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PAPER

Fungal DNA and pet allergen levels in Swedish day care centers and associations with building characteristics

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Pet allergens and mold growth related to damp are common phenomena in day care centers in Sweden but exposure measurements of these factors are lacking. The aim of this study was to investigate the relationship between building construction and indoor environment quality in Swedish day care centers and the potential for exposure to fungi (analyzed by quantitative PCR) and animal allergens (analyzed by ELISA). Measurements were performed in 21 day care centers (103 rooms) from one municipality in Sweden, which were identified as constructions at risk of dampness (85% of the buildings) and with visible damage and mold growth (54% of the buildings). Dust samples were collected using cotton swab and Petri dishes. Total fungal DNA was detected in 99% and 100%, *Aspergillus*/*Penicillium* DNA in 54% and 68%, and *Stachybotrys chartarum* DNA in 4% and 9% of the investigated rooms in cotton swab and Petri dish samples, respectively. The total fungal DNA levels (Geometric Mean, GM) were 4.2×10^6 cell equivalents per m^2 and 2.9×10^5 cell equivalents per m^2 per day in the swab and Petri dish samples, respectively. The concentrations (GM) of cat (Fel d1), dog (Can f1), and horse (Equ cx) allergens were 9.4, 7.2 ng m^{-2} day^{-1}, and 5.0 unit per m^2 per day, respectively. Total fungal DNA levels were higher in risk construction buildings ($p = 0.01$), in rooms with linoleum flooring material ($p = 0.003$), and in buildings with rotating heat exchangers ($p = 0.02$). There were associations between total fungal DNA levels and cat ($p = 0.02$), dog ($p < 0.001$), and horse ($p = 0.001$) allergens. In conclusion, risk constructions, damp constructions, mould growth, fungal DNA, and animal allergens were common exposure factors in Swedish day care centers. Building constructions that represent a high risk for internal dampness should be avoided in the future, and measures to reduce allergen levels should be considered to protect pet-allergic children from asthmatic problems.

1. Introduction

There is a trend that more children enter day care centers in Europe. In 1997, 63% of US children under 5 years of age were in regular child care.[1] In 2002, 74% of all Swedish pre-school children attended day care centers.[2] Dampness and indoor mould growth seem to be common problems in day care centers. In a Finnish study, 70% of the day care centers were said to have water damage and 17% to have mould odor.[3] In a nation wide survey of Swedish day care center study, more than a third of the

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Environmental impact

Indoor allergens and microbial contamination can influence the risk for asthma and allergy, and there is a need for simple methods to monitor indoor levels of these biological agents. This paper has applied simple monitoring methods for these agents, and generated new information about allergen and mould levels in Swedish day care centers. The results of this study show an association between indoor mould contamination and dampness in the building construction, as well as other building factors. The spread of pet allergens implies a need to improve the indoor environment in day care centres. Dust sampling by cotton swab and Petri dish can be a sensitive and convenient method to monitor allergen levels and quantitative PCR is also useful to quantify total indoor fungal concentrations.

buildings were found to have a history of mould growth or building dampness.[4] This risk for dampness is related to building constructions. In Sweden, many day care centers have been constructed during the 1970's and 1980's, when the risk of poor constructions leading to dampness was common.

Building dampness and indoor moulds are risk factors for asthma and respiratory symptoms, as concluded by World Health Organization (WHO).[5] Most epidemiological studies on fungal exposure have been performed in homes. As an example, a Finnish population based incidence study showed that moisture damage and mould growth at home were associated with development of childhood asthma.[6] In a recent review of allergen levels in day care centers, two studies reported quantifiable levels of the allergenic mould *Alternaria alternata* in settled dust in day care centers.[7] Another two studies from Taiwan[8] and Turkey[9] have reported increased mould levels in two day care centers, but in general reports of airborne moulds (filamentous fungi) in day care centres are uncommon. To measure amounts of environmental levels of viable fungi culture methods are generally used. However, culture methods are inevitably affected by culturability and viability of target microorganisms and often underestimate their amounts in environmental samples.[10] Furthermore non-viable mould may harm human health and also needs to be monitored. By using different primers and probes, quantitative Polymerase Chain Reaction (qPCR or sometimes called real time PCR)[11] can detect and quantify both viable and non-viable fungi. qPCR detects DNA-sequences common for most fungi (total fungal DNA),[12] species of fungi DNA (*e.g. Aspergillus*/*Penicillium*),[13] as well as specific sequences (*e.g. Stachybotrys chartarum*).[14] We have previously applied standardized sampling of fungal DNA on cotton swabs, as a simple method to sample dust settled on horizontal surfaces (*e.g.* upper door frames) as a proxy for long term exposure to fungal DNA. The method is sensitive and detected fungal DNA in 91% of samples by qPCR, and we found associations between self-reported dampness/moulds, reported odour, and the amount of total fungal DNA in the swab samples.[12]

Apart from dampness problems, indoor allergen exposures in day care centers have been an area of research. Studies have been conducted worldwide, but the research has been most active in the United States and Scandinavian countries.[7] A number of studies have shown that animal allergens can be present in environments in which no animals reside.[15,16] Cat (Fel d1) and dog (Can f1) allergens are frequently detected in day care centers and schools, but the levels of exposure vary greatly.[7] In Sweden, there are about 280 000 horses and about half a million people (6%) ride horses and this number is steadily increasing.[17] This may cause a wide spread secondary or tertiary allergen contamination in the society. Horse (Equ cx) allergen has been commonly detected in Swedish day care centers[12] and schools.[18]

Exposure to pet allergens in indoor environments may influence allergy and respiratory health. One national survey concluded that cat (Fel d1) and dog (Can f1) allergens are universally present in US homes, and levels that have been associated with increased risk of allergic sensitization are found even in homes without pets.[19] Another US study showed that allergens were common in homes of asthmatic children, and most homes had multiple allergen burdens at levels thought to be associated with sensitization and exacerbation of asthma.[20] One study from Northern Sweden concluded that the school environment appeared to be a major site of exposure to cat and dog allergens.[21] Another Swedish study showed that children with cat allergy had an increased risk of exacerbation of asthma at school, measured as asthma symptoms, peak expiratory flow, and use of asthma medication if they were in classes with many cat owners.[22]

There are many different sampling methods that can be used to assess allergen exposure. The Petri dish sampling method has been applied to measure allergens in schools[18,23,24] and day care centers.[12] It may be a more relevant method than the common reservoir dust collection such as vacuum cleaning. In a study in Chinese schools, allergen levels were low in vacuumed settled dust, but relatively high in Petri dishes, possibly because allergens are transferred directly to the air from the students, without staying in the reservoirs.[23] Moreover, the Petri dish sampling method can collect airborne particles for a longer period (1–4 weeks) than the conventional pumped sampling method.

To date, there is still scarce information on the exposure to airborne moulds (filamentous fungi) and allergens in day care centers. In this study, we expanded the sampling method to include both cotton swab[12] and Petri dish to collect settled dust. Two studies in the US[25] and Singapore[26] evaluated the associations between characteristics of day care center buildings and concentrations of indoor allergens. Rotating heat exchangers are used for energy saving and are common in the Swedish day care center ventilation systems. They have been shown to accumulate volatile organic compounds,[27] but no data on associations with fungal DNA/allergen levels are available.

The aim of this study was to investigate the relationship between selected aspects of quality of pre-school day care building and potential exposure to fungi and animal allergens. The objectives were to quantify exposure to fungi using a quantitative PCR assay; to quantify exposure to animal allergens using immunoassays; and to assess the relationship between building quality characteristics and these measures of allergen and fungi exposure.

2. Materials and methods

2.1. Selection of population

One mid-Swedish municipality (Österåker) was selected because there had been a general survey of the building conditions of all day care centers ($N = 24$) performed by a well-known building inspection company (AK Indoor Air AB, Solna, Sweden). Three of the day care centers were excluded in this study since they were located in school buildings and could be influenced by the school environment. The remaining 21 day care centers (26 separate buildings) were included in the study. Measurements and room inspections were performed in 3–5 randomly selected rooms within each building, depending on the size of the building. A total of 103 rooms were investigated on March–April 2007.

2.2. Building inspection

The day care centre buildings and rooms were inspected and details on construction, building materials and age, type of ventilation and heating system, the amount of open shelves, textiles and the number of pot plants were noted. The room

volume (m^3), shelf (m m^{-3}), textile (m^2 m^{-3})[28] and pot plant (number of potplants per m^3) factors were calculated in each room. Previously, the inspection company had classified the buildings into three groups by a two step procedure. Firstly, according to the type of concrete slab on the ground, basement wall, and outdoor ventilated crawl space, the building was classified as non-risk (level 0) or risk construction. The concept of "risk construction" refers to a building constructed in such a way that it increased risk of high humidity within the building fabric, which may lead to fungi growth or chemical degradation.

Moreover, the risk construction was classified into two levels depending on the absence (risk level 1) or presence (risk level 2) of visible water damage/moulds. The classification information is shown in Table 1.

2.3. Dust samples collection

Dust samples were collected both by cotton swab and Petri dishes. One type of dust samples for fungal DNA analysis was collected by swabbing a 60 cm^2 surface (1 × 60 cm) of the upper half of the door frame around the main entrance to each room in the day care centers. Each swab was rotated slowly and moved 3 times back and forth over the surface. Two samples were collected by dividing the doorframe into left and right sides. The left side was used for fungal DNA analysis and the right side sample was stored for future analysis. Another type of dust samples for fungal DNA and allergen analyses was collected by two Petri dishes in each room, placed on the top of book shelves or similar areas (about 1.5–2.0 m height)[23] and kept open for 30 to 40 days. Each Petri dish has a total surface of 0.0124 m^2 (sum up of 2 sides), and is made of plastic material.

2.4. Analysis of fungal DNA by qPCR

One cotton swab and one Petri dish dust sample from each investigated room were sent to a professional lab (anoZona AB, Uppsala, Sweden) for fungal DNA analysis using the qPCR method. Methods for DNA extraction from the cotton swab dust samples and qPCR procedure and standard curves have been described.[12] Briefly, for DNA extraction, the cotton swabs were cut into 2 ml tubes, diluted with 400 μl of AP1 buffer (DNeasy Plant Mini Kit, Qiagen, Hilden Germany) and vortexed briefly.[12] In contrast, 10 ml of double distilled water was added to the Petri dish, transferred into a 10 ml tube and then centrifuged at 12 000 rpm (8000*g*) for 5 minutes. After complete removal of the supernatant, 250 μl AP1 buffer was added and transferred into a 2 ml tube. After this preparation, the respective target DNA extracts and total genomic DNA from the homogenates were extracted with the DNeasy Plant Mini Kit (Qiagen, Hilden Germany), according to the manufacture's instructions. The DNA extracts were kept at −70 °C until PCR amplification.

Amplification and detection of the DNA extracts for *Aspergillus* or *Penicillium* genera (*Asp*/*Pen*), *Stachybotrys chartarum* (*S. chartarum*) and total fungal DNA were performed on an Mx3000P/MXpro real-time PCR machine (Stratagene, La Jolla, CA, USA) in the TaqMan Master Mix (Applied Biosystems, Carlsbad, CA USA) according to the manufacture's protocols. A partial fungal DNA sequence common for a large number of moulds (Universal Fungal assay 1) was used and described here as total fungal DNA. The primers and probes for Universal Fungal are: Forward Primer 5.8F1: 5′-AACTTTCA ACAACGGATCTCTTGG (SEQ ID NO: 213), Reverse Primer 5.8R1: 5′-GCGTTCAAAGACTCGATGATTCAC (SEQ ID NO: 214) and probe 5.8P1: 5′-CATCGATGAA GAACGCAGCGAAATGC (SEQ ID NO: 215). The species list is available online at http://www.freepatentsonline.com/6387652.html. Primer and probe sequences for *Asp*/*Pen* (assay name: PenAsp1mgb) and *S. chartarum* (assay name: Stac) are available online (http://www.epa.gov/nerlcwww/moldtech.htm). The standard qPCR 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.[12]

Standard curves were created for respective analysis, using total genomic DNA obtained from pure cultures. The DNA extracts from the pure cultures were quantified using limiting dilution analysis.[29] The quantity of the unknown samples was calculated based on the calibration curve of standardized DNA solutions *versus* the corresponding cycle threshold (Ct) value. If the internal control was not detected or the Ct was over a certain value, the samples were diluted further. In respect of the sampling method used in this study, the mould level was expressed as cell equivalent (CE) for each target mould or mould group assuming one DNA copy per cell.[12] The final results were presented as CE m^{-2} for cotton swab and CE m^{-2} day^{-1} for Petri dish dust samples.

Table 1 Principles for classification of risk construction buildings

Classifications principles	Non-risk constructions	Risk constructions	
Concrete slab on the ground	With underlying insulation	With overlying insulation	
Basement walls	With insulation outside	With insulation inside	
Outdoor ventilated crawl space	No	Yes	
Risk construction levels	Non-risk level 0	Risk level 1	Risk level 2
Visible water damage/moulds	No	No	Yes

2.5. Allergen analysis

Phosphate buffered saline buffer (PBS, 3 ml) with 1% bovine serum albumin (BSA) was added to the Petri dish for extraction. Then the liquid was transferred into an Eppendorf tube and centrifuged.[23] The supernatants were stored at −20 °C until analysis. Two-site sandwich ELISA (Enzyme-Linked Immunosorbent Assay) was applied to determine cat (Fel d1), dog (Can f1) (Indoor Biotechnologies Ltd, Manchester, UK), and horse (Equ cx) (Mabtech, Stockholm, Sweden)[30] allergen levels, as previously described[18] by using monoclonal antibodies. Amplified ELISA was used for cat allergen analysis for cases when the allergen levels were lower than 1.0 ng ml^{-1} by the conventional ELISA.[24] The cat and dog allergen levels in Petri dish dust samples were expressed as ng m^{-2} day^{-1}, horse allergen level was expressed as unit per m^2 per day where one unit is equal to one ng protein of a horsehair and dander extract used as standard.[18]

2.6. Statistical methods

Statistical calculations were performed using the Statistical Package for the Social Sciences (SPSS 17.0). The concentrations of fungal DNA and allergens were presented as geometric mean (GM) and geometric standard deviation (GSD). The Mann–Whitney *U*-test was used to analyze fungal DNA, allergen levels and associations with room factors since the data were not normally distributed. In order to reduce the number of statistical testing, assuming that different types of allergens would have similar associations with building characteristics, we analysed associations only for total allergens (by summing up cat, dog and horse allergen values). However, the associations between fungal DNA and allergens were analysed separately for each type of allergens. Associations between continuous variables were tested using the Kendall's tau_*b* rank correlation test. The codes for all the tests with risk construction classifications valuable were coded as the inspection classification levels (0–2).

Within and between day care center buildings variability was evaluated using mixed linear models with a random intercept. Data on total fungal DNA and allergens were log-transformed to get approximately normally distributed variables. In addition, the variance ratios also called 'fold-ranges' within and between buildings ($_{w}R_{0.95}$ and $_{b}R_{0.95}$) were calculated from the variance components of the 97.5th and 2.5th percentiles of the log-normally distributed exposure.[31] As an example: a $_{w}R_{0.95}$ of 3 means that 95% of the mean values for each day care centers can vary with a factor 3 between rooms. A $_{b}R_{0.95}$ of 3 indicates that the 95% of the mean values for the schools are with a range of factor 3. To adjust for the hierarchic structure of data and for mutual adjustment, linear mixed models were used to analyze associations between fungal DNA, allergen levels and building factors. Coefficients (β) with 95% confidence intervals (95% CIs) were calculated.

All tests were two-tailed, and a *p*-value below 0.05 was used to indicate statistical significance.

3. Results

3.1. Building inspection

All day care center buildings were wooden buildings (wooden construction and wooden façade) with mainly one floor level. The mean of the construction year was 1975 (SD = 20). However, there were no building age data for the barracks ($N = 3$) of the day care centers. Most of the buildings had a supply exhaust mixing ventilation system except one building with only a supply ventilation system. In the investigated rooms, most of the rooms were used as dining rooms and had linoleum floor materials. Most of the buildings had a radiator heating system, a rotating heat exchanger and a leaning roof. The mean value of room volume was 86.17 m^3 (SD = 33.23), shelf factor was 0.11 m m^{-3} (SD = 0.07), textile was 0.06 m^2 m^{-3} (SD = 0.05) and pot plant factor was 0.05 potplant per m^3 (SD = 0.09), respectively. The mean value of the Petri dishes height above floor was 1.64 m (SD = 0.22). The details of the building characteristics are shown in Table 2.

3.2. Fungal DNA and allergen levels

Total fungal DNA was detected in nearly all of the dust samples, while *Asp/Pen* DNA was found in more than half of the samples, both for cotton swab and Petri dish dust samples. *S. chartarum* DNA was present in only a few samples (4% in cotton swab and 9% in Petri dish samples). Cat allergen was detected in all samples, while dog and horse were in more than half of the Petri dish dust samples. The detected percentages and the concentrations of fungal DNA and allergens (in Geometric Mean values) are shown in Table 3.

S. chartarum DNA (in both swab and Petri dish samples) was detected in 10 buildings ($N = 13$ rooms), whereas 9 of them were risk construction buildings. The rooms with detectable *S. chartarum* DNA had higher levels of total fungal DNA (GM = 7.83×10^6 CE m^{-2}) than the rooms without (GM = 3.81×10^6 CE m^{-2}) ($p = 0.001$) in the swab samples, but not in the Petri dish samples. The comparison data for 'within' and 'between' building variation of total fungal DNA and allergens are shown in Table 4. There was less variation between buildings than within buildings, particularly for total fungal DNA in Petri dish samples.

3.3. Associations between fungal DNA level in the swab and Petri dish

When comparing the fungal DNA levels in the cotton swab and Petri dish dust samples, there was an association between the two sampling methods for *Asp/Pen* DNA (Kendall's tau_b = 0.18; p = 0.02), but not for other types of fungal DNA. When comparing the amount of cell equivalents of total fungal DNA

Table 2 Building characteristics in 21 Swedish day care centers (N= 26 buildings, 103 rooms)

Building characteristics		N (buildings)	N (rooms)	Percent[a] (%)
Number of floors	1	21	86	81
	2	2	8	8
	3	3	9	11
Risk construction classification	0	4	13	15
	1	8	31	31
	2	14	59	54
Type of floor	Linoleum	—	93	90
	PVC	—	10	10
Heating system	Radiator	—	96	92
	Floor heating	—	7	8
Rotating heat exchanger	Yes	16	66	62
	No	10	37	38
Type of roof	Leaning roof	23	94	88
	Flat roof	3	9	12
Type of ventilation	Supply exhaust mixing	25	100	96
	Supply	1	3	4
Dining room	Yes	—	62	60
	No	—	41	40
Wooden outside	Yes	26	103	100
	No	0	0	0
Barrack	Yes	3	9	12
	No	23	94	88

[a] The percentage data were calculated at building level except for factors at room level (type of floor, heating system, and dining room).

Table 3 Total fungal DNA and allergen levels from swab and Petri dish dust samples in 21 Swedish day care centers ($N = 103$ room)

Dust samples (number) (unit)	Fungal DNA/allergens	Percent[a] (%)	GM[b]	GSD[c]	Range
Cotton swab ($N = 103$)/CE m^{-2}	Total fungal DNA	99	4.2×10^6	2.1	($<1.0 \times 10^4$, 3.2×10^7)
	Asp/*Pen* DNA	53	8.4×10^3	4.5	($<3.0 \times 10^3$, 3.9×10^5)
Petri dish ($N = 101$)/CE m^{-2} day^{-1}	Total fungal DNA	100	2.9×10^5	1.8	(6.0×10^4, 9.8×10^5)
	Asp/*Pen* DNA	68	385	4.4	(<100, 1.6×10^4)
Petri dish ($N = 97$) (for horse allergen: unit per m^2 per day)/ng m^{-2} day^{-1}	Cat allergen (Fel d1)	100	9.4	2.6	(0.9, 78.6)
	Dog allergen (Can f1)	81	7.2	2.9	(1.2, 72.5)
	Horse allergen (Equ cx)	63	5.0	5.8	(0.6, 208.7)

[a] Positive detected values that were above the detection limits. [b] Geometric mean. [c] Geometric standard deviation.

Table 4 Variations within and between buildings of total fungal DNA, allergens, and fold ranges for variation in 21 Swedish day care centers ($N = 26$ buildings)

Fungal DNA/Allergens		Variations within building (%)	Variations between buildings (%)	${}_wR_{0.95}{}^b$	${}_bR_{0.95}{}^b$
Total fungal DNA	In swab samples	57	43	2.7	2.3
	In Petri dish samples	90	10	2.6	1.4
Allergens[a]	Cat allergen (Fel d1)	79	21	4.3	2.1
	Dog allergen (Can f1)	75	25	4.8	2.5
	Horse allergen (Equ cx)	60	40	10.6	6.9

[a] From Petri dish dust samples. [b] Variance ratios ("fold-ranges") within (w) and between buildings (b), calculated from the variance components of the 97.5th and 2.5th percentiles of the log-normally distributed exposure.

sampled by the two methods, the GM level was 2.58×10^4 CE (GSD = 1.95) in the swab samples and 1.25×10^5 CE (GSD = 1.76) in the Petri dish samples.

3.4. Associations between building/room factors and fungal DNA

Using linear mixed models to analyse each exposure separately, there were associations between total fungal DNA in swab samples (e logarithm values) and risk construction classification ($\beta = 0.105$; 95% CI 0.003–0.201), and rotating heat exchanger ($\beta = 0.184$; 95% CI 0.037–0.332). However, there were no associations between total fungal DNA in the swab, type of roof, or barrack. Moreover, there were no associations between total fungal DNA by any of the two sampling methods and other building factors *e.g.* construction year, shelf, textile, and number of pot plant factors. In addition, there was no association between total fungal DNA in the Petri dishes and the placed Petri dishes height.

After mutual adjustment, both risk construction classification ($\beta = 0.106$; 95% CI 0.002–0.194), rotating heat exchanger ($\beta = 0.235$; 95% CI 0.074–0.357), and linoleum floor material ($\beta = 0.273$; 95% CI 0.100–0.487) remained significant variables (the β and 95% CI values were for e logarithm values). Taking anti-log data (e^x) for these adjusted β values, two steps on the risk construction classification level scale were associated with an increased total fungal DNA in the swab samples by a factor 1.24, while the presence of the rotating heat exchanger increased it by a factor 1.26, and linoleum floor material increased it by a factor 1.31.

The data on geometric mean values for total fungal DNA in swab and Petri dish dust samples stratified for building characteristics are shown in Table 5.

3.5. Associations between building/room factors, allergen levels and fungal DNA in Petri dish samples

There was a negative association between total allergen levels (sum up of cat, dog and horse allergen levels) and textile factor (Kendall's tau_$b = -0.19$; $p = 0.008$), but no associations with construction year, shelf, pot plant factors, or placed Petri dishes height. There were associations between total fungal DNA and cat (Kendall's tau_$b = 0.17$; $p = 0.02$), dog (Kendall's tau_$b = 0.29$; $p < 0.001$) and horse (Kendall's tau_$b = 0.24$; $p = 0.001$) allergens. Moreover, there were associations between *Asp*/*Pen* DNA levels and cat (Kendall's tau_$b = 0.19$; $p = 0.007$) and dog (Kendall's tau_$b = 0.17$; $p = 0.02$) allergens.

Table 5 Geometric mean values for total fungal DNA in swab and Petri dish dust samples in 21 Swedish day care centers ($N = 103$ rooms), stratified for building characteristics[a]

Building characteristics		Total fungal DNA in swab samples			Total fungal DNA in Petri dish samples		
		N/room	GM[b]	GSD	*N*/room	GM[c]	GSD
Risk construction classification	0	13	3.0	1.9	13	3.8	1.5
	1	31	3.7	2.5	30	2.8	1.7
	2	59	4.8	1.9	58	2.7	1.9
Type of roof	Flat	9	6.1	2.4	8	2.7	2.1
	Leaning	94	4.0	2.1	93	2.9	1.8
Type of floor	Linoleum	93	4.5	2.0	91	2.8	1.8
	PVC	10	2.1	2.2	10	3.5	1.6
Rotating heat exchanger	Yes	66	4.8	1.9	66	2.9	1.9
	No	37	3.2	2.3	35	2.8	1.7
Barrack	Yes	9	5.5	2.5	8	3.1	1.9
	No	94	4.1	2.1	93	2.8	1.8

[a] GM: Geometric Mean and GSD: Geometric Standard Deviation. [b] $\times 10^6$ CE m^{-2}. [c] $\times 10^5$ CE m^{-2} day^{-1}.

4. Discussion

In this study, we have demonstrated that risk constructions and visible water damage/moulds were common in Swedish day care centre buildings in agreement with previous studies.[3,4,8] There were higher levels of fungal DNA in risk construction buildings than non-risk construction buildings. Moreover, total fungal DNA levels were related to rooms with linoleum floor material and buildings with rotating heat exchangers. The associations remained significant after mutual adjustment. Pet allergens were very common and associated with total fungal DNA. However, the variations of total fungal DNA and allergens were higher within buildings than between buildings. This implied that sufficient rooms must be investigated in exposure studies in order to get a reliable estimate of average levels in a building.

Our study included all day care centers situated in separate buildings within one municipality, and different types of rooms were randomly selected within each building. Thus there was no bias due to selection of buildings or rooms. In addition, the municipality was chosen because a previous survey of all day care centers had been undertaken to assess the damp conditions irrespective of whether complaints had been raised about these buildings. Thus, selection or recall bias was less likely in this study. Sampling of airborne allergens on filters using personal sampling is considered as the gold standard method to assess allergen exposure,[32] but it is practically difficult to use in day care centers over several days. However, the Petri dish sampling method has previously been shown to agree with the results obtained using personal sampling to assess exposure to cat allergens in schools.[33]

There were higher levels of total fungal DNA in swab samples in risk construction buildings, especially those with visible water damages/mould growth (risk level 2). This association remained significant even after mutual adjustment for other building factors. To our knowledge, this association has not been previously demonstrated. The prior risk classification survey from the day care centers (Österåker municipality) showed that most of these buildings are risk construction buildings. A similar high prevalence of risk construction day care center buildings was found in two other surveys in Stockholm area (Anders Kumlin, AK Konsult Indoor Air AB, Solna, Sweden, personal communication). One typical risk construction design is, for example, a concrete slab on the ground overlaying thermal insulation. This design was commonly used for smaller buildings such as day care centers during the 1970's and 1980's. These constructions are often damaged by moisture because the concrete basement structures achieve relative humidity (RH close to 100%) equivalent to that of the surrounding soil. Another typical Swedish risk construction design is the outdoor ventilated crawlspace. In this case, the crawl-space remains a lower temperature than the outdoor air during summer. Crawlspace is ventilated by warm and humid air, the RH reaches levels high enough to support fungal growth.

Linoleum floor materials are commonly used in Sweden. In the swab dust samples, total fungal DNA was significantly higher in rooms with linoleum floor material than those with PVC floors. This is in agreement with a previous day care centre study using the same swab sampling method for fungal DNA analysis.[12] The higher levels of fungal DNA could be due to fungal growth accumulating in the jute material backing of the linoleum material if there was a dampness problem in the floor construction.

There were higher levels of total fungal DNA in the swab dust samples in buildings with rotating heat exchangers than those with plate heat exchanger or no heat recovery system. To our knowledge this is a new finding, with respect to fungal contamination, but it has been shown that rotating heat exchangers can transfer water soluble chemicals from the exhaust air to the supply air.[27,34]

In contrast, in Petri dish samples, the total fungal DNA levels were not significant related to the risk construction classification, floor material or rotating heat exchanger. The Petri dish sampling efficiency could therefore have been influenced by ventilation and air movements in buildings. Moreover, the swab samples were taken from the door frames which were closer to the building constructions. This could be the reason why we found associations only for swab samples.

S. chartarum is a toxigenic fungus that has been associated with human ill health[35] and was detected in 13% of the investigated rooms. Members of the *Stachybotrys* genus exist worldwide, from Finland to the South Pacific Islands.[36,37] In general, the organism is found in soil and strata rich in cellulose.[36] In Sweden, growth may occur in wall paper or on the paper layer of gypsum boards which are commonly used indoors.

Cat, dog and horse allergen contamination was common in the Swedish day care centers, in agreement with other studies.[12,38] Since no pets were kept in the day care centers, the allergens were most likely transferred from the home environment of pet keeping staff or children through clothes or human hair as indicated by some other studies.[39–43] However, we were unable to obtain data on the frequency of pet keeping by the staff or children who attended these day care centers. Some studies have shown that cat and dog keeping is common among families with school children in Sweden.[18,44] The cat allergen levels in the day care centers were lower than school environment using the same Petri dish sampling method.[33,45] There were associations between cat, dog, and horse allergens and total fungal DNA in Petri dish samples, which were in agreement with a previous study of day care centers.[9]

5. Conclusions

In conclusion, in Swedish day care center with visible water damage/moulds, pet allergens, and fungal DNA were common. This indicates that there is a need to improve the construction of these day care centers to avoid the risk factors in construction that result in elevated humidity. The association between the building design and room factors and fungal DNA levels also showed that linoleum flooring and rotating heat exchangers were risk factors for exposure to fungi. Finally, the associations between the levels of allergens and fungal DNA indicated that bio-aerosols may need to be addressed as a hygiene risk factor.

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