

Indoor airborne fungal spores, house dampness and associations with environmental factors and respiratory health in children

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Summary

Background Children living in a damp house are more likely to suffer from respiratory symptoms and it has been suggested that exposure to fungi is an important contributing factor. However, more knowledge about underlying mechanisms for the association are needed.

Objective To identify associations between measures of house dampness, levels of airborne fungal spores, housing factors and health outcomes in children.

Methods Eighty households with 148 children between 7 and 14 years of age were recruited in the Latrobe Valley, Victoria, Australia. Some 36% of participating children were asthmatic. Six sampling visits were made to each house between March 1994 and February 1995 on a 2-monthly cycle. Samples for airborne total and viable fungal spores were collected from bedrooms, living rooms, kitchens and outdoors. A detailed dwelling characterization, using a questionnaire and inspection surveys, was carried out. Skin-prick tests were performed with extracts of common aeroallergens and a respiratory questionnaire was completed for each child.

Results Large airborne fungal spore concentrations were recorded in association with: musty odour, water intrusion, high indoor humidity, limited ventilation through open windows, few extractor fans and failure to remove indoor mould growth. Visible mould growth or condensation evidence was associated with large concentrations of *Cladosporium* spores, but not with large total spore concentrations. *Penicillium* exposure was a risk factor for asthma, while *Aspergillus* exposure was a risk factor for atopy. Fungal allergies were more common among children exposed to *Cladosporium* or *Penicillium* in winter or to musty odour. Respiratory symptoms were marginally more common with exposure to *Cladosporium* or total spores in winter.

Conclusion Indoor exposure to certain fungal genera in winter was a risk factor for asthma, atopy and respiratory symptoms in children. On the other hand, no significant associations were seen between average viable or total spore concentrations and child health. Actual measurements of fungal spores predict health outcomes better than reported dampness.

Keywords: allergy, asthma, dampness, fungal spores, housing factors, indoor air, moulds, respiratory symptoms

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Introduction

Several large epidemiological studies have identified damp living conditions as a major risk factor for respiratory symptoms in children [1–4]. While these epidemiological studies were able to show that the respiratory health of children was adversely affected by damp living conditions, they provided little detail about the underlying mechanism for the association. Since both fungi and house dust mites thrive in damp conditions, an allergic reaction to either or both of these allergens is suggested as an important contributing factor in causing more respiratory symptoms among children residing in damp houses [5]. However, other mechanisms may also be important, as suggested by the results of a Canadian study where such allergies did not appear to account for more than part of the increase in disease [1]. An increased exposure to fungal metabolites such as β -glucans and mycotoxins has been suggested as such an alternative mechanism, as these compounds can have general immuno-suppressant or irritant properties and may therefore adversely influence the respiratory system [6].

While the health risks associated with exposure to dampness are now recognized, there is no reliable objective method of measuring exposure to dampness in a house which is suitable for epidemiology. In most studies, a simple subjective questionnaire has been used to classify houses as damp or affected by fungal contamination. While such classification has been useful in identifying the problem, more specific methods are needed in order to quantify the specific fungal exposure which is relevant to health. Air sampling for spore concentrations has been used in many previous studies to measure fungal exposure, but most studies using these methods have not included both a health assessment of the exposed subjects as well as environmental measurements. This paper presents data from a study where a detailed assessment of indoor environmental factors, including exposure to airborne fungi, was combined with an assessment of health outcomes in children. Consequently, a comparison of different ways of classifying exposure to indoor dampness and fungal exposure could be made and specific factors associated with child health could be identified. The purposes of this paper were to identify indoor environmental factors associated with airborne fungal spore concentrations and, to identify relevant exposure measures associated with respiratory illness in children.

Materials and methods

Eighty households with children between 7 and 14 years of age, residing in the Latrobe Valley, Victoria, Australia, were recruited as volunteers from local schools and doctor's

surgeries. Approval was obtained from the Standing Committee on Ethics in Research on Humans at Monash University (project number 73/93). Forty-three of the households had at least one asthmatic child between 7 and 14 years, diagnosed by a doctor, while the remaining 37 households had only non-asthmatic children. All children in the age-group living in the 80 houses were included as participants, making a total of 148 study children. Their mean age was 10.2 years at the start of the study, and equal numbers of girls and boys were included, i.e. 74 of each gender. Six visits were made to each house over a period of 1 year, with the first in March–April 1994, and the following on a 2-monthly cycle, finishing in January–February 1995.

Determination of airborne fungal spore levels

Air samples were collected for determination of both viable (culturable) and total fungal spore levels after some activity in the room. All samples were collected between 08.00h and 18.00 h. Viable fungal spore samples were collected from bedrooms of study children, living rooms, kitchens, and outside each house during each of the six sampling periods. A one-stage Andersen sampler (Andersen Instruments Inc., Atlanta, Georgia, USA) was operated at a flow rate of 24 L/min for 2 min and malt extract (20 g Oxoid CM057B/L) solidified with technical agar no. 3 (14 g Oxoid LP013B/L) was used as the collection media. No additions for prevention of bacterial growth were used. Samples were incubated for 4–5 days prior to counting the colonies. These counts were converted using the positive hole conversion method [7], and results are expressed as concentrations of colony forming units per cubic meter (CFU/m³). Identification of viable colonies to generic level was performed for the winter (July) and late spring (November) sampling periods.

Total fungal spore samples were collected from bedrooms, living rooms and outside the house during the six sampling periods using a Burkard personal spore trap (Burkard Manufacturing Co., Rickmansworth, UK), operated at a flow rate of 10 L/min for 9 min. Particles in the sampled air were trapped on a thin layer of silicone grease on a microscope slide. Fungal spores were counted on 5% of the total deposition area using a magnification of $\times 1000$ [8]. Results are expressed as concentrations of spores per cubic meter of air (S/m³).

Environmental factors

A detailed characterization was made of each dwelling. During the first sampling visit, both a dwelling questionnaire and a survey were completed. Collected data included the resident's opinion about house dampness, house age, foundation type, cladding type, extent of garden beds,

shading of house, frequency of ventilation through open windows, presence of air conditioners, fans and insulation. In addition, a detailed house survey looking for evidence of water intrusion, condensation, visible mould growth, and musty odour was performed in each sampled room during every sampling visit. Temperature and humidity were measured in all sampled rooms at the time of sample collection.

Health outcomes

A respiratory questionnaire was completed for each child during an interview with a parent during the last visit to each household. The questionnaire was based on the Monash Respiratory Questionnaire for which the reliability and validity has been established in adults [9]. The frequency of eight respiratory symptoms was recorded: cough, shortness of breath, waking due to shortness of breath, wheeze, asthma attacks, chest tightness, cough in the morning, and chest tightness in the morning. A child was considered symptomatic if at least one of these respiratory symptoms was present. In addition, the questionnaire included items about parental asthma, parental allergy, and presence of pets or smokers in the house.

Skin-prick tests were performed for 145 study children (three children were not tested) using 12 common aero-allergens (Hollister Stier, Spokane, WA, USA) including: cat, dog, grass mix no. 7, Bermuda grass, house dust, and house dust mites (*D. pteronyssinus* and *D. farinae*). In addition five fungal extracts were included: *Alternaria alternata* (*A. tenuis*), *Cladosporium* (*Hormodendrum*) *cladosporioides*, *Penicillium* mix, *Aspergillus* mix (for details see Hollister Stier catalogue), and Mould mix A (mixture of *Aspergillus* mix, *Botrytis cinerea*, *Chaetomium indicum*, *Epicoccum nigrum*, *Fusarium vasinfectum*, *Geotrichum candidum*, *Helminthosporium interseminatum*, *Monilia sitophila*, *Mucor racemosus*, *Phoma* spp., *Penicillium* mix, *Aureobasidium* (*Pullularia*) *pullulans*, *Rhizopus stolonifer* (*R. nigricans*), *Rhodotorula glutinis* and *Saccharomyces cerevisiae*). A saline solution was used as a negative control, while a histamine solution (10 mg/mL) was used as a positive control. Tests were performed by a trained technician between August and October 1994. The largest weal diameter was measured 15 min after pricking, and the ratio of the size of the allergen weal divided by the histamine weal was calculated. Tests were considered positive if this ratio was equal to or greater than 0.5 [10].

Statistical methods

Statistical analyses of data were performed using SPSS for Windows version 6.0 (SPSS Inc., Chicago, USA, 1993), with Genstat 5, release 3.1 (Oxford University Press Inc., New York, USA, 1994) being used for logistic regression

modelling [11]. Fungal spore concentration data were positively skewed and the non-parametric Mann–Whitney and Kruskal–Wallis tests were therefore used for comparisons. A $\log_{10}$ -transformation was performed in order to gain a near-normal distribution for further analyses. Multiple linear regression was used to identify risk factors for indoor fungal spore contamination, using forward selection of variables with an entry criterion of $P < 0.05$. Logistic regression was applied to calculate adjusted odds ratios for asthma, allergies and respiratory symptoms with exposure to indoor spores and evidence of dampness. For the logistic regression models the proportion of children with asthma (or allergy or respiratory symptoms) in each house was used as the dependent variable since more than one child was included from some houses and independence between observations could not be assumed for data concerning the individual children. Many associations were tested and there is therefore a risk of finding false significant associations by chance. By reporting both significant and nonsignificant associations and (in some instances) indicating P -values for significant associations, and also by relating the results to previous literature, it is believed that this potential problem has been addressed. If not otherwise stated, associations and correlations were considered significant if $P < 0.05$.

Results and discussion

Fungal spore concentrations

The median indoor viable fungal spore concentration for all samples over the year of study was 812 CFU/m³, with a range from <20 –54 749 CFU/m³. The median total spore concentration was 7778 S/m³, ranging from 667 to 118 222 S/m³. Seasonal variation was apparent with an approximate tripling of concentrations in summer compared with winter for both viable and total spore concentrations indoors as well as outdoors. Spore concentrations indoors were significantly smaller than those outdoors for every sampling period, with the exception of total spore concentrations in early spring (September). *Cladosporium*, *Penicillium*, and yeasts were the three most common colony types, together accounting for 73% of colonies in winter and 86% in late spring. In addition, the genera *Aspergillus* (2.6%), *Cephalosporium* (2.2%), and *Botrytis* (1.1%) were relatively common in winter [8].

Health outcomes

Some 36% of the 148 study children were diagnosed as asthmatic by a doctor. Of the asthmatic children, 83% were atopic (had at least one positive skin prick test), while 48% of non-asthmatics were atopic. The most common skin test

Table 1. Percentage of asthmatic ($n=52$) and non-asthmatic children ($n=93$) with a positive reaction to each of the fungal extracts, with 95% confidence intervals (CI)

Extract	Asthmatic children		Non-asthmatic children	
	%	95% CI	%	95% CI
<i>Cladosporium cladosporioides</i>	23*	12–35	8	2–13
<i>Penicillium</i> mix	15*	6–25	5	1–10
<i>Aspergillus</i> mix	12	3–20	5	1–10
<i>Alternaria alternata</i>	21	10–32	17	10–25
Mould mix A	19	8–30	10	4–16

* Significantly (χ^2 test, $P < 0.05$) higher proportion compared with non-asthmatics.

reactivity in both groups was to the house dust mite, *D. pteronyssinus*, (81% of asthmatics and 39% of nonasthmatics). Some 31% of asthmatic children gave a positive reaction to at least one fungal extract, compared to 23% of non-asthmatic children. A positive reaction to *Cladosporium cladosporioides* was most common among asthmatics, while reactions to *Alternaria alternata* were most prevalent among non-asthmatics (Table 1). A positive reaction to a fungal extract in the absence of a reaction to house dust mite extracts was seen for five non-asthmatic children, but not among asthmatic children. Respiratory symptoms were experienced by 94% of asthmatic children and by 48% of non-asthmatic children.

House characteristics

Evidence of dampness was common in the study houses with visible mould growth present in every house at some time during the study, and evidence of condensation in 92% of houses. Water intrusion was observed in 40% of houses and the investigator experienced musty odour in 67% of houses. The common problem of indoor mould growth is manifested by the fact that 60% of households removed mould growth from the house on a regular basis, yet only 23% of residents considered their house damp (including structural dampness and dampness from condensation). Consequently, while most residents know that mould growth is occurring in the house, they do not necessarily regard this as a sign of a damp house or a problem which should be rectified.

Significant differences between rooms were seen for severity of visible mould growth ($\chi^2 = 67.6$, d.f. = 6, $P < 0.001$), condensation ($\chi^2 = 80.8$, d.f. = 6, $P < 0.001$), and for severity of musty odour ($\chi^2 = 16.3$, d.f. = 4,

Table 2. Median viable (CFU/m³) and total spore concentrations (S/m³) by house variables in 80 houses

Variable	<i>n</i>	Viable spores	Total spores
Open windows, months of the year	2–6	11	854
	6–10	19	1055
	>10	50	1056 [#]
Foundation	Stumps	50	1024
	Slab	30	962
Cracks in cladding	yes	13	1108
	no	66	970
Remove mould growth	yes	31	965
	no	47	1036
Insulation score†	1	38	1110*
	2	12	994
	3	24	933
Extractor fans	1	9	1238
	2	44	1019
	3	26	985

* $P < 0.05$ # $P < 0.10$. † Number of locations with insulation (floor, ceiling, walls).

$P < 0.002$). Bedrooms were consistently associated with the most severe dampness problems and winter was the season when most problems occurred ($P < 0.001$). It can also be noted that bedrooms were cooler ($P < 0.001$) and the relative humidity was higher ($P = 0.006$) compared to other rooms. These results suggest that heating of the bedrooms as well as the living areas could prevent much of the demonstrated problems with indoor mould growth by lowering indoor humidity and the risk of condensation in winter.

Environmental factors associated with fungal spore concentrations

Environmental factors were tested for an association with airborne fungal spore concentrations. Outdoor concentrations of fungal spores were significantly correlated ($P < 0.001$) with indoor concentrations for both viable ($r = 0.41$) and total spores ($r = 0.52$) [8]. Over the year of sample collection, the mean indoor relative humidity (%) was significantly, but weakly correlated with both viable ($r = 0.28$), and total ($r = 0.22$) spore concentrations. However, the correlation was not significant for all sampling periods and was in some cases reversed in direction (data not shown) [8]. In the present study, humidity was measured at the time of sample collection and this may not estimate well the humidity in the microclimate which determines the possibility of fungal growth [12]. This may explain the lack of a strong consistent correlation between humidity and fungal spore concentrations.

Viable and total spore concentrations are related to some housing factors in Table 2. For viable spores, a significant association was seen with insulation score only, suggesting larger concentrations in houses with little insulation. It is possible that houses with less insulation have a more open structure allowing outdoor air to enter the indoor environment, thereby increasing the indoor viable spore concentration. Another possibility is that lack of insulation increases the risk of condensation on indoor surfaces and therefore allows moisture accumulation and fungal growth. Total spore concentrations were significantly larger in association with: stump type foundation, cracks in the cladding, limited ventilation through open windows and failure to remove mould growth from the house. These results suggest that uncontrolled ventilation through cracks in the cladding and through the foundation of the house may increase indoor total spore concentrations, while controlled ventilation through open windows may reduce spore concentrations. No significant associations were seen between spore concentrations and: air conditioning, gas stove presence, damp house (resident's opinion), blocked subventilation, cladding type, soil type, drainage, garden beds around house or house age.

During the house surveys, any evidence of dampness in sampled rooms or presence of an open window at the time of sample collection was recorded. Associations with viable and total airborne spore concentrations were tested separately for each sampling period. Evidence of condensation was significantly associated with larger total spore concentrations in spring only, while no significant association with viable spore concentrations was evident. Visible mould growth was associated with smaller concentrations of viable spores, but was not significantly associated with total spore concentrations. It is very difficult to explain why significantly smaller viable spore concentrations would be found in rooms with visible mould growth. However, one possibility is that rooms without visible mould were more likely to have an open window at the time of sample collection, thus allowing the more concentrated air spora from outdoors to enter the indoor environment. Musty odour, water intrusion and presence of an open window at the time of sample collection were all significantly associated with larger concentrations of viable and total spore levels in at least one season. Previously, large concentrations of airborne fungal spores in association with visible mould growth and in complaint homes have been reported [13,14], but some studies have found no difference in spore concentrations between houses with mould problems and reference houses [15,16]. Suggested reasons for a lack of an association in some studies have included the variable nature of airborne spore concentrations and a variable release of spores from indoor sources [16]. In the present study, data on house dampness were available from six

sampling periods and associations with spore concentrations could therefore be tested for each period. The results were not consistent between sampling periods, confirming the variability of the association and suggesting a substantial seasonal component. However, presence of musty odour or water intrusion in a room were consistently associated with larger indoor fungal spore concentrations.

The two common fungal genera *Cladosporium* and *Penicillium* were tested for associations with environmental factors. Houses with ventilation through open windows for 2–6 months of the year had significantly larger *Cladosporium* concentrations (median 335 CFU/m³) compared with houses with ventilation through open windows for more than 10 months of the year, (median 187 CFU/m³). This suggests that regular ventilation through open windows can significantly reduce indoor *Cladosporium* concentrations, despite outdoor concentrations being larger. A possible explanation for this is a reduction in indoor humidity with more ventilation at suitable times of the day. *Penicillium* concentrations were significantly larger in houses where only the ceiling was insulated (75 CFU/m³), compared with houses with insulation in ceilings, walls and floors (38 CFU/m³). The larger *Penicillium* concentrations in houses lacking wall and floor insulation may point to greater condensation risk and a resulting fungal growth potential in such houses.

Table 3 summarizes associations for *Cladosporium* and *Penicillium* concentrations with measures of dampness in sampled rooms. *Cladosporium* concentrations were significantly larger in rooms with: substantial visible mould growth, substantial condensation evidence, a mean relative humidity <60%, and open windows on most sampling occasions. The larger *Cladosporium* concentration in rooms with visible mould growth or condensation suggests

Table 3. Median *Cladosporium* and *Penicillium* concentrations (CFU/m³) in association with measures of dampness and presence of open windows in rooms (*n* = 293)

Variable		<i>n</i>	<i>Cladosporium</i>	<i>Penicillium</i>
Condensation	substantial	72	984*	144
	none-slight	221	744	144
Visible mould	substantial	67	998*	144
	none-slight	222	458	144
Musty odour	yes	176	816	168
	no	112	768	144
Open windows	yes	164	936*	144
	> 50% of visits	129	720	144
Relative humidity	yes	58	600	216*
	> 60%	235	912*	144

* Significantly (*P* < 0.05) larger concentration.

Table 4. Multiple linear regression results using mean indoor total spore concentrations as dependent variable ($\log_{10}$ -transformed, S/m^3)

Variable	Estimate (B)	SE (B)	T-statistic	P
Mean outdoor spore conc., $\log_{10}$ -transformed	0.391	0.078	5.03	<0.001
Months of the year with open windows*	-0.047	0.020	-2.32	0.02
Remove mould growth†	-0.070	0.029	-2.42	0.02
Substructure, stumps†	0.085	0.030	2.84	0.006
Extractor fans‡	-0.039	0.022	-1.79	0.08
(Intercept)	2.55	0.355	7.18	<0.001)
			R = 0.67 R ² = 0.45	

* Coded as 1 = 2–6 months, 2 = 6–10 months, and 3 = > 10 months. † Dichotomous variables (1 = yes, 0 = no).

‡ Number of sites with a fan (bath, kitchen, toilet).

that a substantial proportion of indoor moulds are members of the *Cladosporium* genus. This is consistent with the finding that damp homes had significant indoor sources of *Cladosporium* in Finland [17], and with evidence of *Cladosporium* growth on damp surfaces in the United Kingdom [14]. The higher *Cladosporium* concentration in rooms with a mean humidity <60% is surprising. A possible explanation for this would be that drier rooms have more ventilation through open windows during sample collection which would lead to a reduced humidity ($P = 0.04$), and also allow entry of outdoor *Cladosporium* spores to the indoor environment (Table 3). It is also possible that dry conditions are more conducive to the dispersal of *Cladosporium* spores. *Penicillium* concentrations were significantly larger in association with a mean humidity above 60% only. In winter, musty odour was associated with larger concentrations of *Penicillium* ($P = 0.03$) and marginally with larger *Cladosporium* concentrations ($P = 0.10$).

A multiple linear regression model predicting mean total spore concentrations in the house was developed (Table 4). After controlling for outdoor spore concentrations, significant risk factors for large total spore concentrations were: a lack of regular ventilation through open windows for much of the year, failure to remove mould growth, and stump type rather than concrete slab foundation. Few extractor fans in wet areas was a marginally significant risk factor. The associations with indoor relative humidity and cracks in the cladding seen in bivariate analyses were no longer significant after adjustments. A linear regression model predicting viable spore concentrations is not presented due to paucity of significant predictors. These results would suggest that removal of mould growth may be an effective way of decreasing indoor reserves of dead spores, thus decreasing airborne total spore concentrations. Extractor fans and ventilation through open windows are likely to decrease the indoor moisture load and thereby lead to a

decreased risk of fungal contamination. The association with stump type foundation was also seen in a previous study in the Latrobe Valley, but in that case it did not remain significant after adjustment for the age of the house [18], while in the present study there was no significant association between house age and total spore levels.

Suggestions for remedial action in houses with fungal problems would include installing more insulation (ceilings, walls and floors), adequate ventilation (via open windows and extractor fans), removal of any visible mould growth and heating of the whole house to avoid high relative humidity and condensation in winter.

Associations with health outcomes

Asthma in children was significantly associated with exposure to *Penicillium* in winter. The odds ratio for asthma with an increase in the exposure to *Penicillium* by 100 CFU/m³ was 1.43 (95% CI 1.03–2.00), adjusted for parental asthma and parental allergy. This estimate was strongly influenced by three cases and if these were excluded, the association was no longer significant. Thus, the association could be a chance finding. However, there are previous reports of an association between *Penicillium* exposure and asthma [19,20] so despite the weak association found in this study, *Penicillium* is likely to be clinically important. No other measure of spore exposure or dampness (musty odour, damp house — resident, damp house — investigator, visible mould growth, condensation evidence) showed a significant association with asthma.

Atopy was significantly associated with exposure to *Aspergillus* spores. The adjusted odds ratio for atopy with an increase by 10 CFU/m³ was 1.48 (95% CI 1.10–1.99), adjusted for gender and parental asthma. No other significant associations between atopy and fungal exposure measures or dampness were seen.

Table 5. Odds ratios (OR) with 95% confidence intervals (95% CI) for fungal allergies with winter exposure to *Cladosporium* and *Penicillium* spores for 145 children, adjusted for parental asthma and gender

Extract	Proportion with positive reaction	Cladosporium + 100 CFU/m ³		Penicillium + 100 CFU/m ³	
		OR	95% CI	OR	95% CI
<i>Cladosporium cladosporioides</i>	13%	1.24	1.00–1.54	1.24	0.92–1.67
<i>Penicillium</i> mix	9%	1.29	1.02–1.62	1.60	1.13–2.18
<i>Aspergillus</i> mix	8%	1.37	1.07–1.76	1.42	1.04–1.95
<i>Alternaria alternata</i>	19%	1.12	0.91–1.36	1.18	0.88–1.57
Mould mix A	13%	1.16	0.95–1.43	1.19	0.88–1.62
<i>D. pteronyssinus</i>	49%	1.02	0.93–1.13	1.22	0.89–1.67
<i>D. farinae</i>	46%	1.03	0.95–1.11	1.32	0.95–1.85
House dust	34%	1.06	0.96–1.17	1.49	1.05–3.17
Dog	16%	1.02	0.91–1.15	1.46	1.09–1.96
Cat	24%	1.08	0.98–1.19	1.21	0.92–1.59
Bermuda grass	31%	1.04	0.94–1.15	1.12	0.85–1.47
Grass mix no. 7	43%	1.03	0.95–1.11	1.12	0.85–1.47

Positive reactions to some extracts included for skin-prick testing were more common with greater exposure to *Cladosporium* or *Penicillium* in winter (Table 5). Associations with spore exposure in late spring were much weaker and never significant (data not shown). As one might expect, a positive reaction to *Cladosporium cladosporioides* was more likely with exposure to *Cladosporium* spores, and a reaction to the *Penicillium* mix extract was more likely with exposure to *Penicillium* spores. However, exposure to these genera was also a risk factor for a positive reaction to other fungal extracts, and surprisingly, *Penicillium* exposure was a significant risk factor for house dust and dog allergies. These latter two associations are likely to be chance findings. The significant associations between allergy and exposure to differing fungal genera may be explained by crossreactivity which is known to occur between species and even genera of moulds [21], so that for example a positive reaction to *Aspergillus* can also occur among those sensitized to *Penicillium*. Furthermore, it should be recognized that the sensitization to fungi for most study children would have occurred well before the exposure was measured in this study which may therefore not closely reflect the exposure at the time of sensitization. Another possibility is that exposure to any fungi indoors could have the potential to increase the risk of allergic sensitization to any allergen. General immunosuppressant properties of some fungal metabolites, such as mycotoxins and volatile organic compounds [22], demonstrate the possibility of a nonspecific effect on the immune system of fungal exposure. Such a general effect of fungal exposure on the

immune system can also explain the significantly increased risk of atopy with exposure to *Aspergillus* spores since several *Aspergillus* species are known to produce mycotoxins [6].

Exposure to *Aspergillus* spores was not a significant risk factor for a positive reaction to the *Aspergillus* mix, and exposure to *Alternaria* spores was not a significant risk factor for a reaction to the *Alternaria alternata* extract (data not shown). A possible reason for this lack of association for *Alternaria* and *Aspergillus* is that exposure to those genera was not as common as to *Cladosporium* and *Penicillium* and the sample size may therefore have been too small to detect an association.

There was some evidence for a greater risk of fungal allergies with exposure to musty odour. The odds ratio for a positive reaction to mould mix A was 4.79 (95% CI 1.00–22.92), adjusted for gender and parental asthma, but a positive reaction to the other fungal extracts was not significantly more likely with musty odour. Furthermore, children exposed to musty odour had a significantly greater number of positive skin-prick tests compared with children not exposed to musty odour ($P = 0.03$), suggesting a more severe allergy with exposure to musty odour. This association between allergy and exposure to musty odour could be attributed to the general immunosuppressant effect of an increased exposure to fungal products (discussed above) in houses with musty odour since this is a non-specific sign of fungal contamination [23].

A marginally significant odds ratio for the presence of respiratory symptoms was seen (adjusted for parental

asthma) with an increase in the indoor *Cladosporium* exposure by 500 CFU/m³ (OR=1.92; 95% CI 0.96–3.80). Similarly, the specific respiratory symptoms of cough (OR=2.11; 95% CI 1.11–4.03) and wheeze (OR=1.58; 95% CI 1.00–2.50) were significantly more common with *Cladosporium* exposure (+ 500 CFU/m³). These findings lend further support to previous reports of *Cladosporium* exposure being associated with respiratory illnesses [24–26]. Total spore concentrations in May (late autumn) were a marginal risk factor for respiratory symptoms (adjusted for parental asthma) with the odds ratio for an increase in late autumn total spores by 5000 S/m³ 1.76 (95% CI 0.97–3.19). On the other hand, mean total spore concentrations were not significantly associated with respiratory symptoms. Interestingly, one previous study has found that total spore exposure in winter is a risk factor for respiratory symptoms [24], suggesting that fungal exposure in this season is important for health. Previous findings of respiratory symptoms such as wheeze being associated with visible mould growth in houses [27] were not supported by this study.

In conclusion, results presented in this paper suggest a large overall effect of fungal exposure on child health (especially in winter). Asthma, atopy and respiratory symptoms were all significantly associated with exposure to one or more genera of fungal spores. On the other hand, average concentrations of viable or total fungal spores were not significantly associated with health outcomes. These results are in agreement with those reported by Strachan *et al.* [27] in that no significant association between total viable mould concentrations and health outcomes were seen despite significant associations with specific genera. This implies that measurements of specific fungal spore genera concentrations predict health outcomes better than total spore concentrations, reported dampness or observed dampness.

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References

- 1 Dales RE, Zwanenburg H, Burnett R, Franklin CA. Respiratory health effects of home dampness and molds among Canadian children. *Am J Epidemiol* 1991; 134:196–203.
- 2 Spengler J, Neas L, Nakai S *et al.* Respiratory symptoms and housing characteristics. *Indoor Air* 1994; 4:72–82.
- 3 Brunekreef B, Dockery DW, Speizer FE *et al.* Home dampness and respiratory morbidity in children. *Am Rev Resp Dis* 1989; 140:1363–7.
- 4 Li C-Y, Hsu L-Y. Home dampness and childhood respiratory symptoms in a subtropical climate. *Arch Environ Health* 1996; 51:42–6.
- 5 Brunekreef B, Verhoeff AP, van Strien RT, van Wijnen JH. Home dampness and childhood respiratory symptoms: the role of sensitisation to dust mites and moulds. In: Samson RA, Flannigan B, Flannigan ME, Verhoeff AP, Adan OCG and Hoekstra ES, eds *Health implications of fungi in the indoor environment*. Amsterdam, Netherlands: Elsevier Science, 1994: 189–99.
- 6 Jarvis BB. Mycotoxins and indoor air quality. In: Morey PR, Feeley JC, Otten JA, eds *Biological contaminants in indoor environments*, ASTM STP 1071, Philadelphia, American Society for Testing and Materials, 1990.
- 7 Andersen AA. New sampler for the collection, sizing and enumeration of viable airborne particles. *J Bacteriol* 1958; 76:471–84.
- 8 Garrett MH, Hooper BM, Cole FM, Hooper MA. Airborne fungal spores in 80 homes in the Latrobe Valley, Australia: levels, seasonality and indoor-outdoor relationship. *Aerobiologia* 1997; 17:121–6.
- 9 Abramson M, Kutin J, Bowes G. The prevalence of asthma in Victorian adults. *Aust NZ J Med* 1992; 22:358–63.
- 10 Meinert R, Frischer T, Karmaus W, Kuehr J. Influence of skin prick tests criteria on estimation of prevalence and incidence of allergic disease in children. *Allergy* 1994; 49:526–32.
- 11 Hosmer DW, Lemeshow S. *Applied logistic regression*. New York, John Wiley & Sons, 1989.
- 12 Flannigan B, Morey PR. Control of moisture problems affecting biological indoor air quality. Ottawa: International Society of Indoor Air Quality and Climate, ISIAQ guideline: Task force I, 1996.
- 13 Li CS, Hsu LY. Airborne fungus allergen in association with residential characteristics in atopic and control children in a subtropical region. *Proceedings of the 7th international conference on indoor air quality and climate*. Nagoya, Japan, 1996; 3:411–6.
- 14 Hunter CA, Grant C, Flannigan B, Bravery AF. Mould in buildings: the air spora of domestic dwellings. *Int Biodeter* 1988; 24:81–101.
- 15 DeKoster JA, Thorne PS. Bioaerosol concentrations in non-complaint, complaint, and intervention homes in the Midwest. *Am Ind Hyg Assoc J* 1995; 56:573–80.
- 16 Nevalainen A, Pasanen A-L, Niininen M, Reponen T, Kalliokoski P, Jantunen MJ. The indoor air quality in Finnish homes with mold problems. *Environ Int* 1991; 17:299–302.
- 17 Pasanen A-L, Niininen M, Kalliokoski P, Nevalainen A, Jantunen MJ. Airborne *Cladosporium* and other fungi in damp vs. reference residences. *Atm Environ* 1992; 26B:121–4.
- 18 Godish D, Godish T, Hooper B, Hooper M, Cole M. Airborne

- mould levels and related environmental factors in Australian houses. *Indoor Built Environ* 1996; 5:148–54.
- 19 Tariq SM, Matthews SM, Stevens M, Hakim EA. Sensitisation to *Alternaria* and *Cladosporium* at the age of 4 years. *Clin Exp Allergy* 1996; 26:794–8.
- 20 Licorish K, Novey HS, Kozak P, Fairshier RD, Wilson AF. Role of *Alternaria* and *Penicillium* spores in the pathogenesis of asthma. *J Allergy Clin Immunol* 1985; 76:819–25.
- 21 Salvaggio J, Aukrust L. Mold-induced asthma. *J Allergy Clin Immunol* 1981; 68:327–46.
- 22 Flannigan B, Miller JD. Health implications of fungi in indoor environments – an overview. In: Samson RA, Flannigan B, Flannigan ME, Verhoeff AP, Adan OCG and Hoekstra ES, eds *Health implications of fungi in the indoor environment*. Amsterdam, Netherlands: Elsevier Science, 1994: 3–28.
- 23 Gravesen S, Frisvald JC, Samson RA. *Microfungi*. Copenhagen: Munksgaard, 1994.
- 24 Godish TJ, Godish DR, Hooper BM, Hooper MA. Latrobe Valley indoor air quality study: health and house variables. In: Morawska L, Bofinger ND and Maroni M, eds *Oxford, Elsevier Science Ltd*, 1995: 211–4.
- 25 Stout C, Kotses H, Carlson BW, Creer TL. Predicting asthma in individual patients. *J Asthma* 1991; 28:41–7.
- 26 Targonski PV, Persky VW, Ramekrishnan V. Effect of environmental molds on risk of death from asthma during the pollen season. *J Allergy Clin Immunol* 1995; 95:955–61.
- 27 Strachan DP, Flannigan B, McCabe EM, McGarry F. Quantification of airborne moulds in the homes of children with and without wheeze. *Thorax* 1990; 45:382–7.