Residential Fungal Contamination and Health: Microbial Cohabitants as Covariates

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An association between symptoms and residential mold growth has been consistently observed in several countries, but the contribution of dust mites and bacterial endotoxins to this relation has not been established. To address this issue, we studied a sample of 403 Canadian elementary school children during the winter months. Reported mold growth was compared to respiratory and nonspecific symptoms before and after adjusting for dust mite antigens and bacterial endotoxin. A 12–50% relative increase in symptom prevalence was associated with reported mold growth both before and after adjusting for subject characteristics, dust mite antigens, and endotoxins. In conclusion, the association between residential fungal contamination and symptoms is not confounded by dust mites or bacterial endotoxins or other known disease-causing agents. *Key words*: bacteria, dust mites, epidemiology, fungus, health. — *Environ Health Perspect* 107(suppl 3):481–483 (1999).
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Questionnaire-based studies from several countries have consistently found an increased prevalence of respiratory symptoms in residents of homes reported to be damp or moldy (*1–5*). The apparent explanation is that airborne spores or mycelia are causing illness through allergic or toxic mechanisms (*6,7*). However, both dust mites and bacterial endotoxins are known disease-causing agents (*8,9*). Thus, mites and endotoxins are plausible confounders of the mold–health association; they may be associated with the exposure of interest (fungus) and are risk factors for disease (symptoms). Williamson et al. (*10*) reported a positive association between physician-diagnosed asthma and objectively measured dampness but not between asthma and mold growth seen by a qualified surveyor. This raises the possibility that dampness causes illness independent of fungus, for example, by increasing the burden of dust mites or Gram negative bacterial endotoxins, both of which may be related to water sources. A recent review of nine population-based cross-sectional studies with objective fungal measurements similarly concluded that future studies must address the influence of copollutants such as endotoxins and dust mites (*11*). This present study investigates the influence of dust mites and bacterial endotoxins on the observed association between reported fungal contamination and symptoms.

Materials and Methods

Study Group

Families of elementary school children in Wallaceburg, Ontario, Canada were sent letters of introduction and then telephoned. Consenting families were consecutively recruited until the target sample size of 400 subjects was obtained. From each home, the one child closest to 10 years of age was studied. The participation rate was low, about 35%, but no differences were found when the sample was compared to population statistics obtained from a Provincial health survey.

Organization of the Data Collection

Home visits occurred during the winter as follows. The parent most knowledgeable about the child's health was asked to complete a questionnaire. Air samples from both the main living area and the child's bedroom were collected over a 14- to 20-hr period at a flow of 1.7–2.0 L/min on endotoxin-free nucleopore filters. The following day, during the second visit, the air samplers were turned off, and a dust sample was vacuumed from the child's mattress and coverings (2 min) and the entire main living area floor (10 min).

The Questionnaire

Exposure questions. The presence of mold growth was defined as a "yes" response to both of the following questions: *a*) Have you ever had mold or mildew growing on any surface inside your present home? and *b*) Did this occur in the past 12 months? In a previous study, test–retest agreement for this two-question combination was 87% [95% confidence interval (CI), 85–90%] with a corresponding Kappa statistic of 0.73 (95% CI 0.68–0.79) (*12*).

Health Questions. To reduce the number of statistical comparisons and probability of type I errors, health questions were summarized as follows:

GENERAL SYMPTOMS. In the past month has this child experienced any of the following: Headaches, muscle aches, fever and chills, nausea, diarrhea, difficulty concentrating, irritability?

IRRITATION. In the past month has this child experienced any of the following: itchy eyes, skin rash or itch, nose irritation?

COUGH OR WHEEZE. Does this child usually cough during the night or first thing in the morning? (or) Has this child ever wheezed during the night in the past 12 months?

CHEST ILLNESS. During the past 12 months, did this child have any chest illness? (and) Did the child have more than one such illness?

ASTHMA. Has the doctor ever said that this child had asthma? (and) Does he/she still have asthma? (or) Does he/she currently take medicine for asthma regularly (usually everyday)?

BACTERIAL ENDOTOXIN. Air samples from the bedroom and living area, and dust samples from the bedroom were analyzed for endotoxin. Air filters were washed in 8 ml of pyrogen-free water for 60 min. Two hundred milligrams of dust were washed in 10 ml of water for 60 min. The water extracts were submitted to a Limulus amoebocyte lysate assay using a chromogenic test kit (Associates of Cape Cod, Woods Hole, MA) (*13,14*) in a kinetic

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assay on an MR 5000 Microplate reader (Dynatech Laboratories, West Sussex, UK). Samples were tested in duplicate in 96-well pyrogen-free plates along with a blank (pyrogen-free sterile water). A standard curve was constructed from a control standard endotoxin. Readings were considered acceptable when the log time to a defined optical density versus log concentration had an $R^2 > 0.98$. The detection limit may be as low as 0.005 endotoxin units per milliliter.

DUST MITE ANTIGENS. Immunoassays using monoclonal antibodies for Der p 1 and Der f 1 were used to detect the presence of *Dermatophagoides pteronyssinus* and *Dermatophagoides farinae* in the bedroom dust samples. These were collected by vacuuming the entire mattress and coverings for 2 min using a Euroclean HEPA-filtered UZ 930 vacuum with a cotton filter (Cambridge, Ontario, Canada) attached at the entry port. Analysis was performed by The Department of Allergy and Clinical Immunology at Johns Hopkins University. Results were based on the World Health Organization international standard extract "National Institute for Biological Standards and Control 82/518" (*15*).

Results

Characteristics of the subjects are presented in Table 1. Of note, parental history of allergic disease was high and yet so was pet ownership and environmental tobacco smoke, the latter being higher in homes where mold was reported to be absent. Median values of dust endotoxin and Der p 1 were higher in homes reporting mold growth (Table 2). There was also a 12-50% relative increase in symptom prevalence associated with mold growth (Table 3). The symptom–mold association was adjusted using BMDP multivariate logistic regression for all the characteristics of the subject and family listed in Table 1. Only smoking contributed to the model at $p < 0.05$. Adjusted odds ratios (OR) remained above 1 for all symptoms except for asthma, and CIs excluded 1 for general and irritative symptoms (Table 4). The symptom–mold association was then adjusted for all the measures of dust mite antigen and bacterial endotoxins listed in Table 2. Only the living area and bedroom Der p 1 contributed to the model at $p < 0.05$. Again, adjusted OR values remained above 1 for all symptoms except for asthma, and CIs excluded 1 for general and irritative symptoms. Controlling for all significant factors did not change the adjusted associations further.

Table 1. Distribution of index child and family characteristics as a function of absence/presence of reported mold/mildew in past 12 months.

Child and family characteristics	Mold/mildew[a] Absent	Present
Child's age, mean years	9.8	10.0
Child's sex, female	54.1%	49.4%
Parental allergies, hay fever, asthma	43.8%	51.1%
Parental education, more than high school	57.9%	67.0%
Pets in home	57.5%	65.0%
Household smokers[b]	54.1%	42.0%

[a] *n* values in the "absent" mold/mildew category vary from 202–208 across variables; *n* values in the "present" category vary from 176–180. [b] $p < 0.05$, χ^2 test.

Table 2. Median (25th, 75th percentiles) of endotoxins and dust mite antigens as a function of absence/presence of reported mold/mildew in past 12 months.

Environmental characteristics	Mold/mildew[a] Absent	Present
Endotoxins		
Living area dust (eu/mg)	160 (40, 890)	270 (45, 835)
Bedroom air (eu/m^3)	4.6 (0.9, 21.8)	2.4 (0.9, 14.3)
Living area air (eu/m^3)	0.7 (0.0, 1.4)	0.7 (0.4, 1.1)
Dust mites		
Bedroom Der f (ng/g)	923 (194, 3582)	1173 (289, 3943)
Living area Der f (ng/g)	642 (201, 3377)	605 (173, 2939)
Bedroom Der p I (ng/g)[b]	337 (91, 1829)	627 (114, 4999)
Living area Der p I (ng/g)[b]	211 (60, 1120)	627 (63, 3802)

eu, endotoxin units. [a] *n* values in the "absent" mold/mildew category vary from 173–205 across variables; *n* values in the "present" category vary from 154–178. [b] $p < 0.05$, Mann-Whitney U-test.

Table 3. Prevalence of symptoms as a function of absence/presence of reported mold/mildew in past 12 months.

Prevalence of symptoms	Mold/mildew[a] Absent	Present
General[b]	66.3%	83.3%
Irritation[b]	72.6%	81.7%
Cough/wheeze[b]	26.2%	38.9%
Asthma	11.1%	14.4%
Chest illness	12.7%	18.9%

[a] *n* values in the "absent" mold/mildew category vary from 183–208 across variables; *n* values in the "present" category vary from 162–180. [b] $p < 0.05$, X^2 test.

Table 4. Unadjusted and adjusted odds ratios for the association between symptom and ever mold or mildew controlling for selected sets of factors.

	Unadjusted		Controlling for subject characteristics		Controlling for dust mites and endotoxins		Controlling for all factors[a]	
Symptom	OR	95% CI	OR	95% CI	OR	95% CI	OR	95% CI
General	2.28	1.31–3.97	2.26	1.29–3.95	2.26	1.27–4.00	2.25	1.26–4.00
Irritation	1.92	1.10–3.35	1.80	1.03–3.16	1.93	1.09–3.42	1.81	1.02–3.24
Cough/wheeze	1.43	0.84–2.43	1.36	0.79–2.33	1.36	0.79–2.35	1.28	0.74–2.23
Asthma	1.04	0.50–2.17	0.96	0.46–2.00	0.98	0.46–2.08	0.91	0.42–1.95
Chest illness	1.54	0.79–3.01	1.51	0.77–2.95	1.55	0.78–3.08	1.51	0.76–3.02

[a] The presence of smokers and continuous measures of bedroom and living area Der p I (ng/g) are included in this set.

Discussion

Similar to previous studies, an association between symptoms and residential fungal growth was detected. The concern about confounding was justified. Theoretically, fungus, dust mites, and Gram-negative bacteria may tend to coexist because of a common need for available water sources. Empirically we found that dust mites were higher in the bedrooms and living areas of houses with more reported mold. Endotoxins were unrelated to reported mold, however, and the levels detected were similar to those reported elsewhere (*16,17*). However, accounting for these agents did not change the magnitude of the ORs, demonstrating the absence of confounding. These findings indicate that it is more likely

that the observed association between symptoms and residential fungus is causal. Further evidence will come from the development of accurate measures of fungal exposure and follow-up studies to determine the long-term effects of chronic exposure at the relatively low levels commonly seen in residential settings.

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