

Dust sampling methods for endotoxin – an essential, but underestimated issue

Abstract Exposure to farming environment in early life has been associated with lower risk for allergic diseases possibly caused by increased exposure to endotoxin. The aims of this study were to compare the reproducibility of different sampling methods for endotoxin, and to determine whether environmental characteristics have different effect on endotoxin levels of different sample types. The reproducibility of sampling methods (bed dust, floor dust, vacuum cleaner dust bag dust, settled dust and air samples) was studied with repeated sampling (five visits during 1 year) in five farming and five urban homes. To examine determinants of endotoxin for different types of dust sample, sampling was conducted once in 12 farming and 17 urban homes. Endotoxin was analyzed using Limulus Amebocyte Lysate assay. Bed dust samples had the best reproducibility (intraclass correlation, ICC = 66%), but the difference between farming and non-farming homes was not clear with this sample type. The reproducibility of floor (ICC = 52%) and settled dust (ICC = 51%) was moderate. With these sample types the difference between farming and non-farming homes was clear. Settled dust had some seasonal variation. Based on this study, the best compromise for sampling for endotoxin appears to be floor dust sample followed by bed and settled dust samples.

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Practical Implications

Endotoxins have been widely measured, even though the validity of different sample types to reflect the endotoxin exposure level of an indoor environment is poorly known. This study shows that bed dust samples have the best reproducibility, but they do not reflect the differences in exposure due to environmental factors such as farming. Floor dust samples with moderate reproducibility may be the best choice for sampling of endotoxin in large field studies.

Introduction

The prevalence of allergic diseases has increased in developed countries, but their risk factors are still largely unknown. Farmers' children have been observed to have lower risk of allergic diseases (Braun-Fahrlander et al., 1999, 2002; Pekkanen et al., 2001), which has been suggested to be due to the challenge to their immune system caused by high exposure to microbial products, especially endotoxin (cell wall component of gram-negative bacteria). This association between endotoxin exposure in the home and reduced risk of atopy has also been shown in some other studies (Gehring et al., 2001, 2002; Gereda et al., 2000), but the results are partly conflicting (Bolte et al., 2003). In urban home environments, high endotoxin levels have been associated with several housing factors including presence of pets, pests, poor housekeeping,

absence of central air conditioning, old floor coverings, steam cleaning and shampooing the carpet, higher occupancy and even season (Bischof et al., 2002; Gehring et al., 2004; Gereda et al., 2001; Heinrich et al., 2001, 2003; Park et al., 2001; Su et al., 2001; Wickens et al., 2003).

There are only a few studies comparing different house dust sampling methods (bed, floor, settled dust). First, the procedure should be reproducible, i.e. repeated samples should be highly correlated (high intraclass correlation, ICC) or in other words, variability between homes should be high compared with within-home variation. It should preferably also distinguish exposure in different environments, i.e. farming and non-farming environment in this case. The method should also be practical and of low cost. According to Park et al. (2000), bed dust endotoxin levels varied more between homes than within homes

(ICC = 0.74–0.76), while the finding was the opposite (ICC = 0.20–0.40) for floor and airborne dust levels. This study suggests that determination of endotoxin from bed dust distinguishes better different home environments. However, endotoxin results of repeated sampling of floor dust correlated moderately ($r = 0.59$) in the study by Heinrich et al. (2003). Concentrations of extracellular polysaccharides from *Aspergillus* and *Penicillium* in floor dust have been noticed to vary less within homes than between homes, while the variations in the repeated measurements of $\beta(1,3)$ -glucans and culturable fungi were similar within and between homes (Chew et al., 2001). According to Verhoeff et al. (1994), viable fungi in mattresses and especially in floor dust vary substantially within homes. To control this variation, Verhoeff and Burge (1997) concluded that dust samples for viable fungi should be collected in duplicate and should be repeated over time within one season and in different seasons. Based on these few and somewhat contradictory results, it is evident that more research is needed in order to find the most reproducible dust sampling method for analyses of microbial components.

The aims of this study were to compare the reproducibility of different methods for sampling of endotoxin, and to determine whether environmental factors affect endotoxin levels differently in different sample types.

Materials and methods

Study sites and sampling strategy

The study material consisted of two populations and sampling procedures (Table 1). All the homes were located in eastern Finland. Endotoxin levels were determined from farming and urban homes using five different sample types: bed dust, floor dust, vacuum cleaner dust bag dust, settled dust and a filter sample collected from the air.

Study on reproducibility of sampling methods and seasonal variation was conducted in five farming homes and five urban homes. In these homes, five

sampling visits were made during a 1-year period. At each visit, bed dust and floor dust samples were collected. The collection of settled dust and vacuum cleaner dust were initiated at the first visit. Thus, the first settled dust and vacuum cleaner samples were obtained at the second visit and the total number of samples was four per year. The sampling visits in each home were approximately 3 months apart. In addition, filter samples from the air were taken during the five visits in the urban homes. The sampling in urban homes of the reproducibility study was made in the homes of research personnel who also collected the samples.

The study material on determinants of endotoxin levels consisted of 12 farming homes (of which five were included in the reproducibility study) and 17 urban homes. This study population was a part of the study where immunological function and microbial exposure of newborn infants were investigated (Keski-Nisula et al., 2003). In the seven farming homes and 17 non-farming homes the sampling for the five house dust sample types was made once. From the five farming homes also included in the study on reproducibility, only the first samples were incorporated to these analysis. All the 29 homes with newborn children were visited by a field worker.

Sampling procedure

Bed dust was sampled from the bed of the mother by vacuuming the whole mattress area for 2 min. Prior to sampling, duvets, blankets and sheets were removed but undersheets or mattress covers were left on the bed. The floor dust was sampled from the rug of the mother's bedroom by vacuuming 1 m² for 2 min. The vacuuming of the bed and floor dust was done with a vacuum cleaner (AEG Vampyr 5030, AEG, Rothenburg, Germany) with a nylon sample sock firmly attached.

Dust bag dust from the vacuum cleaner or from the container of a central vacuum cleaner was obtained from each individual home. In the case of an ordinary vacuum cleaner, the dust sample was collected to a new clean dust bag, while in the case of a central vacuum cleaner, the container was emptied and washed before the sampling was started. Dust bag samples were collected for known periods of time between 2 and 4 months (range 72–127 days).

Samples of settled dust were taken with an open cardboard box (22 × 31 cm). A paper was taped on the bottom of a box, and the box was kept in an undisturbed place on a shelf or on a table in the bedroom of the mother for 2–4 months (range 75–128 days).

Air samples were taken with a glass fiber filter (SKC Inc., Eighty Four, PA, USA) kept in an IOM-cassette (SKC Inc.) attached into a modified pump (AFC 400S; BGI Inc., Waltham, MA, USA). The samples were

Table 1 Numbers of samples obtained from the two substudies

Study/study sites	Number of samples				
	Bed dust	Floor dust	Settled dust	Dust bag dust	Air
Reproducibility and seasonal variation					
Five farming homes	25	25	20 ^a	19 ^a	
Five urban homes	24	24	20 ^a	19 ^a	23
Determinants					
12 ^b farming homes	12	12	11	12	
17 urban homes	17	14	15	15	

^aSample collection was initiated at first visit and thus the first sample could be obtained at the second visit.

^bIncludes the five farming homes above.

taken in bedrooms at the height of 1.5 m and the sampling time of 18–24 h with airflow of 2 l/min was used.

Housing characteristics and lifestyle factors regarding, for example, pets and cleaning habits were asked with a questionnaire. The cleanliness of the home was estimated by the field worker with three levels of category (poor, moderate and good).

Treatment of dust samples before analyses

Dust from beds, floor and dust bags were homogenized by sieving through a sterile strainer (pore size about 1×1 mm) and dried in a desiccator for at least 24 h before weighing. In the case of dust bag dust, a part of the content of the bag was used. The settled dust on the bottom of the collection box was collected for the analyses by vacuuming the dust onto a filter (SKC, 25-mm glass fiber). In both air sampling methods, filters were equilibrated at constant temperature and relative humidity and weighed before and after sampling. Dust samples were stored at 6°C until analysis of endotoxin within 2 weeks.

Analyses of endotoxin

The dust and air samples were extracted with 5–10 ml of non-pyrogenic water. All extracts were centrifuged at 1000 *g* after shaking (1 h) and the supernatants of samples were analyzed in duplicate at multiple dilutions (1:10 to 1:10000) for the presence of endotoxin. Biologically active endotoxin was assayed with a validated kinetic chromogenic *Limulus* Amebocyte Lysate assay (Kinetic QCL; BioWhittaker, Walkersville, MD, USA, LAL lot no. 0L259G). All standard curves were made by reconstituting the endotoxin standard *Escherichia coli* (BioWhittaker, lot no. 0L2580) with non-pyrogenic water. Spiked and diluted samples (1:1000) were analyzed to determine whether inhibition or enhancement was affecting the endotoxin assay. Endotoxin analysis followed The European Standard EN 14031 (2003). Concentrations of samples were calculated by comparing the calibration curve to $V_{\max}$ values of the samples in a certain range with highest accuracy. The results were expressed as Endotoxin Units (EU) per milligram of dust or per cubic meter of air.

Statistical analysis

Endotoxin concentrations were logarithmically transformed for statistical analysis. Values under detection limits ($n = 4$ in reproducibility study and $n = 1$ in study of determinants of endotoxin) were excluded from the analysis. In addition, an extreme value of 610 EU/mg in one dust bag dust was considered an outlier and excluded.

In the reproducibility study, within- and between-home variability in the endotoxin levels was estimated using random effects analysis of variance. To evaluate seasonal variation, a linear mixed effects model with a random home effect and a fixed season effect was applied (spring–summer vs. autumn–winter) (SAS/PROC MIXED; SAS Institute, Cary, NC, USA). Correlations between different sampling methods are presented as Pearson correlation coefficients. Correlations were also calculated taking into account the dependence of observations (Hamlett et al., 2003) (PROC MIXED). These correlations were similar to those presented. SAS statistical package (version 8.2) was used for these analyses.

The differences in distribution of environmental characteristics [floor covering, last time vacuumed (days), number of persons sleeping in the room, last time changed sheets (days), age of mattress (years), level of cleanliness, cat or dog] between farming and urban homes were tested with Fischer's exact test. The effects of environmental characteristics on endotoxin concentrations were analyzed with *t*-test, analysis of variance and linear regression. In the analysis of level of cleanliness, the categories 'poor' and 'moderate' were combined due to the small number in the category 'poor'. The variables, frequency of vacuuming, changing sheets and age of mattress, were each recoded into two groups according to their median values. Analyses were performed with SPSS statistical package version 10.1 (SPSS Inc., Chicago, IL, USA).

Results

Reproducibility of different sampling methods and seasonal variation

The endotoxin concentrations of the reproducibility study are presented in Table 2. The between-home variance in endotoxin levels was greater than the within-home variance for bed dust when all homes were grouped together, and especially for the farming homes, thus producing the highest ICC coefficient values, 66% and 73%, respectively (Table 3). ICC was also reasonably high for the floor dust among the

Table 2 Geometric mean values (GM), geometric standard deviations (GSD) and ranges of endotoxin concentrations (EU/mg) in four types of dust samples of farming homes and urban homes and in air samples (EU/m³) of urban homes

Sample type	Farming homes ($n = 5$)		Urban homes ($n = 5$)	
	GM (GSD)	Range	GM (GSD)	Range
Bed dust ($n_f = 25$, $n_u = 24$)	7.2 (4.2)	0.5–89.0	2.4 (3.0)	0.1–19.0
Floor dust ($n_f = 25$, $n_u = 24$)	23.7 (3.4)	4.0–270.0	8.7 (2.2)	2.1–48.0
Dust bag dust ($n_f = 19$, $n_u = 19$)	39.1 (1.7)	21.0–109.0	18.4 (2.1)	6–63.0
Settled dust ($n_f = 20$, $n_u = 20$)	10.0 (2.7)	2.0–80.0	7.2 (2.2)	1.0–24.0
Air ($n_f = 0$, $n_u = 23$)	–	–	0.43 (2.6)	0.05–3.99

n_f = number of samples in farming homes; n_u = number of samples in urban homes.

group of 'all homes'. In contrast, the within-home variation was greater than the between-home variation for the dust bag dust and for all the sample types of urban homes.

In the reproducibility study ($n = 10$ homes), the highest correlations between different sampling methods were observed between floor dust and bed dust and between floor dust and dust bag dust (Table 4). All other correlations between bed dust, floor dust, dust bag dust and settled dust were also significant. Correlations between air samples and different dust sampling types were not significant. Correlations calculated from the study on the determinants of endotoxin ($n = 29$ homes) were higher than those of the reproducibility study, except the one between bed and floor dusts (Table 4).

Some seasonal variation was observed in settled dust and dust bag dust samples. In settled dust samples, the autumn–winter levels of endotoxin were lower than the

levels of spring–summer ($P = 0.01$). The endotoxin levels in dust bag dust in autumn–winter were marginally higher than those in spring–summer ($P = 0.07$). With other sample types, no significant seasonal variation was found (data not shown).

Determinants of endotoxin levels

The distribution of endotoxin concentrations in different dust sample types in farming homes and urban homes are presented in Figure 1. The endotoxin concentrations of bed, floor and settled dust varied between 1–95 and 0.1–43 EU/mg in the farming and urban homes, respectively. The corresponding ranges for dust bag dust were 1–104 and 0.2–64 EU/mg. The endotoxin concentrations of floor dust and settled dust were significantly higher in the farming homes than in the urban homes ($P < 0.05$) (Figure 1).

The level of cleanliness was lower and the frequency of changing sheets was higher in the farming homes than in the urban homes (Table 5). Otherwise the distribution of environmental characteristics having a possible effect on endotoxin concentrations did not differ between the farming and the urban homes (Table 5). The level of cleanliness was significantly associated with the concentrations of endotoxin in bed dust and in settled dust ($P < 0.05$): the concentrations were higher in homes with lower cleanliness level (Table 6). In addition, association between lower frequency of vacuuming and higher concentration of endotoxin in floor dust was nearly significant ($P = 0.058$). Other characteristics had no significant association with endotoxin levels. When analyzing the simultaneous effect of different factors, farming persisted as a significant determinant when adjusting for

Table 3 Estimated variance components of log-transformed endotoxin concentrations in different sample types of the reproducibility study

Type of sample (n)	GSD			ICC ^a (%)
	Between-home	Within-home	Within/between	
All homes (10)				
Bed dust (49)	3.86	2.00	0.52	66
Floor dust (49)	2.00	1.92	0.96	52
Dust bag dust (38)	1.22	1.41	1.16	36
Settled dust (40)	1.56	1.53	0.98	51
Farming homes (5)				
Bed dust (25)	5.37	1.87	0.35	73
Floor dust (25)	2.20	2.26	1.02	49
Dust bag dust (19)	1.07	1.22	1.14	26
Settled dust (20)	1.95	1.58	0.81	60
Urban homes (5)				
Bed dust (24)	1.78	2.14	1.20	43
Floor dust (24)	1.14	1.62	1.42	22
Dust bag dust (19)	1.01	1.67	1.66	2
Settled dust (20)	1.33	1.49	1.12	42
Air (23)	1.43	1.90	1.33	36

^aICC = $100 \times [\text{between-home variance}/(\text{between-home variance} + \text{within-home variance})]$.

Table 4 Pearson correlations (r) between different sampling methods

	r (P-value)			
	Floor dust	Dust bag dust	Settled dust	Air ^a
Reproducibility study ($n_{\text{homes}} = 10$)				
Bed dust	0.62 (<0.001)	0.41 (0.01)	0.40 (0.01)	0.05 (0.81)
Floor dust		0.59 (<0.001)	0.43 (0.01)	−0.03 (0.90)
Dust bag dust			0.41 (0.03)	0.14 (0.59)
Settled dust				0.36 (0.15)
Determinant study ($n_{\text{homes}} = 29$)				
Bed dust	0.48 (0.01)	0.61 (<0.001)	0.83 (<0.001)	
Floor dust		0.71 (<0.001)	0.61 (0.002)	
Dust bag dust			0.70 (<0.001)	

^aAir samples were taken only in five urban homes ($n = 5$).

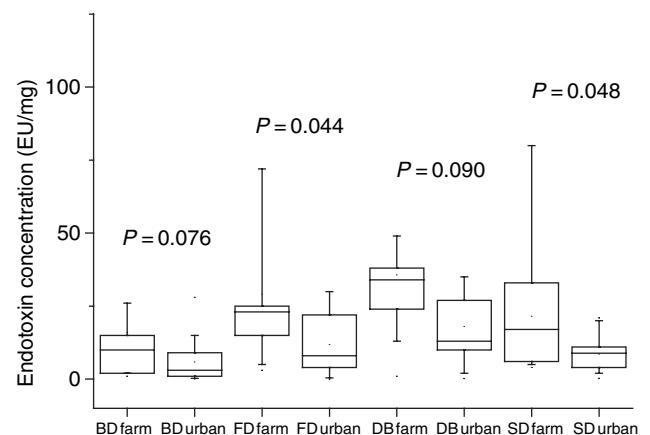


Fig. 1 Endotoxin concentrations in bed dust (BD), floor dust (FD), dust bag dust (DB) and settled dust (SD) in farming ($n = 12$) and urban homes ($n = 17$). The lower and upper boundaries of the box indicate the 25th and 75th percentiles, respectively. The line within the box marks the median, and the whiskers above and below the box indicate the 95th and 5th percentiles, respectively

Table 5 The frequency of housing characteristics of the farming and urban homes

Housing characteristics	Farming homes <i>N</i> (%)	Urban homes <i>N</i> (%)
Floor covering		
Carpeting	1 (8.3)	0 (0)
Hard floor	0 (0)	4 (23.5)
One or more rugs	11 (91.7)	13 (76.5)
Last time vacuumed (days)		
1–4	5 (41.7)	10 (58.8)
>4	7 (58.3)	7 (41.2)
Persons sleeping in the room		
2	1 (8.3)	3 (17.6)
3	9 (75.0)	10 (58.8)
4	2 (11.7)	4 (23.5)
Sheets changed last time (days)*		
0–11	9 (75)	6 (35.3)
>12	3 (25)	11 (64.7)
Age of mattress (years)		
0–5	5 (41.7)	10 (58.8)
>6	7 (58.3)	7 (41.2)
Level of cleanliness*		
Good	4 (33.3)	12 (70.6)
Moderate or poor	8 (66.7)	5 (29.4)
Presence of cat or dog	7 (58.3)	7 (41.2)

**P* < 0.1.

presence of cats or dogs, frequency of vacuuming, changing sheets or age of mattress. Level of cleanliness slightly reduced the effect of farming on endotoxin levels in bed dust, floor dust and settled dust, but not in dust bag dust.

Discussion

Endotoxin levels

The observed median endotoxin levels of bed dust, floor dust and settled dust (4–22 EU/mg) in Finnish farming and non-farming homes were lower than those determined in farming homes in Germany, Switzerland

and Austria (geometric mean for kitchen floor dust: 143 EU/mg; geometric mean for bed dust: 38 EU/mg) (Braun-Fahrlander et al., 2002; von Mutius et al., 2000). The median endotoxin levels of farming homes of this study were even lower or at the same level than those reported from non-farming homes in North America (bed dust: 44 EU/mg, floor dust: 77–105 EU/mg), Germany (floor dust: 28 EU/mg), Estonia (bed and floor dust: 29 EU/mg) or Sweden (bed and floor dust: 14 EU/mg) (Böttcher et al., 2003; Heinrich et al., 2003; Park et al., 2000). The levels observed may be considered typical for Scandinavian climate. However, the absolute levels are difficult to compare, as the methods are not standardized.

Determinants of endotoxin

The concentrations in farming homes were higher than those in non-farming homes, a result similar to the studies of von Mutius et al. (2000) and Braun-Fahrlander et al. (2002). Thus, the clear effect of farming on endotoxin levels was confirmed in this study, especially for floor dust and settled dust. In addition to the farming factor, also the level of cleanliness had a clear effect on endotoxin levels as noted by Bischof et al. (2002). The estimated level of cleanliness was generally lower in farming homes, and adjusting for cleanliness slightly reduced the effect of farming on levels of endotoxin in bed dust, floor dust and settled dust samples. The effect of farming was more prevailing than that of frequency of vacuuming which also appeared to have some influence on floor dust levels in crude analysis. In studies by Bellanti et al. (2000) and Wickens et al. (2003) frequency of vacuuming had no effect on concentrations of endotoxin in floor dust of homes. Several other factors are known to affect endotoxin levels. The presence of pets has been a

Table 6 The geometric mean values (GM) and geometric standard deviations (GSD) of endotoxin concentrations in relation to environmental characteristics

	Bed dust			Floor dust			Settled dust			Dust bag dust		
	GM	GSD	<i>P</i>	GM	GSD	<i>P</i>	GM	GSD	<i>P</i>	GM	GSD	<i>P</i>
Cat or dog												
No (<i>n</i> = 15)	3.2	4.9	0.36	10.6	3.2	0.77	6.5	3.0	0.12	14.0	3.8	0.64
Yes (<i>n</i> = 14)	5.3	3.7		12.2	3.9		12.7	2.7		17.9	3.9	
Vacuumed												
1–4 days ago (<i>n</i> = 15)	4.2	3.7	0.98	7.4	3.7	0.06	8.2	2.2	0.81	15.5	3.7	0.96
≥5 days ago (<i>n</i> = 15)	4.1	4.2		18.6	2.7		9.1	3.9		15.9	4.1	
Sheets changed												
<12 days ago (<i>n</i> = 15)	3.7	3.4	0.65	10.6	3.9	0.75	9.1	2.2	0.79	16.6	3.6	0.82
≥12 days ago (<i>n</i> = 14)	4.7	5.5		12.5	2.9		8.1	4.1		14.7	4.7	
Level of cleanliness												
Good (<i>n</i> = 16)	2.5	4.9	0.04	9.8	3.9	0.52	5.9	3.1	0.02	14.6	3.8	0.73
Moderate/poor (<i>n</i> = 13)	7.6	2.8		13.5	3.0		15.6	2.1		17.5	3.9	
Age of mattress												
<6 years	2.9	2.9	0.12	10.3	4.7	0.70	6.5	3.8	0.16	13.4	4.7	0.53
≥6 years	5.9	3.2		12.5	2.4		12.0	1.8		13.4	4.7	

P = statistical significance.

significant determinant of endotoxin levels in many earlier studies (Bischof et al., 2002; Gehring et al., 2001; Gereda et al., 2001; Park et al., 2001). In the current study, only some, but not significant, effect of pets on endotoxin levels could be seen. The strongest effect was seen in levels of settled dust.

Reproducibility

The between-home variance of endotoxin concentrations was greater than the within-home variance for bed dust in all homes combined and especially in farming homes, and thus reflecting a good reproducibility. This supports the study carried out among faculty, staff and students of a North American university (Park et al., 2000) and suggests that in order to discriminate between individual homes, the house dust samples should be sampled from the bed. However, an important question is how much the bed dust endotoxin reflects factors such as personal hygiene or lifestyle, instead of the effect of the building characteristics or living environment. In two farming homes with high endotoxin concentrations, the levels could be explained by other factors than farming environment: the presence of a cat and the routine changing of the diapers of the child on the bed. Lower level of cleanliness and higher age of mattresses also increased the endotoxin levels in beds. However, settled dust and floor dust samples had a fairly good reproducibility as seen also in floor dust samples by Heinrich et al. (2003). The endotoxin level of floor dust was affected mainly by frequency of vacuuming. Endotoxin level of settled dust was increased by lower level of cleaning and to a certain extent by the presence of a cat or dog. These factors can be readily assessed using a questionnaire. A benefit of a settled dust sample is that it represents the dust that has been airborne better than the dust vacuumed from the floor. However, organizing the sampling of settled dust is more complicated. The box where the dust is sampled can be sent to a home beforehand, but when sampling is finished, the box has to be collected back by a fieldworker.

The within-home variances of endotoxin levels in urban homes were higher than between-home variances for all sample types. This is due to the fact that the differences in endotoxin levels of urban homes are smaller, indicating that more samples of any sample types would be needed.

In addition to environmental determinants, factors related to the sampling or type of the sample cause variation to the concentrations. Dust bag dust represents an integrated sample over a relatively long period of time. It is an inexpensive sample that is technically easy to obtain. It is, however, evident that the content of the dust bag is not totally homogenous. We have noted that a thorough homogenization treatment in

laboratory improves its reproducibility (Hyvärinen, Sebastian, Larsson, Nevalainen, unpublished data). In settled dust and dust vacuumed from floor, there might be some within-room variation. In case of settled dust collection, also the height and the place where the box is situated matters. Due to the complexity of sampling and its critical importance to exposure assessment more validation studies with larger sample size are needed.

Correlations between methods

Endotoxin levels in settled dust samples correlated moderately with the air samples, whereas no correlation was seen between the other three dust types and the air samples. The different dust sample types correlated with each other, which was also shown by Park et al. (2000). Dust bag dust can be considered as a cumulative floor dust sample, whereas bed dust levels can be expected to be affected more by the human himself than by the environment. No or only a weak correlation between airborne and dust-borne endotoxin was also observed by Park et al. (2000, 2001) and by Hines et al. (2003), and this has also been seen in case of viable fungi (Chew et al., 2003; Ren et al., 1999). This may be partly due to the fact that airborne concentrations of microbes and microbial components have wide temporal and spatial variation (Hines et al., 2000; Hyvärinen et al., 2001; Park et al., 2000), and an extensive sampling campaign is needed to really characterize the airborne level of an environment (Hyvärinen et al., 2001). It can be concluded that measuring endotoxin from dust samples appears to be a reproducible and valid method to describe the exposure in the home environment, but for measuring individual exposure personal sampling may be needed (Toivola et al., 2002).

Seasonal variation

Hardly any seasonal variation was found in endotoxin concentrations: only the levels in settled dust samples were significantly lower in autumn–winter than the levels in spring–summer times. This is somewhat unexpected, as wintertime microbial loads are generally low in cold climates. In the study by Park et al. (2000) airborne endotoxin levels were lowest in the winter, while no seasonal influence on endotoxin levels in bed and bedroom floor dust of these North American homes was observed. In Taiwan, endotoxin levels in bed dust were highest in the months of spring and fall (Su et al., 2001), while in Brazil, New Zealand and Germany, highest levels were found in the months of summer (Gehring et al., 2004; Rizzo et al., 1997; Wickens et al., 2003). The effect of season on dust endotoxin levels appears to be still unclear.

Conclusions

Analyzing endotoxin from bed samples had the best reproducibility, but with bed dust samples the difference between farming and non-farming homes was not clear. The reproducibility of floor dust and settled dust was also fairly good and with those sample types the difference between farming and non-farming homes could be observed as well. A sample of floor dust is easier to obtain than that of settled dust, which must be collected for months and which is logistically more difficult. In addition, floor dust samples had no notable seasonal variation and endotoxin concentration of floor dust was affected

only by the frequency of vacuuming, which can be controlled by questionnaire. Thus, based on this study, the best compromise to assess endotoxin levels of homes is floor dust sampling followed by samples of bed dust and settled dust.

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