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Residential Culturable Fungi, (1–3, 1–6)-β-D-glucan, and Ergosterol Concentrations in Dust Are Not Associated with Asthma, Rhinitis or Eczema Diagnoses in Children

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Abstract

Background—Qualitative reporting of home indoor moisture problems predicts respiratory diseases. However, causal agents underlying such qualitative markers remain unknown.

Methods—In the homes of 198 multiple allergic case children and 202 controls in Sweden, we cultivated culturable fungi by directly plating dust, and quantified(1–3, 1–6)-β-D-glucan, and ergosterol in dust samples from the child's bedroom. We examined the relationship between these fungal agents and degree of parent or inspector reported home indoor dampness, and microbiological laboratory's mold index. We also compared the concentrations of these agents between multiple allergic cases and healthy controls, as well as IgE-sensitization among cases.

Results—The concentrations of culturable fungal agents were comparable between houses with parent and inspector reported mold issues and those without. There were no differences in concentrations of the individual or the total summed culturable fungi, (1–3, 1–6)-β-D-glucan, and

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ergosterol between the controls and the multiple allergic case children, or individual diagnosis of asthma, rhinitis or eczema.

Conclusion—Culturable fungi, (1–3, 1–6)-β-D-glucan, and ergosterol in dust were not associated with qualitative markers of indoor dampness or mold or indoor humidity. Furthermore, these agents in dust samples were not associated with any health outcomes in the children.

Keywords

indoor; asthma; allergies; children; dampness; mold

INTRODUCTION

Detectable moisture and moisture-related problems in the indoor environment are associated with respiratory diseases and disorders such as asthma and wheezing in children and adults (Bornehag et al., 2001; Bornehag et al., 2004; Institute of Medicine, 2004; WHO, 2006; WHO, 2009). Yet, in most studies that identified an elevated risk due to such problems, most predictive markers of indoor dampness or mold (IDOM) remain qualitative in nature (Belanger et al., 2003; Iossifova et al., 2009; Pekkanen et al., 2007). Such indicators include qualitatively assessed dampness, visible mold, visible moisture damage of housing structure, or 'moldy' smell (Belanger et al., 2003; Iossifova et al., 2009; Pekkanen et al., 2007). While the evidence is strong enough for an association between IDOM and a wide range of respiratory and allergic diseases, the evidence *does not* yet support the causal role. In addition, the specific dampness-related agents underlying these diseases, and the mechanisms of their action remain unknown (Institute of Medicine, 2004; Mendell et al., 2011).

On the other hand, quantitatively determined concentrations of microbial agents do not show a consistent association with respiratory health outcomes; in some cases, exposure to microbial factors is protective againt asthma-related symptoms and wheezing, particularly for those who are exposed very early in life (Mendell et al., 2011). In particular, culturable mold spore concentrations in air samples from the children's rooms are not related to doctor diagnosed asthma, asthmoid-spastic or obstructive bronchitis, hay fever, atopic eczema or sensitization (Jovanovic et al., 2004). Taken together, the strength of evidence on *quantitatively assessed* home indoor fungal exposure is inconsistent in regards to the development asthma and allergic disease (Bush et al., 2006; Hardin et al., 2003). As a result, while the qualitative markers of IDOM (e.g., signs of mold, presence of moldy or musty odor, visible water damage) have been associated with health outcomes, the critical gap in our knowledge lies in identifying the specific causative agent(s). In order to address these questions, we measured the culturable fungi, (1–3, 1–6)-β-D-glucan, and ergosterol concentrations from the household dust samples of those taking part in the Dampness in Buildings and Health (DBH) study in Sweden. Both ergosterol and (1–3, 1–6)-β-D-glucan are fungal specific cellular markers used for indirect quantification of fungal biomass (Szponar et al., 2000; Chew et al., 2001). Dust is a time-integrated carrier for environmental contaminants, including fungal agents (Munir et al., 1995; Rudel et al., 2003). Quantification of such agents in dust is therefore of assessment importance in indoor exposure, and could indicate a sustained exposure. It is therefore necessary to assess dust as

a potential inhalation and/or ingestion purveyor of exposure to a risk factor asthma and allergy.

In our earlier analysis, we found no association between airborne culturable fungi and allergic diseases in children (Holme et al., 2010). Here, we further investigate a related line of inquiry by examining the culturable fungi, ergosterol, and (1–3, 1–6)-β-D-glucan in dust and the risks of doctor-diagnosed asthma, and multiple allergy symptoms in the same group of pre-school age case-control children. We also examined the relationship between these fungal agents in dust and parental reporting of home moisture issues, as well as the professional inspections of the home environment.

METHODS

Detailed descriptions of the study methods are provided elsewhere (Bornehag et al., 2005c; Holme et al., 2010). Briefly, this study is part of the on-going Dampness in Buildings and Health (DBH) study focusing on the impact of indoorenvironmental factors on asthma and allergy among children in Sweden. The first Phase of the DBH study was a cross-sectional questionnaire investigation of the parents of all children aged 1– 6 years (n = 14,077) in Värmland County, Sweden (Bornehag et al., 2003, 2005). The current study is part of the Phase II investigation, which is a nested case control investigation on 198 symptomatic children and 202 non-symptomatic controls representing 390 households (including 10 sibling pairs).

Case and Control Definition

In order to meet the definition for a *case*, the following conditions were required for the child; (i) in the baseline questionnaire (DBH Phase I), the parent had to report ≥ two symptoms of wheezing, rhinitis, and/or eczema within the last 12 months. Eighteen months later, at DBH phase II the child's parent had to report ≥ two symptoms of wheezing, rhinitis, and/or eczema without a cold within the prior 12-months period. From the pool of DBH phase I, we sought and recruited clinically diagnosed cases of asthma, rhinitis, and eczema, respectively. Diagnostic criteria for asthma include a) at least three wheezing episodes prior to age 2; b) onset of wheezing since age 2; c) an onset of wheezing in addition to other atopic diseases; d) current asthma medication use; and e) clinical diagnosis of asthma at any age. Diagnostic criteria for rhinitis required: (a) ever having allergic rhinitis symptoms; (b) symptoms presentation in the nose and/or eyes following the contact with furred animals or pollen; (c) present use of rhinitis medicine. Eczema case definition required that the child have at least six months of remitting itching and redness in typical body locations. For the *control* children, only those whose parent reported an absence of symptoms during both Phase I and II were invited.

In order to preclude misclassification of exposure history, the cases and controls were further excluded if: (i) the home was renovated or remodeled due to moisture problems; and (ii) family relocated to a new residence since the phase I. The medical examinations of the 400 children as well as the air, dust sample collection, and home inspection were completed during October 2001 and April 2002 (i.e. 18–24 months after phase I). A team of four medical doctors examined all children following a structured anamnesis (Hederos et al.,

2007). Blood samples (n = 387) from all available children were screened for IgE antibodies to 10 airborne allergens (Phadiatop®, Pharmacia & Upjohn Diagnostics, Uppsala, Sweden), including timothy, mugwort, birch, cat, horse, dog, house dust mites (D. pteronyssinus and D. farinae), and two mold genera (Penicillium and Cladosporium). Cut-off value (1.2 kUA/l) was used to define a dichotomous category for IgE-positive status. Institutional ethical committee in Örebro, Sweden, approved the study.

Here we present culturable fungi as colony-forming unit (CFU) per gram dust of the five most prevalent fungal genera as well as yeasts, (1–3,1–6)-β-d-glucan (μg/g dust), and ergosterol (*μ*g/g dust), and total culturable fungi (CFU/g) from the dust sample collected in the child's bedroom. Consistent with our earlier analysis (Holme et al., 2010), we considered three separate definitions of the moisture and fungal problems as below:

1. Parental-reporting of moisture problems (in DBH Phase I questionnaire)

2. Home building (walk-through) assessment by professional inspectors in Phase II

3. Semi-quantitative mold index by the microbiology lab

1. Parental-Reporting of Moisture Problems: Parents qualitatively reported in a questionnaire (DBH Phase I) regarding the signs of chronic moisture issues and mold in the child's, the parent's, or other rooms. We chose the following questions:

- Visible dampness: mold or obvious water stains on the ceiling, walls, or floor in the child's bedroom or the parents' bedroom.
- Floor moisture: Discolored/blackened parquet or cork-flooring; bubbly, loosening, or discolored vinyl or linoleum floor covering in the child's, parents', or other room.
- Moldy odor: Moldy, earthy, or "cellar-odor" sometimes or often within the last 3 months.
- Condensation on windows: visible condensation (≥5cm diameter) during winter in the child and/or parents' bedroom.

2. Home Building Assessment: Six inspectors conducted a visual and olfactory exam of mold and water damage, and collected air and dust samples in the homes. The inspectors were blind to the case–control status of the children. The inspector graded each home on the scale of 0 to 3 regarding four aspects of the mold presence:

- Mold y odor: First impression upon entering the home or in at least one room
- Moldy odor along the skirting board in at least one room of the dwelling
- Discolored damp stains on walls or ceiling in at least one room
- Blackened, bubbly, or loosening floor-covering material, or any other sign of floor dampness in at least one room

Grade of zero indicated no mold problem; grade 1–2 indicated mild issue for possible smell or a small visible indication of moisture; and grade 3 indicated severe issue for clear and strong odor or obvious moisture damage.

3. Semi-Quantitative Mold Index: The genera and quantity of culturable fungi from each house was validated by four microbiologists. The fungi concentration from an air sample (Holme et al., 2010) was compared to the corresponding outdoor concentration and categorized into one of the four groups (range, 0–3) as developed by the Norwegian standard for building survey (Tilstandsanalyse for byggverk, NS 3424.) The score of 0 denote no signs of fungal spores compared to the reference sample taken outdoors; 1 denotes a limited signs of fungal spores; 2 denotes moderate signs of fungal spores; and 3 denotes an obvious signs of fungal spores. Consistent with our earlier analysis, we further reduced the categories into two, in which score of 0–1 denote houses with no fungal contamination, and score of 2–3 denote houses with fungal contamination (Holme et al., 2010).

Ventilation rates of the entire home and of the bedroom of the index-child were measured during one week with a passive tracer gas method (i.e. the homogeneous emission technique) (Bornehag et al., 2005a). This PFT (perfluorocarbon tracer) technique, described in NT VVS 118, has been validated (Nordtest, 1997; Stymne et al., 1994). Temperature (°C) and relative humidity (%) were measured instantaneously during the home visit (VL2000 Temperature & Humidity Sensor, Vaisala, Helsinki, Finland) and continuously at every hour for a week (Mitec Satelite-TH, Mitec Instrument AB, Säffle, Sweden).

Quantification of Microbial Agents in Dust

A single dust samples was collected with a vacuum from the floor in the child's bedroom by six licensed building inspectors. A random subset of the building inspectors visited each home during the heating season (October 2001–April 2002). Regardless of the type of the floor, the inspectors vacuumed at a sampling a rate of 2 minutes/m^2 until a sample of approximately 300 – 500 mg was attained. The dust samples were analyzed for 388 of the 390 families/houses that are participating in this study; two homes did not have dust samples. A 90-mm membrane filter made of pure cellulose was used to collect the dust. The filters were placed in holders made of tyrene-acrylonitrile polymer mounted on a sampler made of polypropylene (Petersen Bach, Bjerringbro, Denmark) connected to a vacuum cleaner. The filters were wrapped in aluminum foil and placed in a polyethylene bag and stored in the refrigerator for 2–3 days following sample collection. The filter was weighed before and immediately after vacuuming under controlled conditions. Before weighing, the filters were conditioned at 23° C and 50% relative humidity. Once the samples arrived in the lab, 30 mg of unsieved dust from each sample was spread directly onto V_8 agar (Vegetable juice, Campbell Soup. Ltd.) plates. The plates were incubated for a week at 26 °C (Gravesen, 1978). To reduce bacterial growth, penicillin and streptomycin were added. Fungal growth was quantified microscopically as CFU/30 mg of dust (Wickman et al., 1992). Fungi were identified at the level of genera. Molds were considered fungal species which have a predominately multi-cellular growth habit characterized by hyphae, while yeasts are species which can adopt a unicellular growth habit (Madigan et al., 2005).

Whenever possible, fungi were identified to species using direct microscopy using established methods (Andersen et al., 2011). After counting fungi/microorganisms, the values were converted to CFUs per gram of dust (CFU/g).

Ergosterol—Upon arrival at the lab, the dust samples were stored at −20 °C prior to analysis. Two to four milligram aliquots of unsieved dust samples were analyzed for ergosterol using gas chromatography-tandem mass spectrometry. In brief, samples were subjected to alkaline hydrolysis following clean-up and derivatization prior to analysis (Sebastian et al., 2003).

(1–3, 1–6)-β-D-glucan—Approximately 30 mg of the unsieved dust sample from each home was used for glucan analysis. Samples were extracted in phosphate-buffered saline plus 0.05% Tween 20 with 1 hr shaking, 1 hr autoclaving (120 °C), followed by centrifugation at 600 xG for 20 min at 4 °C. The concentration of fungal glucan in the supernatants was quantified by enzyme immunoassay (EIA) using mouse anti-(1–3, 1–6)-β-D-glucan monoclonal antibody as the capture antibody, rabbit anti-scleroglucan polyclonal antibody as the second antibody and goat anti-rabbit IgG (Biosource, Inc. Camarillo, CA) as the labeling antibody as previously described (Blanc et al., 2005). The reaction was monitored at 650 nm and was read at 405 nm in a microplate reader (SpectraMax Plus 384, Molecular Devices, Sunnyvale, CA).

Statistical Analysis

Here we present the observed CFUs, for each genera of fungi, and a group of non-specific yeasts separately, and the sum of these groups as total fungi (CFU/g) in the child's bedroom. We identified in total 31 genera. We restricted our analyses to those fungal genera or fungal groups (yeasts) detected in ≥ 30% of 390 homes — *Penicillium* spp., *Alternaria* spp., *Aspergillus* spp., Yeasts, *Rhodotorula* spp., *Trichoderma* spp., and total fungi CFUs. Yeasts include all yeasts other than *Rhodotorula* spp., which were prevalent enough to warrant separation. In the remaining 25 genera, the prevalence among 390 homes was too low to statistically compare between the outcome groups. The Mann–Whitney U-test was used to assess the associations, when the independent variable was coded as a dichotomous variable. If the independent variable had more than two categories, a Kruskal-Wallis test was used. Specifically, we used Kruskal-Wallis test to compare the distribution patterns of culturable fungi among the categories of parent reported mold problems, building inspector ratings, semi-quantitative home mold index. Similar distributions between the respective outcomes (i.e. asthma, rhinitis, or eczema) vs. the controls were compared using the Mann-Whitney's U-test. Both tests non-parametrically compare the distributions underlying the samples without assuming a normal distribution of the main exposure variable. We did not develop any logistic regression model for the exposure-outcome association, because the results of the descriptive and non-parametric tests did not warrant the development of multivariate models. We examined the modification of mold effect by humidity by stratifying the dataset according to quartiles of absolute (g/m^3) or relative humidity (%).

RESULTS

Overall, the CFU concentrations of the six most prevalent mold genera were low in 388 homes (Figure 1). Median concentration of the six genera ranged between 67 CFU/g for *Trichoderma* spp. to 400 CFU/g for *Penicillium* spp. The interquartile ranges for the six genera were: 200—733 CFU/g for *Penicillium* spp.; 100—400 CFU/g for yeasts; 67—200 CFU/g for *Alternaria* spp.; 33—250 CFU/g for *Aspergillus* spp.; 67—233 CFU/g for *Rhodotorula* spp.; and 33—133 CFU/g for *Trichoderma* spp.. In all, except *Penicillium* spp., the 90th percentile value was below 1,000 CFU/g. Accordingly, the distributions of all six genera were highly skewed to the right (Figure 1). While (1–3, 1–6)-β-D-glucan and ergosterol were overall prevalent (n = 367 and 383 homes, respectively), their arithmetic mean and standard deviation were 29 ±97 μg/g and 3.47 ±8.36 μg/g. The 25th, 50th, and 75th percentile of (1–3, 1–6)-β-D-glucan were 3, 6, and 19 μg/g dust. Similar values for ergosterol were 0.63, 1.69, and 3.72 μg/g dust, respectively.

Parental questionnaire reports of mold and moisture-related damages (taken 18–24 months prior to the presentfungal measurements) were not positively associated with any concentration trends for any of the five genera of fungi, but were significantly positively associated with concentration of non-specific yeasts. Yeasts and *Aspergillus* spp. were significantly negatively associated with parental report of dampness and floor moisture respectively (Table 1).

In addition, no positive correlation was observed between the inspectors' rating of mold and moisture-related damages, IDOM issues, and the concentrations of the five fungal genera or yeasts. However, the concentration of *Rhodotorula* spp. was negatively associated with moldy odor (Table 2).

Based on semi-qualitative mold index, the medians of three mold genera (*Penicillium*, *Aspergillus*, *Rhodotorula*) as well as yeasts were higher in the homes with mold issues compared to the homes with no mold issues. The concentration of *Alternaria* spp. was lower and *Trichoderma* spp. was approximately equal for the same comparison. Total fungi was significantly higher in houses with mold issues based on semi-quantitative mold index ($p = 0.047$, Table 3).

Neither (1–3,1–6)-β-d-glucan nor ergosterol was associated with a clear trend in concentration difference, according to the parental reports of IDOM, inspectors' rating of IDOM, and semi-quantitative mold index, respectively (Tables 1, 2, and 3).

When we stratified the homes according to quartile of indoor absolute humidity, the concentrations of the six mold genera were overall non-significantly lower for those within the highest quartile compared to those within the lowest quartile (data not shown). Similar trend was observed when we compared the concentrations of the mold genera in terms of the child's bedroom relative humidity (data not shown).

The concentrations of the mold genera, (1–3,1–6)-β-d-glucan, and ergosterol were not associated with any notable differences, comparing the controls versus the cases, asthma-diagnosed, rhinitis-diagnosed, and eczema-diagnosed, respectively (*all P-values > 0.05*,

Table 4 and Figure 2). Furthermore, when we restrict the analysis to the cases, culturable fungi, (1–3,1–6)-β-d-glucan, and ergosterol were not significantly different between the IgE-sensitized versus the non-sensitized children (data not shown).

DISCUSSION

In the present investigation, we investigated whether culturable fungi, ergosterol, and (1–3,1–6)-β-d-glucan in indoor dust are associated with IDOM rating by parents, professional inspectors, or mold index. We subsequently examined whether the five most common fungal genera and yeasts pose independent risks on the respective diagnosis of asthma, rhinitis, eczema, or multiple allergic symptom presentation. We additionally examined risk of ergosterol and (1–3,1–6)-β-d-glucan on these allergic outcomes.

Overall, CFU counts/g dust for the five most common fungal genera and yeasts in all homes within our investigation were low. The mean ± S.D. and median for total culturable fungi in house dust was 1680±1443 CFU/g and 1267 CFU/g dust, respectively. While directly plating the dust on the agar medium may increase the diversity of observed fungi, this method might have yielded low detected concentration of culturable fungi due to crowding compared to serial dilution method (Verhoeff et al., 1994). Therefore, we interpreted the CFU counts from directly plated dust as semi-quantitative measures of growth intensity rather than quantitative counts of culturable fungi (Yang et al., 2007). As such, our references to concentrations of culturable fungi indicate observed CFUs on the plate.

Ergosterol concentrations from this study are comparable to those found in other studies. In a study of two nursing homes, one with and one without fungal contamination, the range of ergosterol concentrations in dust was between 1.6 and 3.3 ng/mg and 0.5 and 5.7 ng/mg, respectively (Pitkäranta et al., 2008). Similarly, ergosterol ranged from 2—16.5 ng/mg dust in homes not obviously contaminated with fungi; ergosterol concentrations were positively correlated with total CFU in dust (Saraf et al., 1997). Measured (1–3, 1–6)-β-D- glucan concentrations in the current study are low compared to concentrations in dust from the homes of children from five European countries, where median concentrations were over 1000 μg/g (Schram et al., 2005).

Fungal Agents and Health Outcomes

As shown in Table 4, neither the total CFU nor specific fungal genera or yeasts group were associated with an elevated likelihood of being a case, or any specific diagnosis of asthma, rhinitis, or eczema. As stated above, direct plating of dust may have underestimated the true culturable fungi concentrations. At the same time, this method has been validated for its ability to capture diverse mold genera (Wanner et al., 1993).

Several other studies have reported similar absence of association between quantified mold or mold related factors in dust and asthma or allergic disease. Wickman et al., (1992) found that sum of *Alternaria* spp., *Penicillium* spp. *and Cladosporium* spp. spores in house dust was lower among atopic households as opposed to asymptomatic controls. The authors posited that lower concentrations might reflect sanitary measures put in place by families with atopic children to reduce total allergen levels (Wickman et al., 1992). In a case control

study of German children, fungal CFU counts in dust and air were not different between cases and controls. However, the IgE sensitization rate to fungi was higher among cases (9.2%) as compared to controls (4.4%) (Jovanovic et al., 2004). In a prospective study, visually observed mold in the homes of 8 month old children was associated with a positive asthma predictive index (API) at three years of age, while (1–3, 1–6)-β-D-glucan concentration in dust was associated with a negative API at the same age; the protective effect was not significant (Iossifova et al., 2009). In a study of 226 adults with asthma and rhinitis residing in California, using the same methods as in this paper, Blanc et al. (2005) reported (1–3,1–6)-β-d-glucan median concentration of 211 μg/g in house dust (25^{th} – 75^{th} percentile, 124—426 *μg/g*). The authors noted no association between glucan levels and forced expiratory volume (FEV_1) (Blanc et al., 2005). There was also no significant relationship between visible mold, wall dampness or air humidity with FEV_1 (Blanc et al., 2005). At the same time, laboratory studies have shown that (1–3, 1–6)-β-D-glucans could cause or exacerbate allergic symptoms (Douwes, 2005).

Other studies have found an association between fungal concentrations in house dust and adverse health outcomes. In a birth cohort study, dust-borne fungal concentrations were positively associated with the development of allergic rhinitis in the first five years of life after controlling for dampness and several other covariates (Stark et al., 2005). In this investigation, median mold concentrations in dust are not reported, however the 90^{th} percentiles are approximately one to two orders of magnitude above those reported in our investigation (Stark et al., 2005). Reponen et al., (2012) found that both environmental moldiness index, a measure of prevalence of dampness related vs. non-dampness related molds, and the sum of three mold species *Aspergillus ochraceus*, *Apergillus unguis* and *Penicillium variable* measured in home at age 1 year significantly predict asthma at age 7. Respective geometric mean concentrations for these species, in cell equivalents/mg dust, were 6.8, 2.6 and 12.6 for cases and 2.0, 1.0 and 4.0 among controls (Reponen et al., 2012). These findings suggest a lack of species-specific fungal identification may contribute to misclassification of exposure and bias the results toward the null.

Our present observation regarding ergosterol is contrary to other investigations, which observed significantly elevated risks of asthma. For example, in a group of adult employees at a building with a history of water-damage, ergosterol concentration in floor and chair dust samples was significantly correlated with asthma, independent of culturable fungi concentration (Park et al., 2008). In our present investigation, we observed overall similar ranges of ergosterol exposure as those in Park et al. (2008). However, the distributions of ergosterol were consistently lower in all four outcome groups in our investigation compared to that in controls. Inconsistent associations between ergosterol and health outcomes may be partially explained by the non-specificity of this fungal marker (i.e. it is present in innocuous fungi) and the variable concentrations of ergosterol among different fungal species (Pasanen et al., 1999).

The inconsistent association between exposure to household fungus and health outcomes in different studies could in part be due to lack of standard methodology for assessing mold exposure (Mendell et al., 2011). Stark et al., (2005) noted that total fungal concentrations would pool diverse genera into a single exposure variable that may not accurately predict

risk (Stark et al., 2005). In addition, Muller et al., (2002) found significant associations between *Penicillium* and *Aspergillus* exposure with respiratory infections and IgE sensitization to grass respectively. However total CFU in air were not associated with health outcomes (Müller et al., 2002). Species level identification and molecular quantification methods may alleviate some of the inconsistencies.

Our present observation adds to the body of literature and suggests a lack of association between exposure to fungal agents and a range of respiratory outcomes. In a comprehensive review and meta-analysis, Mendell et al., (2011) found that while there is ample evidence that dampness related factors adversely affects health, dust-borne fungal and bacterial burdens had little predictive value for asthma and allergy outcomes, noting that both positive and negative associations were present (Mendell et al. 2011). Other reviews have come to similar conclusions about the role of indoor molds in the development of asthma and allergic disease (Bornehag et al., 2001; Institute of Medicine, 2004; Portnoy et al., 2008).

Culturable Fungi, Ergosterol, (1–3,1–6)-β-d-glucan and Dampness and Building Characteristics

In the present investigation, *Penicillium* was the most common genus, yielding the highest mean concentration. *Alternaria* spp. and *Yeasts* were also frequently detected and tended to be present at elevated concentrations compared to other genera. The low concentrations of culturable fungi and fungal markers found in the current investigation suggest that exposure to fungal material from dust is probably low. Furthermore, poor associations between IDOM and culturable fungi suggest that IDOM do not necessarily indicate fungal exposure. This is supported by two separate observations. First, there is low agreement between parental report of mold and the mold index, as well as the inspector ratings of mold and the mold index (Holme et al., 2010). Second, there is a general lack of association between qualitative IDOM assessments and culturable fungi in dust samples, with the exception of yeasts.

There is poor and inconsistent correlation between IDOM and *quantified* concentrations of fungal agents in the literature. While some studies found an association, other studies could not replicate such findings. Lignell et al. (2008) found that history of moisture damage is significantly associated with concentrations of several bacterial and fungal genera identified from the house dust (Lignell et al., 2008). Reponen et al. (2010) assessed the correlation between perception of mold and quantitative measures of mold in dust. The authors reported that visual perception of mold was not associated with concentrations of mold as measure by (1–3,1–6)-β-D-glucan in dust and air and airborne fungal spores. However, mold odor and environmental relative moldiness index (ERMI) were associated with mold burden (Reponen et al., 2010). Hyvärinen et al., (2006) measured fungal spores in vacuum cleaner dust and found culturable spore counts to be associated with presence of visible mold in the home (Hyvärinen et al., 2006).

Another study reported that airborne spore concentrations (CFU/m^3) are associated with indicators of dampness in homes such as water intrusion, indoor humidity, musty odor, and low ventilation (Garrett et al., 1998). Dekoster and Thorne (1995) demonstrated that airborne mold spore concentrations in complaint homes were two-fold higher than non-complaint homes and four-fold higher than intervention homes (DeKoster et al., 1995).

Genera-level determinations were performed for *Penicillium, Aspergillus, Cladosporium, Alernaria, Fusarium* and yeasts and showed marked differences between specific organisms. *Penicillium* and *Aspergillus* species were over 20-fold higher in the basements of complaint homes than non-complaint homes while other molds were not markedly different. Concentrations of airborne culturable molds were significantly associated with basement humidity levels and a history of a leaky roof (DeKoster et al., 1995). In contrast, in a birth cohort study, no association was observed between home characteristics (e.g., visible mold or water damage) and concentrations of *Alternaria* spp. antigen (Cho et al., 2006). However, use of a dehumidifier and indoor dryer venting were associated with *Alternaria* spp. concentrations (Cho et al., 2006). In German homes, neither the individual mold genera, nor the summed total of all mold genera were associated with relative humidity, temperature, visible mold, carpet, dampness, or ventilation (Jovanovic et al., 2004). Based on short-term air sampling, no association was found between visible mold or moisture damage and CFU concentrations in air (Müller et al., 2002). Such evidence highlights the inconsistent relationship between IDOM and quantifiable fungal agents.

It is also important to consider that indoor mold may be correlated with other multiple proximal correlates of IDOM, including, but not limited to, dust mites, and select synthetic chemicals. For example, indoor humidity increases concentrations of phthalate degradation by-products from PVC (Norback et al., 2000). Previous research within this cohort suggests that propylene glycol and glycol ethers are correlated with indoor dampness (Choi et al., 2010). Thus, the use of indoor dampness as an indirect measure of mold exposure, or vice versa, is problematic. While high levels of excess moisture in the home may precipitate mold growth they are not shown to be consistently correlated enough to suggest equivalency. In our repeated examination of dampness as a risk factor multiple allergic symptoms, we observed an inconsistent role of indoor moisture. While the parental reporting of home dampness was a strong risk factor of multiple allergic symptoms on a cross-sectional examination (Bornehag et al., 2005b), most of such associations disappeared when the same questions were repeated 5 years after the initial examination and the analyses were conducted with a longitudinal design (Larsson et al., 2011). Thus, the causal agent underlying the home dampness and health outcomes remains an open question.

Strengths

Here, we conducted a direct measurement of culturable fungi in dust samples taken from the floor in the children's bedroom. Dust is a major route of exposure for numerous indoor environmental agents, both biogenic and abiogenic (Munir et al., 1995; Rudel et al., 2003). Measurement of the culturable fungi in dust enabled us to examine possibly integrated human exposure in an indoor setting, independent of dampness. Fungi in dust samples are less likely to be influenced by outdoor sources as compared to air samples (Chew et al., 2003). Furthermore, indoor fungal concentrations in air and dust are not always highly correlated (Reponen et al. 2010). Analysis of ergosterol and (1–3,1–6)-β-d-glucan provide non-culture dependent markers of fungal contamination and exposure. Inclusion of these agents provides independent markers of fungal exposure, and thereby, overcomes the limitations of methods for culturable fungi measurement.

Limitations

Potential for selection biases for cases and controls has already been described and discussed (Bornehag et al., 2006). Briefly, participating families were more likely to have health problems, and also more likely to have health promoting factors such as non-smoking parents and higher socio-economic status. However, our earlier investigation revealed that there were no apparent selection biases regarding home dampness measures or other building characteristics (Bornehag et al., 2006). It is also possible that the children in the present study were exposed to allergenic factors in other non-home environment (e.g., school, nursery). The cross sectional nature of the exposure and outcome assessment make temporal relationships impossible to determine, thus, reverse causality is possible. The methods for determining cases and controls selected the most and least symptomatic children respectively for inclusion in the study. This sampling strategy makes identifying associations more likely; however, results are less generalizable. Our case-control selection strategy examines the risk of fungal exposure in a vulnerable segment of the population, compared to very healthy controls. Our present sample selection strategy does not threaten the validity of our conclusion. This is because the expected direction of the bias would be away from the null association. Our present observation of the null association between home fungal exposure and risk in the vulnerable segment of children is not likely to be different from that in the general population.

One limitation of our culture dependent method is that it fails to capture non-culturable fungal spores or other fungal material, which may still contribute allergic response. For example, Douwes et al., found that *Penicillium* and *Aspergillus* specific extracellular polysaccharides isolated from house dust were associated with doctor diagnosed asthma (Douwes et al., 1999). Additionally culture dependent methods select for easily culturable mold spores, and those that grow rapidly (Pasanen, 2001). Direct plating of dust may cause a reduction in apparent CFU compared to plating of serial dilutions, due to inhibitory effects of high fungal density on the plate (Verhoeff et al., 1994). Culture dependent methods underestimate concentrations and diversity of fungus present in the home as compared to molecular quantification methods such as quantitative PCR (Lignell et al., 2008; Pitkäranta et al., 2008). Additionally, qualitative assessments of visual mold may have underestimated its presence due to mold hidden inside walls or building materials. In addition, our reliance on reservoir dust samples as a proxy for airborne bioaerosol exposures might have introduced an exposure misclassification. This is because some of the materials in reservoir dust are never airborne.

This study reports the absence of an apparent association between exposure to culturable fungi, and fungal agents in dust and asthma and allergic disease symptoms. However, geographic variation in this association due to climatic conditions and endemic mold genera are possible. There is evidence that the association between mold and health outcomes is different in warmer and more humid areas (Hamilos, 2010). A review focusing on fungal rhinitis and rhinosinusitis suggested that geographic areas with higher natural fungal spore concentrations have higher rates of fungal allergy (Hamilos, 2010). In contrast, the occurrence of visible mold on building surfaces is less common in Scandinavian countries as compared to warmer climates (Bornehag et al., 2001). It is possible the low levels of mold

present in the homes of this study were not sufficient to elicit asthma or allergic symptoms. Thus, these results may not be generalizable to areas outside of Scandinavian countries.

CONCLUSION

This study demonstrates that culturable fungi, ergosterol, and (1–3, 1–6)-β-D-glucan in dust are not significantly associated with qualitatively determined degree of mold presence. Culturable fungi, ergosterol, and (1–3, 1–6)-β-D-glucan in dust do not pose an independent risk on individual diagnosis of asthma, rhinitis, eczema, as well as the presence of multiple symptoms of allergies.

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Practical Implications

There is no consistent agreement between qualitative indicators of indoor dampness and mold and the level of culturable fungi in dust. Culturable fungi and fungal factors in house dust do not predict asthma or allergic disease outcomes among children.

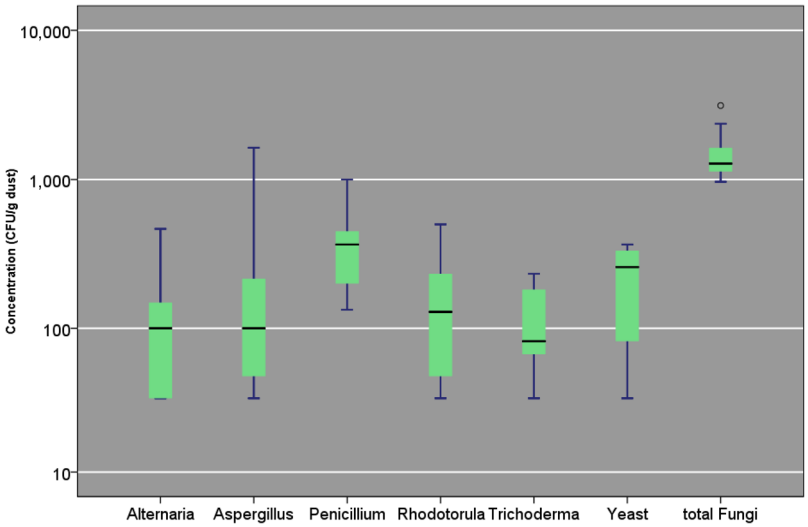

FIGURE 1. Culturable Fungi Concentration (CFU/g) in Home Dust (n=390)

Box and the bar within it show 25th, 50th, and 75th percentile of the concentration; the whiskers show the 5th and the 95th percentiles, respectively. The symbols, ● and *, represent concentrations that are >1.5– and >3–fold of the 75th percentile value.

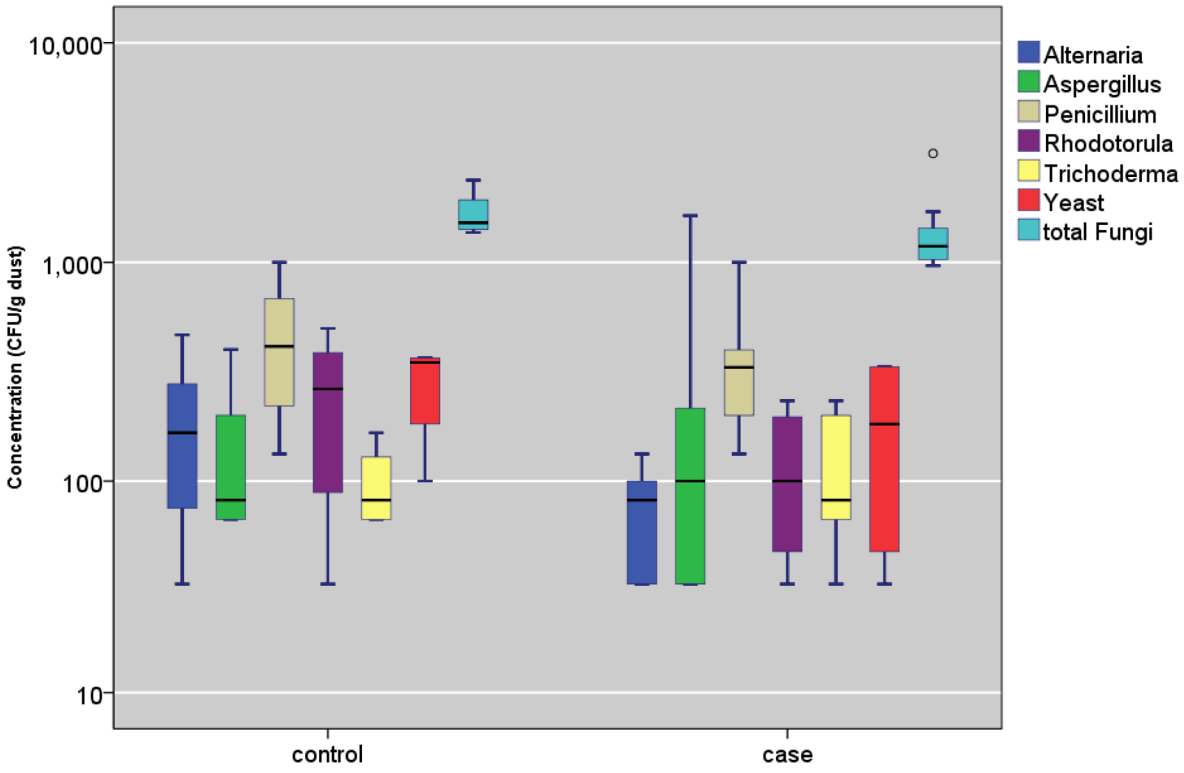

FIGURE 2. Culturable Fungi Concentration Distributions (CFU/g) in Homes of Case and Control Children

Box and the bar within it show 25th, 50th, and 75th percentile of the concentration; the whiskers show the 5th and the 95th percentiles, respectively. The symbols, ● and * represent concentrations that are >1.5– and >3–fold of the 75th percentile value.

Table 1

Distribution of Fungal Genera and Yeasts in Dust Sample and Parent-Reported Dampness Problems.

Parental Report	Unanswered				NO				Yes			
	N	Percentiles			N	Percentiles			N	Percentiles		
		5th	50th	95th		5th	50th	95th		5th	50th	95th	P-value
Visible Dampness													
Penicillium	4	100	417	--	316	67	400	1938	8	100	433		0.980
Alternaria	2	33	117	--	226	33	100	500	6	67	167		0.246
Aspergillus	2	33	500	--	212	33	100	1135	3	33	67		0.240
Yeasts	2	67	683	--	194	33	200	925	7	133	300		0.055
Rhodotorula	2	133	333	--	198	33	100	1142	5	100	233		0.130
Trichoderma	0	--	--	--	129	33	67	233	5	33	67		0.780
Total Fungi	5	467	1067	--	374	392	1267	3808	9	533	2200		0.137
(1–3, 1–6)-β-D-glucan	4	2	6	--	355	1	6	95	8	0	6		0.365
Ergosterol	5	0.626	2.058	--	369	0.079	1.658	9.826	9	96	1.722		0.423
Floor Moisture													
Penicillium	69	50	400	2650	229	67	400	1783	30	85	383	3040	0.768
Alternaria	50	33	117	530	166	33	100	467	18	33	83		0.962
Aspergillus	53	33	100	1360	142	33	100	1412	22	33	67	428	0.043
Yeasts	41	37	233	1480	146	33	200	1143	16	33	233		0.414
Rhodotorula	42	33	167	1667	142	33	100	962	21	33	133	1570	0.202
Trichoderma	26	33	67	308	95	33	67	233	13	33	67		0.792
Total Fungi	85	420	1467	3713	269	367	1267	3933	34	258	1150	4233	0.557
(1–3, 1–6)-β-D-glucan	84	1	6	84	252	1	6	107	31	1	6	85	0.727
Ergosterol	84	0.069	1.756	10.168	265	81	1.680	10.209	34	0.105	1.589	7.264	0.492
Moldy Odor													
Penicillium	94	67	450	3333	212	67	367	1760	22	72	267	2080	0.201
Alternaria	73	33	100	477	145	33	100	500	16	33	67		0.886
Aspergillus	63	33	133	1547	140	33	67	1100	14	33	67		0.646
Yeasts	55	60	300	1953	140	33	200	667	8	100	200		0.421
Rhodotorula	63	33	133	1233	129	33	100	1667	13	33	133		0.442

Parental Report	Unanswered				NO				Yes			
	N	Percentiles			N	Percentiles			N	Percentiles		
		5th	50th	95th		5th	50th	95th		5th	50th	95th	P-value
Trichoderma	40	33	67	198	86	33	67	298	8	33	67		0.645
Total Fungi	114	425	1383	4383	251	353	1233	3767	23	333	1400	4540	0.717
(1–3, 1–6)-β-D-glucan	109	1	6	210	237	1	6	75	21	0	5	67	0.523
Ergosterol	113	0.114	1.716	13.524	247	0.078	1.783	9.966	23	0.019	1.174	6.093	0.099
Condensation													
Penicillium	16	100	383	--	252	67	400	1667	60	67	333	6625	0.793
Alternaria	13	33	167	--	184	33	100	500	37	33	100	350	0.590
Aspergillus	12	33	100	--	164	33	100	1100	41	33	67	1543	0.242
Yeasts	7	67	300	--	160	33	233	1293	36	33	150	1692	0.034
Rhodotorula	13	33	267	--	156	33	100	1158	36	33	100	1802	0.647
Trichoderma	4	33	67	--	105	33	67	233	25	33	67	427	0.646
Total Fungi	19	467	1500	--	300	368	1267	3732	69	383	1400	6417	0.362
(1–3, 1–6)-β-D-glucan	18	1	6	--	284	1	6	96	65	1	7	94	0.902
Ergosterol	19	0.007	1.547	--	296	0.084	1.742	10.003	68	0.083	1.588	9.036	0.445

Concentration units are CFU/g for the fungal and yeasts, μg/g dust for (1–3, 1–6)-β-D-glucan and ergosterol. There were no reported values at the 95th percentile for those with answers for the mold index. These are denoted as --. Kruskal-Wallis test was used to obtain the P-values.

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Table 2

Distribution of the Fungal Genera and Yeasts and Other Markers According to Home Inspector Grading of Dampness Problem in Dwelling.

Inspector Rating	No Evidence				Weak Indication				Strong Indication				P-value
	N	Percentiles			N	Percentiles			N	Percentiles			
		5th	50th	95th		5th	50th	95th		5th	50th	95th	
Moldy Odor													
Penicillium	195	93	400	1987	83	67	400	1907	50	52	333	3333	0.895
Alternaria	139	33	100	567	58	33	83	503	37	33	100	477	0.612
Aspergillus	130	33	100	1633	49	33	67	1333	38	33	100	937	0.592
Yeasts	115	33	200	1667	54	33	250	1275	34	33	167	1250	0.179
Rhodotorula	126	33	133	1632	55	33	133	1080	24	33	67	608	0.023
Trichoderma	81	33	67	323	34	33	67	208	19	33	67		0.567
Total Fungi	235	400	1300	3813	89	367	1300	3867	64	375	1133	4483	0.203
(1–3, 1–6)-β-D-glucan	227	1	7	100	79	1	6	70	61	1	5	131	0.474
Ergosterol	232	0.079	1.940	9.245	87	0.043	0.1355	7.149	64	0.121	1.799	16.063	0.190
Moldy Odor Along Skirting Board													
Penicillium	174	67	367	2233	99	67	400	1667	55	60	400	3333	0.501
Alternaria	115	33	100	667	84	33	83	425	35	33	133	473	0.073
Aspergillus	110	33	67	1355	70	33	67	1082	37	33	133	1633	0.086
Yeasts	101	33	200	1280	65	67	233	1310	37	33	167	3457	0.108
Rhodotorula	110	33	133	1772	67	33	100	720	28	33	100	340	0.527
Trichoderma	66	33	67	298	44	33	67	233	24	33	67	192	0.760
Total Fungi	203	407	1333	3827	122	372	1233	3823	63	340	1200	4740	0.931
(1–3, 1–6)-β-D-glucan	194	1	6	71	116	1	6	179	57	1	6	149	0.637
Ergosterol	202	0.083	1.874	7.988	118	0.065	1.563	10.326	63	0.079	1.358	15.829	0.209
Damp Stain													
Penicillium	243	67	367	1667	72	100	433	3333	13	33	367		0.812
Alternaria	173	33	100	567	50	33	100	463	11	33	67		0.352
Aspergillus	169	33	100	1333	40	33	100	995	8	33	83		0.430
Yeasts	157	33	233	1180	37	33	233	1667	9	67	200		0.781
Rhodotorula	156	33	100	1355	42	33	117	898	7	33	100		0.828

Inspector Rating	No Evidence				Weak Indication				Strong Indication				P-value
	N	Percentiles			N	Percentiles			N	Percentiles			
		5th	50th	95th		5th	50th	95th		5th	50th	95th	
Trichoderma	101	33	67	233	30	33	67	233	3	100	133		0.089
Total Fungi	292	367	1267	3790	81	510	1267	4043	15	400	1267		0.548
(1–3, 1–6)-β-D-glucan	273	1	6	69	79	1	7	139	15	1	6		0.467
Ergosterol	288	0.079	1.563	8.176	80	0.085	2.115	15.742	15	0.032	2.135		0.617
Floor Moisture													
Penicillium	300	67	400	1798	24	75	300	5833	4	167	1367		0.175
Alternaria	221	33	100	500	12	33	150		1	33	33	33	0.271
Aspergillus	200	33	100	1195	15	33	67		2	167	267		0.094
Yeasts	192	33	233	948	11	67	133		0				0.194
Rhodotorula	194	33	100	1175	10	33	83		1	733	733	733	0.393
Trichoderma	128	33	67	233	6	33	33		0				
Total Fungi	359	400	1267	3800	25	220	1400	5927	4	600	2150		
(1–3, 1–6)-β-D-glucan	340	1	6	90	24	1	5	523	3	5	5		0.758
Ergosterol	354	0.093	1.719	9.757	25	0.065	1.200	51.304	4	0.558	1.721		0.752

Concentration units are CFU/g for the Fungal genera and yeasts, μg/g dust for (1–3, 1–6)-β-D-glucan and ergosterol. Kruskal-Wallis test was used to obtain the P-values.

Table 3

Distribution of Fungal Genera, Yeasts, and Other Markers in Dust Sample and Semi-Quantitative Mold Index.

	Mold Index												
	Missing				**No Mold**				**Houses with Mold**				**P**
	N	**Percentiles**			**N**	**Percentiles**			**N**	**Percentiles**			
		5th	**50th**	**95th**		**5th**	**50th**	**95th**		**5th**	**50th**	**95th**	
Penicillium	8	200	383	--	254	67	367	1942	66	45	417	3147	0.259
Alternaria	6	33	133	--	177	33	100	567	51	33	67	327	0.297
Aspergillus	7	33	67	--	169	33	100	1550	41	33	133	1030	0.160
Yeasts	5	100	567	--	155	33	200	1233	43	40	233	1173	0.720
Rhodotorula	3	100	167	--	156	33	100	992	46	33	133	1632	0.255
Trichoderma	3	67	67	--	105	33	67	233	26	33	67	222	0.373
Total Fungi	10	733	1800	--	301	367	1233	3893	77	587	1500	3803	0.047
(1–3, 1–6)-β-D-glucan	8	1	4	--	288	1	6	93	71	1	7	97	0.404
Ergosterol	10	0.079	1.402	--	296	0.083	1.686	10.248	77	0.078	1.734	8.980	0.751

Concentration units are CFU/g for the fungal genera and yeasts, μg/g dust for (1–3, 1–6)-β-D-glucan and ergosterol. There were no reported values at the 95th percentile for those with answers for the mold index. These are denoted as --. Kruskal-Wallis test was used to obtain the P-values.

Table 4

Association between Fungal Concentration, Yeasts and Other Fungal Markers in Dust and Multiple Allergic Symptom Presentation (i.e. case status) or Clinical Diagnosis of Asthma, Rhinitis, or Eczema.

		N	Percentiles			P
			5th	50th	95th	
Penicillium	control	162	67	367	1695	
	case	166	100	400	3147	0.122
	asthma	102	67	400	1922	0.358
	rhinitis	83	73	400	3333	0.263
	eczema	107	80	400	3333	0.270
Alternaria	control	119	33	100	667	
	case	115	33	100	500	0.774
	asthma	68	33	100	470	0.810
	rhinitis	62	33	100	495	0.839
	eczema	77	33	100	470	0.793
Aspergillus	control	119	33	100	1033	
	case	98	33	100	1635	0.701
	asthma	64	33	67	1550	0.292
	rhinitis	56	33	67	2473	0.419
	eczema	64	33	100	1658	0.889
Yeasts	control	97	33	200	717	
	case	106	33	233	1667	0.928
	asthma	64	33	233	1667	0.665
	rhinitis	46	33	250	1003	0.919
	eczema	69	33	200	1483	0.559
Rhodotorula	control	98	33	100	1930	
	case	107	33	100	1013	0.460
	asthma	65	33	100	1367	0.617
	rhinitis	57	33	100	670	0.475
	eczema	77	33	100	1337	0.272
Trichoderma	control	69	33	67	233	

		N	Percentiles			P
			5th	50th	95th	
	case	65	33	67	233	0.560
	asthma	33	33	67	233	0.546
	rhinitis	38	33	67	233	0.484
	eczema	39	33	67	233	0.582
Total Fungi	control	198	367	1283	3767	
	case	190	418	1250	5113	0.973
	asthma	117	330	1200	3973	0.400
	rhinitis	96	385	1200	6272	0.897
	eczema	125	353	1233	4673	0.721
(1–3, 1–6)-β-D-glucan	control	187	1	6	66	
	case	180	1	6	137	0.674
	asthma	112	1	6	108	0.999
	rhinitis	89	1	6	204	0.521
	eczema	119	1	6	69	0.747
Ergosterol	control	195	0.138	1.750	10.367	
	case	188	0.078	1.629	7.572	0.182
	asthma	115	0.079	1.631	7.077	0.346
	rhinitis	94	0.076	1.482	7.083	0.138
	eczema	124	0.083	1.561	7.145	0.101

Concentration units are CFU/g for the mold genera, μg/g dust for (1–3, 1–6)-β-D-glucan, pg/mg for ergosterol. Mann–Whitney U-test was used to obtain the P-values.