

Quantitative PCR analysis of fungal DNA in Swedish day care centers and comparison with building characteristics and allergen levels

Abstract Sweden has had allergen-avoidance day care centers (AADCs) since 1979. The aim of this study was to measure fungal DNA by quantitative polymerase chain reaction (qPCR), a new method, in AADCs and ordinary day care centers (ODCs) and examine associations between allergen levels and building characteristics. Dust samples were collected by swabbing doorframes, vacuum-cleaning, and using Petri dishes. In total, 11 AADCs and 11 ODCs were studied (70 rooms). Total fungal DNA, measured by qPCR in the swab dust, was detected in 89%, *Aspergillus* or *Penicillium* (*Asp/Pen*) DNA in 34%, and *Stachybotrys chartarum* DNA in 6% of the rooms. Total fungal DNA was significantly higher in rooms with linoleum floor ($P = 0.02$), textile carpets ($P = 0.03$), reported dampness/molds ($P = 0.02$) and reported odor ($P < 0.001$) in the buildings, and significantly lower in wooden facade buildings ($P = 0.003$). Reported odor was related to the amount of sieved fine dust, reported dampness/molds and type of building construction. Total fungal DNA was related to cat, dog, horse and total allergen levels ($P = 0.003$) in the day care centers. In conclusion, total fungal DNA is related to reported dampness/molds, reported odor, and type of wall construction. The association between fungal and allergen contamination indicated a general 'hygiene factor' related to biological contaminants.

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Key words: Day care centers; Quantitative PCR; Fungal DNA; Allergen levels; Indoor environment; Building dampness.

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Received for review 28 September 2008. Accepted for publication 5 February 2009.

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Practical Implications

The associations between fungal DNA, reported dampness/molds, and odor support the view that buildings with odor problems should be investigated for possible hidden fungal growth. There is a need to measure fungal biomass in different types of building constructions by monitoring fungal DNA. Analysis of fungal DNA with quantitative PCR can be a fast and practical way to study indoor fungal contamination. Swabbing dust from the doorframe of the main entrance to the room can be a convenient method of sampling dust for fungal DNA analysis. The high prevalence of reported dampness/molds and the common occurrence of fungal DNA indicate the need to improve the indoor environment of Swedish day care centers.

Introduction

Moisture enables reproduction of fungi and bacteria in building materials (Pitkäranta et al., 2008; Samson et al., 1994), and dampness and mold have been associated with health problems such as asthma and

allergy (Bornehag et al., 2001, 2004; Husman, 1996; Jedrychowski and Flak, 1998). Members of the phylogenetically related genera *Aspergillus*, *Penicillium*, and *Paecilomyces* are among the most common fungi in indoor environments (Shelton et al., 2002). Molds pose potential health risks because of their production of

allergens (Shen and Han, 1998; Shen et al., 1999a,b), a wide range of mycotoxins (Hendry and Cole, 1993), and inflammatory substances such as beta-D-glucan (Douwes, 2005).

Air is generally the most common route of fungal exposure (Vesper et al., 2008). After more than a century of little change in the basic microbial collection and analytical techniques, there is a need to develop methods of rapid sampling and detecting of bacteria, fungi, and toxins. Such methods include rapid and miniature assay systems using specific antibodies, protein fingerprints, or DNA that can be applied to collect samples to detect airborne organisms (Tovey and Green, 2005). For simple measurement of mold presence, mycelia can be directly sampled from surfaces by swabbing or lifting with adhesive tape. Such samples can be analyzed by either culture or direct microscopy (Horner et al., 1995). The main disadvantage of these methods is the lack of quantitative correlation of the samples (Tovey and Green, 2005). The benefits of using DNA instead of cultivation-based methods are the speed, accuracy, analytical sensitivity of detection, and the possibility to detect and identify both dead and viable organisms (Amann et al., 1995; MacNeil et al., 1995). The latter benefit is especially valuable in indoor studies because the main hazards of the microorganisms are not dependent on viability (Brasel et al., 2005; Hirvonen et al., 1997).

Molecular methods in fungal studies include conventional or quantitative polymerase chain reaction (qPCR) specific for fungal species or groups (Dean et al., 2005; Gachon and Saindrenan, 2004; Haugland et al., 2004; Vesper et al., 2005). qPCR provides valuable information on the occurrence of the most common indoor fungi and is used for determination of microbes in indoor samples, e.g. house dust and air (Haugland et al., 1999, 2002; Meklin et al., 2004; Rintala and Nevalainen, 2006). This method shows great promise in giving fast quantitative data on the occurrence of the studied organisms (Meklin et al., 2004; Neely et al., 2004). As far as we are aware, no studies have ever dealt with fungal DNA exposure levels at day care centers.

In Sweden, public day care for pre-school children is a legislated right. During the 1990s, there was a focus on the indoor environment at schools and day care centers. National campaigns for allergy prevention and better indoor environments resulted in the creation of special day care centers, called 'allergen-avoidance day care centers' or 'allergy-adapted day care centers' (AADCs). In these special day care centers, neither children nor staff are allowed to have pets at home, and staff members are not allowed to be smokers or use perfumes or cosmetics. General cleaning is enhanced and there is a reduction in the amount of textiles, carpets, open shelves, and pot plants in the rooms. We have previously compared AADCs with matched ordinary day care centers (ODCs) and found a relatively high prevalence of

reported dampness and molds in both types of day care centers (Bröms et al., 2006). The aim of this study was to examine associations between fungal DNA levels in swab dust in Swedish day care centers and selected buildings characteristics, type of day care centers (AADCs and ODCs), measured indoor climate (temperature, RH, CO₂), and levels of furry pet allergens in the investigated rooms. However, differences in allergen levels between AADCs and ODCs, and associations with building characteristics will be analysed in another article.

Study population and methods

Selection of population

In a previous survey, AADCs in Sweden were identified nationwide from technical and environmental sections of all 288 municipalities in Sweden, as well as all outpatient and inpatient paediatric clinics. Seventy-two AADCs and the closest situated ODCs were identified (Bröms et al., 2006). In this study, we selected a subset of matched pairs of day care centers (AADCs and closest ODCs) within near-travelling distance of two major cities in Sweden (Malmö and Göteborg). Three to five rooms in each selected day care center (depending on the size) were investigated for fungal DNA and airborne pet allergens. The rooms were arbitrarily selected. As regards vacuum-cleaning and climate measurement, only three rooms per day care center could be examined because of practical limitations such as time and equipment. In total, 11 AADCs with 33 rooms and 11 ODCs with 37 rooms, in southern and western parts of Sweden, were included in this study. Sampling was performed during the winter season (Jan–Feb) 2007.

Exposure assessment, building inspection and indoor climate measurement

In the previous study (Bröms et al., 2006), a questionnaire was sent to the local directors of all day care centers in 2000. It included three questions on water leakage or flooding, signs of floor dampness, and visible mold growth in any part of the building the last 12 months using previously published questionnaires (Norbäck et al., 1999). Moreover, there was an additional question asking if there had been water leakage or mold growth at any time in the building (irrespective of recall period). There were two yes/no questions on odors in the building, one question asking about moldy odor and the other about other types of odor (Norbäck et al., 1999). A room inspection for signs of water leakage, flooding, damp spots, floor dampness (bubbles under the PVC floor), visible mold growth, moldy odor, and window condensation was performed in the selected rooms ($N = 70$) in 2007. During this time, sampling dust for fungal DNA and

allergen analysis was also carried out. In order to cover the time period from 2000 to 2007, additional data on reported dampness and mold growth were collected by a structured telephonic interview with the local directors, using the same set of questions as in the questionnaire survey.

Details on construction, building materials and age, type of ventilation and heating system, amount of open shelves, textiles, and number of potted plants were noted. The factors of room volume (m^3), shelf (m/m^3), textile (m^2/m^3) and potted plant (number of pot plants/ m^3) were calculated in each selected room (Skov et al., 1990). Temperature (T , $^{\circ}\text{C}$), relative humidity (RH, %), and concentration of CO_2 (ppm) were measured in three rooms of each day care center during normal activities within 50–70 min with Q-TrakTM IAQ monitor (TSI Incorporated, St. Paul, MN, USA), by logging average values over 1 min. The instruments were regularly calibrated by Comfort Control, the Swedish service laboratory for TSI equipment.

Dust collection

The dust samples for analysis of fungal DNA were collected by swabbing a 60-cm^2 surface ($1 \times 60\text{ cm}$) of half of the upper part of the doorframe on the main entrance door to each room in the selected AADCs and ODCs. If the main entrance door had a supply or exhaust ventilation duct above the doorframe, another doorframe without any ventilation duct was selected. Two samples were collected by dividing the doorframe into a left and a right side, whereby the left-side frame was sent for fungal DNA analysis. Sample collection by swabbing was performed with a sterile cotton swab designed for medical use (Copan Innovation, Brescia, Italy; <http://www.copanitalia.com>).

Vacuumed dust was collected in the three rooms in which the climate measurements were made for allergen analysis. Two samples of settled dust were collected in each room by dividing the room into roughly an entrance-side half and a window-side half (Kim et al., 2005; Smedje et al., 1997; Zhao et al., 2006), using a 1200 W vacuum cleaner equipped with a special dust collector (ALK Abello, Copenhagen, Denmark) fitted with a Millipore filter (pore size $6\text{ }\mu\text{m}$). We sieved dust samples through a 0.3-mm mesh screen to obtain the fine dust (Li et al., 1997), weighed the amount of the fine dust collected and stored it at -20°C until extraction.

Airborne dust for allergen analysis was collected in two Petri dishes in each room, placed on the top of open book shelves or similar areas (at about $1.5\text{--}2.0\text{ m}$ height) and kept open for 7 days (Karlsson et al., 2002). After buffer extraction with 3 ml of phosphate-buffered saline (PBS) with 1% bovine serum albumin (BSA), the liquid was transferred into an Eppendorf tube and centrifuged (Zhao et al., 2006) and the supernatants were stored at -20°C until analysis.

Allergen analysis

Two-site sandwich enzyme-linked immunosorbent assay (ELISA) was applied to determine the allergen levels of cat (Fel d1), dog (Can f1) and dust-mite (Der p1 and Der f1) (Indoor Biotechnologies Ltd, Manchester, UK), and horse (Equ c x) (Mabtech, Stockholm, Sweden) (Emenius et al., 2001), as previously described (Kim et al., 2007) for both vacuumed and airborne dust samples by using monoclonal antibodies. The allergen levels were expressed as ng/g dust, except for horse allergen concentration which was expressed as U/g dust, where 1 Unit equalled 1 ng protein of horse hair; dander extract was used as the standard (Allergon, Valinge, Sweden and NIBSC, Hertfordshire, UK).

Amplified ELISA was used for cat allergen analysis in Petri dish airborne dust samples for cases when the allergen levels were lower than 1.0 ng/ml by conventional ELISA. It was completed with a commercial signal amplification kit, basically by following the manufacturer's protocol (Karlsson et al., 2002). The allergen levels in airborne dust samples were expressed as $\text{ng}/\text{m}^2/\text{day}$, by dividing the amount of allergen on the Petri dish by the sampling time, and the total surface area of Petri dish (0.0124 m^2 for sum of both lids) (Kim et al., 2007). Total allergen level (ng/g dust) was calculated by adding cat and dog allergens (ng/g dust) and horse allergens (U/g dust).

Analysis of fungal DNA by qPCR

The cotton swab samples for fungal DNA analysis by qPCR were sent to a professional laboratory and ZonAB (Uppsala, Sweden) which got license from Environmental Protection Agency (EPA), the United States on 'Method of Identifying and Quantifying Specific Fungi and Bacteria' (Patent no. 6387652). For DNA extraction, the cotton swabs were cut into 2-ml tubes, diluted with $400\text{ }\mu\text{l}$ of AP1 buffer (DNeasy Plant Mini Kit; Qiagen, Hilden, Germany) and vortexed briefly. After removal of the swab, the cellular material and cell debris in the sample pellet was disrupted and homogenized by the addition of a tungsten carbide bead (Qiagen) and the subsequent beating and grinding effect of the bead on the sample material when shaken in the micro-centrifuge tube on a TissueLyser (Qiagen) at 30 Hz for 10 min. Total genomic DNA was extracted from the homogenates with the DNeasy Plant Mini Kit (Qiagen) according to the manufacturer's instructions.

Amplification and detection of DNA extractions for *Aspergillus/Penicillium* genera (*Asp/Pen*), *Stachybotrys chartarum* (*S. chartarum*) and total fungal DNA were performed on Mx3000P/MXpro real-time PCR machine (Stratagene, La Jolla, CA, USA) in the TaqMan Master Mix (Applied Biosystem, Carlsbad,

CA, USA) according to the manufacture's protocols. Primer and probe sequences used in the assays for *Asp/Pen* (assay name: PenAsp1mgb) and *S. chartarum* (assay name: Stac) are available online (<http://www.epa.gov/nerlcwww/moldtech.htm>). We analyzed total fungal DNA species from the group consisting of Universal Fungal. The primers are 5.8F1 (SEQ ID NO: 213) and 5.8R1 (SEQ ID NO: 214), and the probe is 5.8P1 (SEQ ID NO: 215). The species list and primers, probes are available online at <http://www.freepatentsonline.com/6387652.html>. The PCR cycling was performed using the following protocol: 50°C for 2 min, 95°C for 10 min, 95°C for 0.25 min, and 60°C for 1 min, 45 cycles.

Standard curves were created for the respective analysis, from total genomic DNA extracts from pure cultures. The DNA extracts from the pure cultures were quantified using limiting dilution analysis (Wang and Spadaro, 1998). For total fungal DNA, *Asp/Pen* and *S. Chartarum*, the slope [cycles/log (copies/ μ l)] of the standard curves was -3.686 , -3.772 , -3.298 , and the intercept (cycles) was 41.71 , 44.09 , 37.44 , respectively. The quantity of the unknown samples was calculated based on the calibration curve of standardized DNA solutions vs. the corresponding cycle threshold (Ct) value. If internal control was not detected or the Ct is over a certain value (37 in this study), the samples were further diluted. As per the sampling method in this study, the mold level was calculated by fungi cell concentrations expressed as cell equivalents (CE) for each target mold or mold group. The final result was presented as CE/m² accounting for the sampling surface areas (60 cm²).

Statistical methods

Statistical calculations were performed using the Statistical Package for the Social Sciences (SPSS 12.0). Summary statistics such as arithmetic and geometric mean values and measures of dispersion were used. The Mann–Whitney *U*-test was used to compare differences in allergen levels between AADCs and ODCs, or associations between allergen levels and building factors, as DNA data were not normally distributed. Associations between continuous variables were tested using the Kendal tau- β rank correlation test. Multiple linear regression was performed to study mutually adjusted associations. All tests were two-tailed, and a *P*-value below 0.05 was used to indicate statistical significance.

Results

Building inspection and climate measurement

All buildings had leaning roofs, 81% were built on a concrete slab without a basement and approximately

Table 1 Characteristics of investigated rooms (*N* = 70) in 11 allergen avoidance day care centers (AADCs) and 11 ordinary day care centers (ODCs)

Building characteristics	AADCs (<i>n</i> = 33) <i>n</i> (%)	ODCs (<i>n</i> = 37) <i>n</i> (%)	Total day care center rooms (<i>n</i> = 70) <i>n</i> (%)	<i>P</i> -value
Floor heating ^a	3 (9%)	3 (8%)	6 (9%)	0.56
Wooden facade buildings ^a	15 (50%)	16 (43%)	31 (44%)	0.45
Basement ^a	6 (18%)	7 (19%)	13 (19%)	0.96
Dining room ^b	16 (31%)	33 (57%)	49 (46%)	0.008**
Observed dampness ^b	0 (0%)	9 (24%)	9 (13%)	0.11
Reported window condensation ^c	0 (0%)	15 (41%)	15 (21%)	<0.001***
Reported dampness ^c	9 (27%)	15 (41%)	21 (34%)	0.24
Any reported odor ^c	0 (0%)	16 (43%)	16 (23%)	<0.001***
Type of floor ^b				
Linoleum	16 (48%)	16 (43%)	32 (46%)	0.73
PVC	14 (42%)	19 (52%)	33 (47%)	
Wood	3 (10%)	2 (5%)	5 (7%)	

P-value * <0.05 , ** <0.01 , *** <0.001 .

^aCharacteristics of the building where the investigated rooms were situated.

^bObservation in the investigated rooms.

^cIn any part of the building where the inspected rooms are situated.

Table 2 Building age and measured indoor climate parameters in investigated rooms in AADCs and ODCs

Parameters	AADCs (<i>n</i> = 33)		ODCs (<i>n</i> = 37)		Total day care center rooms (<i>n</i> = 70)		<i>P</i> -value
	Mean	s.d.	Mean	s.d.	Mean	s.d.	
Year of construction	1985	11	1981	14	1983	13	0.09
Indoor climate							
CO ₂ (ppm)	680	92	630	87	655	92	0.02*
<i>T</i> (°C)	21.7	2.0	21.8	1.1	21.8	1.6	0.56
RH (%)	26	7	27	7	26	7	0.82

P-value * <0.05 , ** <0.01 , *** <0.001 .

half were wooden facade buildings. All had a mechanical ventilation system and most were heated by district heating and waterborne radiators, a few with floor heating. The floor materials in the investigated rooms were either linoleum or PVC; only a few rooms had a wooden floor (Table 1).

There was no significant difference in the year of building construction between the two types of day care centers (Table 2). Mean temperature and RH were similar in both types of day care centers, which were well-ventilated buildings with CO₂ levels below the current standard of 1000 ppm (Burton, 2000; National Swedish Board of Occupational Safety, Health, 2000). There was significantly lower CO₂ levels in ODCs when compared with AADCs, but the mean difference was only 25 ppm (Table 2). The mean of the curtain, carpet, shelf, and pot plant factors for all investigated rooms (*N* = 70) was 0.034 m²/m³ (s.d. = 0.034), 0.014 m²/m³ (s.d. = 0.024), 0.071 m/m³ (s.d. = 0.067), and 0.021 pot plant/m³ (s.d. = 0.037), respectively.

Self-reported data from the local directors showed that some day care centers had a history of dampness, mold growth, and odor, but none of the directors classified the odor as 'moldy odor'. Window condensation and odor in any part of the buildings were more common in ODCs when compared with AADCs, but there were no significant differences in reported dampness/mold between ODCs and AADCs (Table 1). During our inspection in 2007, which covered the selected 70 rooms, we observed signs of dampness in some of the rooms in the ODCs but not in any of the AADCs. Window condensation was not observed in any of the investigated rooms (Table 1).

Buildings with reported odor had significantly more vacuumed fine dust (mean 752 mg; s.d. = 301) when compared with buildings without reported odor (mean 372 mg; s.d. = 491) ($P < 0.001$). In addition, 56% of the buildings with reported odor had reported dampness, when compared with 22% in buildings without reported odor ($P = 0.009$). Moreover, 33% of the stone/brick, but only 13% of wooden facade buildings had reported odor ($P = 0.05$). Finally, 50% of the stone/brick, but 10% of the wooden facade buildings had reported dampness ($P < 0.001$). There was no difference in CO₂ level or RH between stone/brick facade buildings and wooden facade buildings, or between buildings with and without reported odor.

Table 3 Mean levels of total fungal DNA in investigated rooms in relation to building characteristics and room characteristics

Building characteristics		Total fungal DNA ($N = 70$)			
		N	GM ($\times 10^7$ CE/m ²)	GSD	P -value
Reported dampness	No	46	1.02	3.39	0.02*
	Yes	24	1.78	4.07	
Observed dampness	No	61	1.23	3.50	0.69
	Yes	9	1.29	5.18	
Window condensation	No	55	1.18	3.85	0.81
	Yes	15	1.46	3.04	
Any reported odor	No	54	0.93	3.71	<0.001***
	Yes	16	3.22	1.85	
Wooden building ^a	No	36	2.08	2.70	0.003**
	Yes	31	0.81	3.17	
Linoleum	No	33	0.83	3.63	0.02*
	Yes	32	1.60	3.45	
Dining room ^b	No	42	1.20	4.16	0.84
	Yes	28	1.29	2.99	
Floor heating	No	64	1.23	3.79	0.85
	Yes	6	1.30	2.47	
Basement	No	57	1.31	3.51	0.46
	Yes	13	0.96	4.42	
Region of Sweden	South	42	1.21	4.05	0.90
	West	28	1.27	3.16	
Type of day care centers	AADCs	33	0.97	4.14	0.18
	ODCs	37	1.53	3.17	

^aThree rooms with other facade material were omitted.

^bFive rooms with wooden floors were omitted.

P -value * <0.05 , ** <0.01 , *** <0.001 .

GM, geometric mean; GSD, geometric standard deviation.

CE/m² = cell equivalents of DNA per square meter.

Table 4 Description of the four buildings with *S. chartarum* DNA

Buildings characteristics	1	2	3	4
Type of day care center	ODC	ODC	ODC	AADC
Building year	1951	1978	1993	1997
Wall facade	Wood	Stone/brick	Stone/brick	Wood
Basement	NO	NO	NO	NO
Type of floor	PVC	Linoleum	PVC	Linoleum
Reported dampness/mold	YES	YES	NO	NO
Reported odor	NO	YES	YES	NO
Observed dampness/mold, odor	NO	NO	YES	NO
CO ₂ (ppm)	780	740	602	720
RH (%)	25	23	22	19
<i>S. chartarum</i> DNA ($\times 10^5$, CE/m ²)	3.30	0.57	5.73	0.66
Total fungal DNA ^a ($\times 10^7$, CE/m ²)	2.98	5.55	5.94	5.27

^aMean value of total fungal DNA for all 70 rooms was 1.23×10^7 CE/m².

Fungal DNA and total allergen levels

By using the quantitative PCR method, total fungal DNA was detected in 91%, *Asp/Pen* DNA in 34% and *S. chartarum* DNA in 6% of all rooms in the day care centers. The range of total fungal DNA (CE/m²) ranged from $<10,000$ to 8.61×10^7 . The arithmetic mean value was 25 121 for *Asp/Pen* DNA (s.d. = 53,554; max value 307,833). The range of total allergens in vacuumed and Petri dish dust samples was 90 to 11,839 ng/g and 7.70 to 442.26 ng/m²/day, respectively. None of the day care centers had any mite allergens (Der p1 and Der f1) in fine vacuumed dust (<100 ng/g dust). Data on total fungal DNA are given in Table 3. *S. chartarum* DNA was detected in four rooms (6%), in three ODCs, and one AADC. Two of the day care centers (buildings 3 and 4) were present in the same municipality, situated close to each other. Descriptions of the four buildings are given in Table 4.

Associations between fungal DNA, building factors and allergen levels

There were significantly higher levels of total fungal DNA in rooms in day care centers where the local directors had reported a history of dampness, water leakage or mold growth ($P = 0.02$) and odor ($P < 0.001$). There were no associations between total fungal DNA and observed dampness in 2007, and no significant differences in total fungal DNA between ODCs and AADCs. In addition, total fungal DNA was lower in wooden facade buildings when compared with that in stone/brick facade buildings, while it was higher in rooms with linoleum floor when compared with those with PVC (Table 3). Rooms with more carpet area per m³ had higher levels of total fungal DNA. Surprisingly, there was a negative association between RH and fungal DNA but a positive association between reported window condensation and RH ($P < 0.05$). There were associations between total fungal DNA and total allergen levels in vacuumed dust and Petri dish samples (Table 5). The Kendal tau- β correlations of allergens in vacuumed and Petri dish

Table 5 Associations between total fungal DNA levels in selected rooms and building age, room characteristics, indoor climate, allergen levels and total amount of dust

Factors/total fungi DNA	Kendal tau- β	P-value
Year of construction	0.00	0.99
Curtain factor ^a	-0.007	0.93
Shelf factor ^a	0.05	0.59
Carpet factor ^a	0.20	0.03*
Pot plant factor ^a	-0.03	0.78
Indoor climate		
CO ₂	-0.03	0.76
Temperature	0.08	0.35
RH	-0.19	0.03*
Total allergen levels		
In the vacuumed dust	0.22	0.009**
In the Petri dish dust	0.24	0.003**
Total vacuumed dust	0.13	0.11

P-value * <0.05 , ** <0.01 .

^aObservation in the investigated rooms ($N = 70$).

samples were 0.15 ($P = 0.07$) and 0.22 ($P = 0.009$) for cat, 0.19 ($P = 0.03$) and 0.19 ($P = 0.04$) for dog, 0.21 ($P = 0.01$) and 0.19 ($P = 0.04$) for horse, respectively.

The associations between *Asp/Pen* DNA, building and room characteristics were analysed. The analysis was restricted to factors significantly associated with total fungal DNA, as well as all dampness-related factors. There were no significant association between *Asp/Pen* DNA levels and reported dampness/molds, reported odor, reported window condensation, observed dampness/molds, RH, CO₂, carpet factor, or wooden facade. *Asp/Pen* DNA was detected in 21% of rooms with PVC floors, in 39% rooms with linoleum floors, and in 80% rooms with wooden floors. These differences were significant if analysed by chi-squared test for a 2×3 contingency table ($P = 0.02$).

Significant building factors as well as type of day care centers were kept simultaneously in multiple linear regression models. As reported odor and reported dampness were closely correlated, they were initially not kept simultaneously in the models. Model 1 included reported odor, carpet factor, type of floor, type of facade, and type of day care centers. Only reported odor remained significant in the mutually adjusted regression model ($P = 0.001$). Model 2 excluded reported odor on base of model 1 but included reported dampness/molds. Only reported dampness/mold growth remained significant in the mutually adjusted regression model ($P = 0.03$). If both reported odor and reported dampness were kept in the models together with the other factors, reported odor remained significant ($P = 0.001$), while dampness/mold growth were not formally significant ($P = 0.07$).

Discussion

In this study, we have demonstrated that total fungal DNA in swabbing dust samples, detected through a

qPCR method, was related to odor perception and reported dampness/mold growth, and certain building characteristics. Moreover, we found associations between total fungal DNA, cat, dog, horse allergens, and total allergen levels in both vacuumed and Petri dish dust samples.

Most fungal spores are efficiently collected by the most commonly used particle samplers (Burge, 2002). Surface sampling, when used in conjunction with molecular techniques, can help to narrow down the list of potential sources (Maher et al., 2002). We applied swabbing of doorframes as a new method to collect dust samples for fungal DNA analysis in indoor environments. This enables the sampling of dust that had accumulated over a longer period of time (several months), as doorframes are not included in the weekly cleaning routines. Moreover, sampling was done at the same height in all rooms as door areas are standardized. Cotton swabbing has previously been used to sample fungal DNA in patients with fungi in the nasal mucosa (Catten et al., 2001), but we found no previous study applying this method to sample indoor fungal DNA. Most other studies of fungal DNA use either vacuum cleaning of settled dust on floor (Roe et al., 2001) or pumped air sampling (Meklin et al., 2007). These methods might include better defined sampling conditions than swabbing, but the advantage of the swabbing technique is that it is fast and convenient and might provide a better long-term estimate of the indoor fungal DNA contamination.

Our study was a part of a larger day care study in Sweden (Bröms et al., 2006). For practical reasons, we included day care centers that were located within travelling distance from Göteborg in western Sweden and Malmö in southern Sweden. As we studied AADCs and ODCs located close to each other, it is unlikely that there were systematic differences in outdoor fungal levels that would have biased the comparison between the two types of day care centers. Moreover, we found no difference in fungal DNA levels when comparing all day care centers between western and southern Sweden. As the study is a part of a national study, and there was no selection of buildings with health complaints, dampness or microbial growth or a particular building construction, we consider our study to have reasonable external validity.

We compared fungal DNA levels between AADCs and ODCs, as the previous nationwide survey found that dampness/mold growth was more common in ODCs (Bröms et al., 2006). In our subsample, there was no significant difference in reported or observed dampness/molds, or fungal DNA levels between AADCs and ODCs. Moreover, there were no differences in room temperature and RH levels between the two types of day care centers. AADCs had significantly higher CO₂ levels than ODCs; the difference was only 25 ppm though, and would not be of any practical

importance. Previous studies have shown that fungal concentrations may be related to flat roofs that are causing water leakage (Wålinder et al., 2001b) and to the type of ventilation system (Bholah and Subratty, 2002; Fung and Hughson, 2003). None of the buildings had a flat roof, all had a mechanical ventilation system, and the CO₂ levels were below the ventilation standard of 1000 ppm in all rooms.

There was a significant association between a reported history of dampness/mold growth and total fungal DNA levels, but no significant association between observed dampness/mold growth and total fungal DNA in 2007. One reason for the discrepancy could be that the inspection covered only the selected rooms, while the reported dampness/mold growth covered all parts of the building. In addition, inspection only covered current situation, while the reported dampness/covered the history of dampness and mold growth for several years. Hidden microbial growth in the construction may result in fungal emissions to the indoor environment, including odorous and irritant substances and/or allergens (Bornehag et al., 2001). However, hidden microbial growth is difficult to discover at visual inspection, and we had no possibility to do a destructive sampling of building materials for microbial analysis.

There was a strong association between total fungal DNA levels and odor reported by the local directors (Bröms et al., 2006) in the day care centers. In our study, reported odor was related to the amount of fine vacuumed dust, types of buildings (stone vs. wood facade building) and reported dampness/molds. The previous survey (Bröms et al., 2006) reported that dampness and mold growth was common. In most cases, the odor was not classified as fungal odor. However, associations between building dampness and other types of odors, such as stuffy or pungent odor, have been reported previously (Engvall et al., 2001; Roussel et al., 2008). Future studies are needed in which material samples are collected from the construction and analyzed for fungal growth (Wålinder et al., 2001a) and compared with indoor fungal DNA levels.

Water is the primary controlling factor for indoor fungal growth (Pasanen et al., 2000). As expected, we found a positive association between RH measured by us and window condensation reported by the local directors. However, we found a negative association between RH and total fungal DNA levels, but this was not significant after mutual adjustment. The dispersal of spores from a substrate depends on numerous factors (Kuhn and Ghannoum, 2003), and accidental or structural dampness (Roussel et al., 2008). Moreover, RH must be consistently very high (>85%) to lead to fungal growth (Burge, 2002).

We found that buildings with a stone/brick facade had more total fungal DNA than those with a wooden facade in the day care centers, but this difference was

not significant when including reported odor or dampness/molds in the multiple regression models. Other studies have indicated that wooden materials offer more favorable conditions for fungal growth than other building materials (Meklin et al., 2003; Pietari-nen et al., 2008). Water condensation can be particularly problematic inside the structure of walls (Burge, 2002; Pessi et al., 2002), and moisture and mold problems in Swedish buildings may be more frequently located inside the building construction in floors and walls (Bornehag et al., 2001; Roussel et al., 2008). One limitation of our data is that we have no information about the type of wall construction, only the external material. However, most day care centers in our study were small buildings, constructed around 1970–2000, which usually have a wooden construction in Sweden. Thus, in our study, the stone/brick facade day care centers with wooden wall construction may entail an increased risk for dampness and molds, leading to higher fungal DNA levels compared with the wooden facade buildings, as dampness can accumulate behind the brick or stone layer. Dampness problems from this type of construction have been reported by consultant companies in Sweden working with dampness problems in buildings. Thus, our findings indicate that the type of building construction may influence prevalence of odor and dampness problems, and as a consequence influence the indoor levels of fungal DNA, but more studies are needed regarding this issue.

Total fungal DNA was higher in rooms with linoleum floor covering when compared with those with PVC floors, but this difference was not significant when including reported odor or dampness/molds in the multiple regression models. Linoleum floors are commonly used in Sweden. If there is a dampness problem in the floor construction, fungal growth could arise in the jute material on the back of the linoleum material. In the study, rooms with more carpet area per m³ also had higher levels of total fungal DNA. Associations between carpeting and airborne levels of viable fungi have previously been reported (Dharmage et al., 1999; Verhoeff et al., 1994; Wickman et al., 1992).

We found *S. chartarum* DNA in four rooms in four buildings. In Sweden, gypsum board is commonly used in the interior of the wall construction, behind the wall paper surface, and *S. chartarum* can grow on the cellulose paper on the gypsum board. All the four rooms had elevated levels of total fungal DNA (two to five times), three reported dampness/molds or observed dampness/molds, and two were situated in buildings with reported odor. Thus, it seems that sporadic growth of *S. chartarum* can occur in Swedish day care centers, and the health consequence of this exposure needs to be further evaluated.

We found significant associations between total fungal DNA levels, cat, dog, horse allergens, and total

allergen levels, but no association between the total fungal DNA and the total amount of fine dust in the rooms. One limitation of the comparisons is that fungal DNA and allergen levels were sampled by different methods in this study. However, the main aim of the study was to evaluate fungal DNA by the new method using cotton swabs by swabbing doorframe. The other two methods were chosen for allergens because they have been previously used (Kim et al., 2007; Zhao et al., 2006), while vacuum cleaning is the most commonly used method for allergen dust collection.

The association between fungal DNA and allergen levels indicated that there was a hygiene factor more specifically related to bio-aerosols from both allergens and fungi, but not simply related to the total dust level and cleaning conditions. To our knowledge, the association between fungal DNA and allergens has

not been reported previously and it illustrates the need to sample both allergens and microorganisms in epidemiological studies on health effects of indoor bio-aerosols. We were able to demonstrate that total fungal DNA is related to different building characteristics and indoor factors, including reported dampness/molds and reported odor. The observed association between fungal and allergen contamination in the Swedish day care centers indicated a 'hygiene factor' related to different types of biological contaminants.

Acknowledgements

This study was supported by grants from the Swedish Research Council for Environment, Agricultural Sciences and Spatial Planning, CFUG Gävleborg County, and the Swedish Asthma and Allergy Association's Research Foundation.

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