased prevalence of sensitization to cats with high exposure to cat allergen. *J Allergy Clin Immunol* 2001;108:537-9.
- Hesselmar B, Abreg B, Eriksson B, Björkstén B. Does early exposure to cat or dog protect against later allergy development? *Clin Exp Allergy* 1999;29:611-7.
- Roost HP, Kunzli N, Schindler C, Jarvis D, Chinn S, Perruchoud AP, et al. Role of current and childhood exposure to cat and atopic sensitization. *J Allergy Clin Immunol* 1999;104:941-7.
- Custovic A, Simpson BM, Simpson A, Hallam C, Craven M, Brutsche M, et al. Manchester Asthma and Allergy Study: low-allergen environment can be achieved and maintained during pregnancy and in early life. *J Allergy Clin Immunol* 2000;105:252-8.
- Custovic A, Simpson BM, Simpson A, Kissen P, Woodcock A. Effect of environmental manipulation in pregnancy and early life on respiratory symptoms and atopy during first year of life: a randomised trial. *Lancet* 2001;358:188-93.
- Fernandez-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;87:196-200.
- Perzanowski MS, Ronmark E, Nold B, Lundback B, Platts-Mills TAE. Relevance of allergens from cats and dogs to asthma in the northernmost province of Sweden: schools as a major site of exposure. *J Allergy Clin Immunol* 1999;103:1002-4.
- Custovic A, Taggart SC, Woodcock A. House dust mite and cat allergen in different indoor environments. *Clin Exp Allergy* 1994;24:1164-8.
- Custovic A, Green R, Taggart SC, Smith A, Pickering CA, Chapman MD, et al. Domestic allergens in public places. II: dog (Can f 1) and cockroach (Bla g 2) allergens in dust and mite, cat, dog and cockroach allergens in the air in public buildings. *Clin Exp Allergy* 1996;26:1246-52.
- Munir AK, Einarsson R, Dreborg SK. Mite (Der p 1, Der f 1), cat (Fel d 1) and dog (Can f 1) allergens in dust from Swedish day-care centers. *Clin Exp Allergy* 1995;25:119-26.
- Dornelas de Andrade A, Charpin D, Birnbaum J, Lanteaume A, Chapman M, Vervloet D. Indoor allergen levels in day nurseries. *J Allergy Clin Immunol* 1995;95:1158-63.
- Wickens K, Martin I, Pearce N, Fitzharris P, Kent R, Holbrook N, et al. House dust mite allergen levels in public places in New Zealand. *J Allergy Clin Immunol* 1997;99:587-93.
- Berge M, Munir AK, Dreborg S. Concentrations of cat (Fel d 1), dog (Can f 1) and mite (Der f 1 and Der p 1) allergens in the clothing and school environment of Swedish schoolchildren with and without pets at home. *Pediatr Allergy Immunol* 1998;9:25-30.
- Almqvist C, Larsson PPH, Egmar AC, Hedren M, Malmberg P, Wickman M. School as a risk environment for children allergic to cats and a site for transfer of cat allergen to homes. *J Allergy Clin Immunol* 1999;103:1012-7.
- Almqvist C, Wickman M, Perfetti L, Berglund N, Renstrom A, Hedren M, et al. Worsening of asthma in children allergic to cats, after indirect exposure to cat at school. *Am J Respir Crit Care Med* 2001;163:694-8.
- Sarpong SB, Wood RA, Karrison T, Eggleston PA. Cockroach allergen (Bla g 1) in school dust. *J Allergy Clin Immunol* 1997;99:486-92.
- von Mutius E, Braun-Fahrlander C, Schierl R, Riedler J, Ehlermann S, Maisch S, et al. Exposure to endotoxin or other bacterial components might protect against the development of atopy. *Clin Exp Allergy* 2000;30:1230-4.
- Lapa e Silva JR, Possebon da Silva MD, Lefort J, Vargaftig BB. Endotoxins, asthma, and allergic immune responses. *Toxicology* 2000;152:31-5.
- Von Ehrenstein OS, Von Mutius E, Illi S, Baumann L, Bohm O, von Kries R. Reduced risk of hay fever and asthma among children of farmers. *Clin Exp Allergy* 2000;30:187-93.
- Gereda JE, Leung DYM, Thatayatikom A, Streib JE, Price MR, Klinnert MD, et al. Relation between house-dust endotoxin exposure, type 1 T-cell development, and allergen sensitization in infants at high risk of asthma. *Lancet* 2000;355:1680-3.
- Gehring U, Bolte G, Borte M, Bischof W, Fahlbusch B, Wichmann HE, et al. Exposure to endotoxin decreases the risk of atopic eczema in infancy: a cohort study. *J Allergy Clin Immunol* 2001;108:847-54.
- Rizzo MC, Napitz CK, Fernández-Caldas E, Lockey RF, Mimiça I, Solé D. Endotoxin exposure and symptoms in asthmatic children. *Pediatr Allergy Immunol* 1997;8:121-6.
- Michel O, Kips J, Duchateau J, Vertongen F, Robert L, Collet H, et al. Severity of asthma is related to endotoxin in house dust. *Am J Respir Crit Care Med* 1996;154:1641-6.
- 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;163:322-8.
- Santos AB, Chapman MD, Aalberse RC, Vailes LD, Ferriani VP, Oliver C, et al. Cockroach allergens and asthma in Brazil: identification of tropomyosin as a major allergen with potential cross-reactivity with mite and shrimp allergens. *J Allergy Clin Immunol* 1999;104:329-37.
- Arruda LK, Rizzo MC, Chapman MD, Fernandez-Caldas E, Baggio D, Platts-Mills TA, et al. Exposure and sensitization to dust mite allergens among asthmatic children in São Paulo, Brazil. *Clin Exp Allergy* 1991;21:433-9.
- Sopelate MC, Silva DA, Arruda LK, Chapman MD, Taketomi EA. *Dermatophagoides farinae* (Der f 1) and *Dermatophagoides pteronyssinus* (Der p 1) allergen exposure among subjects living in Uberlândia, Brazil. *Int Arch Allergy Immunol* 2000;122:257-63.

37. Munir AK, Einarsson R, Schou C, Dreborg SK. Allergens in school dust. I. The amount of the major cat (Fel d 1) and dog (Can f 1) allergens in dust from Swedish schools is high enough to probably cause perennial symptoms in most children who are sensitized to cat and dog. *J Allergy Clin Immunol* 1993;91:1067-74.
38. Chapman MD, Smith AM, Slunt JB, Vailes LD, Arruda LK. Immunochemical and molecular methods for defining and measuring indoor allergens: in dust and air. *Pediatr Allergy Immunol* 1995;6(suppl 7):8-12.
39. Luczynska CM, Arruda LK, Platts-Mills TAE, Miller JD, Lopez M, Chapman MD. A two-site monoclonal antibody ELISA for the quantification of the major *Dermatophagoides* spp allergens, Der p I and Der f I. *J Immunol Methods* 1989;118:227-37.
40. Ovsyannikova IG, Vailes LD, Li Y, Heymann PW, Chapman MD. Monoclonal antibodies to Group II *Dermatophagoides* spp. allergens: murine immune response, epitope analysis, and development of a two-site ELISA. *J Allergy Clin Immunol* 1994;94:537-46.
41. Chapman MD, Aalberse RC, Brown MJ, Platts-Mills TAE. Monoclonal antibodies to the major feline allergen Fel d I. II. Single step affinity purification of Fel d I, N-terminal sequence analysis, and development of a sensitive two-site immunoassay to assess Fel d I exposure. *J Immunol* 1988;140:812-8.
42. Schou C, Hansen GN, Lintner T, Lowenstein H. Assay for the major dog allergen, Can f 1: investigation of house dust samples and commercial dog extracts. *J Allergy Clin Immunol* 1991;88:847-53.
43. Pollart SM, Smith TF, Morris EC, Gelber LE, Platts-Mills TAE, Chapman MD. Environmental exposure to cockroach allergens: analysis with monoclonal antibody-based enzyme immunoassays. *J Allergy Clin Immunol* 1991;87:505-10.
44. Remillard JF, Gould MC, Roslansky PF, Novitsky TJ. Quantitation of endotoxin in products using the LAL kinetic turbidimetric assay. In: Watson SW, Levin J, Novitsky TJ, editors. Detection of bacterial endotoxins with the Limulus Amebocyte Lysate Test. New York: Alan R. Liss, Inc; 1987. p. 197-209.
45. Custovic A, Simpson B, Simpson A, Hallam C, Craven M, Woodcock A. Relationship between mite, cat, and dog allergens in reservoir dust and ambient air. *Allergy* 1999;54:612-6.
46. Arruda LK, Vailes LD, Ferriani VP, Santos AB, Pomes A, Chapman MD. Cockroach allergens and asthma. *J Allergy Clin Immunol* 2001;107:419-28.
47. Rosenstreich DL, Eggleston P, Kattan M, Baker D, Slavin RG, Gergen P, et al. The role of cockroach allergy and exposure to cockroach allergen in causing morbidity inner-city children with asthma. *N Engl J Med* 1997;336:1356-63.
48. Gold DR, Burge HA, Carey V, Milton DK, Platts-Mills T, Weiss ST. Predictors of repeated wheeze in the first year of life. The relative roles of cockroach, birth weight, acute lower respiratory illness, and maternal smoking. *Am J Respir Crit Care Med* 1999;160:227-36.
49. Finn PW, Boudreau JO, He H, Wang Y, Chapman MD, Vincent C, et al. Children at risk for asthma: home allergen levels, lymphocyte proliferation, and wheeze. *J Allergy Clin Immunol* 2000;105:933-42.
50. 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;107:41-7.
51. Custovic A, Simpson A, Pahdi H, Green RM, Chapman MD, Woodcock A. Distribution, aerodynamic characteristics, and removal of major cat allergen Fel d 1 in British homes. *Thorax* 1998;53:33-8.
52. Ball TM, Castro-Rodriguez JA, Griffith KA, Holberg CJ, Martinez FD, Wright AL. Siblings, day-care attendance, and the risk of asthma and wheezing during childhood. *N Engl J Med* 2000;343:538-43.