

Levels of endotoxin in 390 Swedish homes: determinants and the risk for respiratory symptoms in children

Syed Moniruzzaman^{a*}, Linda Hägerhed Engman^b, Peter James^c,
Torben Sigsgaard^d, Peter S. Thorne^e, Jan Sundell^f and Carl-Gustaf Bornehag^a

^aPublic Health Sciences, Karlstad University, Karlstad, Sweden; ^bTechnical Research Institute of Sweden (SP), Borås, Sweden; ^cHarvard School of Public Health, Boston, USA; ^dAarhus University, Aarhus, Denmark; ^eUniversity of Iowa, Iowa City, USA; ^fTsinghua University, Beijing, China

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Endotoxins are microbiological agents which ubiquitously exist in an indoor environment, and are believed to be causal agents for a number of diseases. This study investigated the indoor levels and determinants of endotoxins and their impact on asthma and allergy diseases among Swedish pre-school children. House dust samples from 390 homes of 198 case children with asthma and allergy and 202 healthy control children were collected in the Dampness Building and Health (DBH) study. House dust endotoxin levels in the child's bedroom and living rooms ranged from 479–188,000 EU/g dust and from 138–942,000 EU/g dust, respectively. Pet-keeping and agricultural activities were significantly associated with the higher endotoxin concentration levels in indoor dust. Endotoxins in the indoor environment did not associate to asthma and allergy diseases in the children. However, we found an association between endotoxins and the presence of disease symptoms in the sub-group of families without indoor pets.

Keywords: asthma and allergy; building characteristics; endotoxins; lifestyle factors; pet-keeping

Introduction

Endotoxins are amphiphilic molecules that make up the outer cell wall of gram negative bacteria, and are believed to be causal agents for a number of diseases. In non-occupational environments, the relation between exposure to endotoxins and development of respiratory symptoms is not clear, where both positive (Michel et al. 1996; Park et al. 2001; Thorne et al. 2005; Tavernier et al. 2006) and negative associations have been reported (Martinez and Holt 1999; Gereda et al. 2000; von Mutius et al. 2000). The previous studies have also identified that this relation varied by types of respiratory disease.

Endotoxins exist ubiquitously in indoor environments. Globally, multiple studies have found high levels of endotoxin in indoor dust in the presence of indoor pets (Bischof et al. 2000; Gereda et al. 2001; Giovannangelo et al. 2007; Thorne et al. 2008). Endotoxins can be imported into indoor environments – endotoxin levels are

*Corresponding author. Email: syed.moniruzzaman@kau.se

also reported to be higher in homes of farming families and in households in which children have regular contact with farm animals (von Mutius et al. 2000; Waser et al. 2004). Studies have also indentified indoor tobacco smoking, the number of persons living in the child's home, and air conditioning as determinants for endotoxin concentration levels (Hasday et al. 1999; Gereda et al. 2001; Gehring et al. 2004; Wickens et al. 2003; Mueller-Anneling et al. 2004).

We found very few studies that have focused on indoor endotoxin levels in the non-occupational Swedish settings and its impact on asthma and allergies. The purpose of the current study was to describe the levels and determinants of endotoxin concentrations in indoor dust in 390 Swedish homes and its impact on asthma and allergic diseases as well as IgE sensitization among pre-school children aged 3–8 years.

Materials and methods

Study design and study population

This study is part of a large investigation the “Dampness in Buildings and Health (DBH)” study that focuses on the importance of indoor environments on chronic illnesses among pre-school children (Bornehag et al. 2004). The DBH study was initiated in Värmland County, Sweden during 2001–2002. All families with a child of 1–6 years in Värmland in 2000 were invited to a baseline questionnaire study, the first step of DBH study. Invitations to participate in the study were sent to the parents of 13,782 children, of which we received responses for 10,851 children, corresponding to a response rate of 79%.

One-and-a-half years after the baseline, a follow-up questionnaire was sent to the families of 1,056 potential case children (9.7% of the total population). Target population was the children reporting at least two symptoms of wheezing, rhinitis and eczema in both questionnaires. Also, we randomly identified 1,100 symptom free children (representing 48.9% of the population) from local primary care clinics. This resulted in 198 cases and 202 controls. The recruitment procedure as well as potential selection bias problems are described in Bornehag et al. (2006).

A medical examination of the 400 children (3–8 years of age) was performed by a team of physicians and nurses during the winter (November 2001 to March 2002), simultaneously with home inspections and sample collection.

A case was defined as a child, whose parent reported at least two symptoms of wheezing, rhinitis, or eczema without a cold, during the preceding 12 months on both the baseline and the follow-up questionnaire. Additional outcomes included asthma, rhinitis and eczema. Respective disease status required physician diagnosis in addition to parent-reported persistent symptoms. Diagnostic criteria for current asthma included a combination of (a) three episodes of wheezing prior to the age of two; (b) first wheezing episode after the age of two; (c) the first episode of wheezing in a child who also suffers from other atopic diseases; (d) asthma medication use; and (e) an earlier clinical diagnosis of asthma. Lung function test was not performed. Rhinitis case definition required: (a) ever having allergic rhinitis symptoms; (b) symptoms presentation in the nose and/or eyes following the contact with furred animals or pollen; (c) present use of rhinitis medicine. Eczema case definition required at least six months of remitting itching and redness over typical body locations. All children with asthma also met the definition of the case. Among the controls, two children were diagnosed with rhinitis and eight were diagnosed with

eczema. Thirteen additional cases were found among the controls. In the present analyses, the 23 misclassified children were excluded from further analysis. The definition of the control status required absence of symptoms without a cold within a year in both rounds of questionnaires. Both cases and controls were excluded if their homes were renovated due to the moisture problems or if they had changed residence since the first questionnaire.

The IgE sensitization status (yes vs. no) of the child was determined by screening all available children's blood sample ($n = 387$) for 10 airborne allergens (Phadiatop[®], Pharmacia & Upjohn Diagnostics, Uppsala, Sweden); timothy, mugwort, birch, cat, horse, dog, house dust mites (*Dermatophagoides pteronyssinus* and *D. farinae*), and two moulds (*Penicillium*, *Cladosporium*). Cut-off value of 1.2 kUA/l was used to define IgE-positive status.

Dust sampling

During the period of October 2001 to April 2002, house dust samples from 390 homes were collected from the floor in the child's bedroom and the living room. The samples were collected from a representative area of hard floor and carpets (i.e., a room with 10 m² hard floors and 2 m² of carpet – sampling 0.5 m² of carpet and 2.5 m² of hard floor. Assessments made “with the eye” by inspector). The dust samples were collected on 90-mm membrane filters made of pure cellulose (VaccuMark disposable nozzle; Petersen Bach, Bjerringbro, Denmark) connected to a vacuum cleaner. House dust samples were packed in a separate plastic bag and then stored in a refrigerator for 2–3 days under standard temperature. Before weighing, the filter samples were conditioned at 23°C and 50% relative humidity.

Endotoxin analysis of house dust samples

Endotoxin was extracted from house dust at 50 mg/ml concentration by shaking for 1 h at 22°C in pyrogen-free water (pfw) with 0.05% Tween-20. Samples were then centrifuged at 600 g for 20 min, aliquot and four serial dilutions were prepared with pfw with Tween in depyrogenated borosilicate glass tubes. These dilutions were assayed in a temperature-controlled micro plate reader using the kinetic chromogenic *Limulus* amoebocyte lysate assay reading each well every 30 sec for 90 min at 405 nm. These values were compared to a 12-point standard curve to yield EU in the sample as previously described (Thorne 2000).

Statistical method

Endotoxin levels were best described by a natural-log (ln) transformed and expressed as geometric mean (GM). Dose-response associations between endotoxin concentrations and health effects were tested by factoring the endotoxin concentrations into quartiles and using simple and multiple regression analyses. For the regression model, both randomly selected one sibling from each pairs were first excluded and then included in the analyses, since this exclusion of siblings did not have significant influence on the results. To control for potential confounding, multivariate models were additionally adjusted for smoking, cleaning frequency, crowdedness and family asthma and allergy history. Lifestyle factors, building characteristics and socio-economic variables with a univariate association with endotoxin were subsequently

tested in multiple logistic regression models for including or excluding a variable from the final model. The regional ethics committee at the university hospital in Örebro approved the study.

Results

Distribution of endotoxin concentration levels in indoor dust

Dust samples from the living room and child's bedroom floors were available for 390 homes. Of these, eight samples from the bedroom and seven samples from the living room were not possible to analyze due to low sample weight or insufficient handling. Dust endotoxin levels in the child's bedroom and living rooms ranged from 479–188,000 EU/g dust and from 138–942,000 EU/g dust, respectively, with geometric mean of 5,700 and 6,100 EU/g dust (Table 1).

Predictors of endotoxin in indoor dust

Table 2 shows the geometric mean of endotoxin concentration levels for different building characteristics and behaviours in the family. Endotoxin levels were found to be significantly higher in homes where pets were kept indoors ($p=0.005$) and in homes with farming life ($p=0.020$). It was further found that homes with a higher frequency of vacuum cleaning had a higher concentration of endotoxin, but such an association was not found for wet cleaning frequency. There were no significant associations between endotoxin concentration levels and construction period, type of dwelling, smoking habits in the family, type of ventilation system, level of the child's bedroom (basement, first floor or higher in the house), flooring materials, family size or type of building and inspector observed odour and moisture problem. Furthermore, there was no significant association between the indoor relative humidity or temperature and endotoxin concentrations in dust (data not shown).

Association between endotoxin exposure and asthma/allergy in children

Table 3 gives the geometric mean concentration and 95% CI of endotoxin in the child's bedroom dust and living room dust by case and control status as well as doctor diagnosed asthma, rhinitis and eczema. It can be seen that there was no significant difference in geometric mean concentrations of endotoxin between cases and controls. However, when the sub-group of homes with no pet-keeping was

Table 1. Endotoxin (LPS) concentrations in dust from the child's bedroom and the living room in 390 homes.

Room	Homes		LPS in settled dust (EU/g dust)							
			Min–max	Percentile				Mean	Median	GM
	<i>n</i>	missing		25%	50%	75%	95%			
Child's bedroom	382	8	479–188,000	3,614	5,324	9,628	26,342	9,138	5,324	5,729
Living room	383	7	138–942,000	3,483	5,731	10,373	27,343	17,912	5,731	6,216

Table 2. Crude association between endotoxins (LPS) in indoor dust and different behaviour in the family as well as building characteristics.

Variable ¹	Child's bed-room				Living room			
	<i>n</i>	LPS GM (EU/g dust)	95% CI	<i>p</i> -value ²	<i>n</i>	LPS GM (EU/g dust)	95% CI	<i>p</i> -value ²
<i>Pet keeping</i> ^Q								
No pet keeping	200	5156	5659–5824	Ref	202	5631	4900–6471	Ref
Yes, pets are kept indoor	149	6741	5798–7836	0.010	148	6615	5659–7731	0.241
Yes, but pets are kept outdoor	28	5517	4120–5824	0.909	28	8902	5853–13540	0.042
<i>Farming</i> ^Q								
No	350	5551	5054–6097	Ref	351	6060	5440–6751	Ref
Yes	32	8095	5846–11209	0.021	32	8219	6353–10633	0.097
<i>Smoker in the family members</i> ^Q								
No	305	5550	5007–6152	Ref	305	6074	5391–6844	Ref
Yes	67	6593	5426–8012	0.149	67	6934	5709–8422	0.329
<i>Vacuum cleaning, child's bedroom (frequency)</i> ^Q								
1 time/week or more frequently	308	6076	5510–6700	Ref	308	6400	5705–7181	Ref
Less than 1 time/week	71	4585	3639–5776	0.016	72	5443	4362–6790	0.215
<i>Wet cleaning, child's bedroom (frequency)</i> ^Q								
1 time/ week or more frequently	116	6095	5254–7072	Ref	117	6152	5331–7099	Ref
Less than 1 time/week	261	5626	5019–6307	0.420	261	6223	5440–7119	0.918
<i>Construction period for the building</i> ^Q								
1984 onwards (ref)	54	5710	4448–7329	Ref	54	7428	5442–10140	Ref
1971–1983	101	5398	4578–6364	0.954	102	5848	4666–7329	0.307
1941–1970	121	5737	4862–6770	1.000	121	5595	4803–6518	0.176
Before 1941	106	6066	5089–7230	0.943	106	6789	5683–8110	0.880
<i>Type of building</i> ^Q								
Single family house	315	5848	5283–6473	Ref	316	6361	5687–7113	Ref
Chain house	23	4182	3010–5811	0.154	23	5210	2898–9364	0.581
Multi-family house	44	5836	4572–7449	1.000	44	5781	4669–7158	0.797
<i>Area of the home</i> [†]								
Downtown	33	4769	3504–6490	Ref	33	7363	4741–11437	Ref

(continued)

Table 2. (Continued).

Variable ¹	Child's bed-room				Living room			
	<i>n</i>	LPS GM (EU/g dust)	95% CI	<i>p</i> -value ²	<i>n</i>	LPS GM (EU/g dust)	95% CI	<i>p</i> -value ²
Suburban	251	5471	4894–6117	0.536	252	5803	5177–6507	0.279
Countryside	98	6856	5745–8183	0.064	98	7005	5617–8736	0.935
Family size (incl. children) ^Q								
1–4 members	267	5453	4908–6057	Ref	267	6212	5437–7096	Ref
5 members and more	114	6488	5441–7736	0.080	115	6236	5431–7160	0.972
Crowdedness person/m ^{2Q}								
1st quartile	100	5990	4774–7212	Ref	100	5274	4558–6102	Ref
2nd quartile	92	5690	4764–6795	0.961	93	5912	4890–7146	0.768
3rd quartile	85	6139	5075–7427	0.996	85	7165	5569–9218	0.096
4th quartile	92	5211	4309–6301	0.576	92	6885	5430–8730	0.159
Ventilation rate in the home (air change rate) ¹								
1st quartile	95	5084	4188–6171	Ref	94	5483	4339–6929	Ref
2nd quartile	91	5540	4634–6622	0.847	92	6327	5200–7698	0.637
3rd quartile	96	5681	4689–6882	0.718	97	6402	5112–8021	0.569
4th quartile	96	6608	5622–7768	0.105	96	6800	5840–7918	0.311
Sign of floor moisture ¹								
No remarks	354	5679	5163–6247	Ref	356	6234	5603–6937	Ref
Mild (grade 1 or 2)	25	6451	4722–8811	0.738	24	6350	4521–8920	0.995
Severe (grade 3)	3	5970	3800–9379	0.994	3	3671	2545–5295	0.589
Mold odour in the room ¹								
No remarks	234	5636	5033–6312	Ref	235	6745	5915–7692	Ref
Mild (grade 1 or 2)	86	6119	4956–7555	0.707	86	5322	4490–6308	0.112
Severe (grade 3)	62	5562	4496–6880	0.993	62	5658	4206–7613	0.379
Dampness ¹								
No remarks	286	5544	5024–6117	Ref	287	6160	5481–6923	Ref
Mild (grade 1 or 2)	81	6476	5163–8122	0.300	81	6190	4903–7815	0.999
Severe (grade 3)	15	5548	3134–9818	1.000	15	7572	5004–11456	0.679

¹Q = parental reports in a questionnaire; I = inspector observations at visit in the home. ²Test of difference made by using ANOVA test. The *p* < 0.05 level of significance is indicated in bold.

Table 3. Endotoxins (LPS) in indoor dust for healthy controls and children with doctor-diagnosed diseases expressed as geometric mean concentration in indoor dust and 95% CIs.

Case status	Child's bedrooms (EU/g dust)			Living rooms (EU/g dust)		
	Controls GM (95% CI) /N	Cases GM (95% CI) /N	<i>p</i> -value ¹	Controls GM (95% CI) /N	Cases GM (95% CI) /N	<i>p</i> -value ¹
<i>All homes</i>						
Case	5620 (4948–6382)/198	5928 (5221–6731)/194	0.554	5966 (5309–6704)/198	6631 (5632–7807)/195	0.291
Asthma		5818 (4925–6874)/119	0.740		6492 (5482–7688)/121	0.396
Rhinitis		6347 (5226–7709)/97	0.285		6368 (5193–7809)/97	0.553
Eczema		5375 (4568–6325)/126	0.665		6534 (5318–8029)/126	0.409
<i>Home with pets kept indoor</i>						
Case	6901 (5758–8270)/99	6434 (4900–8449)/50	0.662	6705 (5806–7744)/98	6440 (4458–9304)/50	0.808
Asthma		6634 (4853–9069)/32	0.829		6276 (4695–8390)/32	0.662
Rhinitis		6578 (4161–10399)/25	0.822		6127 (3650–10288)/25	0.642
Eczema		5756 (4041–8199)/34	0.331		6506 (3941–10739)/34	0.875
<i>Home without pets at all</i>						
Case	4504 (3767–5385)/86	5844 (4982–6855)/122	0.033	5139 (4282–6168)/87	6255 (5155–7588)/123	0.156
Asthma		5621 (4483–7048)/71	0.121		6062 (4867–7551)/73	0.245
Rhinitis		6209 (4946–7794)/63	0.026		6180 (4899–7796)/63	0.208
Eczema		5226 (4284–6375)/80	0.266		6252 (4954–7890)/81	0.183

¹Difference in GM concentration between controls and diseased children made by Student's *t*-test on log-transformed data(Note: Data on pet keeping status are reported by the family and categorized by pet in the residence, pet kept outdoor (excluded) and no pet keeping). The *p* < 0.05 level of significance is indicated in bold.

analyzed separately a significant association was found between a higher concentration of endotoxins and case status. Such an association was not found in the sub-group of homes with pet-keeping; in fact an opposite but non-significant association was indicated.

As shown in Table 4 (a) and (b) and Figure 1, in crude analyses including all homes, non-significant associations were found between quartiles of endotoxins and asthma/allergy. After adjustment for pets, significant associations were found for quartiles of endotoxins and asthma/allergy in all homes, except for eczema-control. In the stratified analyses, when restricted to non-pet-keeping homes, significant associations were found between quartiles of endotoxins and case status, asthma vs. control, rhinitis vs. control and eczema vs. control status even after adjustments for potential confounders. Such associations were not found in pet-keeping families. The significant associations between quartiles of endotoxins and health in non-pet keepers' families were only found for dust from the child's bedroom, not for the living room.

No association was found between endotoxin concentration levels and IgE sensitization against 10 common allergens (data not shown), both in crude data and stratified by pet-keeping status data. The association between endotoxin concentration levels and IgE sensitization was examined only among the cases (atopic vs. non-atopic), since IgE was very seldom present among the controls. The association between IgE sensitization among the cases and endotoxin concentration was still the same after the adjustment of smoking, cleaning habits, crowdedness and family asthma and allergy history.

Discussion

A major limitation of the current study lay in its case-control study design and the inclusion criteria for cases, especially when examining the effects of endotoxin on different diseases, i.e., asthma, rhinitis, and eczema where we could not separate these diseases. Furthermore, the case-control status meant that we compared the healthiest children with the most diseased ones. Finally, the study used a cross-sectional design which created difficulties in assessing if exposure came before health effects were seen.

We found house dust endotoxin levels in the child's bedroom and living room ranged from 479–188,000 EU/g dust and from 138–942,000 EU/g dust, respectively, where children stay most of their time indoor. A potential explanation for the differences of endotoxin concentrations between rooms might be that the living room is the most commonly used place for all family members and out source place of endotoxins. Endotoxin concentration levels in indoor dust, however, differed by home settings and different geographic locations. Differences in endotoxin concentration levels sampled from indoor dust at five locations within homes have been reported by Thorne et al. (2005) with geometric mean concentration ranges from 18.7 EU/mg in the bedding to 80.5 EU/mg in kitchen floor dust in 2,469 samples from US homes.

In a cross-national comparative study, Giovannangelo et al. (2007) found lower endotoxin concentrations in Swedish homes compared to homes in Germany and The Netherlands. A recent study by Thorne et al. (2008) examined predictors of endotoxin in a representative survey of 831 US households. The higher levels observed were likely due to the wide range of climates represented by the US as

Table 4. (A) Crude and adjusted associations between endotoxin (LPS) levels in quartiles in the child's bedroom and case status stratified for pet-keeping.

All homes				No pet-keeping indoors				Pet-keeping indoors						
LPS exposure in quartiles		Crude analysis		Adjusted analysis [§]		Crude analysis		Adjusted analysis [¶]		Crude analysis		Adjusted analysis [¶]		
Case-controls	Range (EU/g dust)	n	ORs	p-value	ORs	p-value	ORs	n	ORs	p-value	ORs	n	ORs	p-value
<i>Case vs. controls</i>														
1st quartile (Ref)	≤3615	98	1		1		1	54	1		1	34	1	
2nd quartile	3616–5315	98	1.39	0.253	1.83	0.277	1.69	54	1.69	0.179	1.86	32	0.63	0.385
3rd quartile	5316–9608	98	1.28	0.390	1.27	0.419	1.99	57	1.99	0.075	1.68	36	0.62	0.353
4th quartile	>9609	98	1.64	0.087	2.00	0.022	3.64	43	3.64	0.004	3.37	47	1.00	0.995
<i>Asthma vs. controls</i>														
1st quartile (Ref)	≤3615	81	1		1		1	44	1		1	28	1	
2nd quartile	3616–5315	77	1.35	0.368	1.44	0.294	1.58	40	1.58	0.312	1.92	28	0.65	0.516
3rd quartile	5316–9608	80	1.34	0.375	1.45	0.283	2.05	43	2.05	0.108	2.32	33	0.81	0.726
4th quartile	>9609	79	1.78	0.080	2.31	0.016	3.70	30	3.70	0.009	3.89	42	1.35	0.590
<i>Rhinitis-controls</i>														
1st quartile (Ref)	≤3615	78	1		1		1	43	1		1	29	1	
2nd quartile	3616–5315	74	1.37	0.359	1.39	0.356	1.71	40	1.71	0.247	1.83	28	0.57	0.384
3rd quartile	5316–9608	68	0.91	0.815	0.92	0.824	1.57	37	1.57	0.336	1.25	28	0.20	0.058
4th quartile	>9609	75	1.79	0.089	2.26	0.024	3.77	29	3.77	0.009	3.22	39	0.91	0.857
<i>Eczema-controls</i>														
1st quartile (Ref)	≤3615	85	1		1		1	46	1		1	32	1	
2nd quartile	3616–5315	86	1.53	0.178	1.49	0.218	2.12	49	2.12	0.075	2.172	29	0.49	0.498
3rd quartile	5316–9608	79	1.12	0.729	1.14	0.681	1.87	44	1.87	0.146	1.671	31	0.37	0.367
4th quartile	>9609	74	1.32	0.403	1.69	0.130	2.72	27	2.72	0.044	2.086	41	0.79	0.790

[§]Adj. for pet-keeping; [¶]Adj. for smoking, cleaning habits, crowdedness and family asthma and allergy history. The p < 0.05 level of significance is indicated in bold.

Table 4. (B) Crude and adjusted associations between endotoxin (LPS) levels in quartiles in the living room and case status stratified for pet-keeping.

LPS exposure in quartile			All homes				No pet-keeping				Pet-keeping					
			Crude analysis		Adjusted analysis [§]		Crude analysis		Adjusted analysis [¶]		Crude analysis		Adjusted analysis [¶]			
			n	ORs	p-value	ORs	p-value	n	ORs	p-value	ORs	p-value	n	ORs	p-value	ORs
Case-controls	Range (EU/g dust)															
1st quartile	≤3508	98	1		1		63	1		1		30	1			
2nd quartile,	3509–5833	99	0.98	0.941	1.02	0.946	51	1.44	0.346	1.20	0.682	38	0.59	0.299	0.58	0.395
3rd quartile	5834–10373	97	0.62	0.098	0.68	0.201	50	1.18	0.668	1.19	0.691	41	0.28	0.017	0.23	0.023
4th quartile	> 10374	99	1.11	0.725	1.22	0.493	46	1.33	0.473	1.34	0.499	39	0.71	0.494	0.42	0.159
<i>Asthma-controls</i>																
1st quartile	≤3508	74	1		1		47	1		1		23	1		1	
2nd quartile	3509–5833	83	1.27	0.468	1.35	0.368	41	1.86	0.151	1.60	0.338	33	0.73	0.607	0.47	0.343
3rd quartile	5834–10373	84	0.78	0.463	0.90	0.763	39	1.38	0.463	1.69	0.310	40	0.48	0.239	0.30	0.116
4th quartile	> 10374	78	1.28	0.463	1.48	0.252	33	1.34	0.523	1.59	0.371	34	0.95	0.934	0.35	0.170
<i>Rhinitis-controls</i>																
1st quartile	≤3508	71	1		1		44	1		1		24	1		1	
2nd quartile	3509–5833	74	1.06	0.866	1.18	0.647	36	1.73	0.235	1.31	0.600	33	0.64	0.452	0.64	0.618
3rd quartile	5834–10373	82	0.81	0.547	0.92	0.827	38	1.56	0.326	1.47	0.458	38	0.30	0.065	0.14	0.038
4th quartile	> 10374	68	1.01	0.998	1.10	0.793	32	1.50	0.393	1.38	0.535	28	0.33	0.112	0.09	0.022
<i>Eczema-controls</i>																
1st quartile	≤3508	80	1		1		53	1		1		24	1		1	
2nd quartile	3509–5833	80	0.95	0.872	1.01	0.973	38	1.21	0.657	1.02	0.959	34	0.72	0.572	0.49	0.318
3rd quartile	5834–10373	87	0.71	0.291	0.83	0.576	41	1.15	0.736	1.19	0.714	40	0.42	0.153	0.26	0.063
4th quartile	> 10374	78	1.04	0.893	1.21	0.564	36	1.21	0.662	1.24	0.649	34	0.83	0.751	0.40	0.196

[§]Adj. for pet keeping; [¶]Adj. for smoking, cleaning habits, crowdedness and family asthma and allergy history. The p < 0.05 level of significance is indicated in bold.

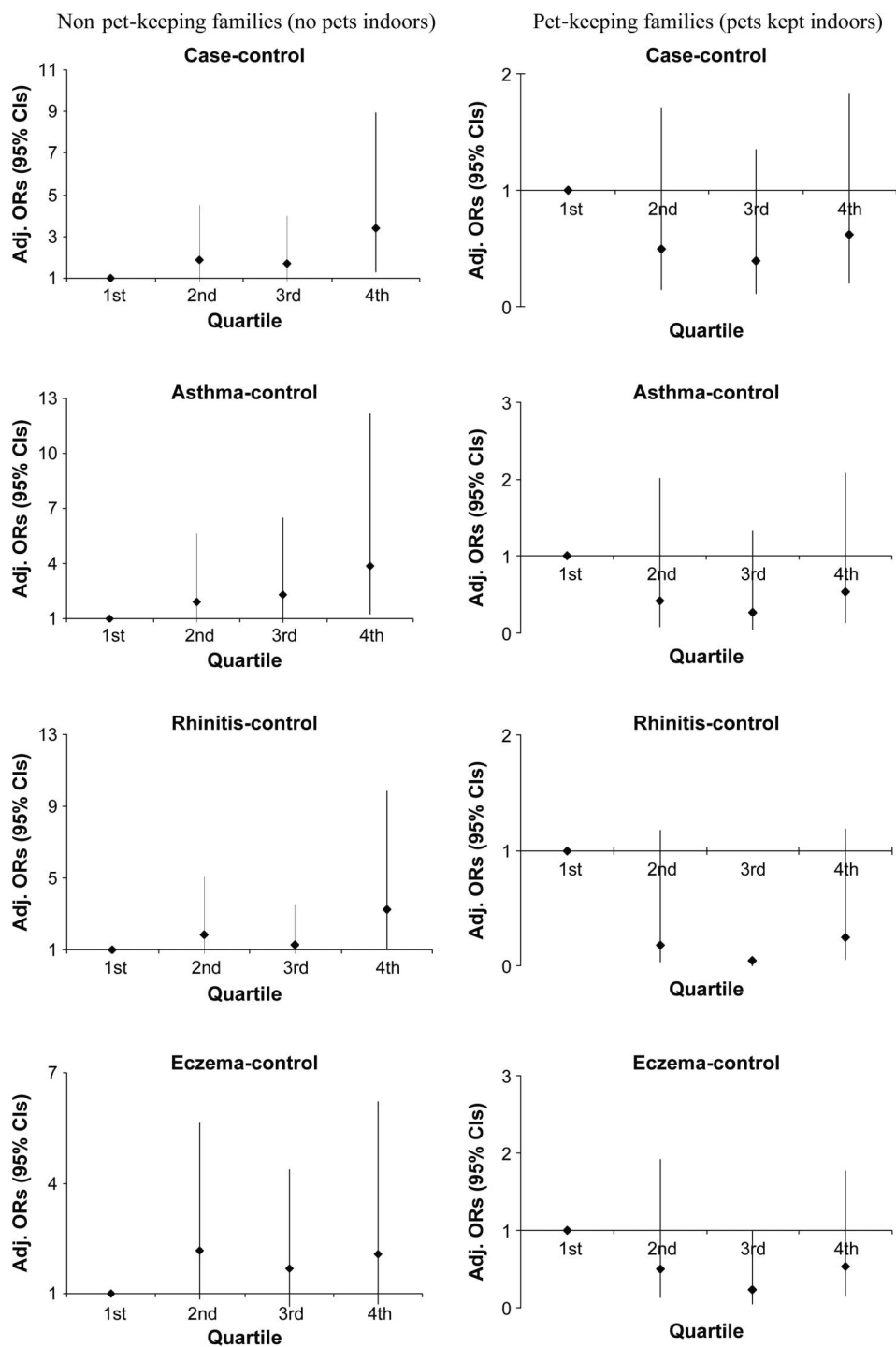


Figure 1. Associations between LPS exposure in quartiles (bedroom) and case status for pet-keeping (indoors) and non pet-keeping families expressed as adjusted odds ratio with 95% confidence interval.

compared to Sweden, the higher utilization of carpeted floors, and the inclusion in the study of homes experiencing cockroach problems. Further explanation for these differences might be that the study populations between countries differ in a number of factors such as household materials and cleaning habits, factors that have been reported to be predictors of endotoxin levels (Park et al. 2001; Waser et al. 2004; Giovannangelo et al. 2007). One important difference between the study by Thorne et al. (2008) and the current study was that reservoir dust was sieved before analyzing endotoxin in the US study but not in the present study. Sieving removes large debris and particles too big to become airborne and be inhaled. If it is not sieved, the collected dust particles are subjected to a biased exposure estimate as 1 particle of material (e.g., food debris, a bit of dog/cat faecal matter, or an insect carcass) can lead to a large exposure misclassification. However, sieving is time-consuming and increases study costs. Comparing endotoxin levels between studies is a difficult task since different dust collection methods and measurement of endotoxin concentration levels are used. Besides these, there are no commonly established analytical procedures for measuring endotoxin levels.

We found higher endotoxin concentration levels in floor dust in homes with indoor pet-keeping, in particular for dog keeping (data not shown), compared to homes with no indoor pet, or pets kept outside. Endotoxin concentration levels, however, were not significantly different between homes without pets and with other pets such as birds, rabbits and hamsters. The current findings on pet-keeping and endotoxin concentrations is consistent with previous studies which have identified pets as an important predictor of indoor endotoxin levels, and demonstrated that the presence of indoor pets, especially cats and dogs, increased the endotoxin levels in indoor dust (Bischof et al. 2000; Gereda et al. 2001; Heinrich et al. 2001; Waser et al. 2004; Thorne et al. 2008). Faecal contamination and pet foods might be the possible sources for indoor endotoxins. Also pets may carry different types of microbes from outdoors to indoor environments. Heinrich et al. (2001) found homes with the presence of dogs or cats for a year period have been shown to have poorer hygienic conditions with a higher load of endotoxins, compared to homes without indoor pets.

The study found that the endotoxin levels in homes with agricultural activities were significantly higher than in non-farming homes, which is in agreement with earlier findings in other studies (von Mutius et al. 2000; Braun-Fahrlander et al. 2002; Hyvärinen et al. 2006). It is likely that direct contact with farming animals or indirect contact from carrying dust (loaded with endotoxins) on clothes and shoes from the stables is a direct source of endotoxins. In addition, there are more endotoxins found in ambient air in rural areas than in urban areas (Mueller-Anneling et al. 2004; Thorne et al. 2009).

Cigarette smoking has been recognized as a source of endotoxin (Hasday et al. 1996, 1999; Park et al. 2001). The US National Survey of Endotoxin in Housing (Thorne et al. 2008) found a significant association of endotoxins with evidence of cigarette smoking in the household. In the present study, we also found a higher geometric mean concentration of endotoxin in homes with smokers, but this difference was not significant. This might be explained by the fact that a relatively low rate smoke indoors in Sweden which give us a low statistical power, especially if they have small children (Johansson et al. 2003, 2004).

The association between endotoxin levels and more frequent vacuum cleaning indoors is relatively contradictory and unclear. Findings from a study from

Germany by Bischof et al. (2000) were in contrast to our study's observation that less frequent vacuum cleaning was associated with higher levels of endotoxin in living room floor dust. A possible explanation of this difference may be that houses with pets are more often cleaned than non-pet-keeping houses; however, our study data did not demonstrate this. In other studies, there has not been an observed effect of cleaning habits on endotoxin concentration levels (Gereda et al. 2001; Wickens et al. 2003). An association between endotoxin concentration and a higher ventilation rate in the home – as indicated in the current study – may indicate that there are outdoor sources of endotoxin. However, Bischof et al. (2002) found that poor ventilation habits (fewer opened windows) were associated with increased endotoxin concentration levels in living room floor dust in Germany. In another study set in Germany, Gehring et al. (2004) found that regular ventilation significantly increased endotoxin concentration levels in the mother's mattress dust samples during the summer when outdoor endotoxin levels are high. These contradictory findings may be based on seasonal variation of ventilation as endotoxins often come from outdoor sources.

In this study we found no significant differences in exposures to endotoxin between healthy and symptomatic children. We found significant associations between endotoxins and asthma/allergy problem after adjusting for pet-keeping, except for eczema and healthy children. However, in homes with no indoor pets, significantly higher levels of endotoxin were found in homes of children diagnosed with a minimum of two possible symptoms of asthma, eczema and rhinitis (Table 3). This finding might be explained by the earlier study using DBH data, where Bornehag et al. (2003) found that pets are not randomly distributed in the population. A "healthy pet-keeping effect" was suggested meaning that families who have pets are those who can tolerate them, and that families where someone reacts or is allergic to pets more often avoid having pets. In a large population-based study set in the late 1980s, Brunekreef et al. (1992) found that families with members who suffered allergic symptoms admitted to having avoided pets. In a prospective birth-cohort (BAMSE) study in Sweden, Almqvist et al. (2003) found selective avoidance of pet ownership depended on the family history of allergic diseases. In addition, there may even be another pet-bias: families with allergic children may choose to have less contact with animals in places other than the home, and thus may bring home less endotoxin on clothing, suggested another pet-related behaviour.

The impact of health-related information about the consequences of pet-keeping and related allergies may also influence family's choice to avoid pets in when children are young. Information from doctors and health authorities has taught the general Swedish population that if they are going to have a baby they should avoid pets if there is a family history of pet allergy (Hesselmar et al. 1999). This means that children at risk of developing allergic diseases – due to asthma and allergy in the family – in general are less exposed to pets at home in Sweden. Our study's finding with the positive association between endotoxins and the presence of diseases in families without pets is not easy to interpret. One possibility is that children from non-pet-keeping families are more sensitive in general – i.e., the families avoid pets because of allergic problems from pets – and therefore maybe react to endotoxin exposure at lower concentrations compared to children from less sensitive families, i.e., families without an avoidance behaviour and/or family history.

Conclusion

This Swedish DBH data demonstrated that house dust endotoxin concentration levels depend on the location sampled within the home. Endotoxin concentration levels increased with pet-keeping and agricultural activities. More people in the family, higher ventilation rates and higher frequency of cleaning were also associated with endotoxin levels but to a lesser degree. Other building-related factors did not predict endotoxin levels indoors including moisture-related problems observed by professional inspectors. Endotoxin concentration levels in indoor dust were not associated with asthma and allergic diseases in the children. However, we found an association between endotoxins exposure and the presence of disease in the sub-group of families without indoor pets.

References

- Almqvist C, Egmar A-C, van Hage-Hamsten M, Berglind N, Pershagen G, Nordvall SL, Svartengren M, Hedlin G, Wickman M. 2003. Heredity, pet ownership, and confounding control in a population-based birth cohort. *J Allerg Clin Immuno*. 111(4):800–806.
- Bischof W, Koch A, Gehring U, Fahlbusch B, Heinrich J, Wichmann HE. 2000. Predictors of high endotoxin concentrations in German homes. *Proc Healthy Buildings*. 1:251–256.
- Bischof W, Koch A, Gehring U, Fahlbusch B, Wichmann HE, Heinrich J. 2002. Predictors of high endotoxin concentrations in the settled dust of German homes. *Indoor Air*. 12(1):2–9.
- Bornehag CG, Sundell J, Hagerhed L, Janson S, and the DBH study group. 2003. Pet keeping in early childhood and airway, nose and skin symptoms later in life. *Allergy*. 58(9):939–944.
- Bornehag CG, Sundell J, Sigsgaard T, Janson S. 2006. Potential self-selection bias in a nested case-control study on indoor environmental factors and their association with asthma and allergic symptoms among pre-school children. *Scand J Public Health*. 34(5):534–543.
- Bornehag CG, Sundell J, Sigsgaard T. 2004. Dampness in buildings and health (DBH): report from an ongoing epidemiological investigation on the association between indoor environmental factors and health effects among children in Sweden. *Indoor Air*. 14(7):59–66.
- Braun-Fahrlander C, Riedler J, Herz U, Eder W, Waser M, Grize L, Maisch S, Carr D, Gerlach F, Bufe A, Lauener RP, Schierl R, Renz H, Nowak D, von Mutius E. 2002. Environmental exposure to endotoxin and its relation to asthma in school-age children. *N Engl J Med*. 347(12):869–877.
- Brunekreef B, Hoek G, Groot B. 1992. Pets, allergy and respiratory symptoms in children. *Int J Epidemiol*. 21(2):338–342.
- Gehring U, Bischof W, Borte M, Herbarth O, Wichmann HE, Heinrich J. 2004. Levels and predictors of endotoxin in mattress dust samples from East and West German homes. *Indoor Air*. 14(4):284–292.
- Gereda JE, Klinnert MD, Prince MR, Leung DYM, Liu AH. 2001. Metropolitan home living conditions associated with indoor endotoxin levels. *J Allergy Clin Immunol*. 107(5):790–796.
- Gereda JE, Leung DYM, Thatayatikom A, Streib JE, Price MR, Klinnert MD, Liu AH. 2000. Relation between house-dust endotoxin exposure, type 1 T-cell development, and allergen sensitisation in infants at high risk of asthma. *Lancet*. 355(9216):1680–1683.
- Giovannangelo M, Gehring U, Nordling E, Oldenwening M, Terpstra G, Bellander T, Hoek G, Heinrich J, Brunekreef B. 2007. Determinants of house dust endotoxin in three European countries – the AIRALLERG study. *Indoor Air*. 17(1):70–79.
- Hasday JD, Bascom R, Costa JJ, Fitzgerald T, Dubin W. 1999. Bacterial endotoxin is an active component of cigarette smoke. *Chest*. 115(3):829–835.
- Hasday JD, Fhibin W, Fitzgerald T, Bascom R. 1996. Cigarettes are a rich source of bacterial endotoxin. *Chest*. 109(3):63S–64S.

- Heinrich J, Gehring U, Douwes J, Koch A, Fahlbusch B, Bischof W, Wichmann HE. 2001. Pets and vermin are associated with high endotoxin levels in house dust. *Clin and Exp Allergy*. 31(12):1839–1845.
- Hesselmar B, Åberg N, Åberg B, Eriksson B, Björkstén B. 1999. Does early exposure to cat or dog protect against later allergy development? *Clin and Exp Allergy*. 29(5):611–617.
- Hyvärinen A, Rönönen M, Tiittanen P, Laitinen S, Nevalainen A, Pekkanen J. 2006. Dust sampling methods for endotoxin – an essential, but underestimated issue. *Indoor Air*. 16(1):20–27.
- Johansson A, Halling A, Hermansson G. 2003. Indoor and outdoor smoking: Impact on children's health. *Euro J Public Health*. 13(1):61–66.
- Johansson AK, Hermansson G, Ludvigsson J. 2004. How should parents protect their children from environmental tobacco-smoke exposure in the home? *Pediatrics*. 113(4):e291–295.
- Martinez FD, Holt PG. 1999. Role of microbial burden in aetiology of allergy and asthma. *Lancet*. 354 (Suppl. 2):SII12–15.
- Michel O, Kips J, Duchateau J, Vertongen F, Robert L, Collet H, Pauwels R and Sergysels R. 1996. Severity of asthma is related to endotoxin in house dust. *Am J Respir Crit Care Med*. 154(6):1641–1646.
- Mueller-Anneling L, Avol E, Peters JM, Thorne PS. 2004. Ambient endotoxin concentrations in PM10 from Southern California. *Environ Health Perspect*. 112(5):583–588.
- Park JH, Gold DR, Spiegelman DL, Burge HA, Milton DK. 2001. House dust endotoxin and wheeze in the first year of life. *Am J Respir Crit Care Med*. 63(2):322–328.
- Tavernier G, Fletcher G, Gee I, Watson A, Blacklock G, Francis H, Fletcher A, Frank T, Frank P, Pickering CA, Niven R. 2006. IPEADAM study: indoor endotoxin exposure, family status, and some housing characteristics in English children. *J Allergy Clin Immunol*. 117(3):656–662.
- Thorne PS, Ansley A, Perry SS. 2009. Concentrations of bioaerosols, odors and hydrogen sulfide inside and downwind from two types of swine livestock operations. *J Occup Environ Hyg*. 6(4):211–220.
- Thorne PS, Cohn RD, Mav D, Arbes SJ, Zeldin DC. 2008. Predictors of endotoxin levels in U.S. housing. *Environmental Health Perspectives*. National Institute of Health. U.S. Department of Health and Human Services. pp 1–39.
- Thorne PS, Kulhankova K, Yin M, Cohn R, Arbes jr SJ, Zeldin DC. 2005. Endotoxin exposure is a risk factor for asthma: The National Survey of Endotoxin in United States Housing. *Am J Resp Crit Care Med*. 172:1371–1377.
- Thorne PS. 2000. Inhalation toxicology models of endotoxin- and bioaerosol-induced inflammation. *Toxicology*. 152(1–3):13–23.
- von Mutius E, Braun-Fahrlander C, Schierl R, Riedler J, Ehlermann S, Maisch S, Waser M, Nowak D. 2000. Exposure to endotoxin or other bacterial components might protect against the development of atopy. *Clin Exp Allergy*. 30(9):1230–1234.
- Waser M, Scheirl R, von Mutius E, Maisch S, Carr D, Riedler J, Eder W, Schreuer M, Nowak D, Braun-Fahrlander C. 2004. Determinants of endotoxin levels in living environments of farmers' children and their peers from rural areas. *Clin Exp Allergy*. 34(3):389–397.
- Wickens K, Douwes J, Siebers R, Fitzharris P, Wouters I, Doekes G, Mason K, Heartfield M, Cunningham M, Carne J. 2003. Determinants of endotoxin levels in carpets in New Zealand homes. *Indoor Air*. 13(2):128–135.

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