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Exposure to Airborne Mould in School Environments and Nasal Patency in Children

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Key Words

Mould · Children's health · School environments

Abstract

Mould in schools has been associated with increased upper airway obstruction in adults. In this study, we investigated the relationship between school indoor mould spore exposure and nasal patency in children. Airborne mould samples were collected in 32 classrooms from 4 primary schools during both summer and winter using a single-stage Anderson sampler. Nasal patency was measured in 275 children in summer and 200 children in winter using acoustic rhinometry. Various mould species were isolated but the most common species found inside the classrooms were *Alternaria*, *Aspergillus niger*, *Cladosporium spp.* and *Penicillium spp.* In the univariate analyses, significant negative correlations were found between exposure to various mould species, particularly *Alternaria*, and rhinometric measures in the children. In multivariate analyses, exposure to *Alternaria* was associated with both decreased mean nasal cross-sectional area ($p=0.001$) and decreased nasal volume ($p=0.026$).

Decreased nasal patency was also associated with exposure to *A. niger* ($p=0.034$) and *Penicillium* ($p=0.043$) in the classrooms. The findings of this study suggest that exposure to airborne mould, particularly *Alternaria*, in school environments may affect the upper airways of children.

Introduction

Moulds are ubiquitous in both indoor and outdoor environments. A broad spectrum of fungi has been identified in indoor environments with *Aspergillus*, *Alternaria*, *Cladosporium* and *Penicillium* the most common microfungi [1,2]. These species of fungi may cause adverse health effects, ranging from subjective symptoms to allergic conditions such as asthma and allergy [3]. Epidemiologic studies have consistently shown that indoor exposure to mould is associated with respiratory symptoms and conditions including wheezing, asthma and rhinitis [4–7].

School and home are the most important indoor environments for children [8]. During school terms, children spend over 6 h a day at school, and therefore, there is a potential for significant exposure to environmental pollutants in school buildings. A number of studies have been conducted to investigate the health effects of mould exposure in school for both children [9–11] and adults [12,13]. These studies have generally reported poor health outcomes, predominantly respiratory and allergic symptoms. Three studies have measured markers of upper and lower airway inflammation in adult school workers. Two of these studies reported significant associations between these markers and mould exposure [12,13] while no association was found in the third [14]. No similar studies have been conducted in children.

Much of the research for mould exposure in schools has been conducted in colder northern European countries [12,13,15]. Although Perth, Western Australia, has a much warmer, drier climate, mould problems have been reported in indoor environments in this city [16]. The aims of this study were to measure airborne mould concentrations in Perth primary schools and investigate the relationship between school indoor mould spore exposure and nasal patency in children.

Subjects and Protocol

Children were recruited from four primary schools in the southern suburbs of Perth, Western Australia, as part of an investigation into the indoor air quality and potential health benefits of an “allergy free” school [17]. The four schools included the “allergy free” school and three neighbouring conventional schools. The “allergy free” school was built using low-emission products and designed to reduce the accumulation of allergens in dust. Several measures were implemented to eliminate dust and hazardous chemicals. These included avoiding dust accumulation (construction of storage cupboards to the ceiling and sloped window sills), improving the indoor–outdoor air exchange through large ventilation vents, avoiding soft furnishings and carpet in classrooms (vinyl flooring) and replacing ceiling insulation that can generate dust particles with sealed insulation products along with paints, adhesives and sheathed particle boards that have lower emissions of volatile organic compounds (VOCs). Two of the neighbouring conventional schools were of an age similar to the “allergy free” school while the third was older. More details of the schools have been presented elsewhere [17]. Our earlier study of indoor air quality in these schools did not show any significant difference in indoor pollutants such as particulate matter,

formaldehyde and VOC between the “allergy free” school and conventional schools. However, there were lower levels of dust mite and pet allergens in the “allergy free” school [17]. Mould was not measured as part of the original study.

This study was a follow-up to investigate the respiratory health, including measurements of acoustic rhinometry (AR), of the children in the four schools. Children who had been given parental consent to be part of the study underwent a respiratory assessment, which included allergy tests and measures of AR. The assessment was conducted at the school during school hours. On the assessment days, airborne viable fungi spores were measured in selected classrooms. Eight classrooms were measured at each school. The study was conducted at each school on two occasions, once in summer and once in winter. The summer measurements were conducted in February/March and the winter tests in June/July. Each testing period was conducted in the second half of the respective school term. The study had ethics approval from the Human Research Ethics Committee of the Princess Margaret Hospital for Children.

Rhinometry was measured in all children on both sampling occasions by the same investigator using the same procedure and equipment (SRE 2000; Rhinometrics A/S, Lyngø, Denmark). Measurements were made according to standard procedure [18]. The procedure has been described by us previously [19]. At least three measurements that varied by less than 10% were taken for each nostril. Measured rhinometric variables included the first and second minimal cross-sectional areas (MCA) from the nasal opening as well as volumes of the nasal cavity. The mean cross-sectional areas were measured from 0 to 22 mm (MCA1) and 23 to 54 mm (MCA2) from the nasal opening. Nasal volumes were calculated for the intervals 0 to 40 mm (VOL1) and 0 to 54 mm (VOL2) from the nostril. Data on nasal dimensions are presented as the sum of the values from the right and left sides [19]. Children were asked if they had had a recent cough or runny nose. If they answered yes, they were not included in the results.

Allergy Testing

Skin reactivity to cow's milk, egg white, rye grass, mixed grass, *Dermatophagoides farinae*, *Dermatophagoides pteronyssinus*, cat dander, dog dander and *Alternaria alternata* was assessed by skin prick tests (SPTs) as described by Pepys [20]. The positive control was histamine sulphate (10 mg·mL⁻¹) and the negative control 0.9% saline. A positive SPT was defined as a weal at least

3 mm in its longest dimension. Atopy was defined as the presence of at least one positive SPT.

Airborne Mould Measurements

Airborne fungi were collected from eight classrooms at each school and the same classrooms were sampled in summer and winter. All sampling in the schools was performed during school hours in order to sample the environment as it is experienced by the children. All classrooms were measured when occupied with the windows closed under representative conditions. A single-stage Andersen N6 400 impaction hole sampler (flow rate $28.3 \text{ L} \cdot \text{min}^{-1}$) was operated for 2 min per sample and all samples were taken in duplicate. The culturable fungi levels were averaged for the duplicate samples. The sampling method was based on the National Institute for Occupational Health and Safety (NIOSH) method for sampling bioaerosols (NIOSH: Method 0800. In Bioaerosol Sampling (Indoor Air)). Outdoor samples were also collected simultaneously at each school. Airborne fungi spores were collected onto malt extract agar plates (2% MEA; Amyl Media Pty Ltd) enriched with chloramphenicol. Colonies were counted and identified after a minimum of 96 h incubation at 21°C ($\pm 2^\circ\text{C}$) utilising the Hughes–Tubaki–Barron system and Saccardo system [21].

Questionnaire

Two questionnaires, housing and respiratory health, were completed by the parents/caregivers of the children. Housing questions were used to gather data on the home environment. Data were collected for smoking in the home, fuel used for cooking and heating, mould problems in the home and distance to busy roads. The respiratory questionnaire was based on the International Study of Asthma and Allergies in Children questionnaire and focussed on the asthma and rhinitis modules [22].

Statistical Analysis

The airborne fungi were measured in a unit of $\text{CFU} \cdot \text{m}^{-3}$ (CFU, colony forming units). The culturable levels of airborne fungi were not normally distributed and could not be transformed; therefore, median and inter-quartile ranges as well as arithmetic means were presented. We compared the levels of fungi in classrooms between winter and summer using Wilcoxon signed-rank test. Individual exposure to fungi was quantified using the airborne culturable fungi levels measured in the classroom.

As several factors have been shown to be significantly associated with AR parameters, we estimated adjusted values of AR parameters after adjusting for age, gender, height, height square and weight. The adjusted levels of AR parameters were calculated by adding the residual values to the means of AR parameters. The residual values were derived in a linear regression model with the corresponding AR parameters as dependent variables and the confounders as independent variables [23]. This adjustment method has been broadly used in the literature [24,25]. The adjusted levels of AR were used for the following statistical analyses. We compared the differences in the adjusted AR parameters during winter and summer in the paired samples using paired-samples *t*-test.

We further employed generalised estimating equations (GEE) techniques [26] to investigate the relationships between airborne fungus exposure and AR measurements. In the GEE analyses, we included individual fungus exposure, school and season as predictors with an independent structure for correlation matrix. In order to investigate the independence of the effects of fungal exposure on nasal patency with several allergic symptoms, we investigated the association after adjusting for current wheezing, hayfever and atopy (SPT). We further adjusted for two important domestic risk factors: house mould exposure and passive smoking. These were included *de novo* based on their potential importance in eliciting inflammatory changes in the upper airways [27]. Other risk factors from the questionnaire, such as heating and cooking fuels, distance from busy roads and attached garages, were not included in the model as they were not associated with any of the rhinometric variables in univariable analyses. All the statistical analyses were conducted using SPSS program (SPSS 16.0 for PC).

Results

A total of 376 children (including 190 girls, mean age 8.3 years (95% CI: 8.0–8.5 years) CI, confidence interval) took part in the study over the two seasons. Among them, 275 children had AR measurements done during winter and 200 children were tested during summer. Ninety-nine of these children were tested on both occasions. The age and sex mix was similar for each school and representative of the school student population for the individual schools. Table 1 presents the demographic characteristics of the children, including the prevalence of atopy and allergic symptoms, as well as domestic risk factors.

Table 1. Demographical characteristics, allergic symptoms and domestic risk factors

	Winter		Summer	
	<i>n</i>	%	<i>n</i>	%
Sex (M/F)	140/135	50.9/49.1	105/95	52.5/47.5
Current wheezing (yes/no)	33/239	12.1/87.9	30/165	15.4/84.6
Current hayfever (yes/no)	54/221	19.6/80.4	44/155	22.1/77.9
Current eczema (yes/no)	49/225	17.9/82.1	36/164	18.0/82.0
Atopy (SPT) (yes/no)	102/165	38.2/61.8	77/120	39.1/60.4
Passive smoking (yes/no)	114/161	41.5/58.5	88/112	44.0/66.0
Mould patch at home (yes/no)	20/255	7.3/92.7	15/185	7.5/92.5
	Mean	95% CI	Mean	95% CI
Age (years)	8.3	7.9–8.6	8.2	7.8–8.6

Table 2. The presence (%) of fungi species identified in the classrooms during summer and winter

	Winter		Summer	
	Indoors	Outdoors	Indoors	Outdoors
Deuteromycetes				
<i>Acremonium sp.</i>	22.6	69.2	0	25
<i>Alternaria sp.</i>	61.9	69.2	50	66.7
<i>Aspergillus sp.</i>	18.7	23.1	18.7	16.7
<i>Aspergillus niger</i>	64.7	38.5	66.2	25
<i>Aureobasidium sp.</i>	14.1	7.7	0	8.3
<i>Botrytis sp.</i>	0	15.4	10.9	8.3
<i>Cladosporium sp.</i>	81.2	92.3	96.9	83.3
<i>Monilia sp.</i>	0	0	3.1	0
<i>Nigrospora sp.</i>	15.6	7.7	7.8	16.7
<i>Paecilomyces sp.</i>	29.7	53.8	0	6.7
<i>Penicillium sp.</i>	81.2	53.8	96.9	100
Hyphomycetes				
<i>Phialophora sp.</i>	1.6	0	0	0
<i>Sporobolomyces sp.</i>	1.6	0	0	0
Yeast	68.7	31.8	21.9	41.7

Note: presence (%) = number of samples identified for the species/total number of samples.

Airborne Mould in Classrooms

A total of 128 airborne fungi samples were collected in the 32 classrooms during winter and summer. A range of fungi species were identified in these samples. The species and the percentage of samples they were identified are presented in Table 2. There was no significant difference in the fungal species in classrooms between summer and winter. The oldest school had the highest total airborne fungi and the difference was statistically significant from each of the other three schools in summer but not winter. There was no difference between the “allergy free” school and the other similarly aged conventional schools in either summer or winter (data not shown).

Only species that occurred with sufficient frequency for the statistical analyses (>50%) in both summer and winter were investigated for the associations with nasal patency in school children. These included: *A. alternata*, *Aspergillus niger*, *Cladosporium spp.* and *Penicillium spp.*

Table 3 presents the average levels of the four common culturable fungi and total culturable fungal colonies in the classrooms. Thirteen and twelve outside fungi samples were also collected during winter and summer, respectively. The medians of total airborne fungi were 141.4 (interquartile range: 106.0–185.5) CFU·m⁻³ and 353.4 (304.8–485.9) CFU·m⁻³, respectively, during winter and summer. The spectrum of fungal species outdoors was slightly different from indoors with *Acremonium*, *A. alternata*, *Cladosporium spp.*, *Paecilomyces spp.* and *Penicillium spp.* in winter and *A. alternata*, *A. niger*, *Cladosporium spp.*, *Paecilomyces spp.*, *Penicillium spp.* and yeast in summer being the outdoor predominant fungal genera.

AR Parameters

Four parameters of AR measurements were investigated in this study, namely MCA1, MCA2, VOL1 and

Table 3. Four common fungus levels (CFU·m⁻³) found in classrooms during winter and summer

	Classrooms (<i>n</i> = 64)				Outside (<i>n</i> = 12)	
	Mean	Median	Interquartile range	Presence (%)	Median	Interquartile range
Winter						
<i>Alternaria</i>	25.1	17.7	0–35.3	61.9	17.8	0–17.8
<i>Aspergillus niger</i>	46.4	17.7	0–35.3	64.7	0	0–17.8
<i>Cladosporium</i>	51.6	35.3	17.7–70.7	81.2	35.3	26.5–41.2
<i>Penicillium</i>	83.1	53.0	35.3–88.4	81.2	17.8	0–35.3
Total	344.8	282.7	212.0–402.0		141.4	106.0–176.7
Summer						
<i>Alternaria</i>	20.2	8.8	0–35.3	50.0	35.3	0–53.0
<i>Aspergillus niger</i>	34.0	17.7	0–35.3	66.2	0	0–13.3
<i>Cladosporium</i>	130.6	106.0	70.7–159.0	96.9	106.0	57.4–212.0
<i>Penicillium</i>	119.0	106.0	70.7–159.0	96.9	176.7	53.0–265.1
Total	346.2	318.1	247.4–419.7		353.4	304.8–486.0

Table 4. Rhinometry parameters in children during winter and summer

		Raw values				Adjusted values			
		<i>n</i>	Mean	95% CI		<i>N</i>	mean	95% CI	
				Lower	Upper			Lower	Upper
<i>Winter</i>									
	MCA1 (cm ²)	275	0.945	0.929	0.962	273	0.945	0.930	0.960
	MCA2 (cm ²)	275	0.914	0.890	0.937	273	0.914	0.893	0.935
	VOL1 (cm ³)	275	5.648	5.528	5.767	273	5.648	5.540	5.755
	VOL2 (cm ³)	275	7.911	7.725	8.097	273	7.911	7.741	8.082
<i>Summer</i>									
	MCA1 (cm ²)	200	0.833	0.814	0.851	179	0.833	0.815	0.850
	MCA2 (cm ²)	200	0.840	0.816	0.863	179	0.840	0.817	0.862
	VOL1 (cm ³)	200	5.259	5.132	5.386	179	5.259	5.138	5.380
	VOL2 (cm ³)	200	7.423	7.218	7.627	179	7.423	7.223	7.622

VOL2. Table 4 presents the raw and adjusted levels of the four variables measured during winter and summer. There were 99 subjects having paired samples for adjusted AR measurements. There were significant differences in MCA1, MCA2, VOL1 with children having larger values in winter related to those in summer.

The Associations of Rhinometry Parameters and Individual Airborne Fungus Exposure

As *A. alternata*, *A. niger*, *Cladosporium spp.* and *Penicillium spp.* were the common species isolated in the school classrooms, we only investigated the associations of the four different fungi as well as total culturable fungi counts with nasal patency in this study. Other species were isolated and did not occur with sufficient frequency for the analyses. However, they were included in the calculation of total culturable fungi levels.

First, we correlated the adjusted AR parameters with individual fungal exposure in classrooms during winter and summer (Table 5). Generally, these correlations (75%)

were negative with significant correlations between log values of *A. alternata* and the adjusted VOL1 ($p=0.031$) and between log values of *A. niger* and the adjusted MCA2 ($p=0.038$) during winter and between log values of *A. alternata* and the adjusted MCA1 ($p=0.005$) during summer. However, a positive correlation between log values of *Cladosporium spp.* and the adjusted MCA2 ($p=0.037$) appeared to be significant during winter.

To accommodate individual correlations for the measurements in the same individual during winter and summer, GEE techniques were employed for further regression analyses (Table 6).

Exposure to *A. alternata* in classrooms was associated with decreased nasal patency in children with significant regression coefficients of -0.014 (95% CI: -0.022 to -0.005 ; $p=0.001$) and -0.066 (-0.123 to -0.008) for adjusted MCA1 and VOL1 (as dependent variables), respectively. A marginally significant decrease was also found for exposure to *A. niger* and adjusted MCA2 ($p=0.034$) and exposure to *Cladosporium spp.* and

Table 5. The correlations between fungus exposure and adjusted rhinometry parameters

	Adjusted MCA1		Adjusted MCA2		Adjusted VOL1		Adjusted VOL2	
	Coefficient	<i>p</i>	Coefficient	<i>p</i>	Coefficient	<i>p</i>	Coefficient	<i>p</i>
Winter (<i>n</i> = 247)								
<i>Alternaria</i>	−0.038	0.55	−0.107	0.10	−0.137	0.031	−0.109	0.088
<i>Aspergillus niger</i>	−0.077	0.23	−0.132	0.038	−0.097	0.13	−0.101	0.11
<i>Cladosporium</i>	−0.082	0.20	0.133	0.037	0.074	0.25	0.073	0.25
<i>Penicillium</i>	−0.094	0.14	−0.040	0.53	−0.118	0.065	−0.088	0.17
Total mould	−0.114	0.07	−0.020	0.75	−0.021	0.74	−0.013	0.84
Summer (<i>n</i> = 174)								
<i>Alternaria</i>	−0.212	0.005	−0.061	0.42	−0.051	0.51	−0.033	0.67
<i>Aspergillus niger</i>	0.143	0.060	−0.028	0.71	−0.024	0.76	−0.010	0.90
<i>Cladosporium</i>	0.037	0.63	0.064	0.40	0.034	0.65	0.012	0.88
<i>Penicillium</i>	−0.026	0.74	−0.058	0.45	−0.033	0.66	−0.017	0.82
Total mould	0.032	0.67	0.006	0.93	−0.012	0.87	−0.005	0.95

Notes: Natural log values of fungus levels were used for the Pearson correlations.

Table 6. The coefficients of fungus exposure for adjusted rhinometry parameters derived by regression analysis using GEE techniques

Adjusted		Coefficient	<i>p</i>	95% CIs	
				Lower	Upper
<i>Alternaria</i>					
	MCA1	−0.014	0.001	−0.022	−0.005
	MCA2	−0.009	0.112	−0.020	0.002
	VOL1	−0.066	0.026	−0.123	−0.008
	VOL2	−0.080	0.089	−0.172	0.012
<i>Aspergillus niger</i>					
	MCA1	−0.002	0.51	−0.010	0.005
	MCA2	−0.011	0.034	−0.021	−0.000
	VOL1	−0.040	0.17	−0.097	0.016
	VOL2	−0.056	0.22	−0.146	0.034
<i>Cladosporium</i>					
	MCA1	0.002	0.63	−0.007	0.012
	MCA2	0.012	0.094	−0.002	0.025
	VOL1	0.032	0.431	−0.048	0.112
	VOL2	0.047	0.47	−0.080	0.175
<i>Penicillium</i>					
	MCA1	−0.011	0.043	−0.021	−0.000
	MCA2	−0.004	0.63	−0.020	0.012
	VOL1	−0.073	0.117	−0.164	0.018
	VOL2	−0.087	0.231	−0.230	0.056
<i>Total mould counts</i>					
	MCA1	−0.012	0.43	−0.047	0.024
	MCA2	−0.009	0.703	−0.053	0.036
	VOL1	−0.064	0.58	−0.290	0.162
	VOL2	−0.108	−0.470	0.253	0.56

adjusted MCA1 ($p=0.043$). However, we did not find a significant influence of total fungal exposure measured by total colony counts of fungi on nasal patency in children.

Further GEE regression analyses after adjusting for current wheezing, current symptoms of hayfever and atopy showed that the effect of exposure to *A. alternata* on nasal patency was independent of these current allergic conditions in children. The confounding effects of passive

smoking and fungi exposure in domestic environments were also insignificant (Table 7).

Discussion

In this study, we found an association between exposures to airborne *A. alternata* in classrooms and

Table 7. The coefficients of fungus exposure for adjusted rhinometry parameters derived by regression analysis using GEE techniques after adjusting for current wheezing, hayfever, atopy, passive smoking and mould patch at home

	Adjusted	Coefficient	<i>p</i>	95% CI	
				Lower	Upper
<i>Alternaria</i>	MCA1	−0.014	0.001	−0.023	−0.006
	MCA2	−0.009	0.10	−0.020	0.002
	VOL1	−0.067	0.020	−0.124	−0.011
	VOL2	−0.084	0.069	−0.174	0.006
<i>Aspergillus niger</i>	MCA1	−0.003	0.39	−0.010	0.005
	MCA2	−0.010	0.069	−0.020	0.001
	VOL1	−0.039	0.19	−0.098	0.020
	VOL2	−0.053	0.27	−0.146	0.040
<i>Cladosporium</i>	MCA1	0.002	0.74	−0.008	0.012
	MCA2	0.014	0.045	0.000	0.028
	VOL1	0.043	0.31	−0.039	0.126
	VOL2	0.061	0.36	−0.069	0.192
<i>Penicillium</i>	MCA1	−0.014	0.008	−0.025	−0.004
	MCA2	−0.006	0.53	−0.023	0.012
	VOL1	−0.081	0.091	−0.175	0.013
	VOL2	−0.097	0.19	−0.242	0.048
<i>Total mould counts</i>	MCA1	−0.014	0.46	−0.050	0.022
	MCA2	−0.012	0.63	−0.060	0.036
	VOL1	−0.089	0.46	−0.326	0.148
	VOL2	−0.155	0.42	−0.530	0.220

decreased nasal patency in children. These associations were observed even at relatively low levels of mould in classrooms. Decreased nasal patency associated with mould was independent of common allergic conditions, such as wheezing, hayfever and atopy. These findings are consistent with previous research in adults [12,13] exposed to moulds at school and, although the changes were small and not related to clinical outcomes, highlight the potential importance of school environments on children's health.

For children, schools are one of the most important environments for exposure to environmental pollutants [8]. Common indoor pollutants, such as formaldehyde, VOCs and particulate matter, have been shown to exist in classrooms at levels that can be harmful to the health of children [28,29]. A number of studies have found that mould levels in schools are associated with adverse respiratory and allergic health effects in both adults [12–14] and children [9–11,15,30–33]. These studies have predominantly been conducted in Nordic countries. Very few studies on the health effects of indoor mould have been conducted in Australia [34,35] and none have been

done in schools. This study suggests mould in schools is a ubiquitous problem, irrespective of climate.

In this study, we used AR as an objective test to measure upper airway changes in children exposed to mould. AR has been used extensively by a Swedish research group investigating the effect of the school environment, including mould, on occupants' health [12,36,37]. The results of our study are mostly consistent with their findings in adults [12]. In that study, however, there was an association between nasal patency and both *Aspergillus spp.* and total mould exposure in the adult school personnel [12]. In our study, *Aspergillus* was only weakly associated with upper airway changes, while *Alternaria* was a more important species. Some small changes were also observed as a result of exposure to *Penicillium*, but these were not consistent. Further, we did not find a significant effect of total mould exposure.

AR is becoming more accepted in the clinic as an objective, non-invasive technique for assessing nasal airway obstruction [38]. Although AR has predominantly been used to assess the upper airway responses to therapeutic agents [39], it has also been used to investigate

changes in the nasal mucosa with exposure to natural [40] and experimental irritants [41]. Furthermore, as discussed above, AR has been used in epidemiological studies for measuring the response of both adults [12,36,37] and children [42] to air pollutants. To reduce variability in measurements, only one assessor of AR was used in this study.

Alternaria is one of the common fungi indoors. In this study, over half of the samples collected in classrooms had airborne viable *Alternaria* spores (Table 2). *Alternaria* antigens have also been found regularly in homes [43], suggesting that *Alternaria* is ubiquitous in indoor environments. *Alternaria* sensitivity has been shown to be a risk for asthma [44,45] and exposure to *Alternaria* has been associated with asthma symptoms [46]. Potential pathogenicity and allergenicity of *Alternaria* [3,44,45] may be related to the underlying mechanisms of *Alternaria*-induced airway inflammation, particular in the upper airways, although much more research needs to be conducted to clarify the biological plausibility. This study provides data to support the hypothesis that *Alternaria* may contribute to upper respiratory inflammation even at low levels of exposure. The findings most likely represent an acute response rather than a chronic effect as the monitoring and nasal assessments were relatively contemporaneous.

It is timely to investigate the association between mould exposure and its adverse effects on children's health. A solid guideline for fungal indoor exposure, particularly for specific types of and groups of fungi is yet to be formulated, although several thresholds have been recommended [47–49]. Our study suggests that it is a challenge to set a reasonable threshold level for mould exposure in school environments as even low levels of exposure in classrooms may be detrimental to children's health. This and similar studies highlight the importance of trying to control mould levels in indoor environments. However, for moulds which have predominantly outdoor sources, such as *Alternaria*, this will be difficult. This may be particularly so in countries such as Australia where most schools rely on natural ventilation. In this study, as there was little evidence of moisture and visible mould damage in the classrooms, much of the *Alternaria* most likely infiltrated from outdoors. Reducing ventilation in schools may have consequences for the build-up of other pollutants and allergens, while retrofitting schools with mechanical ventilation will be prohibitively expensive. Therefore, these findings need to be replicated and extended before recommendations can

be made, although controlling obvious indoor sources of mould remains an important strategy.

There are limitations for this study. First, participation was voluntary and may have contributed to a self-selection bias. However, based on age and sex, the children were representative of the school populations. Self-selection may also have occurred due to concerns about the school environment. However, there was little obvious sign of mould or water damage in the classrooms and it is unlikely that children participated because of a concern about mould exposure. Second, it is possible that other factors or combination of factors in the school or home environment, which were not measured in this study, could have contributed to the upper airway congestion in the children. For example, particulates and chemicals in the air at schools have been associated with decreased nasal volumes in the Swedish studies [12,35,36]. We did measure formaldehyde and nitrogen dioxide as part of this study but concentrations were very low in all classrooms and were not associated with nasal patency (data not shown). Other pollutants were not measured. Information on the home environment was only available via questionnaire. We did not find that any of the factors, including smoking in the home, heating and cooking appliances, proximity to main roads, the presence of pets and attached garages, affected nasal patency in these children. However, we did not have indoor air pollution concentrations or meteorological conditions in the children's homes to fully assess this. Finally, no corrections were made for multiple testing. However, we have described all statistical analyses carried out and agree with Rothman [50] that there is little to be gained by adjustment, provided all the analyses are described when the study is reported. It is generally agreed that Bonferroni corrections for multiple tests are overly conservative and increase the probability of type II errors. However, even we use the Bonferroni corrections, our findings for *Alternaria* and adjusted MCA1 ($p = 0.001$) are still significant as we conducted 20 tests (five mould variables multiplied by four rhinometry parameters) considering a corrected significance of 0.0025 (0.05/20).

In the analyses, we controlled for respiratory allergic diseases such as asthma and rhinitis. We also controlled for atopy in the absence of disease. Atopy without disease was not associated with any measure of nasal patency, which is in agreement with a previous study in adults [51]. However, it is possible that children with specific sensitisation to mould allergens may have been more likely to have a response to mould exposure. Upper and lower airway inflammation, as measured by exhaled nitric oxide, is elevated in sensitised asthmatic and rhinitic children when

exposed to the relevant allergen [52,53]. There were too few children (10 in summer and 21 in winter) to explore this relationship. We only tested for a single mould allergen in this study and may have missed a number of other mould sensitised children, albeit only those without allergic disease. As these children are most likely to have been randomly distributed among the classrooms, we do not feel this affects the overall results, but it does exclude being able to investigate the relationship between sensitisation, exposure and upper airway changes.

Conclusion

In this study, we have observed that exposure to airborne *Alternaria* in school environments is associated

with decreased nasal patency in children. There is also a suggestion that exposure to airborne *A. niger* and *Penicillium* in classrooms is associated with upper airway changes in children. These observations agree, to some degree, to research in Northern Europe where the climate is quite different and adds to support to the need for the introduction of guidelines for indoor mould levels.

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