Quantification of airborne moulds in the homes of children with and without wheeze

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Abstract

A population survey of 1000 7 year old children found a significant excess of wheeze among children whose homes were reported to be mouldy (odds ratio 3.70, 95% confidence limits 2.22, 6.15). The airborne mould flora was quantified by repeated volumetric sampling during the winter in three rooms of the homes of 88 children. All of these had previously completed spirometric tests before and after a six minute free running exercise challenge. Total airborne mould counts varied from 0 to 41 000 colony forming units (CFU)/m³, but were generally in the range 50-1500 CFU/m³, much lower than the concentrations found outdoors in summer. The principal types of fungi identified are all known to be common out of doors, and most were found on at least one occasion in most of the homes. Median and geometric mean total mould counts were not related to reports of visible mould in the home, or to a history of wheeze in the index child. The heterogeneous group of non-sporing fungi (mycelia sterilia) were the only airborne fungi present at significantly higher concentrations in the homes of wheezy children (geometric mean 2.1 v 0.7 CFU/m³). A non-significant increase in total mould counts was observed in the homes of children with a 10% or greater decline in FEV₁ after exercise (geometric mean 354 v 253 CFU/m³). Questionnaire reports of mould in the home may be a poor indicator of exposure to airborne spores. The total burden of inhaled mould spores from indoor sources is probably not an important determinant of wheeze among children in the general population. Although the association with mycelia sterilia could be a chance finding, these non-sporing isolates may include a potent source of allergen.

The possibility that fungal spores normally present in outdoor air might have a causal role in asthma and hay fever was first proposed before the second world war and became widely accepted in the 1950s.^{1,2} A review in 1981, however, pointed out that much of the evidence was anecdotal and published reports were inadequate and controversial.³ Type 1 cutaneous hypersensitivity reactions to mould extracts provide the best evidence of a causal

link, but many asthmatic patients who have cutaneous hypersensitivity to fungi are also allergic to pollen or house dust mites,^{2,4} so the independent contribution of mould exposure to their symptoms may be difficult to determine. In a detailed longitudinal study of eight Dutch asthmatic subjects known to be allergic to moulds pulmonary complaints were more common when outdoor mould spore concentrations were high, but no association was found with indoor concentrations.⁵

Evidence of an association between asthma and indoor exposure to mould comes from a case-control study of 72 adults with asthma and 72 age and sex matched control subjects.⁶ Nineteen cases and nine controls reported visible mould on the walls of their homes, and there was a significantly higher prevalence of positive radio allergosorbent test (RAST) responses to *Penicillium* among patients reporting mould in their homes. These and other results⁷ suggest that domestic mould spores may be capable of invoking species specific type 1 hypersensitivity in a small proportion of asthmatic adults.

A recent cross sectional study of children and their parents in selected areas of Glasgow, Edinburgh, and London⁸ found no association between reports of mould in the home and wheeze among adult respondents. A strong relation, however, was found between reported mould growth and wheeze in children, and total airborne mould spore counts were higher in the homes of wheezy children. A survey of a random sample of 7 year old children in Edinburgh⁹ found that the prevalence of wheeze was three times greater among children from homes reported to have visible mould growth than among children from allegedly non-mouldy homes. This confirmed earlier findings in the north west of the city.^{10,11} Reporting of symptoms was validated by measuring exercise induced bronchoconstriction in the children. Much of the association between mould and wheeze could be attributed to biased case ascertainment, but there were nevertheless more children with exercise induced bronchoconstriction among the group from mouldy homes.⁹

The reporting of visible mould in the home may differ between asthmatic and non-asthmatic individuals, owing to a tendency for asthmatic patients (or their parents) to seek an identifiable cause for the complaint in their home environment. In the Edinburgh study⁹ the possibility of differential reporting of housing conditions was initially evaluated by measurements of temperature and relative

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humidity in the child's bedroom. No differences were found between the bedroom conditions of children with and without wheeze.¹² Ambient humidity may be a poor indicator of conditions in specific indoor microenvironments, where penetrating moisture or condensation on to cold surfaces promotes the growth of moulds.¹³ The study was therefore extended to measure exposure to airborne mould spores among children with and without wheeze. We postulated that, if substantial reporting bias had occurred, the reports of mould growth by parents of wheezy children would be associated with lower levels of measured exposure than similar reports by parents of unaffected children.

Methods

POPULATION SURVEY

The original questionnaire survey and medical examinations have been described elsewhere.⁹ Briefly, a random sample of children in their third primary year (age 6½ to 7½ in September 1986) were chosen. Parents were asked to complete a postal questionnaire that inquired about recent respiratory symptoms experienced by the child and conditions in the home, with particular reference to dampness and visible mould growth. The study received ethical approval from the Lothian Health Board and Lothian Regional Council department of education.

With parental consent, children were examined by one author (DPS) at school during the first half of 1987, using the American Thoracic Society protocol.¹⁴ A "Compact" pneumotachograph (Vitalograph Ltd, Buckingham) was used to record forced expiratory spirometers before and after a six minute free running exercise challenge. The minimum of two satisfactory recordings of forced expiratory volume in one second (FEV₁) taken five and 10 minutes after exercise was compared with the pre-exercise recording. Bronchial lability was defined as the difference between the post-exercise and pre-exercise FEV₁ divided by the pre-exercise FEV₁.

At the conclusion of the mycological survey in March 1988 the parents of each child were asked to complete a short questionnaire relating to respiratory symptoms during the previous year. No further measurements of ventilatory function or bronchial lability were made.

SELECTION OF HOMES

One third of the children in the main survey were selected for continuous measurement of temperature and relative humidity in their bedroom for seven days, from January to April 1987. This sample was stratified by estimated bedroom relative humidity, to include all the most humid bedrooms and a one in four sample of the remainder.¹² The present study was based on a subsample of this group. An attempt was made to include all the children with wheeze, and all those whose parents had reported visible mould in the home in the original questionnaire. A random sample of one in eight of those with neither wheeze nor mould were

also asked to participate. Families who had moved house since the questionnaire survey were excluded from the study.

During the six months October 1987 to March 1988 each home was visited four times (at approximately monthly intervals) for sampling of air in three rooms with a volumetric Andersen sampler.¹⁵ In each home samples were taken in the living room and the child's bedroom. If visible mould was a problem elsewhere in the house (21 homes), a third sample was taken in that room (other bedroom nine, bathroom 11, hallway one). Otherwise, the third sample was taken in the kitchen (67 homes).

MYCOLOGICAL METHODS

A six stage Andersen 1ACFM Viable Particle Sampler (Andersen 2000 Inc, Atlanta, Georgia, USA) was used to sample air for viable mould spores and yeasts. The intake orifice was placed about 45 cm above floor level, and the instrument was operated according to the manufacturer's instructions except that 9.0 cm disposable plastic Petri dishes were used as collection plates. Each plate contained 27 ml sterile malt agar (2% (w/v) malt extract, 1.2% Agar No 3 (Oxoid)) with 20 units/ml benzylpenicillin and 0.05 mg/ml streptomycin sulphate to inhibit bacterial growth. After 85 litres of air had been sampled over three minutes the plates were incubated at 25°C for seven days. Counts of colony forming units (CFU) were computed from the numbers of colonies developing on the plates, correction being made by the "positive hole" method recommended by the manufacturer.¹⁵ For identification, fungi were isolated on to potato dextrose agar (Oxoid) plates incubated at 25°C, and identified by reference to standard texts¹⁶ and by comparison with reference strains.

Isolates of mycelial fungi (that is, the vegetative, non-sporing hyphae), which because they did not sporulate in culture on either medium could not be identified, were plated out on 3% malt extract tannic acid agar to test for production of diphenol oxidase.¹⁶ Those that were positive were classified as basidiomycetes capable of causing white rot of wood. Those that did not sporulate, did not produce diphenol oxidase, and did not show the clamp connections characteristic of the class Basidiomycetes family were categorised together as mycelia sterilia—that is, non-sporing fungi.

STATISTICAL ANALYSIS

Statistical analysis was performed by means of the Statistical Analysis System (SAS).¹⁷ In quantitative comparisons a logarithmic transformation was used for fungal counts to correct for the positively skewed distribution. For this purpose, zero counts were treated as 0.1 CFU/m³. Observations from the "no mould and no wheeze" group were assigned a weight of 8 in the calculation of geometric means, to allow for the sampling fraction of 1/8 in this group. Weighting was also used in comparisons of means by one way analysis of variance, the GLM procedure in SAS¹⁷ being used.

Table 1 Relation between reported mould and wheeze in the population survey and the derivation of the study sample

	No of children with				
Symptom report Nov 1986:	Wheeze past year		No wheeze past year		
Home condition Nov 1986:	Mould	No mould	Mould	No mould	Odds ratio (95% CL) for wheeze v mould
Group:	A	B	C	D	
Questionnaire survey (9)	27	96	62	815	3.70 (2.22-6.15)
Bedroom monitored (12)	15	30	38	247	3.25 (1.60-6.60)
Target sample	15	30	38	31	
Moved	2	5	6	5	
Refused	1	2	1	1	
No contact	1	0	2	0	
Achieved sample	11	23	29	25	

Results

POPULATION SURVEY

The original questionnaire survey obtained usable data from 1000 (91%) of the 1095 children selected as a random sample of the population. Table 1 shows the numbers of children who were reported by their parents to have wheezed in the past year, and to have visible mould growth in the home. The association between mould and wheeze was highly significant ($p < 0.0001$), and was largely independent of other social and housing variables.⁹ Among the 330 children whose bedroom conditions were monitored for temperature and relative humidity during January–April 1987¹² the association was of similar strength (table 1).

SAMPLE SELECTION

Among the target sample for the present study, 18 families were excluded because they had moved house, five refused to participate in further studies, and no visits could be arranged with three families who initially agreed to participate. The overall response rate for sampling of airborne fungi was therefore 77% (88 of the 114 homes in the target sample). The response rate did not differ substantially in the four "wheeze-mould" groups (table 1).

Although the sample was chosen on the basis of questionnaire data obtained one year earlier, 29 (85%) of the 34 children with wheeze reported in the questionnaire were still wheezing during the winter of 1987–8. Our field-workers observed mould growth in few of the homes that they visited. This probably reflects the exceptionally warm winter conditions during the period of the survey. The incidence of mould growth was higher, however, in the homes reported to be mouldy in 1986; five of 11 homes in group A (wheeze and mould); two of 23 homes in group B (wheeze and no mould); five of 29 homes in group C (mould and no

wheeze); and one of 25 homes in group D (no mould and no wheeze).

DISTRIBUTION OF TOTAL MOULD COUNTS

Table 2 shows the distribution of total (all species) counts of colony forming units obtained from each air sample, by sampling site and wheeze-mould group. There was a wide range of values, but only three samples (two from group B, one from group C) had more than 10 000 CFU/m³ of air. Six air samples contained no viable moulds. In relation to the variation between homes in each group, the median counts for each group at each sampling site were remarkably similar.

TYPES OF MOULD ISOLATED

Nearly 50 species or genera of fungi were identified in air samples. Table 3 shows the principal types isolated in each wheeze-mould group. Yeasts were isolated on most occasions, but were not included in the total mould count. Spores of *Penicillium* spp and *Cladosporium* spp were isolated from at least one room in all homes on every occasion. Together they accounted for about half of the total mould count. Only the heterogeneous group of airborne mycelia sterilia appeared to bear any relation to the questionnaire reports of visible mould in the home, being particularly evident in the homes of children with wheeze and visible mould growth (table 3). Less common fungi included various *Aspergillus* species, most frequently *A. glaucus*, *A. fumigatus*, *A. nidulans*, and *A. terreus* were occasionally isolated, but never in large numbers.

MOULD COUNTS, WHEEZE, AND BRONCHIAL LABILITY

Weighted geometric mean mould counts for children with and without a history of wheeze and with and without exercise induced bronchospasm are shown in table 4. The differences

Table 2 Distribution of total airborne mould counts (CFU per m³) by site of sampling and subjects grouped according to the reporting of wheeze and mould, all visits combined

Room sampled:	Living room				Child's bedroom				Other room†			
	Yes	Yes	No	No	Yes	Yes	No	No	Yes	Yes	No	No
	Yes	No	Yes	No	Yes	No	Yes	No	Yes	No	Yes	No
Wheeze:	A	B	C	D	A	B	C	D	A	B	C	D
Mould:												
Group*:												
Minimum	47	35	0	12	0	12	12	0	59	0	0	0
Lower quartile	177	153	153	129	106	130	141	106	177	153	152	130
Median	294	271	283	271	200	212	224	247	247	283	269	271
Upper quartile	553	659	483	789	330	447	424	459	412	636	445	883
Maximum	2895	38 594	4531	6038	3072	2695	41 265	7168	5049	36 946	3637	9534
No of samples	41	91	112	95	41	91	111	95	41	91	111	96

*As in table 1.

†See text for details.

Table 3 Principal types of fungi identified in air samples, by wheeze-mould group

	Number of homes in which the fungus was isolated							
	At any one visit				At each visit			
	Yes Yes A	Yes No B	No Yes C	No No D	Yes Yes A	Yes No B	No Yes C	No No D
Wheeze reported: Mould reported: Group*								
Yeasts†	11	23	29	25	10	21	28	25
<i>Penicillium</i> spp	11	23	29	25	11	23	29	25
<i>Cladosporium</i> spp	11	23	29	25	11	23	29	25
<i>Sistotrema brinkmanii</i>	11	23	28	24	5	10	12	11
White rot basidiomycetes	11	23	28	25	5	7	14	5
<i>Mycelia sterilia</i>	11	22	28	23	8	5	7	4
<i>Aspergillus versicolor</i>	11	21	28	21	2	2	5	1
<i>Aureobasidium pullulans</i>	11	18	22	15	0	1	0	2
<i>Botrytis cinerea</i>	7	15	16	18	0	0	0	1
No of homes visited	11	23	29	25	11	23	29	25

*As in table 1.

†Yeasts were excluded from the total mould counts.

between geometric mean counts for all species combined were small and inconsistent; counts were slightly lower in the homes of wheezy children than in the homes of non-wheezy children, but somewhat higher in the homes of children with exercise induced bronchospasm than in the remainder of the sample; these differences, however, could easily have occurred by chance.

The geometric mean counts for the five most common groups of moulds in the homes of children with and without wheeze and with and without exercise induced bronchoconstriction are shown in table 4. For *Penicillium* spp, *Cladosporium* spp, *Sistotrema brinkmanii*, and white rot basidiomycetes the differences were non-significant and in opposing directions for wheeze and bronchial lability. Non-sporing mycelia sterilia were generally found in low concentrations, but were present at higher levels in the homes of both the wheezy children and those with exercise induced bronchoconstriction. When the homes of wheezy and non-wheezy children were compared, the difference in weighted geometric mean counts of mycelia sterilia was significant ($p < 0.01$), but this significance test should be interpreted in the light of the multiple comparisons that could have been made in table 4.

Discussion

This study is the first to compare, in both quantitative and qualitative terms, the indoor mould flora of samples from homes of symptomatic and symptomless individuals from the

general population. The viable mould counts that we obtained from three minute air samples may not adequately reflect peaks and troughs of exposure but, to put them in perspective, only three of the samples in our study approached the levels of 10 000–50 000 CFU/m³ that are typical of a summer garden.^{19,20} Occupational exposures that are implicated in allergic alveolitis, such as the handling of mouldy hay, are associated with airborne spore burdens of a different order of magnitude, reaching 1 600 000 000/m³ at times.²¹

Until recently there was little published information on the extent to which indoor mould growth affects the composition of the domestic air spora in the United Kingdom.^{22,23} A survey in 60 occupied council houses in London and three Scottish towns¹⁸ found, as we did, considerable differences in the total airborne spore burden, not only between homes but also with repeated sampling within homes. This variation is probably related to domestic activity, as spores are found in large numbers in house dust (100 000–1 000 000/g dust.²⁴ Homes with visible mould had somewhat higher airborne mould counts, generally in the range 1000–2000 CFU/m³, with skewing of the distribution towards high values.¹⁸ This suggests that mould growth on walls does increase the average indoor spore burden (which is usually much higher than the outdoor burden in winter), though even these higher concentrations are modest by comparison with summer outdoor concentrations. The lower spore counts in our study may reflect the mild winter conditions that we encountered; but our sample of mouldy homes, being based on questionnaire responses, probably included fewer severe cases of mould growth than homes that have been the subject of complaint to local authorities¹⁸ or private landlords.

Although valuable in comparing different homes, volumetric sampling may underestimate the true exposure of mobile people to fungal spores. Spores may be released from house dust by movement in the room and enter convection currents around the warm body, which are estimated to contribute 10% of inspired air.²⁵ The count of viable spores or colony forming units of any one species, or of all species combined, may be of limited clinical and epidemiological relevance if spores from different species have different allergenic potential,²⁶ or if hypersensitivity reactions can also be provoked by non-viable spores—which predominate, for example, in the air of some agricultural environments.²⁷

Many of the types of indoor mould fungi isolated here and in the above studies,^{18,22,23} however, were similar to those normally found out of doors, so mould growth in most homes seems unlikely to influence the quality of the indoor fungal flora to an important extent. Furthermore, most of the species that were identified indoors in the present study, including the highly allergenic spores of *Botrytis* and *Aspergillus* spp,²⁶ were isolated on at least one occasion in most homes. This suggests that most people are exposed to some extent to a wide range of fungi in the indoor air, quite

Table 4 Geometric mean* airborne mould counts (CFU per m³), all visits combined, by history of wheeze and degree of exercise induced bronchial lability during 1986–7

Room sampled	Fungus	Wheeze in past year			Bronchial lability > 10%†		
		Yes	No	(t†)	Yes	No	(t†)
Living	Total	304	311	−0.08	381	282	+1.52
Child's	Total	223	229	−0.07	285	206	+1.28
Other	Total	309	314	−0.04	407	278	+1.67
Pooled samples	Total	276	281	−0.07	354	253	+1.63
	<i>Penicillium</i> spp	39	55	−0.78	62	49	+0.75
	<i>Cladosporium</i> spp	16	12	+0.46	12	13	−0.30
	<i>Sistotrema brinkmanii</i>	5.1	3.4	+0.63	3.1	3.8	−0.18
	White rot basidiomycetes	2.5	1.3	+1.45	1.0	1.7	−1.70
	<i>Mycelia sterilia</i>	2.1	0.7	+2.84	1.0	0.7	+1.11
No of children		34	54		26	62	

*Log counts weighted for the different sampling fractions in groups A–D.

†Student's *t* test with 88 degrees of freedom.‡Reduction in FEV₁ of > 10%, of pre-exercise value, at either five or 10 minutes after six minute free running exercise challenge.

apart from their shared exposure to airborne spores out of doors.

Fewer asthmatic individuals have cutaneous hypersensitivity to extracts of mould fungi than to house dust mite, but such reactions are not uncommon.² Reported prevalence estimates vary from 3–4% in Sweden to 80% in the United States.³ Among 656 asthmatic patients attending an immunological outpatient clinic in London, 13% had positive skin test responses to *A. fumigatus* and 18% to other fungal species.⁴

We found that the concentration of airborne moulds in the home varies widely between houses so that, if indoor moulds were of aetiological relevance in childhood asthma, we would expect airborne mould counts to be higher in the homes of wheezy children. The fact that little difference was found is in striking contrast to the results of the original questionnaire study. The discrepancy appears to arise partly from bias in the reporting of symptoms⁹ and partly because mild to moderate visible indoor mould growth has only a minor influence on the airborne mould flora inside the home, compared with dust raising household activities such as cleaning. We set out to investigate the possibility that reporting of mould is more likely where a child has asthma. The absence of any difference between median counts of colony forming units in mouldy homes with and without a wheezy child (groups A and C) may not be remarkable as the airborne spore burden differed so little between mouldy and non-mouldy homes.

Mycelia sterilia were cultured more frequently from air samples taken in the homes reported to be mouldy, and were present in higher concentrations in the homes of wheezy children. Further studies of these heterogeneous fungi are required to confirm these associations. Particular attention is being paid to them in continuing studies of both viable and non-viable airborne moulds.

The appearance of mycelia in air samples is consistent with several explanations. Firstly, the air samples may include spores from species that do not sporulate in vitro on the culture media used, so that they cannot be assigned to species or genus. Alternatively, fragments of mycelium from fungi that are not currently sporulating may become detached from their substrate and wafted into passing air currents. Thirdly, the substrate itself may become airborne and carry non-sporulating mould fungi with it. Faecal particles from the house dust mite *Dermatophagoides pteronyssinus* are known to become airborne and contain potent allergens, so the association between non-sporulating fungal isolates and wheeze could perhaps reflect the occurrence of moulds on mite faeces. Before any link can be confirmed between mycelia sterilia and childhood asthma, the source, nature, and allergenicity of the various components should be clarified.

The conclusions to be drawn from these findings are limited by the methodological difficulties of quantifying fungi in indoor air and by the relatively small number of homes studied, but they are strengthened because the

relation of our study sample to the general population is well defined. Epidemiological data such as these cannot exclude the possibility of clinically important idiosyncratic reactions to domestic mould spores in a few susceptible individuals, nor do they address the possible effects on health of exceptionally high mould spore concentrations in homes severely affected by mould.²⁵

On the other hand, they are relevant to assessing the importance of damp, mouldy housing as a public health problem and to the setting of building standards.²⁸ The confirmation of differential reporting of both symptoms and housing conditions in questionnaire data suggests that further investigation of the link between respiratory disease and damp, mouldy housing should be based on valid and objective measurements, preferably in samples that can be related to the general population.

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