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Predictors of airborne endotoxin concentrations in inner city homes

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ABSTRACT

Few studies have assessed in home factors which contribute to airborne endotoxin concentrations. In 85 inner city Baltimore homes, we found no significant correlation between settled dust and airborne endotoxin concentrations. Certain household activities and characteristics, including frequency of dusting, air conditioner use and type of flooring, explained 36–42% of the variability of airborne concentrations. Measurements of both airborne and settled dust endotoxin concentrations may be needed to fully characterize domestic exposure in epidemiologic investigations.

1. Introduction

Endotoxin, ubiquitous in the environment, is a biologically active lipopolysaccharide (LPS) that is a component of the outer membrane of Gram negative bacteria. Several epidemiologic investigations have shown a modest effect of settled dust home endotoxin exposure on asthma morbidity (Michel et al., 1996). Measures in settled dust are easy to collect and have shown little variability over time (Park et al., 2001); however, inhalation is the exposure route for most agents impacting the respiratory tract. Therefore, settled dust endotoxin concentration may not be ideal for estimation of inhalation risk. In human experimental exposure studies, inhaled endotoxin has been linked to adverse asthma health effects, and (Boehlecke et al., 2003; Michel et al., 1996) recently, indoor airborne endotoxin concentrations were associated with wheezing in infants (Dales et al., 2006). Horick et al. (2006) showed that airborne endotoxin concentrations, estimated based on settled dust endotoxin concentrations, and household characteristics and activities, were a better predictor of asthma morbidity than settled dust endotoxin concentrations. These studies emphasize the relevance the indoor airborne endotoxin concentrations may have to respiratory health. The purpose of our study was to assess the association between settled dust and airborne endotoxin concentrates in inner city Baltimore homes, and to identify characteristics in the home that may predict elevated airborne endotoxin concentrations.

2. Methods

This study was approved by the Johns Hopkins University Institutional Review Board. Written informed consent was obtained from all participants. We measured airborne and settled dust endotoxin concentrations in 85 homes in inner city Baltimore. We evaluated the homes of subjects who were recruited as part of a cohort study to investigate environmental risk factors for asthma morbidity in children living in the inner city. This subset of homes included all homes of children with asthma recruited between December 2007 and 2009, and housing characteristics were similar to all homes ($n=203$) of subjects recruited as part of the parent study (Table 1). A trained environmental health technician inspected the home using an inspection checklist to document housing characteristics and condition of the house (Hansel et al., 2008; McCormack et al., 2008). Home occupants completed a standardized daily activity diary that detailed common household activities, including smoking, cleaning, and cooking activities that occurred in the home during the 72 h monitoring period.

Air monitors were set up in a bedroom and dust samples were collected from the bedroom floor. Airborne particulate matter (PM_{10}) samples were collected continuously for 72 h and gravimetric analysis was conducted as previously described (Hansel et al., 2008; McCormack et al., 2008). We measured in home temperature and humidity concurrently using a HOBO temperature and humidity data logger (Onset Corporation, Pocasset, MA). House dust samples were collected from the bedroom floor on an unwoven fabric collector inserted into the nozzle of a standard portable vacuum by vacuuming a 2.2 m^2 area near the bed for approximately 3 min. Air and dust samples were extracted for endotoxin analysis in sterile, pyrogen free water containing 0.05% Tween 20 for 1 h at 22 °C with

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Table 1
Home and bedroom characteristics.

	% of homes		p
	Homes included in endotoxin analysis ($n=85$)	Homes not included in endotoxin analysis ($n=118$)	
Type of home			
Row home	46.9	49.2	0.93
Detached	22.2	24.6	
Apartment	13.6	12.7	
Row—end of group	11.1	7.6	
Duplex	6.2	5.9	
Visual cleanliness			
Average	71.6	72.0	0.95
Above average	28.4	28.0	
Type of heating			
Forced air, Standard filter	60.8	62.1	0.99
Radiator	29.1	28.5	
Forced air, Unknown filter	3.8	4.3	
Baseboard/space heater	3.8	2.6	
Forced air, No filter	2.5	2.6	
Presence of pets			
Cat	21.5	22.6	0.86
Dog	16.5	20.9	0.44
Flooring			
Wall to wall carpeting	50.6	51.3	0.93
Linoleum	24.1	23.5	0.93
Other	25.3	25.2	0.99
Pests			
Evidence of mouse droppings	13.8	12.9	0.87
Evidence of cockroaches	8.8	7.8	0.80
Moisture			
Any mildew	6.3	6.9	0.86

continuous shaking. Extracts were centrifuged and supernatants were transferred into pyrogen free cryotubes. Endotoxin levels were analyzed using a kinetic limulus amebocyte lysate assay (Thorne et al., 2009). The concentrations of airborne and settled dust endotoxin were expressed in EU/m^3 and EU/mg, respectively. The detection limit of airborne endotoxin concentrations was 0.024 EU/ml. Samples with concentrations below the detection limit were assigned a value of half of the detection limit.

Descriptive statistics were used to characterize housing characteristics and endotoxin concentrations. Activities from a time activity diary were dichotomized according to whether participants performed the activity 0–3 times per week or 4 or more times per week. Spearman correlation coefficients were examined to compare settled dust and airborne endotoxin concentrations. Kruskal Wallis, the Wilcoxon rank sum test and Cuzick's test for trend across ordered groups were used to test for differences in housing characteristics and daily activities that may predict airborne endotoxin concentrations. Bivariate linear regression models were performed with $\log_{10}$ transformed airborne endotoxin concentrations as the dependent variable. All housing characteristics and activities in the homes that were associated with airborne endotoxin concentrations with a p-value <0.05 were included in a multivariable linear regression model. Season of sampling and temperature and humidity of the home were added as potential confounders of the relationship between air conditioner use and endotoxin concentration. In sensitivity analyses, all variables that contributed at least 1% to the variance in airborne endotoxin concentrations in bivariate analyses were included in a multivariable model, regardless of statistical significance. A p-value of <0.05 was considered statistically significant.

3. Results

Homes ($n=85$) were studied in nine contiguous zip codes within Baltimore City representing the neighborhood of East Baltimore; approximately half were row homes. During the sampling period, the average temperature of the homes was 24.4 °C, while the average relative humidity was 35.6%. Most homes were heated by forced air with a standard filter (61%). House pets were uncommon, with 17% of homes having a dog and 22% of homes having a cat. Nearly half of all bedrooms contained wall to wall carpeting and one quarter had linoleum flooring. Most participants reported the presence of active smoking in the home (53%) during the air monitoring period. Additionally, participants reported frequent (greater than or equal to four times during the monitoring period) cleaning activities: sweeping (54.4%), wet mopping (32.9%), vacuuming (24.0%) and dusting (11.3%).

The mean ($\pm$ standard deviation) settled dust endotoxin concentration was 64.6 ± 113.0 EU/mg [median 31.7 EU/mg, range: 4.8–643.5 EU/mg] and the mean airborne endotoxin concentration was 0.13 ± 0.26 EU/m^3 [median 0.05 EU/m^3, range: 0.001–1.675 EU/m^3]. There was no significant correlation between settled dust and airborne endotoxin concentrations [$r=0.1$, $p=0.55$]. General home characteristics, such as the type of home, general cleanliness and type of heat used, were not significantly associated with airborne endotoxin concentrations. Homes with a dog had higher airborne endotoxin concentrations (0.21 ± 0.33 EU/m^3) compared to homes without a dog (0.12 ± 0.25 EU/m3), but this difference did not reach statistical significance ($p=0.17$). Among characteristics of bedrooms, wall to wall carpeting was associated with higher and linoleum flooring with lower airborne endotoxin concentrations. Of recorded household activities, frequent dusting and air conditioning use were associated with lower levels of airborne endotoxin concentration (Table 2).

Though airborne endotoxin concentrations tended to be higher during the fall and spring seasons (0.19 EU/m^3 spring [$n=14$],

Table 2
Association between housing and bedroom characteristics and home activities with airborne endotoxin concentration.

General home characteristics	Percent change [95% CI]	Variance (%)
Condition of dwelling (above average vs. average)	−12 [−59, 91]	0.1
Type of heating		
Radiator	–	
Forced air with standard filter	−38 [−72, 37]	6.0
Forced air (no or unknown filter)	−67 [−93, 58]	
Baseboard/space heater	−83 [−98, 16]	
Presence of dog	104 [−22, 425]	2.7
Presence of cat	−31 [−71, 64]	0.9
Season of sampling	–	
Winter	–	
Spring	78 [−55, 608]	3.6
Summer	−7 [−75, 255]	
Fall	83 [−99, 512]	
Temperature (°C)	7 [−5, 20]	1.6
Humidity (%)	−2 [−6, 2]	1.6
Bedroom characteristics		
Flooring	–	
Wall to wall carpeting	–	
Linoleum	−66 [−86, −20]*	7.7
Other	−39 [−74, 43]	
Mouse droppings	−11 [−68, 151]	0.06
Evidence of cockroach	−11 [−74, 224]	0.03
Any mildew	15 [−74, 401]	0.04
Home activities (% with frequent activity)[a]		
Dusting (11%)	−81 [−94, −46]*	11
Sweeping (54%)	−15 [−58, 73]	0.3
Vacuuming (24%)	35 [−41, 202]	0.6
Wet mopping (33%)	−48 [−75, 11]	3.6
Air conditioning (33%)	−63 [−82, −23]*	8.7
Air purifier (6%)	−73 [−94, 15]	4.0
Humidifier use (8%)	−15 [−78, 226]	0.08

* $p<0.05$.
[a] Activities from a time activity diary were dichotomized according to whether participants performed the activity 0–3 times per week or 4 or more times per week (frequent).

Table 3
Multivariable model predicting airborne endotoxin concentration.

	Percent change [95% CI]	p
Frequent dusting[a]	−79 [−93, −38]*	0.005
Frequent air conditioning use[a]	−70 [−87, −28]*	0.007
Bedroom flooring	–	–
Wall to wall carpeting (reference)	–	–
Linoleum	−73 [−88, −40]*	0.002
Other	−65 [−86, −15]*	0.02
Season of sampling	–	–
Winter (reference)	–	–
Spring	82 [−59, 694]	0.43
Summer	32 [−74, 557]	0.73
Fall	51 [−60, 475]	0.53
Temperature (°C)	8 [−5, 22]	0.23
Humidity (%)	1.0 [−3, 6]	0.68

* $p<0.05$.
[a] Activities from a time activity diary were dichotomized according to whether participants performed the activity 0–3 times per week or 4 or more times per week (frequent).

0.15 EU/m^3 fall [$n=45$], 0.04 EU/m^3 summer [$n=18$] 0.07 EU/m^3 winter [$n=8$]; $p=0.39$), home temperature ($r=0.15$, $p=0.18$) and humidity ($r=0.11$, $p=0.34$) were not significantly associated with airborne endotoxin concentrations. In multivariable analysis, frequency of dusting, air conditioner use and type of bedroom flooring remained independently associated with airborne endotoxin concentrations. (Table 3) These housing characteristics and home activities explained approximately 36% of the variance of measured airborne endotoxin concentrations. Additionally, when home characteristics and activities which contributed at least 1% to the variance of airborne endotoxin concentrations in bivariate analyses were included in multivariable analyses, there was only marginal improvement in the predictive ability in airborne endotoxin concentrations. The housing characteristics and home activities still only explained 42% of the variance in measured airborne endotoxin concentrations.

4. Discussion

Our results show that there is little correlation between settled dust and airborne endotoxin concentrations measured over a 72 h period in inner city Baltimore homes. These results underscore the importance of measuring both airborne endotoxin and settled dust endotoxin levels to assess the potential adverse health effects of endotoxin exposure on respiratory disease. This is particularly important when determining risk of acute or varying exposure levels. In addition, we found that type of bedroom flooring, and frequent dusting and air conditioner use were significant predictors of airborne endotoxin concentrations. They could represent future targets for remediation strategies, if concentration of airborne endotoxin is convincingly shown to adversely affect human health.

Several studies have highlighted the importance of inhalable organic material in respiratory disease. Occupational studies have linked indoor concentrations of airborne endotoxin to adverse respiratory health effects in adults, including increased bronchial hyperresponsiveness, lung function decline, and obstructive airway disease (Eduard et al., 2009). Similarly, airborne fungi have been associated with increased asthma morbidity (Bundy et al., 2009). In a recent study, airborne mouse allergen concentrations were associated with increased healthcare utilization for asthma and were more strongly associated with increased airway inflammation than were mouse allergen measurements taken from settled dust on the bed or floor (Torjusen et al., 2010). These studies demonstrate the potential importance of assessing airborne concentrations of endotoxin, fungi and allergens to comprehensively determine adverse effects of exposure on respiratory health.

Most epidemiological studies of endotoxin and risk of respiratory disease, to date, have used endotoxin measured in settled dust as the indicator of exposure. However, endotoxin measured in settled dust is an uncertain surrogate for inhalation exposure. Settled dust concentrations may be good surrogates for long term exposure but poor surrogates for short term inhalation risk. Conversely, a short term air sample, in which endotoxin concentrations may show higher variability over time compared to settled dust samples (Dassonville et al., 2008; Park et al., 2000) may not be an appropriate measure to estimate longer term inhalation exposure and chronic disease risk. As a result, the use of settled dust or airborne measures alone might produce non-differential exposure misclassification, which may bias results towards the null hypothesis in epidemiological studies. Given that our findings showed settled dust and airborne endotoxin concentrations to be poorly correlated, it may be necessary to measure both airborne and settled dust endotoxin concentrations to accurately estimate the adverse effect of endotoxin exposure on airway and respiratory health.

Compared to endotoxin in settled dust, there is paucity of data on airborne endotoxin in homes. The concentrations of airborne endotoxin in our study were slightly lower compared to other studies in residential areas (Dales et al., 2006; Dassonville et al., 2008; Park et al., 2000, 2001). The cause of the variation in concentrations between studies may be attributable to differing residential environments, including differing climates, home characteristics, and activities, as well as to differences in sampling method, and extraction, collection, and storage of endotoxin samples across studies. Our study found a lack of correlation between settled dust endotoxin concentrations and airborne endotoxin concentrations; however, we found an association between several household characteristics and activities and airborne endotoxin concentration. To our knowledge, previous studies have not assessed predictors of in home airborne endotoxin concentrations in an exclusively low income urban environment. In addition to the association between settled dust and airborne endotoxin concentrations not reaching statistical significance, the very low correlation ($r=0.1$) between dust and airborne concentrations support the findings from previous studies that show that settled dust concentrations explain little, if any, of the variation in airborne endotoxin concentrations (Horick et al., 2006; Park et al., 2001; Sohy et al., 2005). In our study, frequent use of an air conditioner (room or central) during the air monitoring period was associated with lower airborne endotoxin concentrations. This finding was independent of season of sampling, temperature, or humidity. Filters that are commonplace in air conditioners may lower airborne particulate matter in general, resulting in lower airborne endotoxin concentrations in the home. Characteristics of the bedroom floor also predicted airborne endotoxin concentrations: in particular, wall to wall carpeting was associated with higher endotoxin concentrations, while linoleum flooring was associated with lower concentrations. Carpeting may provide a favorable microenvironment for the growth of Gram negative bacteria, and, subsequently, endotoxin production. Additionally, the presence of wall to wall carpeting may lead to higher dust loading, and, though not statistically significant, its presence was associated with higher settled dust endotoxin concentrations (data not shown). Among household activities, frequent dusting was associated with lower airborne endotoxin concentrations. Similarly, frequent mopping tended to be associated with lower concentrations of airborne endotoxin. These results suggest that frequent cleaning activity reduces endotoxin, and a potential intervention to lower indoor airborne endotoxin concentrations should be directed toward cleaning behavior.

The presence of dogs in the home was found to be associated with higher airborne endotoxin concentrations in previous studies (Park et al., 2001; Platts Mills et al., 2005). We found a tendency for homes with a dog to have higher airborne concentrations; however, this association did not reach statistical significance. The small number of homes with a dog ($n=13$) or cat ($n=18$) likely limited our ability to identify a significant difference in endotoxin concentrations between homes with and without pets. Our study also failed to reveal a significant relationship between self-reported number of cigarettes smoked in the home and airborne endotoxin concentrations; however, objective measures such as airborne nicotine may be more reliable indicators of smoking behavior but were not available in this study. An additional limitation to our study was that endotoxin load in settled dust could not be assessed accurately due to the dust sampling protocol which instructed the environmental technician to increase vacuuming time if adequate dust was not collected during the 3 min vacuuming period.

In summary, our study found a lack of correlation between settled dust endotoxin concentrations and airborne endotoxin concentrations; we also found several household characteristics and activities to be associated with airborne endotoxin concentration. The results of this study point to the need for a more comprehensive assessment of endotoxin exposure that includes both settled and airborne measures in epidemiological studies. Cleaning practices, type of flooring, and air conditioner use accounted for some of the variability in airborne endotoxin concentrations in bedrooms; however, a large part of the variability of in home airborne endotoxin concentrations remains unexplained. Given the lack of ability to predict airborne endotoxin concentrations reliably and the likely importance of inhalation to respiratory disease, settled dust measures and airborne endotoxin concentrations should both be considered for routine measurement in future epidemiological studies investigating the respiratory health effects of indoor endotoxin exposure.

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