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USF INTERNAL MEDICINE & PEDIATRIC DIVISIONS OF ALLERGY AND IMMUNOLOGY

Another key historical article of scientific impact in the medical literature is “House dust mite, cat, and cockroach allergen concentrations in daycare centers in Tampa, Florida” by Fernández-Caldas E, Codina R, Ledford DK, Trudeau WL, and Lockey RF, *Annals of Allergy, Asthma & Immunology*, 2001.

From Richard F. Lockey, MD:

This article appeared in the *Annals of Allergy, Asthma, & Immunology* in 2001.

This past week, for Wednesday morning conference, we learned about aeroallergen contamination of schools. This paper indicated 21 years ago that mite, cat, and cockroach allergens are present in 40% of daycare centers in Tampa at concentrations associated with sensitization and the onset of allergic symptoms. Indoor aeroallergens are universally present in the indoor environment. These include dust mite and cockroach debris, but in many cases, also cat, dog, and other animal emanations. Cat and dog dander are no longer just primarily limited to homes.

With warm regards,

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House dust mite, cat, and cockroach allergen concentrations in daycare centers in Tampa, Florida

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Background: Allergen exposure in early childhood is a risk factor for sensitization and the development of asthma. Studies performed in Europe, New Zealand, and Singapore indicated the presence of indoor allergens in childcare centers and schools. However, the importance of indoor allergens in daycare centers in humid and warm regions of the world is not known.

Objective: To measure total mite counts, Der p 1, Der f 1, Fel d 1, and Per a 1 allergens in dust samples and mite allergen airborne concentrations in daycare centers in Tampa, Florida, United States.

Methods: Twenty daycare centers were surveyed for mite, cat, and cockroach allergens in Tampa, FL. One dust and two air samples (one during the day and one during the night) were collected in each center. Dust samples were extracted and analyzed for mite (Der p 1 and Der f 1), cat (Fel d 1), and cockroach (Per a 1) allergens. Mite airborne allergen concentrations were analyzed by RAST inhibition and expressed in standardized mite allergen units per m³ of air (AU/m³).

Results: Mites were identified in 15 samples, and concentrations ranged from 10 to 1,200 mites/g (298 ± 355.2). The most prevalent mite species was *Dermatophagoides pteronyssinus* (Der p 1). Der p 1 and/or Der f 1 were detected in 10 daycare centers. Der p 1 was detected in eight centers and ranged from 1 to 21.8 $\mu\text{g/g}$ of dust (5.4 ± 6.9); Der f 1 was detected in 3 centers and ranged from 0.2 to 2.1 $\mu\text{g/g}$ of dust (1.3 ± 0.9). Per a 1 and Fel d 1 were detected in all centers in small quantities; Per a 1 ranged from 8 to 1,806 ng/g (263.1 ± 449.7) and Fel d 1 from 0.2 to 120 U/g of dust (16.6 ± 31.7), respectively. Airborne mite allergen was detected in 18 centers and ranged from 0.01 to 2.7 AU/m³ during the day (0.2 ± 0.6) and from 0.01 to 0.12 AU/m³ during the night (0.06 ± 0.03), $P = 0.001$.

Conclusions: Mite, cat, and cockroach allergens are present in daycare centers in Tampa, FL. Mite allergen concentrations exceeded levels that have been associated with sensitization and symptoms in allergic subjects in 40% of these centers.

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INTRODUCTION

Allergen exposure in early childhood is a risk factor for sensitization and development of asthma.^{1,2} The increase in number of homes with two income sources and single-parent families has

resulted in a larger number of children attending daycare centers for 8 or more hours a day in the United States. Studies in other countries have investigated allergen concentrations in public institutions, including childcare centers and schools.^{3–10} Zhang et al⁵ performed a study in Singapore, a tropical country, investigated the presence of indoor allergens in schools and daycare centers. This study demonstrated the presence of low levels of mite, cat, dog, and cockroach in most schools and daycare centers, but in some cases, the levels exceeded the threshold values

for sensitization. Similar results were reported by Dornelas de Andrade et al⁴ in 30 French day nurseries, but allergen levels in homes were higher than those measured in daycare centers. Studies from Sweden and New Zealand demonstrated the presence of higher levels of cat and dog allergens in schools than those found in homes without pets.^{7–9} In a study performed in British schools, 65% of classrooms had Bla g 2 levels greater than the threshold that causes sensitization.⁶ Sarpong et al³ found similar results; they detected the presence of Bla g 1 at potentially significant concentrations in some inner-city schools in Baltimore, MD.

High levels of mite allergen and several mite species have been described in house dust samples collected in Tampa homes.¹¹ High specific IgE levels to *Dermatophagoides pteronyssinus*, *Alternaria alternata* and *Blatella germanica* are prevalent among asthmatic children treated in emergency rooms in Tampa.¹² This study demonstrated that 90% of the asthmatic children evaluated for an acute exacerbation of their asthma in a pediatric emergency room in the Tampa Bay area are sensitized primarily to mite allergens.

The purpose of this study was to survey 20 daycare centers in Tampa, to identify mite species in dust samples, and to quantify mite, cat, and cockroach allergen levels using immunochemical methods.

MATERIALS AND METHODS

Selection of Daycare Centers

Managers of daycare centers were asked to participate in the study and allow the collection of dust and air samples. A questionnaire, including in-

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quiries about the age of the building, floor covering, and the use of insecticides was used. Twenty centers agreed to participate in the study.

Collection and Analysis of Dust Samples

Dust samples were collected in the playroom area using a 1.7 peak horsepower vacuum cleaner. This hand-held vacuum cleaner incorporated a polytetrafluoroethylene filter that retains particles larger than 0.3 μm . After collecting dust from 1 m^2 for 2 minutes, the filters were folded, transported to the laboratory in sealed plastic containers and stored at 4° C until analyzed. Mites were counted and identified using methods previously described.¹¹ Total mite counts in each sample were expressed as mites/g of dust.

Extraction of Dust Samples and Allergen Measurement

Dust samples were extracted 1:20 wt/vol with 0.2 mol/L ammonium bicarbonate for 16 hours at 4° C and centrifuged at 8,000 rpm for 20 minutes. The supernatants were centrifuged, preserved in 50% glycerin, and stored at -20° C until analysis. Der p 1, Der f 1, and Fel d 1 were quantitated by ELISA using methods previously described.^{13,14} Per a 1 was measured using a sandwich ELISA. The standard curves and controls were maintained in 50% glycerin and di-

luted in phosphate buffered saline. The assay was based on a monospecific rabbit antibody preparation reactive with determinants shared by Per a 1 and Bla g 1 from American and German cockroaches. The sensitivity was 0.2 ng Lowry protein of Per a 1 equivalents per milliliter, corresponding to 1 ng of Per a 1 equivalents per gram of dust. The assay did not react with non-cockroach-allergen sources.¹⁵

Collection and Extraction of Air Samples

Mite airborne allergens were collected using an Air Sentinel air sampler (Rupprecht & Patashnick, Albany, NY)¹⁶ and Teflon filters (DuPont, Wilmington, DE). Two air samples were collected in each daycare center. The Air Sentinel was placed in the main playroom area and two samples were collected from 8 AM to 6 PM and from 6 PM to 8 AM with an air flow of 14 m^3/hour . The inlet of the machine was placed 1.5 meters above the floor. The exposed sides of the filters were extracted with 1 mL 0.1 mol/L phosphate buffer, pH 7.5, for 16 hours by soaking and gentle mixing. The supernatants were transferred to Brinkman tubes (Brinkman Instrument Co., Westbury, NY), centrifuged at 6,000 rpm, the pellets discarded, and the supernatants preserved at -20° C in 50% glycerin. Airborne mite allergen levels were

measured by a RAST inhibition assay using methods previously described.¹⁷ A standardized extract of *D. pteronyssinus* (ALO, Dayton, OH) containing 10,000 allergy units (AU)/mL was used as a reference standard. A serum pool prepared with sera of six patients with high titers of specific IgE to *Dermatophagoides* spp. was used. Airborne cockroach and cat allergens were not measured, because sera pools with high titers of specific IgE to these allergens were not available.

Statistical Analysis

Student's *t* test, regression analysis, and mean and standard deviations were calculated using a Macintosh SE computer (Apple Computer Inc, Cupertino, CA) and the Statview program (Brain Power Inc, Ventura, CA).

RESULTS

Only four daycare centers had linoleum floors. The rest of the buildings had wall-to-wall carpets. Mites were identified in 15 daycare centers and ranged from 10 to 1200 mites per gram of dust (298 ± 355.2 ; Fig 1). Six different mite species were identified and the most prevalent were *D. pteronyssinus*, found in 10 samples, and *D. farinae* in 5 (Table 1). Ten dust samples had more than 100 mites per gram of dust.

Mite numbers, Der p 1 and Der f 1, Per a 1, and Fel d 1 levels showed a scattered distribution. Der p 1 and/or Der f 1 were detected in 10 centers (Fig 2); Der p 1 was detected in eight centers and the levels ranged from 1 to 21.8 $\mu\text{g}/\text{g}$ of dust (5.4 ± 6.9). Der f 1 was detected in three centers and

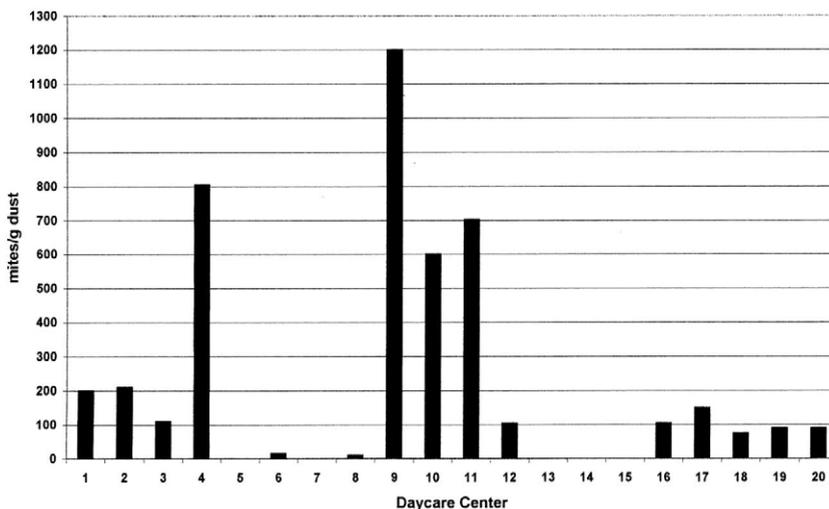


Figure 1. Mite numbers in dust samples

Table 1. Qualitative and Quantitative Analysis of 20 Dust Samples Collected in 20 Daycare Centers in Tampa, Florida

Species	Found in	(%)	Range*
<i>D. pteronyssinus</i>	10	50	10-1200
<i>D. farinae</i>	5	25	50-100
<i>Blomia tropicalis</i>	1	5	50
<i>Cheyletus malaccensis</i>	1	5	100
Mesostigamata	1	5	50
Oribatida	1	5	50

* mites per gram of dust.

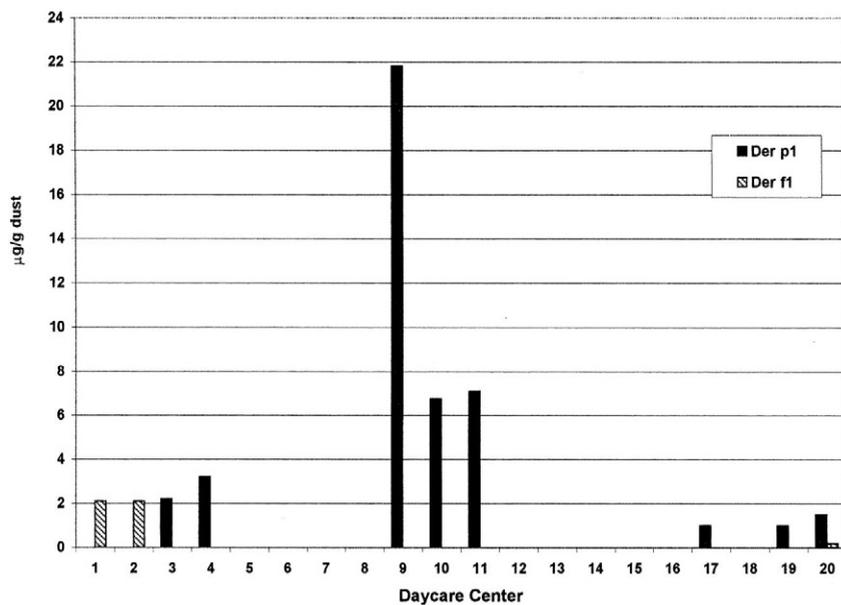


Figure 2. Mite allergen (Der p 1 and Der f 1) levels in settled dust

ranged from 0.2 to 2.1 $\mu\text{g/g}$ of dust (1.3 ± 0.9). Ten centers had undetectable levels of mite allergens. Eight daycare centers (40%) had mite allergen levels $>2 \mu\text{g/g}$ of dust.

Per a 1 was detected in all dust samples and ranged from 8 to 1,806 ng/g (263.1 ± 449.7 ; Fig 3). Fel d 1 allergen was detected in small amounts in all centers ranging from 0.2 to 120 U/g of dust (16.6 ± 31.7).

Airborne mite allergens were detected in 18 centers and ranged from 0.01 to 2.7 AU/m^3 during the day (0.2 ± 0.6) and from 0.01 to 0.12 AU/m^3 during the night (0.06 ± 0.03), $P = 0.001$ (Fig 4). The highest mite aeroallergen level, 2.7 AU/m^3 , was measured during the day in the center that had the highest Der p 1 allergen concentration in dust (21.8 $\mu\text{g/g}$ of dust).

DISCUSSION

This study demonstrates the presence of mites and indoor allergens (Der p 1, Der f 1, Per a 1, and Fel d 1) in 20 daycare centers in Tampa. Experimental and epidemiologic studies suggest that the threshold level for sensitization to group 1 mite allergens might be $<2 \mu\text{g/g}$ of dust and that any level should be considered a risk factor for

sensitization in genetically predisposed individuals.¹⁸ Therefore, mite allergen exposure should be reduced as much as possible in daycare centers. Group 1 mite allergen levels were $>2 \mu\text{g/g}$ of dust in 40% of the centers and airborne mite allergens were detected in 80% of them. Threshold mite aeroallergen levels for sensitization or symptoms have not been established, but an aeroallergen concentration of 2.7 AU/m^3 should be considered high, as it was recorded

in the daycare center with the highest mite allergen level in the carpet (21.8 $\mu\text{g/g}$ of dust). This level was recorded during the day, when the center was occupied. Mite aeroallergen levels recorded during the night in the same center were significantly lower. These data suggest that mite allergens become airborne because of reservoir disturbance during daily activities.

Mite allergens in air samples were detected in some daycare centers in which Der p 1 and Der f 1 allergens in dust samples were below the detection levels of the techniques. Mites other than *D. pteronyssinus* and *D. farinae* were found in some daycare centers, and their allergens could be detected by RAST-inhibition, but not by ELISA using monoclonal antibodies.

Cat allergen levels $>8 \mu\text{g}$ of Fel d 1 per gram of dust are also considered a risk factor for sensitization. Cat allergen levels were consistently below this suggested threshold of sensitization, although Fel d 1 was detected, in small amounts, in all centers. These data are in agreement with previous reports, ie, that cat allergen levels in day care centers are low, as compared with those measured in homes with a cat.^{4,5} However, studies performed in Sweden and New Zealand detected Fel d 1 levels in high concentration in school classrooms.⁷⁻⁹

Per a 1 was detected in all daycare centers in variable quantities. Thresh-

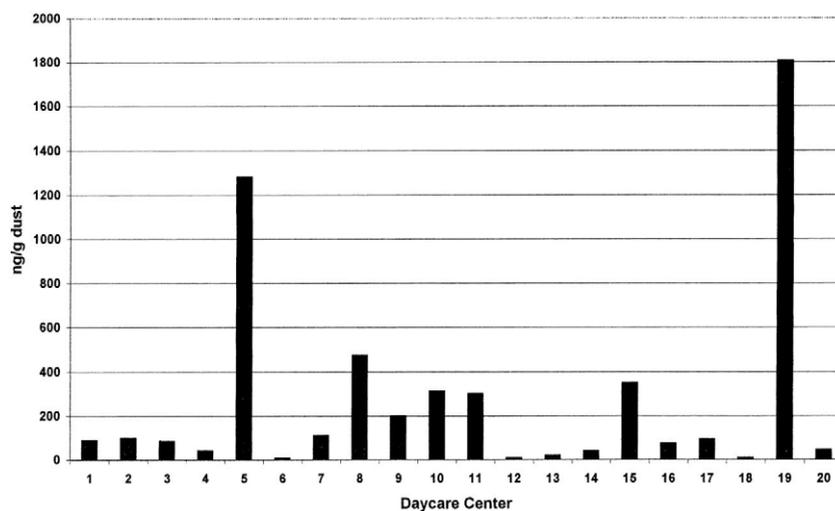


Figure 3. Cockroach allergen (Per a 1) levels in settled dust

old levels of Per a 1 for cockroach sensitization have not been established. Per a 1 is a prevalent allergen in Tampa, FL. The presence of Per a 1, using the same assay, was verified in 73 house dust samples collected in Tampa, FL. Only two samples were below the detection limit (<1 ng Per a 1 equivalent per gram of dust) and the remaining 71 samples contained 2 ng to 200 $\mu\text{g/g}$ of dust. Most samples contained between 10 ng and 10 μg of Per a 1/g dust.¹⁵ The levels detected in the daycare centers in Tampa are between these domestic limits. Studies performed in Singapore, Baltimore, and the United Kingdom found high levels of Bla g 1 and Bla g 2 in schools.^{3,5,6} Because the assay used in this study also detects Bla g 1, the levels measured may also reflect exposure to *B. germanica*. It can be concluded that exposure to cockroach allergens in daycare centers in Tampa is similar to what occurs in homes. Mold exposure and humidity problems in the buildings were not evaluated, but it has been suggested that exposure to molds and humidity problems in schools are also an important risk factor for developing respiratory problems.^{19,20}

Sixteen of the daycare centers had carpeted floors. Renovation of daycare centers and installing ventilated floors, which protect the floor material from moisture, has been recommended to decrease cat and dog allergen exposure.¹⁰ The removal of carpets in daycare centers has been recommended to avoid the accumulation of dust and allergens. Similar suggestions have been made in other studies.²¹ However, the risk-benefit from injuries has to be considered when carpets are removed. Perhaps a soft pliable tile or linoleum may be an ideal floor cover for daycare centers.

The prevalence of asthma and sensitization to indoor allergens in children attending daycare centers in the United States is unknown. However, high rates of asthma prevalence have been reported in school children in the United States. Sixteen percent of students in a stratified-cluster random sample of 3,670 children in the 7th and 8th grades had asthma. Prevalence

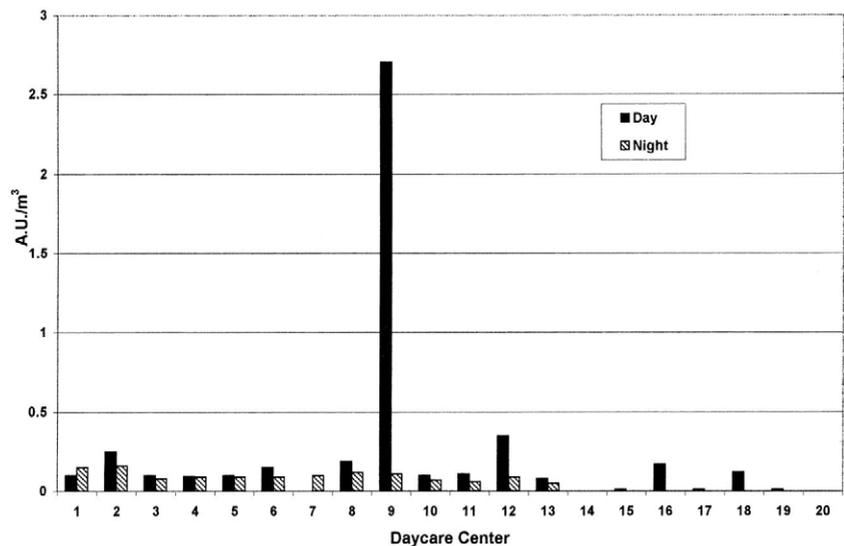


Figure 4. *D. pteronyssinus* aeroallergen concentrations

rates were significantly higher in schools with >98% African-Americans. Rates were also associated with a low median income.²²

The importance of indoor air quality on respiratory symptoms of the preschool child is unknown. However, the improvement in symptoms of allergic children after removal from environments with a large allergen burden and the association of respiratory diseases with allergens and irritants suggest that air quality plays an important role.

There is conflicting information about the benefits of attending daycare centers and the future development of allergic diseases.^{23–25} A study performed in the former East Germany suggests that daycare center attendance, and therefore, early infections, may decrease the likelihood of developing allergic diseases later in life.²³ However, the European Community Respiratory Health Survey found no such association between daycare attendance and adult atopy.²⁵

CONCLUSION

This study demonstrates that indoor allergens are prevalent in daycare centers in Tampa, and that in many instances, mite concentrations exceed known risk levels for sensitization and for causing allergic respiratory symptoms.

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