Dustborne *Alternaria alternata* antigens in US homes: Results from the National Survey of Lead and Allergens in Housing

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Background: ***Alternaria alternata*** **is one of the most common fungi associated with allergic disease. However,** ***Alternaria*** **exposure in indoor environments is not well characterized.**
Objective: The primary goals of this study were to examine the prevalence of ***Alternaria*** **exposure and identify independent predictors of** ***Alternaria*** **antigen concentrations in US homes.**
Methods: Data for this cross-sectional study were obtained from the National Survey of Lead and Allergens in Housing. A nationally representative sample of 831 housing units in 75 different locations throughout the United States completed the survey. Information on housing and household characteristics was obtained by questionnaire and environmental assessments. Concentrations of ***A alternata*** **antigens in dust collected from various indoor sites were assessed with a polyclonal anti-*** ***Alternaria*** **antibody assay.**
Results: ***Alternaria*** **antigens were detected in most (95% to 99%) of the dust samples. The geometric mean concentration, reflecting the average** ***Alternaria*** **concentration in homes, was 4.88 μg/g (SEM, 0.13 μg/g). In the multivariable linear regression analysis, the age of the housing unit, geographic region, urbanization, poverty, family race, observed mold and moisture problems, use of dehumidifier, and presence of cats and dogs were independent predictors of** ***Alternaria*** **antigen concentrations. Less frequent cleaning and smoking indoors also contributed to higher** ***Alternaria*** **antigen levels in homes.**
Conclusion: Exposure to ***A alternata*** **antigens in US homes is common. Antigen levels in homes are influenced not only by regional factors but also by residential characteristics. Preventing mold and moisture problems, avoiding smoking indoors, and regular household cleaning may help reduce exposure to** ***Alternaria*** **antigens indoors. (J Allergy Clin Immunol 2005;116:623-9.)**

Key words: *Alternaria alternata, allergen, antigen, indoor, exposure, asthma, allergy*

Exposure to the fungus *Alternaria alternata* is an important risk factor for asthma and allergic rhinitis.[1-5] Severe asthma and acute, sometimes life-threatening exacerbations of asthma have been associated with *Alternaria* sensitivity and increased airborne concentrations of *Alternaria* spores.[6-10]

Alternaria spores are common aeroallergens in many regions of the world, especially in warm inland climates, but also in arid regions.[9,11] *Alternaria* exposure is often assessed by outdoor spore counts, because most intense exposure is likely to occur outdoors.[12-14] Nonetheless, fungal spores can enter a home from outdoor air via ventilation or infiltration, or they can be carried in by occupants.[15,16] Infiltration may have less importance for *Alternaria* spores because of their large size (23-34 μm × 7-10 μm).[17] The indoor environment may also become a secondary source of exposure if fungal spores colonize interior or building materials.[15,16] Although indoor fungal levels tend to reflect the levels found outdoors, housing characteristics and occupants' behavior can affect exposure levels considerably.[15,16,18,19]

Because of the complexity of fungal exposure assessment, few studies have assessed exposure to *Alternaria* or other fungal allergens in indoor environments.[20] Fungal allergen extracts have largely remained uncharacterized and nonstandardized, unlike other common allergens derived from cat, dog, dust mite, cockroach, or pollens.[21,22] Exposure to fungal allergens is traditionally estimated by indirect methods, considering spores as indicators of the presence of allergens.[23] However, allergen content in spores may vary, and fungal allergens may also be carried by means other than intact spores (eg, hyphael fragments).[24-26] Therefore, spore counts may not accurately reflect allergen exposure levels. Recent advances in molecular biology and immunology have facilitated progress in qualifying and quantifying fungal

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Abbreviations used
MSA: Metropolitan statistical area
NSLAH: National Survey of Lead and Allergens in Housing

allergens, especially *Alternaria* allergens.[16,22,27] The National Survey of Lead and Allergens in Housing (NSLAH) is the first population-based study that measured antigenic components of *A alternata*, including allergens, in US homes using a polyclonal anti-*Alternaria* antibody assay.

This article presents nationally representative estimates of dustborne *A alternata* antigen levels at multiple household sites and identifies independent predictors of *Alternaria* antigen concentrations in US homes.

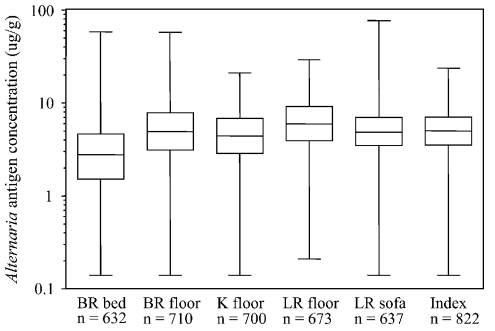

FIG 1. Distributions of *Alternaria* antigen concentrations in US homes. *Box plots* display the minimum and maximum values and the 25th, 50th, and 75th percentiles. *BR,* Bedroom; *K,* kitchen; *LR,* living room; *Index,* house index.

METHODS

Study data

The data for this study were collected as part of the NSLAH. This cross-sectional study, which was conducted from 1998 to 1999 by the National Institute of Environmental Health Sciences and the US Department of Housing and Urban Development, used a complex, multistage design to sample the US population of permanently occupied, noninstitutional housing units that permit children. The study protocol was approved by the National Institute of Environmental Health Sciences Institutional Review Board in 1998. The sampling frame of 1404 primary sampling units consisted of metropolitan statistical areas (MSAs), counties, or groups of counties. MSAs included areas with a large population nucleus and adjacent communities having a high degree of economic and social integration with the area. Every area in the 50 states and the District of Columbia was assigned to a primary sampling unit. A nationally representative random sample of housing units was drawn from 75 randomly selected primary sampling units. In all, 831 housing units were surveyed. A detailed description of the methodology for the survey has been previously published.[28]

At each home, a trained interviewer obtained information on housing characteristics and the occupants' household via questionnaire. A copy of the questionnaire can be found online (www.niehs.nih.gov/airborne/research/risk.html). Environmental data were also acquired by inspection and sample collection. Detailed, well-defined protocols for all aspects of data and sample collection were used throughout the study.[28] Briefly, single surface dust samples were collected from a bed (all bedding layers, pillow, and mattress or mattress cover), a sofa, or a chair, and from bedroom, living room, and kitchen floors, as previously described.[28] Vacuumed dust samples were collected by using a Eureka Mighty-Might 7.0-ampere vacuum cleaner (Eureka Co, Bloomington, Ill) modified to collect dust into a 19 mm × 90 mm cellulose extraction thimble (Whatman International, Ltd, Maidstone, United Kingdom). Each sampling site was vacuumed for 5 minutes. For bedding samples, all bedding layers were vacuumed for a total of 2.5 minutes, the primary sleeping pillow for 30 seconds, and the mattress or mattress cover for 2 minutes.

At the laboratory, dust samples were sieved through 425-μm pore grating and divided into 100-mg aliquots of fine dust. Dust aliquots were extracted in borate buffered saline (pH 8.5), 2 mL per 100 mg dust extracted. After extracts were centrifuged, supernatants were decanted and stored at −20°C. Concentrations of the *A alternata* antigens were measured with a competitive inhibition ELISA using a commercially prepared polyclonal rabbit anti-*Alternaria* antibody (lot #ZA4-4L; Greer Laboratories, Inc, Lenoir, NC) and *Alternaria* antigen standard (lot #XPM1-X10; Greer Laboratories, Inc).[20,24] Briefly, antigen standard at 1 μg/mL in bicarbonate buffer, pH 9.6, was added to 96-well Immunlon 4HBX plates (VWR Scientific, Willard, Ohio) and incubated overnight at 4°C. Unbound antigen was washed away (with phosphate buffered saline, pH 7.4), and the plate was blocked with BSA. After washing, anti-*Alternaria* antibody, along with either dilutions of unknown samples or dilutions of the antigen standard, were combined in the wells and incubated overnight at 4°C. Unbound material was washed away, and peroxidase-labeled goat antirabbit IgG (Sigma Chemical, St Louis, Mo) was added to the wells and incubated for 1 hour. Excess antibody was washed away and substrate added; color change was measured kinetically at 405 nm by using an OptiMax plate reader (Molecular Devices, Sunnyvale, Calif). Reaction rates of the unknown sample were plotted against those of the antigen standard to determine concentration. Optimal assay dilutions were determined empirically by using dilution matrices. The assay detects major *Alternaria* antigens, including the most common allergen, Alt a 1.[29] For most samples, the lower limit of detection of the assay was 0.14 μg/g sieved dust.

Statistical analyses

Statistical analyses were conducted by using SUDAAN (version 8.0; Research Triangle Institute, Research Triangle Park, NC), and Taylor series linearization methods were used to adjust SEs for the complex survey design. Sample weights were applied to all estimates to account for housing selection probabilities, nonresponse, and poststratification. A detailed description of statistical weighting for the NSLAH is described elsewhere.[28]

In the statistical analysis, *Alternaria* antigen concentrations were log-transformed because the distributions were skewed to the right. Samples with concentrations less than the detection limit were assigned ½ the value of the detection limit. Samples having insufficient amount of dust for analysis were considered missing (Fig 1). We used Spearman rank correlation coefficients to evaluate associations between antigen concentrations. We calculated a house index (ie, the mean of all sampling location concentrations) to represent the average *Alternaria* antigen concentration in the household.

Descriptive statistics of *A alternata* antigen concentrations were generated. Median and mean (geometric) concentrations were estimated for each level of selected household characteristics. Comparisons of the log-transformed means were assessed with ANOVA by using Wald *F* statistics. Characteristics in Tables I and II with *P* values less or equal to .25 were selected to multivariable