Residential Fungal Contamination and Health: Microbial Cohabitants as Covariates

Robert E. Dales and David Miller

1University of Ottawa, and Air Quality Health Effects Research Section, Health Canada, 2Institute of Biochemistry, Department of Biochemistry, University of Ottawa, Ottawa, Ontario, Canada

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).

http://ehpnet1.niehs.nih.gov/docs/1999/suppl-3/481-483dales/abstract.html

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 every day)?

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

This article is based on a presentation at the International Conference on Indoor Mold and Children held 21–24 April 1998 in Alexandria, Virginia.

Address correspondence to R. Dales, Ottawa General Hospital, 501 Smyth Road, Ottawa, Ontario, K1H 8L6, Canada. Telephone: (613) 737-8198. Fax: (613) 737-8141. E-mail: rdales@ogh.on.ca.

The authors thank J. White, Canada Mortgage and Housing Corporation, Canada; and C. Dulong, statistician and epidemiologist, for their assistance. This study was supported by Canada Mortgage and Housing Corporation, Canada; Program on Energy Research and Development, Federal Government, Canada; and Health Canada.

Received 3 September 1998; accepted 2 December 1998.
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 BMDF 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 | 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. \( * p < 0.05, \chi^2 \) 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 | Absent | Present |
|--------------------------------|--------|---------|
| Endotoxins                    |        |         |
| Living area dust (eu/mg)      | 160 (40, 880) | 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, 3962) | 1173 (288, 3943) |
| Living area Der f (ng/g)      | 642 (201, 3377) | 605 (173, 2593) |
| 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. \( * \) n values in the “absent” mold/mildew category vary from 173–205 across variables; \( n \) values in the “present” category varied from 154–178. \( * p < 0.05, \text{ Mann-Whitney } \text{ U-test.} \)

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

| Prevalence of symptoms | 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%  | 19.9%   |

\( * \) n values in the “absent” mold/mildew category vary from 183–208 across variables; \( n \) values in the “present” category varied from 162–180. \( * p < 0.05, \chi^2 \) test.

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

| Symptom | Unadjusted OR | 95% CI | Controlling for subject characteristics OR | 95% CI | Controlling for dust mites and endotoxins OR | 95% CI | Controlling for all factors 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 |

\( * \) 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 sympotms 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.

References and Notes

1. Waegemaekers M, Van Wageningen N, Brunekreef B, Boleij J. Respiratory symptoms in damp homes. Allergy 44:1–7 (1989).
2. Martin CJ, Platt SD, Hunt SM. Housing condition and ill health. Br Med J 294:1125–1126 (1987).
3. Brunekreef B, Dockery DW, Speizer FE, Ware JH, Spengler JD, Ferris BG. Home dampness and respiratory morbidity in children. Am Rev Respir Dis 140:1363–1367 (1989).
4. Dales RE, Zwanenburg H, Burnett R, Franklin CA. Respiratory health effects of home dampness and moulds among Canadian children. Am J Epidemiol 134:196–203 (1991).
5. Verhoeef AP, van Strien RT, van Wijnen JH, Brunekreef B. Damp housing and childhood respiratory symptoms: the role of sensitization to dust mites and moulds. Am J Epidemiol 141:103–110 (1995).
6. Flannigan B, McCabe EM, McGarry F. Allergic and toxigenic micro-organisms in houses. J Appl Bacteriol 70(suppl):618–738 (1991).
7. Tobin RS, Baranowski E, Gilman A, Kuiper-Goodman T, Miller JD, Giddings M. Significance of fungi in indoor air: report from a working group. Can J Public Health 8(suppl):S1–S30 (1987).
8. Sporn R, Holgate S, Platts-Mills T, Cogswell J. Exposure to house-dust mite allergen (Der p I) and the development of asthma in childhood. N Engl J Med 323:502–507 (1990).
9. Michel M, Kips J, Duchateau J, Vertongen F, Robert L, Collet H, Pauwels R, Sergyels R. Severity of asthma is related to endotoxin in house dust. Am J Respir Crit Care Med 154:1641–1646 (1996).
10. Williamson IJ, Martin CJ, McGill G, Monie RDH, Fennerly AG. Damp housing and asthma: a case-control study. Thorax 52:229–234 (1997).
11. Verhoeef AP, Burge H. Health risk assessment of fungi in home environments. Ann Allergy Asthma Immunol 78:544–546 (1997).
12. Dales RE, Schweitzer I, Bartlett S, Raizenne M, Burnett R. Indoor air and health: reproducibility of respiratory symptoms and reported home dampness and moulds using a self-administered questionnaire. Indoor Air 4:2–7 (1994).
13. Remillard JF, Roslansky PF, Novitsky TJ. Quantification of endotoxin using the LAL kinetic turbidimetric assay in an incubating microplate reader. LAL Update 10:1–5 (1992).
14. Dawson ME. Endotoxin standards and CSE potency. LAL Update 11:2–5 (1993).
15. Hamilton RG, Chapman MD, Platts-Mills TA, Adkinson NF. House dust aeroallergen measurements in clinical practice: a guide to allergen-free home and work environments. Immunol Allergy Practice 14:9–26 (1992).
16. Michel O, Gianni R, Duchateau J, Vertongen F, Le Bob B, Sergyels R. Domestic endotoxin exposure an clinical severity of asthma. Clin Exp Allergy 21:441–448 (1991).
17. Milton, DK, Johnson DK, Park J-H. Environmental endotoxin measurement: interference and sources of variation in the Limulus assay of house dust. Am Ind Hyg Assoc J 58:861–867 (1997).