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Airborne Endotoxin and β-D-glucan in PM_1 in Agricultural and Home Environments

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ABSTRACT

Objectives: Concentrations of microbial cell wall components such as endotoxin and β-D-glucan in airborne submicrometer particle size fraction (PM_1, defined as particles with median aerodynamic diameter, $d_a \leq 1$ μm) have not been well characterized. In this study, airborne concentrations of endotoxin and β-D-glucan among different size fractions were quantified in two distinctly different environments: farms and homes. Effect of microbial source (farm type for farms and mold damage for homes) on size-fractionated concentrations was investigated.

Methods: Airborne endotoxin and β-D-glucan were collected on 7 farms and 184 suburban homes using NIOSH two-stage sampler, a cyclone air sampler that fractionates airborne particles into three size fractions: ≤ 1 μm, 1–1.8 μm and ≥ 1.8 μm.

Results: Geometric means (geometric standard deviations) of airborne total endotoxin concentration on farms and in homes were 1.20×10^4 (7.57) EU/m^3 and 2.67 (3.82) EU/m^3, respectively. Similar values of airborne total β-D-glucan concentrations were 1.80×10^3 (152.88) ng/m^3 and 1.91 (2.83) ng/m^3, respectively. Relative proportions of PM_1 endotoxin, however, were significantly higher ($p < 0.001$) in home samples (7.9%) than in farm samples (0.3%). Likewise, the proportion of PM_1 β-D-glucan was significantly higher ($p < 0.001$) in homes (28.2% vs. 2.3%). Farm type significantly influenced concentrations of PM_1 β-D-glucan ($p < 0.05$) and total β-D-glucan ($p < 0.001$).

Conclusions: The results of this study demonstrate higher proportions of PM_1 endotoxin and β-D-glucan in homes compared to farms. These data further emphasize the importance of conducting size-selective air sampling for microbial exposure assessment in homes.

Keywords: Bioaerosol; Farms; Moldy buildings; Size-Selective sampling; Fine particles.

INTRODUCTION

Personal exposure to microbial biomarkers including gram-negative bacterial endotoxin and fungal (1-3)-β-D-glucan (β-D-glucan) in the occupational and residential environment has been proposed to mediate a variety of adverse health effects (Rylander, 2008). Among cotton textile workers, prolonged exposure to endotoxin in the occupational environment has been associated with intermittent respiratory symptoms (e.g., chest tightness) that improve following intervention (Wang *et al*., 2003). Strong associations have also been identified between total airborne β-D-glucan and the deterioration of lung function in residential and occupational environments (Thorn and Rylander 1998; Jacob *et al*., 2002; Gehring *et al*., 2004). Studies conducted within the last decade have shown that exposure to endotoxin and β-D-glucan in infancy may be protective against development of atopy or recurrent wheeze in infants born to atopic parents (von Mutius *et al*., 2000; Dales *et al*., 2006; Iossifova *et al*., 2007).

Recent studies have reported the occurrence of endotoxin and β-D-glucan in the PM_1 particle size fraction in homes (Kujundizc *et al*., 2006; Reponen *et al*., 2007; Salares *et al*., 2009) and occupational environments (Madsen *et al*., 2006a; Wang *et al*., 2007; Madsen *et al*., 2009). PM_1 is defined as the mass concentration of particles having the median aerodynamic diameter, d_a, up to 1 μm. Particles of fungi (fragments) may represent up to 56% of the total airborne particles in the outdoor environment (Green *et al*., 2006), and *in vitro* chamber experiments demonstrated that the concentration of fungal fragments can be about 500-fold higher than the concentration of intact spores (Cho *et*

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al., 2005). Compared to larger bacterial and fungal propagules (PM_{10}, $d_a \leq 10$ μm), PM_1 remains aerosolized for longer durations, penetrates deeper into the terminal airways, and exposures may exacerbate adverse health effects (Schwartz *et al*., 1996; Churg and Braurer, 1997). For example, stimulation of bronchial epithelial cells due to submicron particles is associated with significantly higher release of inflammatory mediators and tissue damage compared to stimulation by particles ranging in diameter between 1.0–2.5 μm. (Huang *et al*., 2003). PM_1 has also been shown to contain fungal antigens (Górny *et al*., 2002) and mycotoxins (Brasel *et al*., 2005). Furthermore, the respiratory dose of microbial PM_1 particles has been estimated by computer modeling to be up to 250-fold higher than that of spores (Cho *et al*., 2005). The results of these studies demonstrate that PM_1 microbial particles are likely to contribute significantly to the airborne biomass in the environment and may contribute to the exacerbation of adverse health effects.

The majority of microbial particles in PM_1 are derived from the fragmentation of spores, hyphae, or intact vegetative cells of fungi and fragmented cells and intact cells of bacteria (Gorny *et al*., 2002; Green *et al*., 2006). The fragmentation may occur by various abiotic and biotic processes (Reponen *et al*., 1995; Papagianni *et al*., 1999; Moore, 1988; Andersen, 2008). Abiotic factors that characterize microbial fragment dispersal include surface, air, and anthropogenic disturbances over surfaces colonized with microbial growth (Górny *et al*., 2002; Górny *et al*., 2003; Cho *et al*., 2005). Furthermore, the aerosolization of fragments has been shown to differ between fungal species (Górny *et al*., 2002; Seo *et al*., 2008). To date, these factors remain uncharacterized and require further study.

We hypothesized that size distributions and concentrations of airborne microbial components are dependent on the type of microbial source and the diversity of microbial species. Few studies have explored the comparative distribution of microbial PM_1 particles in agricultural and residential environments. Each of these environments is influenced by various occupational and anthropogenic disturbance regimes that may fragment and disperse colonized bacterial and fungal species. In this study, airborne concentrations of endotoxin and β-D-glucan among different size fractions were quantified in two distinctly different environments: farms and homes. The effect of potential microbial source (farm type on farms and mold damage in homes) was also investigated.

MATERIALS AND METHODS

Sampling Sites

Two different environments – farms and residential homes – were evaluated in this study. Farm samples were collected in a sub-set (n = 7) of agricultural sites included in a larger study (n = 9) that aimed to assess the efficacy of respirators used in agricultural environments (Cho *et al*., 2010). A total of seven farms were selected for inclusion in the present study and these were located in southern Kentucky (USA). Three farming categories were represented: swine farms (n = 3), grain handling bins (n = 3), and a horse farm (n = 1). The farms were typical of those located in the East-Central US with respect to the size and type of livestock (Cho *et al*., 2010). A total of 19 size-selective air samples were collected on farms and analyzed for airborne endotoxin and β-D-glucan.

Size selective home samples were collected from 184 homes participating in an ongoing birth cohort study (Cincinnati Childhood Allergy and Air Pollution Study) (LeMasters *et al*., 2006). The presence or absence of visible fungal contamination and water damage on various surfaces within the home was noted during each home inspection using a checklist as previously described (Cho *et al*., 2006). The homes were divided into three categories based on the results of the home inspection: Category 0 (no visible mold or moldy odor), Category 1 (low mold: reported history or observed water damage, observed moldy odor, or visible mold area ≤ 0.2 m^2), and Category 2 (high mold: visible mold area > 0.2 m^2) (Cho *et al*., 2006; Reponen *et al*., 2010).

Air Sampling

The NIOSH two-stage air sampler [BC212, described by Lindsley *et al*. (2006)] was used in this study for collecting airborne particles from the farm and home environments. In brief, the sampler separates particles into three size fractions. It consists of two conical 1.5 mL sterile microcentrifuge tubes (USA Scientific, Inc., Orlando FL), a back-up filter (polycarbonate filter, 37 mm diameter, 0.8 μm pore size, Millipore, Billerica, MA), and a polypropylene filter support (SKC Inc., Eighty Four, PA). The physical collection efficiency of the collection filter was determined similarly as described in Burton *et al*. (2007). The minimum efficiency was found to be 92% for 90 nm particles. The first tube collects particles with $d_a \geq 1.8$ μm (coarse particles) at a flow rate of 3.5 L/min. The second tube collects particles ranging from 1.0 to 1.8 μm (intermediate fraction), and the back-up filter collects particles with $d_a \leq 1$ μm (PM_1).

Prior to sampling, the polycarbonate filters obtained from the manufacturer (unused filters) were found to contain varying levels of β-D-glucan, ranging from less than the LOD to 360.45 pg/filter. Therefore, prior to loading the filters on the samplers, they were pretreated with 0.05% Tween 80 solution, vortexed for 2 minutes, ultrasonicated for 15 minutes (Fisher Scientific Mechanical Ultrasonic Cleaners FS20, Pittsburgh, PA) and then allowed to air dry. This treatment reduced β-D-glucan from the unused filters to below the limit of detection (data not shown). The desired flow rate through the sampler was maintained by a BGI 400 personal sampling pump (BGI, Inc, Waltham, MA). The air flow rate through the samplers was calibrated prior to sampling and verified after the sampling using a flow calibrator (Defender 510 H, Bios Intl. Corp., Butler, NJ).

The environmental monitoring was done inside partial or full enclosures on farms and indoors in homes. The air samplers in the farm environment were placed approximately one meter above the ground and within 1–

10 meters of the main activity areas of the farmers. The air samples were collected while the workers conducted regular occupational activities that included sweeping or air-blowing dust from the floor of the horse/swine farms using brooms and powered air-blowers or handling corn inside the grain handling bins. In the homes, the samplers were placed one meter above the ground in the child's primary activity room, which was either the living room (n = 143) or bedroom (n = 41), as specified by parents/guardians. The families were instructed to continue normal activities throughout the duration of home sampling.

The average sampling duration was 3 hours in the farming environments (ranging from 2.5 to 6 hours) and 24 (± 1) hours in the home environments. The ambient particle concentration was shown to influence the collection efficiency of the NIOSH two-stage sampler. Longer sampling times at high ambient particle concentrations were identified to decrease the collection efficiency of the sampler and lead to particle bounce. To overcome this confounding variable, different sampling times were empirically tested in each environment. The ambient particle concentration was concurrently measured using an optical particle counter (OPC; Model 1.108, Grimm Technologies, Inc., Douglasville, GA) during sampling with the NIOSH two-stage sampler. During the preliminary experiments, spore bounce from the second NIOSH two-stage sampler cyclone onto the back-up filter was observed when the concentration of airborne particles with an optical diameter > 1 μm entering the sampling system exceeded a threshold of 8×10^6 particles. As a result of these preliminary findings, the sampling intervals on farms were chosen based on the overall concentration of ambient particles with an optical diameter > 1 μm. Sampling duration was adjusted to assure that the number of collected particles remained below 8×10^6 to mitigate spore bounce and ensure that the quantification of endotoxin and β-D-glucan on the back-up filter was representative of the PM_1 size fraction. In contrast to farms, lower particle concentrations were encountered in the residential environments. Also, it was not possible to continuously monitor the particle concentrations in homes for extended periods of time because it was not practically feasible for sampling personnel to stay at home sampling sites for the entire 24-hour sampling duration. Preliminary experiments showed negligible spore bounce over a 24 hour sampling duration in homes. This sampling time for home samples was selected to enable the collection of measurable quantities of endotoxin and β-D-glucan in PM_1. Following sample collection, the samples were then transported to the laboratory and stored at 4°C until sample extraction. The extraction took place in 24–48 hours after completion of sampling.

The diversity of fungal species present on farms was determined by collecting fungal bioaerosols non-size-selectively on a polycarbonate filter (25 mm diameter, 0.8 μm pore size, SKC Inc.) that was placed inside a clear styrene filter cassette (SKC SureSeal, 2-section, 25 mm diameter) as previously described (Cho *et al*., 2010). In homes, a Button Inhalable Aerosol Sampler (SKC Inc.) was used to collect fungal bioaerosols in parallel with the NIOSH two-stage sampler (Reponen *et al*., 2010). In both cases, 25-mm polycarbonate filters were used for the sample collection (pore size of 3 μm; GE Osmonics, Inc., Minnetonka, MN). Relative humidity and temperature were also recorded at the start and end of the sampling period for each sample.

Analytical Protocols

Following air sampling, the three size fractions from the NIOSH two-stage sampler were extracted with 0.05% Tween (1.3 mL for the two microcentrifuge tubes and 5 mL for the back-up filter), vortexed for 2 minutes, and ultrasonicated for 15 minutes. The extracts were then aliquoted, stored at –20ºC, and analyzed within 3 to 7 days. Aliquots of 500 μL were used for separate analysis of endotoxin or β-D-glucan. Samples for β-D-glucan analysis were further diluted with 500 μL of (0.06N) NaOH solution. Serial (one-tenth) dilutions of the extracts were then used in the assay to determine the concentrations of endotoxin and β-D-glucan in each size fraction. The analyses of the extracts were conducted using the kinetic chromogenic *Limulus* Amebocyte Lysate (LAL) assay using commercially available Pyrochrome (endotoxin) and Glucatell (β-D-glucan) kits from Associates of Cape Cod (East Falmouth, MA) as previously described (Adhikari *et al*., 2009). Endotoxin and β-D-glucan in different size fractions were expressed as EU/m^3 and ng/m^3 after accounting for the volume of extracts in each fraction and the sampling flow rate.

The limit of detection (LOD) for the endotoxin assay was 0.053 EU/mL. The LOD for the β-D-glucan assay was 2.538 pg/mL. The LOD for the final air concentration varied depending on the actual flow rate and sampling time. For example, sampling at 3.5 L/min for 24 hours resulted in the following LOD-values for endotoxin: 0.052 EU/m^3 for the PM_1 fraction and 0.014 EU/m^3 for intermediate and coarse particle size fractions. Corresponding LODs for airborne β-D-glucan were 3 pg/m^3 for the PM_1 fraction and 1 ng/m^3 for intermediate and coarse particle size fractions. The percentages of home samples below the LOD for endotoxin were 31%, 7% and 2% for the PM_1, intermediate and coarse fractions, respectively. The corresponding percentages of home samples below the LOD for β-D-glucan were 18%, 4% and 1%. In contrast, only one farm sample had endotoxin in the PM_1 fraction below the LOD, and one farm sample showed undetectable β-D-glucan in the coarse fraction.

To determine the spore bounce in the PM_1 fraction, spores were enumerated as well as morphologically identified and quantified by analyzing a 2 mL suspension, which was obtained from the PM_1 fraction collected with the NIOSH two-stage sampler. In addition, for determination of spore concentration and fungal diversity in ambient air, spores were similarly enumerated from the non-size-selective and inhalable samples. Sample extracts were processed following the procedure described by Adhikari *et al*. (2003). Briefly, the suspensions were filtered through membrane filters (mixed cellulose esters, hydrophilic, 0.45

μm, 13 mm, Millipore, Billerica, MA). After air drying, the filters were cleared with acetone vapor and stained with lactophenol cotton blue (Becton Dickinson & Co., Sparks, MD) and subsequently visualized using bright field microscopy (400 × and 1000 × magnifications). Spores were counted in forty random fields. The concentration of spores that had bounced from the intermediate stage to the back-up filter was quantified and compared to the total airborne spore concentration.

Data Analysis

For farms and homes, total concentrations of endotoxin and β-D-glucan were calculated by adding together the corresponding fractional concentrations. Concentrations below the LOD were divided by two before calculating the total concentrations. PM_1 endotoxin values for homes were not normally distributed even after log transformation of these skewed data. Therefore, non-parametric analysis was used to describe this exposure variable. Other concentrations of endotoxin and β-D-glucan were $\log_e$-normally distributed, after adjustment for farm type and mold category, as determined by Shapiro-Wilk tests. Geometric means (GMs), geometric standard deviations, 95% confidence intervals (95% CI), minimum and maximum values of total and size-fractionated concentrations were calculated, except median was used for PM_1 endotoxin in homes. Bar charts showing geometric means and 95% confidence intervals by farm type and home category as well as by the particle size fraction were drawn to visualize differences.

Total concentrations of log transformed endotoxin and log transformed β-D-glucan were compared among farms and homes. Each exposure variable was analyzed separately by unpaired t-tests. Relative humidity and temperature were also analyzed to investigate differences between farms and homes. Total concentration of endotoxin and β-D-glucan were compared among farm types and mold categories of homes by analysis of variance. Analysis for farms and homes were done separately for each exposure variable. Unpaired t-test was performed to compare farms and homes with respect to each size fractions of endotoxin and β-D-glucan.

The proportion of analyte in each size fraction, compared to the total concentration, was calculated and expressed as a percentage. Exploratory analysis had shown that the distribution of the proportions was not normal and was unable to be transformed to approximate normality. Therefore, medians and interquartile (IQ) ranges of size-fractionated percentages of endotoxin and β-D-glucan on farms and in homes were obtained and visually presented in bar charts. For each size fraction, farm and home medians were compared by the Wilcoxon Rank Sum test. Spore bounce was shown to increase the amount of β-D-glucan quantified in the PM_1 size fraction; a potential confounding variable that could lead to the over estimation β-D-glucan. To overcome this potentially limiting factor, the statistical analyses were repeated to account for the influence of spore bounce on the quantification of β-D-glucan in the PM_1 size fraction. The percent of the total ambient spore concentration detected in the PM_1 fraction (i.e., relative proportion of bounced spores) was calculated for each farm and home. Percents were categorized into three levels for farms and homes separately: < 5%, 5–10% and > 10%. The number of farms and homes in each spore bounce category were obtained. The equality of β-D-glucan median concentrations in homes versus farms was tested by the Wilcoxon non-parametric test.

Finally, associations between log transformed airborne concentrations and temperature and relative humidity were investigated by linear regression analysis for total concentrations and censored regression analysis for PM_1 concentrations of endotoxin and β-D-glucan.

RESULTS

Differences between Farms and Homes in Total Concentrations, Temperature, and Relative Humidity

Geometric means (GMs) of total concentrations of airborne endotoxin and β-D-glucan on farms were approximately three to four orders of magnitude greater than in homes ($p < 0.001$). There were no significant differences in temperature and humidity between farms and homes ($p > 0.05$) (Table 1). Total endotoxin and β-D-glucan concentrations by farm type and mold category are shown in Fig. 1. Endotoxin GMs were 4.66×10^4, 2.49×10^3 and 7.04×10^3 EU/m^3 for grain handling bins, horse farms and swine farms, respectively. For the same farm types, β-D-glucan concentrations were 1.50×10^5, 3.73×10^1 and 3.11×10^2 ng/m^3. Endotoxin GMs were 2.02, 2.81 and 3.33 EU/m^3 in home categories, 0, 1, and 2, respectively. The corresponding β-D-glucan concentrations were 2.15, 1.75 and 2.62 ng/m^3. Statistically significant differences were observed with the total β-D-glucan concentrations between the farm types ($p < 0.001$). Post hoc analysis indicated that β-D-glucan concentrations in grain handling bins were significantly higher than in the two other farm types.

Size-selective Data on Airborne Endotoxin and β-D-glucan

GMs of endotoxin and β-D-glucan concentrations in PM_1 samples obtained on farms were 25.6 EU/m^3 and 24.4 ng/m^3, respectively. In homes, the median concentration for PM_1 endotoxin was 0.1 EU/m^3, whereas the GM for PM_1 β-D-glucan was 0.3 ng/m^3 (Fig. 2). The airborne endotoxin and β-D-glucan concentrations in each particle size range were significantly higher on farms than in homes ($p < 0.001$). Analyses showed significant difference for PM_1 β-D-glucan among farm types ($p < 0.05$) with marginally significant differences ($p = 0.05$) only between grain handling bins and horse farms. There were no significant differences for PM_1 endotoxin between farm types in these analyses. For homes, the effect of mold category on the concentrations of endotoxin or β-D-glucan was not statistically significant.

Median Percentage Differences between Farms and Homes

The concentration of airborne endotoxin and β-D-glucan in each particle size range was expressed as the percentage of the total concentration (Fig. 3). Overall, the percentages

Table 1. Means and standard deviations (SD) of concentrations of total[a] endotoxin, total β-D-glucan, relative humidity and temperature.

Variables	Mean (SD)		p-value [b]
	Farms (n = 19)	Homes (n = 184)	
Total endotoxin (EU/m^3)[c]	1.20×10^4 (7.57)	2.67 (3.82) [b]	< 0.001
Total β-D-glucan (ng/m^3)[c]	1.80×10^3 (152.88)	1.91 (2.83) [b]	< 0.001
Relative humidity (%)	43.4 (11.2)	41.1 (11.3)	0.39
Temperature (°C)	24.6 (6.6)	23.8 (2.4)	0.59

[a] Total concentration of endotoxin or β-D-glucan in the three size ranges (< 1.0, 1–1.8, > 1.8 μm) added together
[b] P-values testing the differences between farms and in homes based on unpaired t-tests
[c] Geometric means and geometric standard deviations

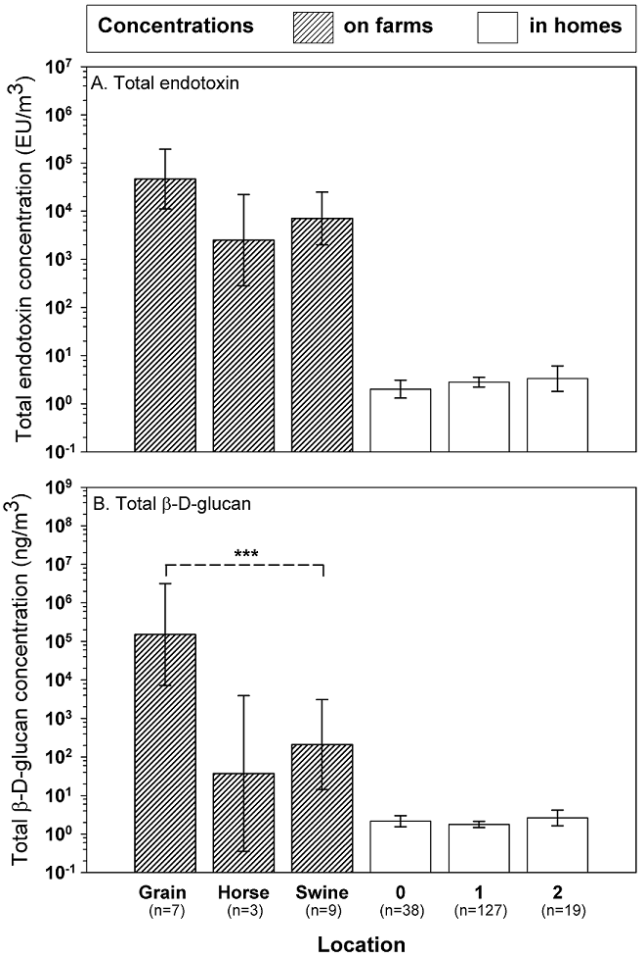

Fig. 1. Total concentration of endotoxin and β-D-glucan quantified on farms categorized by farm types (grain, horse or swine) and in homes categorized by mold category (0 = no mold, 1 = low mold or 2 = high mold). The bars represent the geometric means of the respective concentrations; the error bars represent the 95% confidence intervals (CIs). The values have been adjusted for farm type and home category. Level of significance is represented by horizontal lines across the top of the bars (*** = $p < 0.001$).

of endotoxin and β-D-glucan in PM_1 were significantly higher in homes than on farms. The median percentages of endotoxin and β-D-glucan in the PM_1 fraction on all farms taken together were 0.3%, and 2.3% respectively, whereas in homes the respective percentages were: 7.9% and 28.2% (Fig. 3, $p < 0.001$). Coarse fractions ($d_a \geq 1$ μm) derived from farm environments had a significantly higher percentage ($p < 0.001$) of endotoxin and β-D-glucan compared to those from homes.

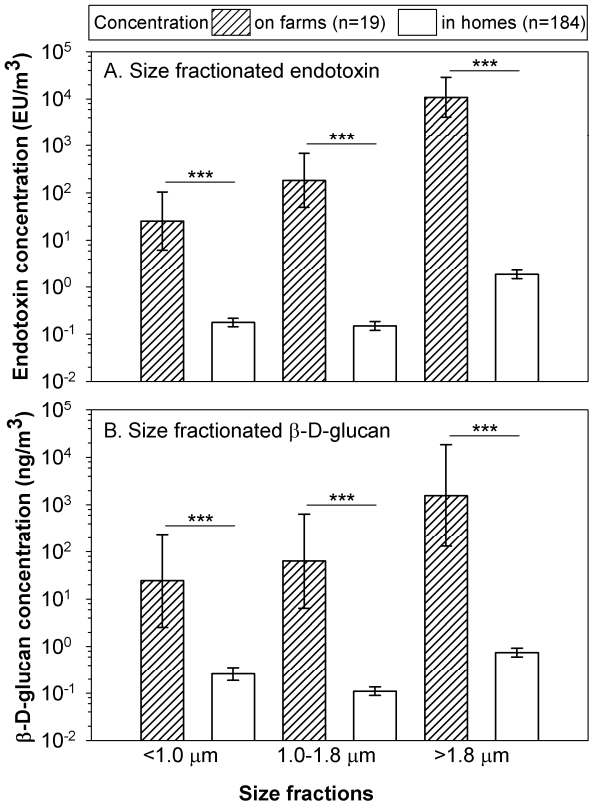

Fig. 2. Fractional concentration of endotoxin and β-D-glucan quantified in farm and home samples. The bars represent the geometric means (GMs) of the respective fractional concentrations; the error bars represent the 95% CI [except for PM1 (< 1 μm) endotoxin in homes, in which case the median concentration was used instead of GM]. Unpaired t-tests of $\log_e$-transformed endotoxin and β-D-glucan concentrations for each size fraction were performed to examine difference between mean values across all farms and homes. Level of significance is represented by horizontal lines across the top of the bars (*** = $p < 0.001$).

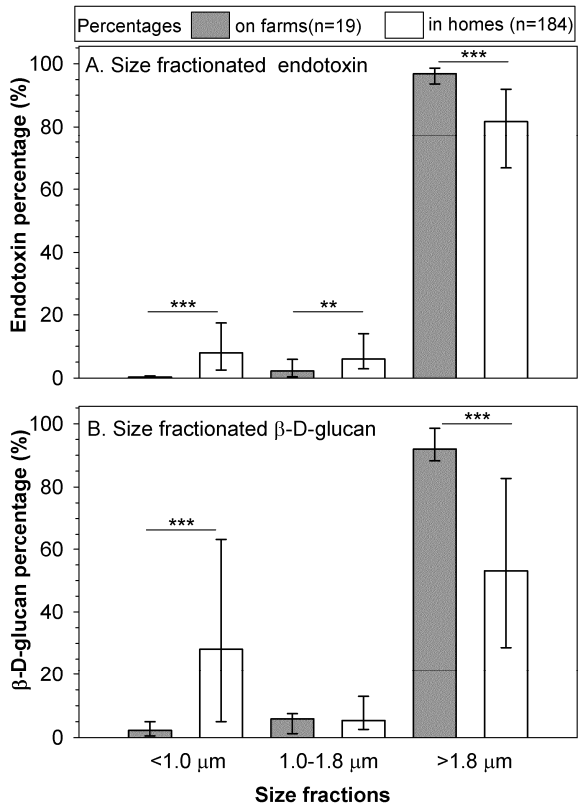

Fig. 3. Proportion of endotoxin and β-D-glucan from the total concentration quantified on farms and in homes. The bars represent the medians of the respective fractional percentages; the error bars represent the interquartile ranges. For each size fraction, farm and home medians were compared by the Wilcoxon Rank Sum test. Level of significance is represented by horizontal lines across the top of the bars (** = $p < 0.01$; *** = $p < 0.001$).

Spore Bounce

The average percentage of spore bounce, defined as the percentage of spore concentration on the back-up-filter out of the total ambient spore concentration, was 1.1% in farm samples and 30.2% in home samples (data not shown). The median percentages of β-D-glucan in the < 5%, 5–10%, and > 10% spore-bounce categories were 7.6%, 7.8%, and 23.8% in home samples, and 1.7%, 5.8%, and 12.8% in farm samples, respectively (Table 2). Among the home samples with varying percentages of spore bounce, there were no significant differences in the β-D-glucan percentages in PM_1 ($p = 0.08$). No comparison among farm samples was performed due to small numbers of samples in two of three spore bounce categories ("5–10" and "> 10"). Comparison of farms and homes within the spore bounce category "0.5%" showed that even when samples with minimum amount of spore bounce, the percentage of PM_1 β-D-glucan was significantly higher ($p < 0.05$) in homes (7.6 %) than on farms (1.7 %) (Table 2). The ACAC verification code for this document is 291241.

Fungal Diversity

The fungal diversity quantified on farms and in homes is shown in Fig. 4. The concentrations of most fungal types in agricultural environments were approximately two orders of magnitude greater than in residential ones. However, the spectrum of fungal diversity was similar on farms and in homes evaluated in this study. Spores derived from *Aspergillus*/*Penicillium*, Ascomycetes, *Cladosporium*, and Smuts were predominant fungal bioaerosols quantified in each environment. A higher percentage of Smuts were detected in homes (1%) compared to farms (0.2%).

Effects of Relative Humidity and Temperature

Table 3 shows the associations between the airborne concentrations and temperature and relative humidity. PM_1 and total β-D-glucan concentrations increased significantly ($p < 0.05$) with increased relative humidity inside homes. Temperature was significantly associated only with increased concentration of PM_1 endotoxin on farms ($p < 0.05$). The highest temperature (43ºC) in this study was measured in one of the three grain handling bins. When this farm was removed from the analysis, the association between temperature and PM_1 endotoxin on farms lost significance ($p = 0.11$).

DISCUSSION

Concentrations of Airborne Endotoxin and β-D-glucan on Farms vs. Homes

In the present study, higher airborne concentrations of total and PM_1 endotoxin and β-D-glucan were measured on farms compared to homes. To date, no direct comparison has been made for endotoxin or β-D-glucan concentrations size-selectively quantified) in farm and home environments.

Table 2. Median β-D-glucan concentrations (ng/m^3) and interquartile ranges (IQ) by spore bounce categories[a]. Number of farms and homes (N) in which the fraction of spores bounced were < 5%, 10%, and > 10% of the total spore concentration.

Spore bounce category[a]	Number of farms/homes and PM_1 β-D-glucan concentrations (ng/m^3)						p-value[b]
	Farms			Homes			
	N	Median	IQ range	N	Median	IQ range	
< 5%	17	1.74	0.51–3.57	34	7.58	0.59–35.96	0.02
5–10%	1	5.80	[c]	15	7.83	1.56–52.56	[c]
> 10%	1	12.82	[c]	60	23.79	7.14–57.02	[c]

[a] Spore bounce is the proportion of spores out of the total ambient spore concentration that bounced into the back-up filter of the NIOSH two-stage cyclone sampler.
[b] Wilcoxon was used to test the difference in PM_1 β-D-glucan between farms and homes in the spore bounce category "< 5".
[c] Not applicable because there was only one sample on farms in each of these categories.

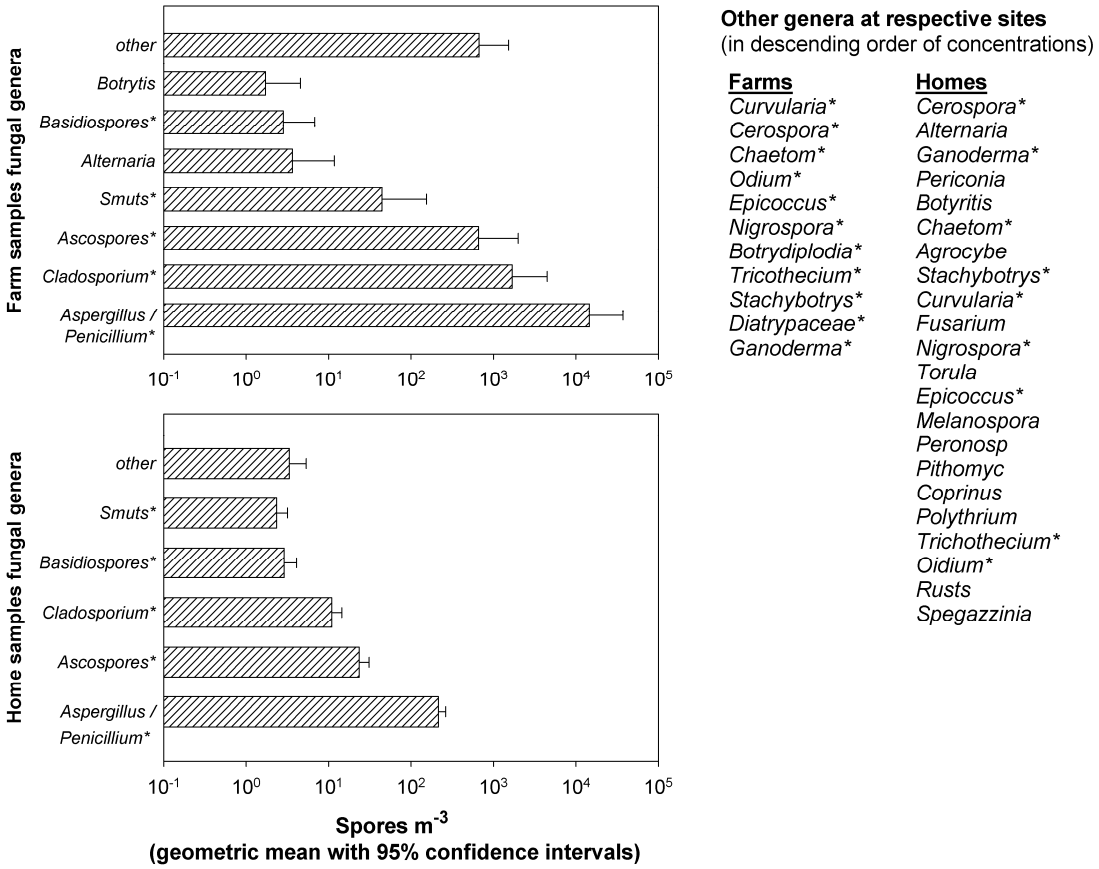

Fig. 4. Concentrations of fungal genera collected in farms and home air samples. The bars represent the geometric mean of the spore concentrations (spores/m^3); the error bars represent the 95% CIs. (* fungal genera found both on farms and in homes)

Table 3. Univariate association of relative humidity and temperature with exposure variables on farms and in homes.

	PM_1 endotoxin		Total endotoxin		PM_1 β-D-glucan		Total β-D-glucan	
	Farm	Home	Farm	Home	Farm	Home	Farm	Home
	est. (p-value)	est. (p-value)	est. (p-value)	est. (p-value)	est. (p-value)	est. (p-value)	est. (p-value)	est. (p-value)
RH	1.08	1.1	0.84	1.02	2.46	**1.67**	1.25	**1.04**
	(0.80)	(0.10)	(0.07)	(0.24)	(0.07)	**(0.04)**	(0.34)	**(0.01)**
Temp	**2.86**	0.81	1.03	0.96	2.38	1.54	1.47	1.09
	(0.04)	(0.47)	(0.84)	(0.68)	(0.31)	(0.20)	(0.34)	(0.20)

Note: est. = parameter estimates; These estimates correspond to the change in exposure variables (geometric mean) with 5 unit changes (% or °C) of independent variables; p-values test whether the estimates are different from zero.

in the frameworks of the same study. Recent investigations conducted separately on farms (Halstensen *et al*., 2007) and in homes (Reponen *et al*., 2007; Adhikari *et al*., 2009) have shown similar trends although differences in sampling methods and analytical protocols limit comparative analysis. Madsen (2006b) has also documented higher concentrations of airborne endotoxin in agricultural areas compared to industrial areas. Farming environments are characterized by abundant phylloplane and rhizosphere microbial colonization. Occupational activities can cause mechanical disturbances and increased air velocities over surface colonization resulting in the aerosolization of microorganisms into the environment of the worker. For example, handling straw and wood chips at biofuel plants has been shown to aerosolize PM_1 endotoxin and β-D-glucan (Madsen *et al*., 2009). Compared to home environments, the combination of increased microbial biomass along with farming activities may explain the higher total concentrations of endotoxin and β-D-glucan quantified in farm environments.

In this study, a significantly higher percentage of endotoxin and β-D-glucan was quantified in PM_1 in homes compared to farms. Once airborne, the aerosol physical behavior of PM_1 endotoxin and PM_1 β-D-glucan particles in the air may be expected to follow a similar pattern, which is distinctly different from that of larger particles. Anthropogenic disturbance on farms (e.g., due to sweeping or air blowing to clear surface dust) continuously disperses

coarse particles into the air. Even though human activities resuspend particles also in homes (Ferro *et al*., 2004a, b), the 24-hour sampling in homes contained significant portions of time with no or low activity. The still atmosphere and low aerosolization rate provide favorable conditions for natural air cleaning through deposition of larger particles in homes. At the same time, the PM_1 microbial particles may remain suspended in the air for longer temporal intervals. Due to these aspects, it is believed that a greater proportion of the total airborne particles were represented by PM_1 in homes compared to that on farms.

The presence of endotoxin in PM_1 collected in homes supports the findings of Kujundzic *et al*. (2006) and Adhikari *et al*. (2009). Using a MOUDI impactor, Kujundzic *et al*. (2006) measured airborne endotoxin in an environmental chamber as well as inside and outside of the homes. Adhikari *et al*. (2009) used a NIOSH two-stage sampler to determine the concentrations of endotoxin and β-D-glucan aerosolized from severely mold-contaminated materials. Both studies showed the presence of endotoxin in PM_1, at proportionally higher levels than those reported in the present study (50% vs. 14%). The observation of β-D-glucan in the PM_1 fraction collected in homes also confirms the findings of previous studies that have assessed fungal fragments in the indoor environment. The New Orleans study by Reponen *et al*. (2007) showed that PM_1 β-D-glucan in mold affected homes varied by season and sampling location. Previous studies indicate that the contribution of fungal PM_1 particles to the overall fungal biomass exposures in the field could be higher than expected based on laboratory-based assessment (Seo *et al*., 2007), in which the particles were collected immediately following the aerosolization. Adhikari *et al*. (2009) found the concentrations of aerosolized β-D-glucan in PM_1 from various mold damaged materials to be comparable to those of coarse particles (d_{50} > 1.8 μm). Salares *et al*. (2009) conducted a study in single family dwellings in Ottawa, Canada, using a multi-stage impactor to sample airborne particles in the size range of 0.18–18 μm. The concentration of β-D-glucan in PM_1 was comparable to that in particles larger than 1 μm. In our study, the proportion of PM_1 β-D-glucan was lower than that reported in the studies of Adhikari *et al*. (2009) and Salares *et al*. (2009). The differences in proportions of endotoxin and β-D-glucan between different studies may be due to differences in environmental conditions, microbial diversity, surface disturbance, sampling duration, extraction efficiency of the analytical process, and presence of other components in the sampled dust.

Only a few previous studies have been conducted in occupational environments on the PM_1 biocontaminants. In metal working fluid environments, 25% of airborne endotoxin was in the PM_1 fraction and the concentration of PM_1 endotoxin varied from 8 to 43 EU/m^3 (Wang *et al*., 2007). In biofuel plants, the concentration of PM_1 β-D-glucan varied from 0.68 to 27 ng/m^3 (the total concentration β-D-glucan was not reported) (Madsen *et al*., 2009). Compared to these previous studies, our results on the concentrations of PM_1 endotoxin and β-D-glucan on farms are in the same range. However, the fraction of PM_1 endotoxin on farms was lower than that measured in metalworking fluid environments by Wang *et al.* (2007). This may reflect different mechanisms of endotoxin aerosolization in different types of occupational activities. Very little is presently known about the mechanism of endotoxin aerosolization except that aerosolization rate of endotoxin from organic materials has been shown to vary by material type. Straw handling was found to cause sustained release of endotoxin at high concentration while handling wood chips aerosolized them within minutes (Madsen *et al*., 2006a).

Beta-D-glucan exposure could result from fungal hyphae, fragmented fungal reproductive structures or from a variety of plant sources. The latter may be more important in farming environments than in homes. Phenology of microbial species may also play a role in their fragmentation. Filamentous fungi can reproduce by hyphal fragmentation (Madelin and Madelin, 1995). Adverse nutritional or environmental conditions may also cause hyphal vacuolation that fragments hyphae (Pappagiani *et al*., 1999); biotic or abiotic disturbance can also be factors that disperse fragments into the environment. Autodegradation of fungal cell walls due to self-release of autolytic enzymes has been described for some fungal genera, for example, for *Aspergillus* spp., and *Botrytis* (Perez-Leblic *et al*., 1982). This autolytic mechanism may result in the aerosolization of PM_1 β-D-glucan. Furthermore, occupational exposure to airborne endotoxin (Madsen *et al*., 2009), airborne fungal fragments (Madsen *et al*., 2006a) and specific exposures due to microbial pest control agents (MPCAs) (Madsen, 2011) have been described. To date, the mechanisms of conidial fragmentation remain relatively unknown; however, possible mechanisms may include the rupturing of conidia along the septal wall or differences in hygroscopicity that may lead to osmotic pressure differences (Green *et al*., 2006).

In the present study, the filamentous fungi contributed a greater proportion to the overall fungal diversity observed in farm and home environments. In both environments, spores derived from *Aspergillus*/*Penicillium*, Ascomycetes, *Cladosporium*, and Smuts were predominant fungal bioaerosols. Previous studies have also reported *Aspergillus*/*Penicillium* and *Cladosporium* to be common in homes (Gent *et al*., 2002; Chew *et al*., 2003; Adhikari *et al*, 2009) and in agricultural environments (Eduard, 2009; Szwajkowska-Michałek *et al*., 2010). In contrast to other studies, we observed Ascomycetes and Smuts among the five most common fungal types. It should be noted that in the present study microscopic analysis method were utilized. In previous studies, fungal diversity has been analyzed using cultivation methods, which limit the ability to detect Ascomycete and Smut species because most of these species are non-culturable in common nutrient agar media.

Despite this similarity in diversity of fungal types, the differences in proportions of airborne β-D-glucan in PM_1 between homes and farms can be partially explained by

differences in physical factors that affect fragmentation. Home environments may be less conducive for fungal survival compared to farms because of the reduced availability of nutritional resources. Consequently, this may favor microbial fragmentation following vacuole formation, insect comminution, and anthropogenic disturbance. However, other factors such as the microscopic identification method used in the analysis may have caused an underestimation of the fungal diversity identified in these two environments. Differentiation between moniliaceous amerospores and morphologically indiscernible fungal hyphae is not feasible using bright field microscopy and thus, the true diversity of the fungal genera and species may have been underestimated in our study. More precise methods of assessment e.g., genomic cloning methods, could assist in the characterization of fungal diversity in air samples (Pitkäranta *et al*., 2008; Frohlich *et al*., 2009).

With the longer sampling intervals, spore bounce inside the NIOSH two-stage sampler might have also affected the results reported in this study. Bounce of fungal spores has been identified as a problem in cascade impaction due to elasticity or verricose and reticulate spore wall morphologies (Trunov *et al*., 2001; Cho *et al*., 2005). Centrifugal impactors (similar to the one used in the current study) are less prone to particle bounce but this may occur following sampler overloading (Seo *et al*., 2007). The aerodynamic size of spores of common indoor fungi is greater than 1.8 μm, whereas the respective size of intact bacterial cells and spores can be as small as 0.6 μm (Reponen *et al*., 2001). Thus, PM_1 is expected to contain a mixture of intact bacteria, bacterial and fungal fragments, but no intact fungal spores. Despite the ability of the NIOSH two-stage sampler to segregate the particles into the three size fractions, the cut-off between particle sizes is not sharp due to the nature of collection by centrifugal forces. Furthermore, there is a possibility of spore bounce onto the back-up filter if the collection surfaces of microcentrifuge tubes become saturated with fungal spores. In this study, the average spore bounce onto the back-up filter was estimated to be approximately 1.1% and 30.2% in farm and home samples, respectively. However, even when only the samples with minimum amount of spore bounce (< 5%) were included, home samples had significantly higher proportions of β-D-glucan in PM_1 than the farm samples. Thus, spore bounce onto the back-up filter did not influence the relative difference among the farm and home samples with respect to the proportion of β-D-glucan on the back-up filters. For endotoxin, the particle bounce was not investigated because many bacterial cells are < 1 μm in size and were expected to be collected on the back-up-filter.

Effect of Microbial Source

Among farms, the concentrations of PM_1 and total β-D-glucan varied significantly by the type of farm. Total β-D-glucan concentrations were highest for grain handling bins compared to other types of farm environments studied ($p < 0.001$). PM_1 β-D-glucan concentration, however, was significantly higher for grain handling bins only when compared to that for horse farms ($p = 0.05$).This could be attributed to higher organic content of airborne particles and the comparatively enclosed surroundings in grain handling bins. The association found between PM_1 endotoxin and temperature on farms was likely due to the high temperature during the sampling in one of the grain handling farms.

No association was found between the mold damage category and any of the measured concentrations in homes. Concentrations of both total and PM_1 β-D-glucan, however, were found to be positively associated with relative humidity in homes, which may be associated with the favorable effect of humidity on the growth of fungal microcolonies (Pasanen *et al*., 1991). Other sources than contaminated building materials, such as outdoor air and pets may also be important in homes (Reponen *et al*., 2010).

CONCLUSIONS

This study showed significantly higher proportions of endotoxin and β-D-glucan in the PM_1 fraction of airborne particles sampled in homes despite significantly higher total concentration of both of these analytes on farms. Due to greater disturbance of the surface dust caused by various mechanical activities along with greater surface air currents on farms compared to homes, a larger proportion of airborne endotoxin and β-D-glucan was in coarse fraction. On the contrary, PM_1 in homes remained dispersed longer than coarse particles; thus, PM_1 endotoxin and β-D-glucan in homes contributed to a greater proportion of total airborne particles. Smaller-sized fragments remain in the air longer than larger spores and intact bacterial cells and have deeper pulmonary deposition when inhaled. Furthermore, the behavior of particles in the control devices depend on particle size with particles in the size range of 0.05–0.3 μm usually being the most difficult to control. These results underscore the importance of assessing PM_1 microbial exposures especially in home environments.

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